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STUDIES IN MGULD METABOLITE CHEMISTRY

by

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Department of Chemistry

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada.
August, 1967
ABSTRACT

Part I of the thesis describes the study of the yellow pigments produced by *Claviceps purpurea* (ergot). In addition to ergoflavin, five other pigments have been characterized and structural proposals have been made for these. Two of these pigments, secalonic acids B and C are dimeric biphenyl derivatives of a tetrahydroxanthone unit, having the gross structure XLIV. Secalonic acid B is a symmetrical dimer of unit LXXV, and secalonic acid C is a combination of one secalonic acid A unit LXXIV with one secalonic acid B unit LXXV. Ergochrysins A and B are biphenyl derivatives LXXXIV and LXXXV, each consisting of one ergoflavin unit joined to a unit of secalonic acid A or B, respectively. Ergoxanthin has been shown to have the gross structure of a lactonized seco-ergochrysin, XCIII.

Part II of the thesis describes a study of the functional groups of the antibiotic primycin, a metabolite of *Streptomyces primycini*. Primycin is a monosubstituted guanidine derivative (as the sulphate salt). It possesses two carbon-carbon double bonds and a reactive keto function, as well as a number of hydroxyl groups and six or seven
C-methyls. Alkaline fusion of primycin gives n-caproic acid. Acidic hydrolysis yields D (-) arabinose and an aglycone, norprimycin hydrochloride, C$_{53}$H$_{102}$N$_{3}$O$_{14}$Cl. Alkaline hydrolysis studies are described.
ACKNOWLEDGMENTS

I should like to thank my supervisor, Professor P. de Mayo, for suggesting the problems and for his guidance and encouragement during their solution.

Thanks are also due to Professor J.B. Stothers for his aid with the n.m.r. spectroscopy described herein, and to Dr. Y.S. Chen and Dr. M. Hirst for their contributions to the chemistry of the ergot pigments, and primycin, respectively.

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GENERAL INTRODUCTION

MOULD METABOLITES

Those compounds generally defined by the organic chemist as natural products are the so-called secondary metabolites, the primary metabolites being fats, carbohydrates, and proteins. Many of these secondary metabolites serve no known function to the organism that produces them, and have been described as "metabolic accidents" (1). Some may be important, however, as protection agents against disease or predators, and it has been suggested (2) that some play a role in the oxidation-reduction mechanisms of the organism. Such compounds have long attracted the attention of scientists on account of the challenge presented in the enormous diversity of structures, and the importance of some of these metabolites in medicine.

The moulds, or fungi, are well known for the production of secondary metabolites, many of which are valuable antibiotics. The miracle drug, penicillin, was first obtained from a culture of Penicillium notatum (3). The actinomycetes, microorganisms which resemble bacteria in some respects, as well as possessing mould-like properties such as the production of mycelia, have been the source of numerous
important antibiotics (4), including streptomycin (3), chloramphenicol (4), the tetracyclines (5), macrolides (6), and actinomycins (7).

The diversity of structures found in mould metabolites is almost without limit, but some trends may be noted. Steroids and the terpenoids, while produced by moulds, are not as wide-spread and diversified as is the case in the higher forms of life, such as the animal kingdom, or plants possessing chlorophyll (8). The most common steroid in moulds is ergosterol, and several closely related steroids, whereas cholesterol is not found, although several of the postulated progenitors (9) of this compound, such as lanosterol, have been isolated from fungal sources.

In addition, alkaloids, wide-spread among the higher plants (10), are less common amongst moulds, nitrogenous compounds being for the most part limited to amino sugars, with the exception of the ergot alkaloids.

Prevalent amongst mould metabolites are phenolic, and highly oxygenated alicyclic compounds, exemplified by the tetracyclines (5), and the numerous anthraquinone derivatives (11). Examination of the oxygenation patterns of these compounds has led to the postulation, primarily by Birch (12, 13), of a biogenetic scheme based on the cyclization of poly-α-keto chains derived by linear condensation of acetic acid, and the term "acetogenins" has been coined (11)
to characterize such compounds. The study of mould metabolites is thus largely a study of acetogenins.

**Biosynthesis of Acetogenins**

The first step in the biosynthesis of acetogenins is the formation, from acetic acid, of acetoacetyl coenzyme A. As has been shown for fatty acid synthesis, acetyl coenzyme A is carboxylated through the action of biotin, adenosine triphosphate, and magnesium ion (14), leading to malonyl coenzyme A, the active methylene compound in the subsequent Claisen condensation. In fatty acid biosynthesis, the malonyl residue is then transferred to a new enzyme system (15), fatty acid synthetase, and then condensation with a second molecule of acetyl coenzyme A occurs, with concomitant decarboxylation. Subsequent reduction of a keto group, dehydration, and hydrogenation forms the chain extended carboxy enzyme system, which then transfers the fatty acid residue back to coenzyme A.

In acetogenin biosynthesis, however, some modification of this scheme is necessary, since the polyacetaate chain produced is not reduced during chain extension. Hendrixson (11) has suggested the involvement of a polyacetylsynthetase, viewed as a linear succession of sulfhydryl sites, which hold the malonyl residues in close proximity enabling a succession of Claisen condensations. These sulfhydryl
Fig 1 Utilization of Acetate in Biosynthesis
groups then stabilize the resultant keto groups by hydrogen bonding. The reactions involved are outlined in Fig. 1. The product obtained from a series of such condensations is a polyacetyl chain, bearing keto groups on alternate carbons.

Alternatively, condensation of acetoacetyl coenzyme A with another molecule of acetyl coenzyme A may occur by attack at a carbonyl of the former to yield ω-hydroxy-ω-methyl glutaryl coenzyme A. Reduction of this produces mevalonic acid, the precursor of the terpenoids. Reviews of this process are available (9, 11), and it will not be further discussed here.

Cyclization of a polyacetate chain I may occur by two routes, A or B, route A leading to the acylphloroglucinols II and B forming the orsellinic acid derivatives III. In general it is found that most metabolites of the lower organisms (fungi and lichens) originate by the orsellinic acid cyclization B. Aside from these two fundamental routes, it may be expected that cyclizations in long chains could occur in more than one way by preliminary folding of the chain in different manners. For example, one mode of folding of the seven-unit polyacetyl chain IV leads to griseofulvin V, and griseoxanthone VI, whereas an alternative folding VII leads to alternariol VIII (11).

The introduction of 'extra' functions into acetogenins may occur by substitution at methylene carbons prior to
Acylphloroglucinols

Orsellinic acids
cyclization. Groups commonly involved in such substitutions are methyl, methylene, hydroxyl, and glycosidyl, as well as isopentenyl although this substituent is largely restricted to metabolites of higher organisms than the fungi. Insertion of oxygen is involved in the biogenesis of the numerous anthraquinonoid derivatives, exemplified by the biogenesis of endocrocin (11), IX.

Another mechanism whereby methyl substituents can appear in a mould metabolite is by the utilization of propionate in place of acetate in the linear condensation process. Carboxylation of propionate is known (14), and its utilization would lead to methyl groups on alternate carbons of the polyketo chain. The macrolide erythromycin X has been shown to arise from propionate by labelling experiments (16), and labelled methionine was not incorporated into the skeleton, although it formed the methyls in the sugars attached.

Conceivably other acids than acetic may comprise the terminus of the polyacetate chain. The only ones observed, however, are a few benzoic and cinnamic acids, bearing patterns of ring hydroxylation paralleling that in shikimic acid, their precursor. The shikimate derived acids are not acetogenins, as their precursor is sugar-derived. The large class of plant pigments named the flavonoids is derived by utilization of shikimic acid, and the details of their biosynthesis may be found elsewhere (11, 17). It should be
noted that phenyl-C$_3$ units derived from shikimic acid are rare in microbial metabolites.

Many of the fungal metabolites are dimeric compounds, apparently derived by oxidative coupling of the monomeric phenolic compounds, either before or after cyclization of the polyacetate chain. Examples of this type of compound are some pigments isolated by Shibata's group (18), and the ergot pigments, to be discussed in detail later.
PART I

THE ERGOT PIGMENTS
(a) **Introduction to Ergot**

Ergot, botanically known as *Claviceps purpurea*, is a fungus which thrives principally on rye. Rye ergot (*Secale cornutum*) consists of brown-violet, horn-shaped sclerotia which project from the ripe ears of infected rye in place of the rye grains.

Although the sclerotia are quite conspicuous, their injurious effects on health went long unnoticed (19). Ingestion of substantial quantities of ergot may lead to the affliction known as ergotism, which may take the gangrenous form, and/or the convulsive form, the latter being particularly accentuated by a deficiency of vitamin A in the victim. Ergot has, over the centuries, been responsible for the deaths of many thousands of people, many of whom regarded their fate as some kind of divine punishment. In fact, the medieval "epidemics" termed St. Anthony's fire, feu sacré, etc. were apparently caused by the consumption of ergot.

More recently, it has been recognized that the unpleasant effects are due to the presence in the fungus of a series of closely related alkaloids. Six separate alkaloids (and their C-8 epimers) have been characterized and their structures solved, largely due to the efforts of Stoll et al (20).

These alkaloids, XI and XII, are all derivatives of D-lysergic acid XIII, the synthetic precursor of the in-
famous hallucinatory drug lysergic acid diethylamide-25 (LSD) XIV (21). Ergometrine XII has medicinal importance because of its oxytocic effects (21). Alkaline hydrolysis of the alkaloids gives lysergic acid and isolysergic acid, the C-8 epimer; these two compounds are readily interconvertible even by hot water. The alkaloids based on isolysergic acid are practically physiologically inert (their names end in -ine, e.g. ergotamine). Laboratory cultivation of *Claviceps purpurea* gives only small amounts of the aforementioned alkaloids, and instead leads to the production of a new series of bases based on the clavine nucleus XV, with the exception of chanoclavine XVI (22).

In addition to the pigments to be described later in detail, numerous other compounds are found in ergot (19, 20, 22, 23). Included amongst these are vitamin D₂ XVII, ergosterol XVIII, the carbohydrate trehalose XIX, and numerous bases including ergothioneine XX. Recently Whalley et al (24) have obtained from this fungus two aliphatic amides, called cerebrins, of the interesting structures XXI and XXII.

The diversity of structure amongst these secondary metabolites of *Claviceps purpurea* is indeed a testimony to the powers of synthesis of this lowly mould.
Ergotamine \( \text{CH}_3 \) \( \text{CH}_2\text{C}_6\text{H}_5 \)
Ergosine \( \text{CH}_3 \) \( \text{CH}_2\text{CH(CH}_3\text{)}_2 \)
Ergokrystine \( \text{CH(CH}_3\text{)}_2 \) \( \text{CH}_2\text{C}_6\text{H}_5 \)
Ergokryptine \( \text{CH(CH}_3\text{)}_2 \) \( \text{CH}_2\text{CH(CH}_3\text{)}_2 \)
Ergocornine \( \text{CH(CH}_3\text{)}_2 \) \( \text{CH(CH}_3\text{)}_2 \)

Ergometrine \( \text{NHCHCH}_2\text{OH} \)
Lysergic acid \( \text{OH} \)
Lysergic acid diethylamide \( \text{N(C}_2\text{H}_5\text{)}_2 \)
\[ R', R'' = H, CH_3, CH_2OH, OH \]
\[ R''' = H \text{ or } OH \]
(b) **Introduction to the Pigments**

The study of the pigments comprising 1-2% by weight of the sclerotia of ergot has a history of nearly one hundred years. However, as much of the early work was uninformative, it will be dealt with briefly. In 1877, Dragendorff and Podwyssotski (25) isolated a violet compound called sclererythrin, the occurrence of which is limited to the walls of the cortical hyphae. Its characteristic visible spectrum has frequently been utilized in spectroscopic detection of ergot (26, 27, 28). Other early isolated pigments were scleriodin (25, 29), sclerocrystallin (25), scleroxanthin (25, 29) and ergoxanthein (30, 31), all of which were poorly characterized and whose structures remain unknown.

In 1897, Jacobi (32) reported on a yellow crystalline pigment, termed ergochrysin, which was considered neutral and assigned the formula C_{21}H_{22}O_{9}. Hydrolysis gave an acid, C_{21}H_{24}O_{10} indicating that ergochrysin possessed a lactonic function.

Kraft (33), in 1906, extracted from ergot a new yellow pigment named secalonic acid. This material analyzed for C_{14}H_{16}O_{6}, gave a red-brown colour with ferric chloride, and dissolved in aqueous sodium carbonate with effervescence. On prolonged treatment with alkali, secalonic acid gave, apparently, a dicarboxylic acid, and was considered, therefore, like Jacobi's (32) ergochrysin, a lactone.
Later Barger (31) reported the isolation of a pigment preparation assumed to be ergochrysin. Moreover, this author suggested that ergochrysin, secalonic acid, and sclerocrystallin were identical, a view supported only by molecular weight determinations.

An additional yellow ergot dye was obtained in 1912 by Freeborn (34), working in Barger's laboratory, as yellow needles, m.p. 338° (dec.). The compound, named ergoflavin, analyzed for $\text{C}_{15}\text{H}_{14}\text{O}_7\cdot\text{H}_2\text{O}$ in elemental analysis and molecular weight studies, and gave a green colour with ferric chloride. Acetylation with acetic anhydride and sulphuric acid gave a tetraacetate, m.p. 231°, from which ergoflavin could be regenerated on saponification. In a later study of ergoflavin, Barger (31) found that dilute alkali afforded a colourless, crystalline hydrate, $\text{C}_{15}\text{H}_{16}\text{O}_8$, from which ergoflavin could be readily obtained upon dehydrating. Furthermore, whereas alkaline fusion had led to unidentified phenolic material (34), boiling 50% sodium hydroxide produced no further change in ergoflavin beyond the hydrated stage. In this respect, therefore, ergoflavin differed fundamentally from ergochrysin (8) and secalonic acid (33), as these pigments were extensively decomposed in alkaline solution.

In 1932, Bergmann (35) reported his studies on some ergot pigments. The $\text{C}_{15}$ formula was confirmed for ergoflavin (34), and five active hydrogens were detectable by the Zerewitinoff method. Acetylation with acetic anhydride
and pyridine furnished a pentaacetate, m.p. 244°, which is presumably the same as Freeborn's acetate (34). From the behavior in alkaline solution, Bergmann concluded that ergoflavin possessed a lactonic function, thus apparently relating the pigment in this respect to ergochrysin (32) and secalonic acid (33).

More fruitful, fortunately, were his studies on the structure of ergochrysin. Bergmann, like Barger (31), concluded that ergochrysin and secalonic acid were identical, but on molecular weight (Rast) and analytical evidence the C\textsubscript{48}H\textsubscript{48}O\textsubscript{12} formula (33) was doubled to C\textsubscript{28}H\textsubscript{28}O\textsubscript{12}. With acetic anhydride and pyridine, a colourless acetate, m.p. 240°, considered to be a decaacetate, was obtained. Upon distillation from zinc dust, or simply by heating, Bergmann isolated an "acid", m.p. 92°, C\textsubscript{9}H\textsubscript{10}O\textsubscript{3}, which exhibited a violet ferric chloride colour, and gave a monoacetate. However, its structure was not elucidated. Under alkaline fusion conditions were produced oxalic acid, acetic acid, 3-hydroxy-5-methylbenzoic acid XXIII, resorcinol, and 2,4,2',4'-tetrahydroxydiphenyl XXIV. Bergmann established that this latter product was not an artifact derived by dimerization of resorcinol since under the alkaline fusion conditions, resorcinol gave only a small amount of 3,5,3',5'-tetrahydroxydiphenyl XXV. A nitro derivative formulated as C\textsubscript{16}H\textsubscript{15}NO\textsubscript{9} was obtained with cold nitric acid. The transformations achieved by Bergmann are summarized in Fig. 2.
Fig 2 Transformations Achieved by W. Bergmann
Later Stoll et al. (36), in an investigation of Hungarian ergot, isolated secalonic acid together with a very similar pigment differing only in melting point, and optical rotation, and having somewhat lower solubility. Secalonic acid and the new pigment, chrysergonic acid, were considered to be different from ergochrysin (31, 32, 35). They analyzed for $\text{C}_31\text{H}_{30-32}\text{O}_{14}$ and $\text{C}_32\text{H}_{30-32}\text{O}_{14}$ respectively, possessed two methoxyls and 6-7 active hydrogens (Zerewitinoff). In pyridine their specific rotations underwent changes reaching terminal values after several days at room temperature; the same occurred in aqueous alkali but faster. The ultraviolet spectra indicated that secalonic and chrysergonic acid were very closely related, as did the parallelism in the chemical behavior. Thus, under vigorous acetylation conditions, colourless products, m.p. 205-206°, $\text{C}_{17}\text{H}_{16}\text{O}_7$, and m.p. 237-246°, $\text{C}_{15}\text{H}_{16}\text{O}_7$ respectively were formed. Strangely, however, Stoll has reported that these compounds possessed no acetyl functions, although the methoxyls were retained. Milder acetylation gave acetates containing ca. 40% acetyl. From alkaline fusion of both pigments were isolated $2,4,2',4'$-tetrahydroxybiphenyl XXIV, and methylsuccinic acid.

The next important contribution to the chemistry of the ergot pigments was made by Whalley et al. in 1958 (37), concerning the structure of ergoflavin. The pigment was considered to have the formula $\text{C}_{30}\text{H}_{26}\text{O}_{14}$, possessed two C-methyls but no methoxyl group (34), and could not be readily
hydrogenated. The green ferric chloride colour (34) was attributed to an o-hydroxycarbonyl system. Potassium hydroxide fusion gave acetic acid, methylsuccinic acid, and the familiar 2,4,2',4'-tetrahydroxybiphenyl XXIV, while potassium permanganate oxidation afforded only methylsuccinic acid. Ergoflavinic acid, C_{30}H_{30}O_{16}, the product of aqueous alkali (Barger's "hydrate" (31)), having υ max. 1740 cm⁻¹, could be converted into a dimethyl ester with diazomethane, but attempted methylation or acetylation led instead to the corresponding ergoflavin derivatives. Whalley considered this behavior indicative of two γ-lactonic functions in the molecule, this view being supported by the infrared spectrum, υ max. 1790 cm⁻¹.

Upon acetylation, ergoflavin formed a hexaacetate, m.p. 248-249°, undoubtedly identical with Freeborn's (34) and Bergmann's (35) acetates, having no hydroxyl absorption in the infrared spectrum. However, two of the hydroxyls in ergoflavin were apparently hindered, since only a tetratosylate could be prepared. Under methylaing conditions, ergoflavin tetramethyl ether was obtained, from which a diacetate was prepared. Hence, apparently ergoflavin possessed 4 phenolic and 2 alcoholic hydroxyls.

Further structural information was gleaned by Whalley in the subjection of ergoflavin tetramethyl ether to barium hydroxide degradation. A dimethyl ether of 3,3'-diacetyl-2,4,2',4'-tetrahydroxybiphenyl XXVI was obtained, which after methylation and reduction gave XXVII identical with a
product synthesized by Ullmann coupling 3-ethyl-1-iodo-2,4-dimethoxybenzene XXVIII. This observation indicated the presence of carbonyl functions at the positions corresponding to C-3 and C-3' in ergoflavin; however, the usual carbonyl derivatives could not be prepared. The occurrence in pairs of the functional groups in ergoflavin was considered indicative that the molecule is symmetrical, produced in nature by oxidative coupling of two identical units. The above described transformations are summarized in Fig. 3.

In 1961, Franck et al (41, 42) reported the isolation of secalonic acid and chrysergonic acid, together with two red ergot pigments previously isolated by this group, endocrocin IX and clavorubin XXIX, later revised to XXX (38, 39, 40, 42). These latter pigments are of interest in connection with biosynthesis of the yellow ergot pigments, (see later).

Franck considered that his secalonic acid and chrysergonic acid were the same compound, and that the differences between the two preparations observed by Stoll (36) were due to impurities and to the instability of the pigments in pyridine solution. Franck's secalonic acid analyzed for C_{52}H_{50-34}O_{14}, possessed two C-methyls, six active hydrogens, and two methoxyls, apparently present as aliphatic carboxymethoxyl functions from the infrared spectrum, \nu_{\text{max}} 1738 cm^{-1}, and from the loss of methoxyls on alkaline treatment.
Fig 3 Ergoflavin Transformations
Acetylation of secalonic acid in the cold gave a colourless secalonic acid hexaacetate, m.p. 150°, whereas vigorous acetylation apparently involved more complex, but uninterpreted, transformations. With diazomethane, a non-acidic dimethyl secalonic acid, m.p. 236-238°, green ferric colour, was produced.

The ultraviolet spectrum of secalonic acid was very similar to that of 2-methyl-5-hydroxychromone XXXI; however, the acidity, $pK_{\text{DMF}} = 8.5$, required the placing herein of two other hydroxyl groups. These could not be in the aromatic rings of the biphenyl system, since the n.m.r. spectrum ($\text{NaOD/D}_2 \text{O}$) showed a total of two pairs of ortho aromatic protons. Allowing C-6 or C-8 for the biphenyl bridge no other position was available. Ultraviolet and acidity correlations suggested that a hydroxyl should be placed at C-3, and thus the skeleton of secalonic acid was considered to be XXXII. The biphenyl coupling was assigned to the 8,8'-positions on account of the failure of secalonic acid to give an indophenol dye (Gibbs reaction) with 2,6-dibromobenzoquinonechloroimide XXXIII (44, 45). The simplicity of the aromatic absorption in the n.m.r. spectrum indicated that secalonic acid was a symmetrical molecule. Hence, part-structure XXXIV was suggested for secalonic acid (and chrysergonic acid).
(c) **Structures of the Pigments**

As is evident in the foregoing discussion, the yellow ergot pigments are all structurally closely related, and there has been much controversy over the identity or non-identity of several of the pigments. Part of this confusion is due to the difficulty of separation of such closely related substances. However, the recent development of thin layer chromatography (t.l.c.) as an analytical tool has, at least in part, eliminated such difficulty. In addition, we had at our disposal the use of nuclear magnetic resonance spectroscopy (n.m.r.), which had only been first applied to this problem by Franck (42) in 1962, and even then its full potential was not exploited.

The crude pigment mixture from a Portuguese ergot drug was supplied by Sandoz (Switzerland). As received, the material had been defatted with petrol, and freed from alkaloids by precipitating from methanolic solution with aqueous tartaric acid. After preliminary removal of some chloroform-insoluble material, the crude mixture showed, on t.l.c., the presence of six main pigments with much smaller amounts of others. These pigments were numbered in position on the plate I - VI, the smaller number referring to the faster moving component. Solution of the mixture in

*I wish to thank Dr. H. Schwartz (Sandoz) for a generous gift of the pigment mixture.*
chloroform-benzene led to the separation of the pigment designated VI. The remaining material was applied to a column of silicic acid, the chromatogram developed, extruded and divided into sections. From these sections, by crystallization or by repeated chromatography, the pure pigments were eventually isolated. Approximately 28% of the crude pigment weight was eventually isolated in pure crystalline form in the following proportions: I: 4.5%; II: 48.8%; III: 7.1%; IV: 8.3%; V: 22.4%; VI: 8.9%.

Pigment VI, m.p. $\geq 360^\circ$, analyzed for $C_{30}H_{26}O_{14}$, and this formulation was confirmed mass spectrometrically\(^a\). It was identified as ergoflavin by infrared and ultraviolet spectra and optical rotation data, as well as by conversion into the hexaacetate (34, 35, 37), the tetramethyl ether (37) and ergoflavinic acid (35, 37). Although some structural evidence had been accumulated, before definitive conclusions could be drawn, we were kindly informed by Professor W.B. Whalley of his chemical findings (46), and also, in collaboration with Dr. G.A. Sim, of the results of the x-ray investigation leading to XXXV as the stereostructure of ergoflavin (47, 48). Therefore, only the results obtained by n.m.r. spectroscopic examination of ergoflavin and its derivatives will be discussed, since these were essential to our structural elucidation of the other pigments.

\(^a\) I would like to thank Prof. K. Biemann (M.I.T.) for the mass spectra reported in this work.
The 60 Mc.p.s. spectrum of ergoflavin in pyridine (Fig. 4) clearly revealed the symmetrical nature of the structure. It exhibited four rather broad signals at 1.17 (6H), 2.16 (6H), 4.56 (2H), and 5.55 (2H) p.p.m. (All peak positions are given relative to internal TMS). The highest field band ($\nu_{1/2}$-9 c.p.s.) is to be attributed to two identical CH$_3$CH groups. The two low field multiplets are assigned to the protons on C-5 and C-8 respectively. By double irradiation, it could be demonstrated that both these nuclei were spin-coupled to protons absorbing at 2.15 p.p.m. Thus, upon irradiating 144 c.p.s. to higher field, the 4.56 p.p.m. multiplet exhibited some sharpening, although still slightly broadened. The behavior of the 5.55 p.p.m. multiplet was analogous on irradiating 198 c.p.s. upfield. Unfortunately no significant change could be observed upon reversing this process, i.e., irradiating the low field protons (individually) while examining the 2.16 p.p.m. multiplet.
Fig 4

NMR Spectrum of Ergoflavin in Pyridine
The n.m.r. spectrum of ergoflavin was also studied in dimethyl sulphoxide-d₆ solution. In this medium the protons lost in the pyridine solvent bands could be observed. The presence of a simple AB pattern due to two identical pairs of ortho-aromatic protons (Table 2) attests to the symmetrical nature of ergoflavin. Also a sharp singlet (2H) at 11.77 p.p.m. is presumably due to the hydrogen-bonded phenolic protons. Double irradiation experiments analogous to those conducted on the pyridine solution were carried out, and similar results obtained.

Clearly defined doublets (J \approx 6 c.p.s.) are observed for the CH₃CH groups in the spectrum of ergoflavin tetramethyl ether (in CDCl₃). Observation of this band while irradiating 62 c.p.s. downfield showed the doublet collapsing to a broadened singlet. In addition, irradiation 189 c.p.s. upfield caused the 5.40 p.p.m. doublet to collapse, whereas irradiation 117.5 c.p.s. to high field of the doublet at 4.17 p.p.m. led to its collapse. Similar observations were made on ergoflavin hexaacetate.

The fastest moving pigment, designated pigment I,

This solvent was chosen because all ergot pigments, except pigment I, were readily soluble therein, and no decomposition was expected in contrast to pyridine solutions (which were examined immediately after adding the solvent).
m.p. 254°, resembled but differed from the secalonic acid isolated by Kraft (33), Stoll (34), and Franck (41, 42), and also from that later reported (49) by Whalley. Subsequently Franck et al (50) revealed that their secalonic acid was a mixture of two closely related substances termed secalonic acids A and B. In addition they found the yellow pigment preparation named chrysergonic acid (36, 40) to be a 2:1 mixture of secalonic acids A and B. That pigment I was identical with secalonic acid B was indicated by a comparison of their physical properties (see experimental). Moreover, on t.l.c. (both in the t.l.c. system commonly employed by us, and using Franck's method (50) involving oxalic acid-imregnated Kieselgel), pigment I showed a single zone of \( R_f \) value equal to that of the faster running component in an authentic sample of chrysergonic acid, supplied to us by Professor Whalley\(^a\). Franck's nomenclature has been adopted during this study.

Secalonic acid B has the empirical formula \( \text{C}_{32}\text{H}_{50}\text{O}_{14} \), as shown by analysis and, later, mass spectrum (50). It contains two methoxyls, which the infrared spectrum indicates are present as esters.

Secalonic acid B gives a red-brown colour with ferric chloride, in contrast to the green colour exhibited by ergoflavin. The acidic (42) function was, therefore, probably enolic. That secalonic acid B was, in fact, an enolized \( \beta \)-diketone was shown in the following manner.

\(^a\) At this time, Prof. Whalley informed us that his secalonic acid was secalonic acid A.
A reaction typical of enolizable β-diketones is the formation of pyrazoles (51) upon treatment with hydrazine derivatives, e.g. XXXVI $\rightarrow$ XXXVII. On treatment of this pigment with methanolic hydrazine hydrate, an amorphous product was obtained, having now a green ferric chloride colour. Although this material could not be obtained crystalline, nor its purity established firmly, some observations were made concerning its composition. In contrast to expectations, the material was basic. Pyrazoles are weakly-basic compounds (52), since protonation would destroy the aromatic nature of the ring. Thus it was suspected that the carbomethoxyl function(s) had reacted with hydrazine, forming the basic (53) hydrazide, XXXVIII. Support for this view was found in the fact that the crude product reacted readily with acetone producing material which exhibited in the n.m.r. spectrum (CDCl$_3$) two (broad) singlets at 2.23 and 2.49 p.p.m. This may be compared with acetone semicarbazone XXXIX, which has singlets at 2.04 and 2.16 p.p.m. However, the change in the ferric chloride colour indicated that a pyrazole had been formed, and this was confirmed by permanganate oxidation of the hydrazine product to pyrazole - 3,4,5-tricarboxylic acid XL (54, 55), isolated in 42% yield based on two β-dicarbonyl systems in the pigment. This is considered good evidence for the presence of two such systems since the yield of this
acid was only ca. 60% in its synthesis by the oxidation of 3,4,5-trimethyl pyrazole XXXVII.

It was concluded that secalonic acid B was an enolized $\alpha$-diketone, in contrast to the earlier report of Franck (42) that "secalonic acid" was an enolic $\alpha$-diketone.

The enolic nature of secalonic acid B was further characterized by its reaction with diazomethane to give a dimethyl ether, m.p. 237-239°. For their dimethyl secalonic acid, Franck et al (42) record m.p. 236-238°, and thus these compounds are likely identical. The dimethyl ether gave a green ferric chloride colour, and on treatment with dilute hydrochloric acid overnight at room temperature was completely converted into secalonic acid B, thus indicating its enolic nature.

Acetylation of secalonic acid B gave a mixture from which a colourless, optically inactive product, m.p. 203-205°, could be isolated by preparative t.l.c. Bearing in mind the previous obtention of the biphenyl XXIV through alkaline fusion of secalonic acid (35, 36), this acetylation product was assigned the structure XLI, based on the following observations$^2$.

The compound analyzed for $C_{44}H_{38}O_{18}$, with two methoxyls present. A benzophenone-like structure was indicated by a

$^2$ The same compound has been obtained by Whalley by acetylation of secalonic acid A, and the same structural conclusions were reached (49).
comparison of the ultraviolet spectrum of the acetate (Fig. 5) with that of o-benzoyl-benzoic acid methyl ester XLII. Although the long wave-length maxima do not coincide, the overall shapes of the curves are impressively similar. The infrared spectrum possessed bands at 1770, 1723, and 1675 cm⁻¹, as expected for phenolic acetates, aliphatic carbomethoxyl, and benzophenone carbonyl, respectively. The n.m.r. spectrum (CDCl₃) possessed a pair of identical AB quartets = 7.10, 7.45 p.p.m., Jₐ₋ₜ = 8.3 c.p.s., indicating the presence of two pairs of ortho aromatic protons. In addition to these signals, the n.m.r. spectrum showed two multiplets at 7.19 and 7.56 p.p.m., each with a half-band width (N₀/₂) of ca. 3 c.p.s. The latter observation indicated the presence of two pairs of aromatic protons in a meta orientation, and these would not be present in the same rings as the above ortho protons, since the AB pattern was not further split. Also visible in the n.m.r. spectrum of this acetate were singlets for the acetyl groups at 1.81 (6H), 1.92 (6H), and 1.99 (6H) p.p.m., and singlets at 2.41 (6H) and 3.69 (6H) p.p.m. for aromatic methyls and carbomethoxyls, respectively.

Since secalonic acid B has been shown to be an enolic -diketone, it follows that on acetylation the enolic hydroxyl is acetylated. This requires that the acetoxy group in ring A be ortho to the ketonic bridge. The general properties of the pigment (see later) also dictate that the carbomethoxyl group be situated ortho to this bridge. The absence of a second pair of vicinal aromatic protons, but
Ultraviolet Spectra: Bibenzophenone Acetate (—)
o-Benzoylbenzoic acid methyl ester (—–)

Fig 5
presence of meta protons, requires that the methyl group in this ring be para to the bridge. Hence, only structure XLI can be written for this acetate. This is presumably the same compound, m.p. 205-206°, as previously obtained by Stoll (36) by vigorous acetylation of secalonic acid, although it was reported that the compound possessed no acetyl groups.

From these facts, part-structure XLIII follows for secalonic acid B leaving the location of the remaining hydroxyl function uncertain. In pyridine solution the n.m.r. spectrum of secalonic acid B (Fig. 6a) showed a doublet at 1.17 p.p.m. (6H). Also a sharp 6-proton singlet appears at 3.57 p.p.m., a signal at 4.55 p.p.m. (2H) with \( W_{1/2} = 3.5 \) c.p.s.) and a complex series of bands in the region 120-190 c.p.s. The relative simplicity indicated the symmetry of the structure. The highest field band \( W_{1/2} = 9 \) c.p.s.) is attributed to two identical \( \text{CH}_3\text{CH} \) groups and the sharp singlet arises from two identical methoxyl groups. The lowest field band may be attributed to the C-5 proton (ergoflavin numbering) which is only weakly coupled to a neighbouring nucleus. Double irradiation showed that this proton and the methyl protons are coupled to the same proton absorbing near 2.50 p.p.m., since simultaneous irradiation 122 c.p.s. upfield from the methine absorption served to sharpen this peak (at 4.55 p.p.m.), whilst irradiation 80 c.p.s. to low field of the methyl absorption gave an analogous result. These observations require that the methyl and hydroxyl
Fig 6  N.M.R. Spectra in Pyridine
groups be vicinal. Thus, these spectral data are consistent with part-structure XLIII, with the hydroxyl group placed at C-5 or C-7 (the numbering being the same as in ergoflavin see XXXV and XLIII). In either case, the obtention of methyl-
succinic acid (36, 49) with alkali may be readily rational-
ized, as may the formation of the benzophenone XLI on acety-
lation. Of formulations XLIV and XLV, the former is preferred for two reasons.

First, the methylene protons of the secalonic acid unit appear at 2.54 p.p.m., significantly lower field than the cor-
responding absorption in ergoflavin (2.16 p.p.m.). This is consistent with the presence of an allylic methylene group in the molecule. In addition, the proton on the carbon bearing the hydroxyl group in secalonic acid B has virtually the same chemical shift (4.55 p.p.m.) as the corresponding proton in ergoflavin (4.56 p.p.m.) indicating that the environment of this proton is the same in the two compounds.

Secondly, in the infrared spectra of secalonic acid, the allylic methylene absorption (56) appears at lower frequency (near 1435 cm\(^{-1}\)) than the corresponding absorption in ergo-
flavin (1475 cm\(^{-1}\), both in KBr\(^{\circ}\).

The absence of a signal for a proton at C-10 in the n.m.r. spectrum of secalonic acid B requires that this position be filled by the remaining functional group - the carbo-
methoxy group - as was concluded from the bibenzophenone acetate spectrum. Thus, structure XLIV is established for
secalonic acid B.

Support for this structural assignment was derived from a study of the ultraviolet spectral characteristics of secalonic acid B in 0.1 N alkali at room temperature. The quantitative results are given in Table 3. Immediately after dissolution in alkali, the ultraviolet spectrum (Fig. 7) exhibits the bathochromic shift typical of enolic compounds (57), the intense absorption maximum being shifted from 340 m$\mu$ to 364 m$\mu$. However, the spectrum changed over a period of 80 hours. At this point the absorption, in alkali or upon acidification, was essentially the same as the corresponding absorption of ergoflavinic acid XLVI which is that of a di-chromanone, and may be compared with that of 2-hydroxy-6-methoxyacetophenone XLVII. Thus the extension of conjugation through the $\beta$-dicarbonyl section of the molecule was being removed. Although this could be explained by simple hydration of the enolic system, this should be a reversible process. Since secalonic acid B cannot be recovered from alkaline solution, the spectral changes are more likely due to cleavage of the $\beta$-dicarbonyl system, e.g. XLVIII$\rightarrow$XLIX. It should be pointed out that the long wave-length maximum of XLVII does not coincide with that of ergoflavinic acid XLVI, $\lambda_{H_2O}$ 362 m$\mu$.$\text{max}$

However, diphenyl coupling commonly causes small red-shifts
Fig 7 Absorption Spectra

Secalonic acid B (—), after alkali added (・・・), after 80 hr. in alkali (—), acidified after 80 hr (—.—).

Ergoflavinic acid (・・・), 2-Hydroxy-6-methoxy-acetophenone (・・・).
in such compounds even though the chromanone chromophores are insulated from each other. For example, hemiergoflavin L, the ozonolysis/decarboxylation product of ergoflavin (58), has $\lambda_{\text{max}}$ 370 m$\mu$ (c.f. 380 m$\mu$ for ergoflavin); moreover, 3,3'-diacetyl-4,4'-dihydroxy-2,2'-dimethoxydiphenyl LI exhibits $\lambda_{\text{max}}$ 346 m$\mu$ as against $\lambda_{\text{max}}$ 335 m$\mu$ for the monomer XLVII (58).

Following the alkaline treatment titrimetrically revealed the presence in the product of six acidic functions. Two of these were phenolic, as shown by titration with two different indicators. With methyl red (pH range 4.4-6.0), an uptake of ca. 4 equivalents of alkali was indicated, whereas using phenolphthalein (pH range 8.3-10), somewhat greater than 5 equivalents were apparently consumed. This difference is attributable to the lower acidity of the phenolic functions, which would not be ionized in the pH range where methyl red exhibits its colour change.

The structure XLIV deduced for secalonic acid B corresponds to that suggested by Whalley (49) for "secalonic acid", and it allows ready rationalization of the several reactions observed involving especially ring C. The genesis of the bi-benzophenone XLI on acetylation may be depicted as LII$\rightarrow$LI$\text{III}$ $\rightarrow$ XLI (58). Also the formation of methyl m-cresotinate LIV on pyrolysis (49) or in a mass spectrometer (59) is in accord with formula XLIV for this pigment, as is the isola-
tion of methylsuccinic acid LV and α-hydroxy-β-methylglutaric acid LVI (59), as well as the corresponding ketone LVII (60) on ozonolysis.

A discussion of the stereochemistry of secalonic acid B will be postponed until the structure of secalonic acid C has been discussed, since the two compounds are stereoisomers and their stereochemistry is best discussed as a unit.

Pigment II resembles secalonic acid B in general properties, but differs in some respects, and also differs from secalonic acid A reported by Franck (50). Following Franck's nomenclature, this pigment has been termed secalonic acid C (61).

The ultraviolet spectra of acids B and C are identical, and their infrared spectra (Fig. 8) suggest a close relationship. In agreement with this, vigorous acetylation of secalonic acid C gave a mixture from which the bibenzophenone XLI could be isolated.

The enolic nature of this pigment manifested itself in many ways. It gave a red-brown colour with ferric chloride, and in the infrared spectrum, very intense bands at 1592 and 1615 cm\(^{-1}\) suggested the presence of an enolized α-diketonic system. In agreement, reaction with hydrazine followed by permanganate oxidation gave pyrazole-3,4,5-tricarboxylic acid XL, in approximately the same yield as did secalonic acid B.

A dimethyl ether, m.p. 257-259°, could be prepared with
Fig 8  Infrared Spectra
diazomethane, and was readily hydrolyzed back to the starting material under mild acidic conditions, thereby indicating its enolic nature.

Additionally, a second acetate, formed together with the bibenzophenone, was obtained on milder acetylation of secalonic acid C. This compound was different from Franck's secalonic acid hexaacetate (42), and was apparently an enol hexaacetate since under mild acidic treatment it was converted into another (unstable) product, having a red ferric chloride colour and intense absorption in the infrared spectrum at 1610cm⁻¹.

The proton resonance spectrum of secalonic acid C in pyridine solution (Fig. 6b) indicates that the pigment contains one secalonic acid B unit, since signals appear at 1.16, 3.53 and 4.54 p.p.m. (in relative intensities 3:6:1) together with a complex pattern at 120-190 c.p.s. resembling that in the spectrum of acid B. The behavior on double irradiation paralleled that of secalonic acid B as well. However, the second unit differs from the 'B' unit in that absorption attributable to the C-5 protons now appears at higher field, 4.12 p.p.m., and is a doublet with J~10 c.p.s. Also the methyl signal appears as a broadened band at 1.16 p.p.m., as compared with a doublet in secalonic acid B. Double irradiation experiments showed that these protons (1.16 and 4.12 p.p.m.) were mutually coupled to a proton at 2.78 p.p.m.
It was evident at this point that the C-5 and C-6 protons in the 'C' unit were situated trans-diaxially, as required (62) by the large coupling constant.

Additional evidence of the differences between the two substituted diphenyl units in secalonic acid C was observed in the n.m.r. spectra in DMSO-$d_6$, and in CDCl$_3$ solution*. In DMSO-$d_6$, the aromatic protons give rise to two overlapping AB patterns (Table 2) showing that the two units for this acid are different. Also there appear two sharp singlets at 11.60 and 11.72 p.p.m. attributable to two, slightly different phenolic protons, deshielded by hydrogen bonding with the chromanone carbonyls. These appear at 11.69 and 11.84 p.p.m. in CDCl$_3$ solution. That these are the phenolic protons rather than the enolic ones is inferred by analogy with the phenolic protons of ergoflavin, having a chemical shift of 11.77 p.p.m. in DMSO-$d_6$ and 11.84 p.p.m. in CDCl$_3$. Support for this conclusion will be presented later (pg. 88).

However, in dimethyl secalonic acid C, these phenolic protons absorb at 13.12 and 13.29 p.p.m. Four part-structures can be considered for this derivative LVIII, LIX or the unsymmetrical LX or LXI. While it is not possible to specify with certainty which of these enol ether structures is correct, LIX is suggested as being most likely from the following considerations. In the unsymmetrical structures, the phenolic protons would be expected to have chemical

* As mentioned previously, secalonic acid B was too insoluble in this medium for determination of the spectrum.
shifts differing by a large amount, whereas the difference is only 0.17 p.p.m. The enolic form LXII conjugated with the benzene ring would be expected to be more stable (63), although the influence of the phenolic group has not been studied. Moreover it cannot be stated with certainty which of the two enolic forms would react more rapidly with diazomethane (64). However, the downfield shift of the phenolic protons in the ether could be accounted for in terms of increased nucleophilicity of the ring B ether oxygen as a result of replacement of a proton by a methyl group, as in LIX.

The enolic protons of secalonic acid C appear at 13.75 and 13.98 p.p.m. in CDCl$_3$. However, in the n.m.r. spectrum in DMSO-$d_6$, no absorption for these enolic protons was found, although a search was made as low as 50 p.p.m. below TMS. In an attempt to resolve this anomaly, the changes in the low field region of the spectrum were observed while adding DMSO dropwise to a solution of secalonic acid C in CDCl$_3$. On adding dry$^*$ DMSO, the enolic absorption bands, sharp at first, slowly underwent broadening without change in chemical shift, so that when the solvent mixture composition became roughly 1:1, these peaks had a height/$W_{1/2}$ ratio of $\approx 1$, as opposed to $\approx 20$ in pure CDCl$_3$. They still, however, integrated correctly for

$^*$ Vacuum distilled from calcium hydride.
two protons. The phenolic proton absorption did not undergo any change during this treatment (allowing for dilution effects). Surprising, however, was the observation that addition of DMSO containing a trace of water caused rapid disappearance of the enolic absorption. Presumably the DMSO-d$_6$ used in this study was wet, even when freshly opened, and this accounts for the failure to observe this absorption. Although this effect does not seem to have been reported in the literature, it has been observed in this laboratory for some (but not all) model $\alpha$-dicarbonyl compounds; no explanation can be advanced for it at this time.

In addition to the changes observed in the enolic proton absorption on adding DMSO, there appeared two new signals (1H each) between the carbomethoxyl band and the aromatic bands. The chemical shifts of these were dependent on the solvent composition, and reached final values of $\approx 5.6$ and $\approx 5.8$ p.p.m. at high (ca. 50%) DMSO concentration. These are assigned to the C-5 hydroxyl protons, which absorb at 2.92 and 3.18 p.p.m. in CDCl$_3$ (disappearing on equilibration with D$_2$O).

There is, then, a multitude of evidences that secalonic acid C is an unsymmetrical dichromanone derivative of formula XLIV, and it seemed likely that it could be constructed of one unit of secalonic acid B coupled to a unit of the secalonic acid A described by Franck (50) and also studied by Whalley et al (49, 65). Support for this hypothesis was
obtained through a comparison of optical rotation data, and n.m.r. spectra of the three acids. Since secalonic acid A was not available in this laboratory, the data of Franck (60) have been used.

Comparison of the specific rotations in pyridine and chloroform of secalonic acids A, B, and C shows that $[\alpha]_D$ of acid C is roughly the average of those of acids A and B:

<table>
<thead>
<tr>
<th>Compound</th>
<th>$[\alpha]_D$ (pyridine)</th>
<th>$[\alpha]_D$ (chloroform)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>our data</td>
<td>Franck</td>
</tr>
<tr>
<td>Secalonic acid A</td>
<td>—</td>
<td>-202°</td>
</tr>
<tr>
<td>Secalonic acid B</td>
<td>+183°</td>
<td>+202°</td>
</tr>
<tr>
<td>Secalonic acid C</td>
<td>-14°</td>
<td>-10°</td>
</tr>
</tbody>
</table>

Franck has also studied the circular dichroism (CD) of these pigments, and has observed that the CD of secalonic acid C is very close to a summation of those of acids A and B.

Similarly, a comparison by Franck (60) of the n.m.r. spectra of the three compounds in pyridine-$d_5$ showed that the spectrum of secalonic acid C corresponded very well to a summation of the spectra of A and B.

There remains to be described the stereochemistry of the secalonic acids. Knowledge of the stereochemistry of secalonic acids A and B immediately gives that of C. Since most of the work in this connection has been carried out by Franck, the arguments involved will be but briefly presented.

The structural formula XLIV derived for the secalonic
acids possesses three centers of asymmetry - C-5, C-6 and C-10 - making a total of eight possible stereostructures for each unit, and sixteen for the unsymmetrical secalonic acid C. However, C-9, as in LII, does not constitute a center of asymmetry, as earlier suggested (49), since the \( \alpha \)-dicarbonyl system has been shown to be essentially completely enolized.

The absolute configuration at C-6 has been established for secalonic acids A and B (59) through the isolation of optically active methylsuccinic acid on permanganate degradation. The results and conclusions are tabulated below:

<table>
<thead>
<tr>
<th>Compound</th>
<th>MethyIsuccinic acid</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secalonic acid A</td>
<td>R(+) LXV</td>
<td>R LXIV</td>
</tr>
<tr>
<td>Secalonic acid B</td>
<td>S(-) LXVII</td>
<td>S LXVI</td>
</tr>
</tbody>
</table>

The configuration at the carbinol center, C-5, could be ascertained for secalonic acid A from the n.m.r. spectrum in pyridine. The C-5 proton shows a doublet at 4.18 p.p.m.; the coupling constant of 10.5 c.p.s. requires that this proton and the neighbouring C-6 proton be situated trans-diaxially (62). This is in accord (67) with its absorption at higher field than the corresponding (equatorial) proton in ergoflav in 4.60 p.p.m. Thus, the stereochemistry of ring C in secalonic acid A can be expanded to LXVIII. Franck (60) has confirmed this assignment by establishing the absolute con-
figuration of the ozone-derived α-hydroxy-β-methylglutaric acid as LXIX.

For secalonic acid B the situation is not so simple. The C-5 proton has a half-band width of only ~4 c.p.s. indicating a dihedral angle with the C-6 proton of ca. 60°. Therefore, three stereochemical possibilities exist for ring C in this pigment, namely LXX - LXXII. Franck has eliminated LXX on the theory that the diaxially substituted ring would flip to the diequatorial conformer, but of course this is impossible when ring C is fused to the chromanone ring B. Although firm evidence is not available, configuration LXXII is favoured since the chemical shift of the C-5 proton is almost identical with that of the corresponding proton in ergoflavin LXXIII, indicating a similar chemical environment.

The final asymmetric center to be clarified is that bearing the carbomethoxy group, i.e. C-10. The configuration of this center in secalonic acids A and B was obtained by Franck (59, 60) with the aid of CD and mass spectrometry. Ergoflavin and secalonic acid B show a positive Cotton effect at 330 mλ, whereas secalonic acid A exhibits a negative Cotton effect here. Studies on model compounds indicated that the configuration at C-10 had the majority of the influence on the nature of the circular dichroism. Therefore, secalonic acid B must have the same configuration at C-10 as does ergoflavin, namely LXXV, and secalonic acid A the opposite configuration LXXIV. In the mass spectra of acids A and B, the
Ergoflavin

Secalonic acid A

Secalonic acid B
ratio of the peak at m/e 579 (M - COOCH₃) to the molecular ion is greater for secalonic acid A than for secalonic acid B, a fact considered by Franck (60) as supporting evidence that in acid A, the carbomethoxy1 and hydroxyl groups have the less stable cis-orientation, whereas in B they are trans-oriented.

The question of the position of biphenyl coupling will be discussed as a unit after the description of the other pigments.

Pigments III and V appeared to be closely related. They analyzed for C₃₁H₂₈O₁₄, exhibited red-brown ferric chloride colours, and possessed a single methoxy1 group. In the infra-red spectra (Fig. 9), these pigments have absorption for &omicron; -lactone, aliphatic carbomethoxy1, and &sigma;-dicarbonyl functions. Their ultraviolet spectra are identical. Also the ultraviolet spectra of solutions in alkali (or on acidification) after 80 hours were identical with the spectrum of ergoflavinic acid. Thus it seemed likely that we were dealing with dichromanone pigments similar in nature to those previously discussed.

In a comparison of these pigments with those previously reported, it became evident that pigment V was the substance ergochrysin (35, 49), and this was confirmed by direct comparison with an authentic sample supplied by Professor Whalley.

In the proton resonance spectra, it was apparent that
Fig. 9  Infrared Spectra (KBr)
Fig 10  N.M.R. Spectra in Pyridine

A  Pigment III (Ergochrysin B)
B  Pigment V (Ergochrysin A)
both pigment III and pigment V contained ergoflavin units. The observed signals for the spectrum of pigment III in pyridine (Fig. 10) appear at 1.17 (6H), 2.21 (1H), 3.57 (3H), 4.54 (2H) and 5.53 (1H) p.p.m. together with a broad pattern 130-170 c.p.s. Thus, all of the signals present in the n.m.r. spectrum of ergoflavin are found in the spectrum of pigment III, and their behavior on double irradiation paralleled the behavior observed for ergoflavin. From a comparison of the spectra in Fig. 10, the second unit in pigment III corresponds to that in secalonic acid B. The chemical shift for the methine proton in this unit at 2.54 p.p.m. was determined by double irradiation experiments analogous to those performed on secalonic acid B.

Further support for the presence of an ergoflavin unit in pigment III was derived from the n.m.r. spectrum in DMSO-d$_6$ solution, in which the four bands characteristic of the aliphatic protons in an ergoflavin unit are found at 1.03, 2.03, 4.18 and 5.26 p.p.m. On double irradiation they exhibited the expected behavior (cf. ergoflavin). The aromatic protons appear as two separate AB patterns (Table 2). One of these clearly arises from the ergoflavin unit, while the other corresponds closely with one of those observed for secalonic acid C. Additionally, a singlet due to two phenolic protons is found at 11.76 p.p.m. However, like secalonic acid C, pigment III shows no signal attributable to an enolic proton in DMSO-d$_6$ solution.
Pigment V in pyridine exhibits absorption at 1.17 (6H), 2.27 (1H), 2.85 (1H), 3.57 (3H), 4.18 (1H, doublet, \( J \approx 9.6 \) c.p.s.), 4.60 (1H) and 5.56 (1H) p.p.m. Thus, like pigment III, this pigment appeared to possess one ergoflavin unit, and this was supported by decoupling experiments. In addition, the second unit of pigment V appeared to be the same as the second unit of secalonic acid C, namely the A unit. This could be seen by comparison of the n.m.r. spectra of secalonic acid C and pigment V in pyridine and DMSO-\( d_6 \), and was upheld by the appropriate decoupling behavior. In DMSO-\( d_6 \), the aromatic protons of pigment V appear as two overlapping AB patterns, whose parameters agree closely with the appropriate patterns in the ergoflavin and secalonic acid C spectra (Table 2). Similarly the phenolic hydroxyls here show two bands, corresponding to the ergoflavin (11.79 p.p.m.) and secalonic A (11.69 p.p.m.) units.

From these considerations, pigment III and V could tentatively be assigned the gross structure LXXVI, and it seemed likely that pigment III possessed one unit of secalonic acid B, whereas pigment V possessed one unit of secalonic acid A. Chemical confirmation of these conclusions was then sought.

The \( \alpha \)-diketonic system, already signalled by the ferric reaction, infrared and ultraviolet spectra, was confirmed in both pigments as before by treatment with hydrazine and oxidation. This gave the pyrazole-3,4,5-tricarboxylic acid XL
obtained from the secalonic acids, but in approximately half the yield, indicating the presence of but one such unit. Under these conditions, ergoflavin gave no detectable amount of this degradation product.

Vigorous acetylation of pigments III and V gave the same hexaacetate. Based on the behavior of the secalonic acids, it was expected that this compound would have structure LXXVII, and the following evidence supports this. The infrared spectrum shows the expected absorption bands, and the ultraviolet spectrum appears approximately as a superimposition of the spectra of the bibenzophenone XLI and ergoflavin hexaacetate:

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (mμ)</th>
<th>ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergoflavin hexaacetate</td>
<td>238</td>
<td>43,500</td>
</tr>
<tr>
<td></td>
<td>260 (infl.)</td>
<td>34,000</td>
</tr>
<tr>
<td></td>
<td>339</td>
<td>6,300</td>
</tr>
<tr>
<td>Bibenzophenone XLI</td>
<td>234</td>
<td>36,000</td>
</tr>
<tr>
<td></td>
<td>289 (sh.)</td>
<td>8,350</td>
</tr>
<tr>
<td>Acetate from pigment III and V</td>
<td>238</td>
<td>32,900</td>
</tr>
<tr>
<td></td>
<td>264 (infl.)</td>
<td>18,700</td>
</tr>
<tr>
<td></td>
<td>294 (infl.)</td>
<td>5,450</td>
</tr>
<tr>
<td></td>
<td>333</td>
<td>2,000</td>
</tr>
</tbody>
</table>

The n.m.r. spectrum (CDCl<sub>3</sub>) of the acetate exhibited bands for the aliphatic protons at 1.00 (J ~ 6 c.p.s.; CH<sub>3</sub>CH-), 1.55, 1.97, 2.02, 2.14, 2.18 (six CH<sub>3</sub>COO), 2.39 (CH<sub>3</sub>-Ar), 3.69 (CH<sub>3</sub>O), 5.03, 5.77 p.p.m. (J ~ 5 c.p.s., two CH-0Ac), as well as a complex pattern between 100-150 c.p.s.
(3H). The absorption in the aromatic region (Fig. 11) was informative since the pattern observed for the bibenzophenone XLI is duplicated together with an additional AB quartet which must arise from the aromatic protons of the ergoflavin portion of this molecule. The absorption at unusually high field (1.55 p.p.m.) by one of the acetyl groups requires comment, since neither bibenzophenone XLI nor ergoflavin hexaacetate have absorption here. This phenomenon must be attributed to a new interaction between the two biphenyl units, perhaps with one acetyl methyl becoming situated over the plane of a benzene ring, thereby being shielded through the ring current effect (67).

Professor Whalley has also obtained this acetate on vigorous acetylation of ergochrysin (49); their identity has been demonstrated by direct comparison of samples. Numerous transformations achieved on the compound included hydrobromic acid degradation, leading to carbon dioxide, m-cresotinic acid XXIII, and the phenol LXXVIII which gave hemiergoflavin-2-carboxylic acid LXXIX on ozonolysis. This compound has also been obtained by ozonolysis of ergoflavin (58) and ergochrysin (65), and it proves the presence and stereochemistry (including biphenyl coupling position) of the ergoflavin unit in ergochrysin (pigment V).

The structure LXXVI derived for pigments III and V has also been deduced by Whalley et al (49, 65) in their studies
Fig 11
NMR Spectra: Aromatic Protons
(c.p.s. from TMS)
LXXVI \[ \xrightarrow{\text{Ac}_2\text{O}, \text{NaOAc}} \]
LXXVII \[ \xrightarrow{\text{HBr}} \]
LXXVIII \[ \xrightarrow{\text{O}_3} \]
LXXIX
LXXX
on ergochrysinin, and a number of transformations were carried out which support this structure. On methylation an enol ether, depicted as LXXX could be prepared, as well as a trimethyl ether LXXXI. This was oxidized at the C-5 positions to trimethyl ergochrysinone LXXXII; on vigorous acetylation this gave an acetate which, on hydrobromic acid degradation, afforded 2,5-dihydroxy-3-methylbenzoic acid LXXXIII. This sequence provides an elegant demonstration of the presence of a hydroxyl at C-5 in the secalonic acid unit, an uncertainty in our structural elucidation of the secalonic acids.

Having thus established structure LXXVI for pigments III and V, it remains to be shown conclusively that a secalonic acid $B$ unit is present in pigment III and a secalonic acid $A$ unit in pigment V. Comparison of the molecular rotations of the pigments in pyridine solution bears this out:

<table>
<thead>
<tr>
<th>Compound</th>
<th>$[\alpha]_D$ (pyridine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secalonic acid $A$</td>
<td>-1290°</td>
</tr>
<tr>
<td>Secalonic acid $B$</td>
<td>+1170</td>
</tr>
<tr>
<td>Ergoflavin</td>
<td>+628°</td>
</tr>
<tr>
<td>Pigment $V$</td>
<td>-326°</td>
</tr>
<tr>
<td>Pigment $III$</td>
<td>+674°</td>
</tr>
</tbody>
</table>

It can be seen that the molecular rotation of pigment

* Franck's data (68).
III is roughly the average of the $[\mathcal{M}]_D$ values for secalonic acid B and ergoflavins. Similarly $[\mathcal{M}]_D$ for pigment V corresponds to an average of those of secalonic acid A and ergoflavins.

Therefore, on the basis of the molecular rotation data and n.m.r. spectral correlations, pigment V has been renamed (61) ergochrysin A and pigment III renamed ergochrysin B, the letters referring to the species of secalonic acid present in each pigment. Franck et al (68, 69, 70) have reported the isolation of two pigments designated ergochrysin A and B. Although small differences in melting point exist between Franck’s preparations and the pigments described here, they are undoubtedly the same materials, and these workers concur with us in the structures derived for these pigments.

In a study of the CD of the ergochrysin A, Franck found that the CD of ergochrysin A corresponded to a summation of those of ergoflavins and secalonic acid A. Likewise, the CD of ergochrysin B corresponded to a summation of the CD of ergoflavins and secalonic acid B. Furthermore, the observed rotations of the methylsuccinic acid obtained upon permanganate degradation corresponded to expectations:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methylsuccinic acid obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secalonic acid A</td>
<td>$R$ (†)</td>
</tr>
<tr>
<td>Ergochrysin A</td>
<td>$RS$ (†)</td>
</tr>
<tr>
<td>Ergoflavins</td>
<td>$S$ (−)</td>
</tr>
<tr>
<td>Compound</td>
<td>Methylsuccinic acid obtained</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Ergochrysin B</td>
<td>S (-)</td>
</tr>
<tr>
<td>Secalonic acid B</td>
<td>S (-)</td>
</tr>
</tbody>
</table>

Thus, we can write stereostructures LXXXIV and LXXXV for ergochrysin A (pigment V) and ergochrysin B (pigment III), respectively, with only the position of biphenyl coupling in the secalonic acid units remaining uncertain (see later).

The remaining pigment, the fourth, has been termed ergoxanthin (71). The best analysis for ergoxanthin indicated the empirical formula $C_{31}H_{28}O_{14}$. $H_2O$ and the molecular weight was confirmed mass spectrometrically. The presence of a single methoxyl group was indicated in the n.m.r. spectrum and confirmed by analysis.

Comparison of the n.m.r. spectrum of ergoxanthin in DMSO-d$_6$ with that of ergoflavin (Fig. 12) clearly indicated the presence, in this substance, of one ergoflavin unit, and this was confirmed by the double irradiation technique. The gross structural problem was, therefore, entirely concerned with the nature of the second, "xanthin", unit.

The ultraviolet spectrum of ergoxanthin was closely similar to that of ergoflavin, although the long-wavelength

* The original material to which this name was applied was too ill-defined to be recognizable if re-encountered (30).
LXXXIV
Ergochrysin A (Pigment V)

LXXXV
Ergochrysin B (Pigment III)
maximum of the former was to slightly lower wavelength (373 mμ as opposed to 380 mμ for ergoflavin). Also, the ultra-violet spectral changes in dilute alkaline solution paralleled those observed for ergoflavin (Table 3), and the results excluded the presence of a β-diketonic system, a fact already signalled by the green ferric chloride colour given by ergoxanthin.

Strong absorption at 1793 cm⁻¹ in the infrared spectrum (Fig. 13) suggested the presence of a β-lactone grouping, as required for the ergoflavin unit in the molecule. However, comparison of the intensity of absorption in that region with that of ergoflavin (both taken in dioxane solution) appeared to require the presence of two β-lactonic functions in ergoxanthin. In addition there could be discerned comparatively weak absorption at 1739 cm⁻¹ compatible, for instance, with the presence of an ester or cyclopentanone. That this absorption was, in fact, due to an aliphatic carbomethoxyl group was indicated by the following observations.

Ergoxanthin exhibited considerable stability to acid, but after six days refluxing with hydrochloric acid in ethanol, aside from 51% recovered starting material there was isolated 34% of an acid (described below) and 15% of a substance which appeared to correspond in all properties with ergoxanthin except in that a methoxyl group had been replaced by an ethoxyl function. This was readily evident from the n.m.r.
spectrum, and confirmed by ethoxyl determination. The acid referred to above was obtained more simply by the following method. Dissolution of ergoxanthin in dilute sodium hydroxide at room temperature and standing overnight, then acidification gave a product, presumably analogous to ergoflavinic acid (37). This material on refluxing with acetic acid gave the aforementioned acid. These conditions lead to lactone-reformation in ergoflavinic acid. However, the product obtained was not ergoxanthin, but brief treatment with diazomethane regenerated ergoxanthin. This acidic product, demethylergoxanthin, still possessed two γ-lactonic functions, as judged by infrared intensity measurements, but analysis and n.m.r. spectrum confirmed the loss of a methoxyl group. It thus appeared that ergoxanthin was a methyl ester. The behavior of this substance under acidic conditions then became readily explicable as ester interchange.

Ergoxanthin gave, under mild conditions, a tetraacetate, the infrared and ultraviolet spectra of which are very similar to those of ergoflavin hexaacetate (Table 4). Of these four acetyl functions, three must be attributed to the ergo-

| Table 4 |
|------------------|------------------|------------------|------------------|
| **Comparison of Ergoxanthin and Ergoflavin Derivatives** |
| Tri-OCH₃-ergoxanthin | Tetra-OCH₃-ergoflavin | Ergoxanthin tetraacetate | Ergoflavin hexaacetate |
| IR | 1798 | 1806 | 1810 | 1812 |
| Spectrum | 1770 | -- | 1780 | -- |
| (CHCl₃) | 1747 | -- | 1760 | 1757 |
flavin unit; the "xanthin" unit therefore contains but one acylable hydroxyl group. Since the unit contains seven oxygen atoms, all have been accounted for in the following functional groups, if the \( \delta \)-pyrone unit is assumed present:

![Diagram of chemical structure]

- one \( \delta \)-lactone
- one carbomethoxyl

Alkaline hydrolysis of the tetraacetate led to the formation of demethylergoxanthin which on methylation regenerated ergoxanthin. Thus apparently this acetylation had not been accompanied by aromatization or other complex transformation.

On standing with diazomethane in ethereal solution for
24 hours, ergoxanthin gave a trimethyl ether comparable in properties with ergoflavin tetramethyl ether (Table 4). Again one hydroxyl function must be ascribed to the "xanthin" unit, and, in view of its reaction with diazomethane, must be acidic. This is presumably the phenolic hydroxyl, which manifests itself in the n.m.r. spectrum of ergoxanthin (DMSO-d$_6$) at 11.80 p.p.m., and at 11.84 p.p.m. in CDCl$_3$ solution. Under mild conditions the trimethyl ether gave a monoacetate, which exhibited no hydroxyl absorption in the infrared spectrum.

At this point some chemical observations were made which are significant in that the behavior is not paralleled in the chemistry of ergoflavin. This behavior was particularly informative as to the nature of the "Xanthin" unit.

Whilst acetylation under mild conditions gave, as described, a tetraacetate in which no hydroxyl function could be discerned in the infrared spectrum, under vigorous conditions ergoxanthin yielded a pentaacetate. The ultraviolet spectrum of this acetate indicated an extension of conjugation through an increase in extinction of ca. 10,000 near 243 m$\mu$ as compared with the tetraacetate. The fifth acetyl function introduced was shown to be of a different nature from the other four by its behavior when exposed to acid, whereupon, under mild conditions, specific hydrolysis took place with regeneration of the tetraacetate. The compound was, therefore, an
enol acetate, and this conclusion is strongly supported by an examination of the n.m.r. spectra of the two acetates.

Ergoxanthin tetraacetate, amongst other absorption, has one-proton signals at 5.07 p.p.m. (\(W_x = 5\) c.p.s.) and 5.80 p.p.m. (doublet, \(J \sim 5\) c.p.s.) due to the C-5 (CH-OAc) and C-8 (lactone terminus) protons of the ergoflavin unit. A doublet (\(J \sim 4\) c.p.s.) at 4.46 p.p.m. is attributed to the lactone terminus in the "Xanthin" unit, since it is little shifted from its position in the n.m.r. spectrum of ergoxanthin (4.44 p.p.m.) on formation of this acetate. Additionally, however, there appeared a slightly broadened two-proton singlet at 3.03 p.p.m. This signal was also obvious in the n.m.r. spectra (CDCl\(_3\)) of ergoxanthin (3.08 p.p.m.), the trimethyl ether (3.08 p.p.m.), and its monoacetate (3.07 p.p.m.). On formation of the pentaacetate, this signal disappeared and was replaced by a sharp one-proton singlet at 5.45 p.p.m., as well as absorption due to another acetyl function.

In conjunction with the ultraviolet spectral considerations, these observations require partial structures LXXXVI and LXXXVII for the tetraacetate and pentaacetate, respectively, the signals at 5.45 p.p.m. and 3.03 p.p.m. being attributed to vinyl proton and to methylene protons adjacent to carbonyl respectively. The chemical shift of these methylene protons may, for example, be compared with that of 2.99 p.p.m. in 5-methoxy-2-carbomethoxychromanone LXXXVIII\(\alpha\).

\(\alpha\) I should like to thank Mr. J.L. Charlton for this compound.
Also in contrast with ergoflavin, under forcing conditions ergoxanthin gave a 2,4-dinitrophenylhydrazone. That this was not a hydrazide was indicated by a study of the infrared spectrum, which indicated the continued presence of two γ-lactones and the carbomethoxyl group (also visible in the n.m.r. spectrum), as well as possessing the expected DNP bands. The ultraviolet spectrum was that expected for a DNP of an α,β-unsaturated ketone, \( \lambda_{\text{max}}^{380} \text{ m} \) (\( \varepsilon = 26,000 \)), and was totally unlike the spectrum of N-acetyl-N'-2,4-dinitrophenylhydrazone LXXXIX (72), \( \lambda_{\text{max}}^{334} \text{ m} \) (\( \varepsilon = 13,100 \)). This reaction supports partial structure XC for ergoxanthin.

The formulation XCI has been suggested (73) as a plausible alternative to LXXXVII for the pentaacetate. The vinyl proton here might appear as singlet if the coupling with neighbouring protons were small. However, the chemical shift of this proton might be expected to be to lower field (the fumaric ester olefinic proton absorbs at 6.84 p.p.m.). Furthermore the specific hydrolysis of one acetate in XCI may be interpreted as supporting LXXXVII as against XCI, as may the infrared spectrum of this acetate which exhibits absorption \( \lambda_{\text{max}} \text{1698 cm}^{-1} \) only for the carbonyl of the ergoflavin unit.

The n.m.r. spectrum of ergoxanthin in DMSO-d\(_6\) (Fig. 12) exhibits, in addition to the signals characteristic of ergoflavin, a signal at 4.63 p.p.m. (1 proton, \( J \approx 3.5 \) c.p.s.). This is evidently the signal found at 4.53 p.p.m. in the tetraacetate, which was assigned as a lactone terminus proton.
Fig 12  N.M.R. Spectra in DMSO-d$_6$
Upper: Ergoxanthin. Lower: Ergoflavin
Double irradiation experiments showed that this proton was coupled to a proton absorbing at 2.92 p.p.m. Since certain of the methyl protons are also coupled to a proton at 2.92 p.p.m., it may be presumed that this latter proton is situated on a carbon bearing a methyl group. (The methyl protons are also coupled with a proton at 2.00 p.p.m., which is the location of the corresponding proton in the ergoflavin nucleus). In conjunction with the requirement that, aside from the carbon atoms constituting part-structure XC, both a γ-lactone and a carbomethoxyl group must be incorporated into the "xanthin" nucleus, the above results require part-structure XCII. Since the group must be attached through carbon, and no other carbons are available, combination of XC and XCII gives XCIll as the structure of ergoxanthin.

The proximity of the carbomethoxyl group and the lactone terminus in XCII is shown in the n.m.r. spectra (pyridine) through a downfield shift in this terminus proton from 4.68 p.p.m. to 4.94 p.p.m. on converting ergoxanthin into de-methylergoxanthin. Additionally, on converting the tetra-acetate into the pentaacetate, the chemical shift of this proton underwent a paramagnetic shift of 0.05 p.p.m., which indicated some change in the environment of this proton since the shifts in the other methine protons of the "flavin" nucleus are only ca. 0.01 p.p.m.

The structure arrived at is intuitively satisfying since it may be simply derived from an ergochryisin by cleavage of the β-diketone and lactonization: XCIIV ⇔ XCVII. Two ergo-
chrysins, A and B, were available, and each was allowed to react completely with five equivalents of alkali. The resultant product on acidification was refluxed with acetic acid (lactone cyclization) and methylated with diazomethane. Both ergochrysins gave a number of products, and those with $R_F$ values approximating that of ergoxanthin were isolated. That from ergochrysin B (pigment III) had an infrared spectrum showing a general similarity to ergoxanthin, but was clearly different. That from ergochrysin A gave material with an infrared spectrum (Fig. 13) of truly impressive similarity to that of ergoxanthin, and, in the absence of other evidence, identity might have been claimed. However, close inspection of the t.l.c. plates of the purified product showed that the partially synthetic material persistently had a slightly lower $R_F$ value (0.36 as against 0.40) than ergoxanthin, and in fact whereas ergoxanthin is crystalline, the new product was not. This new compound has therefore been termed $\psi$-ergoxanthin.

Its n.m.r. spectrum ($\text{CDCl}_3$) was essentially identical with that of ergoxanthin, with the important exception that the signal at 4.44 p.p.m. (lactone terminus) was replaced by a one-proton signal at 4.72 p.p.m., indicating for example a different orientation of this group with respect to the carbomethoxyl group.

The similarity of the spectra of ergoxanthin and $\psi$-ergoxanthin can be interpreted as supporting the gross structure
Fig 13

Infrared Spectra (CHCl₃)

Upper: Ergoxanthin

Lower: γ-Ergoxanthin
XCIII and it is therefore unlikely that ergoxanthin could be an artifact derived from either ergochrysin A or B on isolation. In view of the vigorous conditions required to effect the conversion of ergochrysin A into Ψ-ergoxanthin, the likelihood of such a process occurring under the innocuous isolation conditions seems very remote. The existence of a third ergochrysin (which must be required to be more sensitive as regards hydrolytic cleavage) cannot, however, be rigorously excluded.

In addition to the six pigments described above, Franck (68, 69, 70) has recently reported the isolation from ergot of four new yellow pigments. These were shown to be dimeric combinations of a new xanthone unit with the secalonic acid A, secalonic acid B, and ergoflavin units, as well as the dimer of the new unit itself. This new unit was that of ergoflavinic acid methyl ester, since heating each of these new pigments in acetic acid led to the loss of methanol with the formation of previously known pigments containing ergoflavin units.

Franck has invented a new system of nomenclature for the yellow ergot pigments. They are all termed ergochromes; their monomeric units and biphenyl coupling are designated by two letters and two numerals. The secalonic acid A unit is designated A, the secalonic acid B unit designated B, the ergoflavin unit C, and the methyl ergoflavinate unit D. Thus
for the above described pigments (except ergoxanthin) the
equivalent names are:

Secalonic acid A  Ergochrome AA
Secalonic acid B  Ergochrome BB
Secalonic acid C  Ergochrome AB
Ergochrysin A    Ergochrome AC
Ergochrysin B    Ergochrome BC
Ergoflavin       Ergochrome CC (2,2')

The new pigments are then designated AD, BD, CD, and DD.
Heating these in acetic acid gave, respectively, ergochrysin
A (AC), ergochrysin B (BC), and the latter two ergoflavin
(CC). Hence the structures of these new pigments are XCVIII
- CI with the biphenyl coupling assigned to the 2,2' positions.

In view of the present number of ergot pigments known,
this proliferation of nomenclature seems unnecessary, and the
older system has been used herein. However, in the event of
the isolation of further related ergot pigments, Franck's sy-
stem may become more desirable.

The final point to be discussed is the nature of the
biphenyl coupling in the various ergot pigments. The pres-
ence of pairs of vicinal aromatic protons in the pigments
requires that the coupling be either ortho or para to the
phenolic groups.

In an effort to distinguish ortho from para coupling, the
Gibbs test (44) as modified by King et al (45) has been applied.
XCVIII
Ergochrome AD (2,2')

XCIX
Ergochrome BD (2,2')

C
Ergochrome CD (2,2')

Cl
Ergochrome DD (2,2')
The formation of an indophenol dye CII with 2,6-dichloroquinonechloroimide CIII is considered evidence of a free position para to a phenolic group. In this work, all pigments gave a positive test, measured spectroscopically, in the same time and under the same conditions, and, moreover, the intensities achieved are of the expected order of magnitude. These findings are, however, in conflict with those of Whalley (49) and Franck (42, 74) for the secalonic acids, negative reactions being reported for these pigments, including "chrysergonic acid". An authentic sample of chrysergonic acid, provided by Professor Whalley, in our hands, gave a positive reaction. This discrepancy may be due to some minor variation in technique and/or reagents. However, the undoubtedly positive nature of the test is not considered as definite evidence of a free para position in the pigments in any case, since it is quite possible that under the conditions of the reaction (pH 9.2), where there is an acidic proton as in the secalonic acids and ergochrysin, \( \beta \)-elimination may readily occur with the liberation of the second phenolic group, as in CIV \( \Leftrightarrow \) CVI. This change is analogous to the chalcone-flavanone interconversion (75).

In this context, the known (36, 42, 49) "mutarotation" of secalonic acid in pyridine is relevant. This rotation change has been confirmed for secalonic acids B and C and ergochrysin A and B, using an elevated temperature to speed up
the process. However, the transformation is complex since t.l.c. reveals a number of products. Originally (49) this effect was tentatively attributed to an inversion at C-9 in the non-enolic form, e.g. CIV, but in view of the enolic nature of the \( \alpha \)-dicarbonyl system, more probably an inversion at C-10 is involved, following \( \alpha \)-elimination and re-addition. Under such circumstances, the phenoxy group undergoing Michael addition may be different from that eliminated. Since such transformations may occur under conditions of general acid or base catalysis, it is therefore unnecessary that transformation products of these pigments (e.g. acetates or ethers not made with diazomethane) have the same diphenyl junction as the original pigment, or need provide structural evidence regarding this point of attachment.

By treatment of ergochrysin with hot pyridine, Professor Whalley's group has obtained a compound denoted isoergochrysin. In the n.m.r. spectrum of this compound in CDCl\(_3\) solution, there appears two one-proton singlets at 11.43 and 11.78 p.p.m., and a one proton singlet at 13.69 p.p.m. Since ergochrysin, and therefore isoergochrysin, has only one \( \alpha \)-dicarbonyl system, this last peak must be that of the enolic hydroxyl, the others being the phenolic absorption. These observations support the assignment of peaks in the case of the secalonic acids.

The signals for the aromatic protons in the n.m.r spectra of the six pigments were studied in an effort to gain insight into the diphenyl junction problem. In each case an AB pattern was observed, but small chemical shift differences
between the two possible arrangements: H-C$_2$-C$_3$-H and H-C$_3$-C$_4$-H were not unlikely. In ergoflavin, and in all pigments believed to contain an ergoflavin unit (Table 1) very good agreement was observed for the chemical shifts. This strongly suggests that the biphenyl junction of this unit is at C-2 in all cases, as has been established for ergoflavin itself by x-ray means (47).

Secalonic acid B was too insoluble for determination of the spectrum in DMSO-$d_6$, but in secalonic acid C, two separate AB patterns are observed. The differences in the chemical shifts of the aromatic protons in the two rings could theoretically be attributed to a difference in the preferred conformation of the otherwise freely-rotating carboxymethoxyl group because of hydrogen bonding. However, although the ergochryssins, A and B, also contain one each of the two different diastereiomic secalonic acid units, the aromatic proton patterns are almost identical, one of their AB patterns being very close to one of those for secalonic acid C. The fact that one of the AB patterns of secalonic acid C is not exhibited by the ergochryssins may mean that a different biphenyl coupling exists in the former pigment as compared with that existing in the ergochryssins. However, this evidence is weak, and the nature of the biphenyl coupling in the secalonic acids must be considered unsettled, although the problem is under study by Professor Whalley's group.

Professors Whalley and Franck have also observed positive Gibbs reactions for the ergochryssins (49, 68) and the
biphenyl coupling has been assigned to the 2,2' positions. This requires that Franck's ergochromes AD-DD also be coupled here, since, as mentioned, these new pigments have been converted into the ergochrysin or ergoflavin.

The biphenyl coupling in ergoxanthin may be assigned to the 2,2' positions from the following considerations. The aromatic chemical shifts in CDCl₃ solution are 6.61, 6.66, 7.47, and 7.52 p.p.m. Two of these are due to the ergoflavin unit. In ergoxanthin tetraacetate, these chemical shifts are 7.02, 7.11, 7.42, and 7.42 p.p.m. Thus, one of the protons in each aromatic ring has exhibited a paramagnetic shift of ca. 0.4 p.p.m. in the conversion of the phenol to its acetate. In a study of some phenolic compounds, Polonsky (76) has shown that acetylation of phenols leads to downfield shifts in the ortho, meta, and para protons of 0-0.2, 0, and 0.4-0.5 p.p.m., respectively. If ergoxanthin possesses two protons para to the phenolic groups, it must be coupled in the 2,2' positions.

Whalley has accumulated some evidence showing that the diphenyl system in ergoflavin is non-planar. This is clearly shown for ergoflavin tetramethyl ether bis-p-iodebenzoate by the x-ray results (47). Also, in the n.m.r. spectrum, a diamagnetic shift of 0.36 p.p.m. for the 1-methoxy signal of ergoflavin tetramethyl ether relative to the signal for 9,9'-dimethoxyergoflavin indicates a large dihedral angle between the benzene rings, so that the 1-methoxy group in one half of the molecule is shielded by the aromatic ring in the other
half (65). It seems likely that biphenyl non-planarity is also prevalent amongst the other ergot pigments and their derivatives.

(d) **Biogenesis**

Professor Whalley (65) has suggested that the ergot pigments may be derived by the biochemical equivalent of a Baeyer-Villiger oxidation sequence of the type CVII $\rightarrow$ Cx (Fig. 14). Dimerization of Cx by oxidative coupling, with some minor structural changes would give the ergot pigments. Structure CVIII may not necessarily be derived from an actually pre-formed anthraquinone derivative, and possibly alternative routes can lead to either the yellow pigments or the red anthraquinone derivatives found in ergot (38). In any case, the precursor is undoubtedly acetate-derived, as indicated by the substitution patterns in the pigments.

Franck, following Whalley's suggestion, has reported some tracer experiments along these lines (77). Methyl-labelled sodium acetate was incorporated into secalonic acid C, in accordance with expectation (Fig. 14), lending support to the biogenetic scheme. An alternative mode of coiling CX1 of the polyacetate precursor is equally compatible with the labelling pattern observed, but the co-occurrence of endocrocin CVII and secalonic acid provides circumstantial evidence for the former pathway.
Fig 14  Biogenesis of the Ergot Pigments
EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. The solvents for IR and UV spectra and rotations are indicated parenthetically. Unless otherwise indicated, the measurements were determined on a Varian DP-60 instrument in the solvents noted (5-10% w/v). Both CDCl₃ and DMSO-d₆ were obtained from Merck, Sharp and Dohme, Ltd. The spectra were calibrated by the usual side-band method and the audio oscillator was continuously monitored with a Hewlett-Packard 522B frequency counter. NMR data, except for spectra determined on a Varian A-60 instrument, are averages of at least two scans in each of increasing and decreasing field direction. The data for the original six pigments in pyridine and DMSO-d₆ are averages of five scans in each direction. The double irradiation experiments were accomplished with an NMR Specialties, Inc., Model PD-60 homonuclear spin-decoupling unit. Kieselgel was used for thin-layer chromatography.

Preliminary Separation of the Mixed Pigments

The mixed pigment concentrate (40 g) was exhaustively extracted with cold chloroform (500 ml), the filtered solution concentrated to 100 ml and filtered through a column of silica gel (200 g, B.A. reagent), the column being eluted with further amounts of chloroform (ca. 5 l.) until the eluent became nearly colourless. Thin layer chromatography (TLC) of a small portion of this extract (eluant: CHCl₃-HOAc (9:1)) showed six spots
of \( R_F \) and ferric chloride colour as follows: 0.65 (red-brown), 0.54 (red-brown), 0.45 (red-brown), 0.40 (green), 0.29 (red-brown), and 0.17 (green).

The chloroform eluate was concentrated to 100 ml, benzene (100 ml) added, and the solution cooled. A precipitate of crude pigment VI (1.0 g)\(^\circ\) was collected and washed with chloroform. The filtrate was concentrated and added to a column of silicic acid (750 g, B.A. reagent, sieved <100 mesh was activated for 3 days at 100°C) in chloroform-benzene (1:1). The column was then eluted with chloroform-benzene (1:1), (ca. 100 l, recycling the solvent) over two weeks. The eluting solvent was then changed to chloroform-benzene (7.3), whereupon 3 red bands (very little material) were eluted and discarded. Solvent was passed until the first yellow band reached the bottom of the column (ca. 50 l). The solvent was then drained and the column extruded by air pressure. This material was divided into 7 equal fractions from which the pigments were extracted with a mixture of chloroform and acetone. The further separation of the pigments was followed at all stages by TLC.

Fractions 1-3 (numbered from the top of the column) containing pigments III, IV, and V were combined, dissolved in chloroform and concentrated to 25 ml. A yellow precipitate of pigment V slowly separated, was collected and washed

\(^\circ\) The six pigments were designated I-VI in order of decreasing \( R_F \) value on TLC.
with chloroform. Repetition (5 times) of this procedure gave moderately pure pigment V (2.52 g). The mother liquors from this separation were evaporated and the residue taken up in ether. This solution was concentrated to 25 ml and cooled giving crystalline pigment IV (940 mg). The mother liquors were again evaporated to dryness and taken up in chloroform. Addition of carbon tetrachloride gave crude pigment III (300 mg).

Fraction 4 was dissolved in chloroform, carbon tetrachloride was added, and - upon seeding - pigment II was obtained (see below). The mother liquors from this crystallization were chromatographed on silicic acid (70 g) and eluted with chloroform-benzene (7.3). After extrusion, extraction of the slowest - moving one-third of the column gave, on evaporation and crystallization from chloroform-carbon tetrachloride, crude pigment III (800 mg total, combined with the material from fractions 1-3).

Fraction 5 consisted of nearly pure pigment II (2.90 g).

Fraction 6 and 7 contained pigments I and II. The extract was dissolved in ethyl acetate and concentrated to 15 ml. On standing, crystals slowly separated and were removed from the viscous solution by centrifugation. Washing with chloroform and acetone gave pigment I (510 mg). The mother liquors were evaporated and dissolved in chloroform. Addition of carbon tetrachloride gave crystalline pigment II. A total of (5.50 g) pigment II was obtained from all fractions.
**Pigment I** (Secalonic acid B)

The pigment was crystallized from dioxane-water to give prisms. The physical constants are recorded below and compared with those for Secalonic acid B (42, 50). (Found: C, 59.65; H, 5.30; O, 35.00; OMe, 9.61. Calc. for C_{32}H_{30}O_{14}: C, 60.19 H, 4.73; O, 35.08; OMe (2), 9.71%).

<table>
<thead>
<tr>
<th>Property</th>
<th>Pigment I</th>
<th>Secalonic acid B</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.p.</td>
<td>254-255°</td>
<td>254-256° (dec.)</td>
</tr>
<tr>
<td>$[^{156} \sigma]_D$ (CHC$_3$)</td>
<td>$^+16$° (c, 0.5)</td>
<td>$^+196°$</td>
</tr>
<tr>
<td>$[^{183} \sigma]_D$ (pyridine)</td>
<td>$^+183°$ (c, 0.91)</td>
<td>$^+194°$</td>
</tr>
<tr>
<td>$\lambda_{max}$ (KBr)</td>
<td>1742, 1613cm$^{-1}$</td>
<td>1738, 1607</td>
</tr>
<tr>
<td>$\lambda_{max}$ (EtOH)</td>
<td>214 (ε=18,900)</td>
<td>220 (ε 34.8)</td>
</tr>
<tr>
<td>242 (16,000)</td>
<td>242 (ε 33.5)</td>
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</tr>
<tr>
<td>262 (14,000)</td>
<td>338 (ε 54.3)</td>
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</tr>
<tr>
<td>340 (30,400)</td>
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<td></td>
</tr>
<tr>
<td>377 (7,550)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FeCl$_3$ colour red-brown red-brown

**Pigment II** (Secalonic acid C)

This was crystallized from chloroform-carbon tetrachloride as prisms, m.p. 159-161°, $[^{23} \sigma]_D$ + 23° (c, 1.31, acetone), $[^{23} \sigma]_D$ + 14° (c, 2.58, CHC$_3$), $[^{14} \sigma]_D$ - 14° (c, 0.99, pyridine),
$\nu_{\text{max}}$ 1740, 1615, 1592, 1567 cm$^{-1}$ (CHCl$_3$), $\lambda_{\text{max}}$ 210 (20,000), 242 (18,500), 261 (15,300), 340 (33,500), 375 (7,800) m$\mu$. The pigment gave a red-brown colour with ferric chloride.

(Found: C, 59.86; H, 4.51; OCH$_3$, 9.55. Calc. for C$_{32}$H$_{30}$O$_{14}$: C, 60.19; H, 4.73; OCH$_3$ (2), 9.71%).

**Pigment III (Ergochrysin B)**

This was crystallized as prisms from chloroform-carbon tetrachloride, m.p. 196-199$^\circ$, $[\alpha]_D^\circ$ + 82$^\circ$ (c, 1.36, acetone),

$[\alpha]_D^\circ$ + 127$^\circ$ (c, 1.05, pyridine), $\nu_{\text{max}}$ 1798, 1737, 1613 cm$^{-1}$ (CHCl$_3$), $\lambda_{\text{max}}$ 208 (20,000), 241 (16,500), 266 (17,000) 336 (17,000), 374 (8,000) m$\mu$. It gave a red-brown ferric chloride colour. (Found: C, 59.27; H, 4.42; OCH$_3$, 4.78. Calc. for C$_{31}$H$_{28}$O$_{14}$ = C, 59.62; H, 4.52; OCH$_3$ (1), 4.97%).

**Pigment IV (Ergoxanthin)**

This was crystallized from 95% ethanol to give needles, m.p. 185-188$^\circ$, $[\alpha]_D^\circ$ + 124$^\circ$ (c, 1.40, CHCl$_3$), $[\alpha]_D^\circ$ + 138$^\circ$

(c, 0.91, pyridine), $\nu_{\text{max}}$ 1793, 1739, 1644, 1622 cm$^{-1}$ (CHCl$_3$), $\lambda_{\text{max}}$ 209 (25,400), 268 (23,600), 373 (8,000) m$\mu$. It gave a green ferric chloride colour. (Found: C, 58.19; H, 4.31; O, 37.51; OCH$_3$, 5.45. M. wt. determined mass spectrometrically = 624. Calc. for C$_{31}$H$_{28}$O$_{14}$H$_2$O: C, 57.95; H, 4.71; O, 37.35; OCH$_3$ (1), 4.83%. M. wt. = 624.6).

**Pigment V (Ergochrysin A)**

This crystallized from chloroform in plates, m.p. 198-200$^\circ$ and 260-265$^\circ$, $[\alpha]_D^\circ$ - 37$^\circ$ (c, 1.47, acetone), $[\alpha]_D^\circ$ - 52$^\circ$
(c, 0.96, pyridine), $\gamma_{max}$ 1805, 1750, 1615 cm$^{-1}$ (KBr),
$\gamma_{max}$ E+OH 208 (19,900), 243 (16,400), 270 (16,700), 336 (16,300),
374 (7,300) m. The compound gave a red-brown colour with
ferric chloride. (Found: C, 59.76; H, 5.15; O, 35.12; OCH$_3$,
4.87. M. wt. 624 (mass spec.). Calc. for C$_{31}$H$_{28}$O$_4$: C,
59.62; H, 4.52; O, 35.86; OCH$_3$ (1), 4.97%. M. wt. 624.6).

Pigment V was identical with the ergochrysin (ergochrysin A)
isolated by Whalley (49) in m.p., mixed m.p., UV and IR spectra,
specific rotation, and $R_f$ value.

**Pigment VI (Ergoflavin)**

This crystallized from methanol in needles, m.p. $> 360^\circ$ (dec.), 
[54]$_D^\theta + 37^\circ$ (c, 1.87, acetone), [54]$_D^\theta + 103^\circ$ (c,
0.97, pyridine), $\gamma_{max}$ 1795, 1645, 1620 cm$^{-1}$ (KBr), $\gamma_{max}$
E+OH 207 (25,000), 241 (19,300), 279 (20,000), 380 (7,300) m.
The compound gave an olive green colour with ferric chloride.
(Found: C, 59.53; H, 4.56; O, 35.79, M. wt: 610 (mass spec.).
Calc. for C$_{30}$H$_{26}$O$_4$: C, 59.02; H, 4.29; O, 36.69%; M. wt:
610.5). The properties of the compound agreed in all respects
with those of ergoflavin reported by Whalley (37).

**Pigment I dimethyl ether (Dimethyl secalonic acid)**

The pigment (76 mg) in ethyl acetate (20 ml) was treated
with ethereal diazomethane and the mixture allowed to stand
3 hours. After evaporation of solvent the product was
Table 1

NMR Spectra Chemical Shifts (ppm from TMS)

<table>
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<tr>
<th>Compound</th>
<th>Solvent</th>
<th>C₅H₁₀</th>
<th>C₆H₁₀</th>
<th>C₇H₂</th>
<th>C₈H</th>
<th>CH₃</th>
<th>OCH₃</th>
<th>Other</th>
<th>Hydroxyl</th>
<th>Aliph.</th>
<th>Phenol</th>
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<td>C₅H₅N</td>
<td>4.55</td>
<td>2.50</td>
<td>120-170</td>
<td>1.17</td>
<td>3.57</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>Secalonic acid C</td>
<td>C₅H₅N</td>
<td>4.12</td>
<td>2.50</td>
<td>120-170</td>
<td>1.16</td>
<td>3.53</td>
<td>—</td>
<td>—</td>
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<td>Ergochrysin B</td>
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<td>4.56</td>
<td>2.21</td>
<td>120-170</td>
<td>5.53</td>
<td>1.17</td>
<td>3.57</td>
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<td>Ergoxanthin</td>
<td>C₅H₅N</td>
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<td>2.9</td>
<td>120-170</td>
<td>5.53</td>
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<td>C₉H₂</td>
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<td>Ergoflavin</td>
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<td>2.15</td>
<td>5.55</td>
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<td>C₉H₂</td>
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<td>1.08</td>
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<td>6.04</td>
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<td>6.52</td>
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<td>11.77</td>
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* determined by double irradiation
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<th>Compound</th>
<th>Solvent</th>
<th>$\delta_A$</th>
<th>$\delta_B$</th>
<th>$J_{AB}$ (cps)</th>
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<td>6.82</td>
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<td>6.51</td>
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<td>Compound</td>
<td>Solvent</td>
<td>δ_A</td>
<td>δ_B</td>
<td>J_{AB} (cps)</td>
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<td>6.66</td>
<td>7.53</td>
<td>8.4</td>
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<tr>
<td>Ergoflavin</td>
<td>Acetone</td>
<td>6.75</td>
<td>7.65</td>
<td>8.8</td>
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<tr>
<td>Ergoflavin Tetramethyl ether</td>
<td>DMSO-d₆</td>
<td>7.12</td>
<td>7.65</td>
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<tr>
<td>Ergoflavin Tetramethyl ether</td>
<td>CDC1₃</td>
<td>7.06</td>
<td>7.59</td>
<td>8.7</td>
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<tr>
<td>Ergoflavin Hexaacetate</td>
<td>CDC1₃</td>
<td>7.11</td>
<td>7.43</td>
<td>8.6</td>
</tr>
<tr>
<td>Bibenzophenone acetate (from Secalonic acids)</td>
<td>CDC1₃</td>
<td>7.10</td>
<td>7.45</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.19</td>
<td>7.56</td>
<td>8.3</td>
</tr>
<tr>
<td>Benzophenone acetate (from Ergochrysins)</td>
<td>CDC1₃</td>
<td>7.11</td>
<td>7.40</td>
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<tr>
<td></td>
<td></td>
<td>7.14</td>
<td>7.47</td>
<td>7.8</td>
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<tr>
<td></td>
<td></td>
<td>7.16</td>
<td>7.56</td>
<td>8.3</td>
</tr>
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</table>

A-60 Spectrum.

The above values are the actual chemical shifts, as calculated from the line positions using the expression

\[
(1-3) = (2-4) = \sqrt{(\delta_B - \delta_A)^2 + J_{AB}^2}
\]  \hspace{1cm} (67).
<table>
<thead>
<tr>
<th>Pigment</th>
<th>Neutral (95% EtOH)</th>
<th>Alkaline&lt;sup&gt;a&lt;/sup&gt; (immediate)</th>
<th>Alkaline&lt;sup&gt;b&lt;/sup&gt; (standing)[hr.]</th>
<th>Acidified&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Alkali&lt;sup&gt;d&lt;/sup&gt; consumption (equ.)</th>
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<tbody>
<tr>
<td><strong>I, Secalonic Acid B</strong></td>
<td>214(18900)</td>
<td>247(22300)</td>
<td>274(17700)</td>
<td>254(16300)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>242(16000)</td>
<td>364(29100)</td>
<td>392(9000)</td>
<td>268(15400)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>262(infl.) (14000)</td>
<td>340(30400)</td>
<td>377(infl.) (7550)</td>
<td>[80 hr.]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>210(20000)</td>
<td>246(20300)</td>
<td>274(18800)</td>
<td>253(16400)</td>
<td>5.30</td>
</tr>
<tr>
<td></td>
<td>242(18500)</td>
<td>362(29800)</td>
<td>394(10300)</td>
<td>356(6300)</td>
<td>(phenolphthalein)</td>
</tr>
<tr>
<td></td>
<td>341(33500)</td>
<td>375(infl.) (7800)</td>
<td>[80 hr.]</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208(20000)</td>
<td>227(19300)</td>
<td>274(14700)</td>
<td>252(15400)</td>
<td>4.60</td>
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<tr>
<td></td>
<td>241(16500)</td>
<td>362(15800)</td>
<td>393(8600)</td>
<td>268(13200)</td>
<td>(phenolphthalein)</td>
</tr>
<tr>
<td></td>
<td>266(17000)</td>
<td>400(infl.) (9800)</td>
<td>[80 hr.]</td>
<td>3.16</td>
<td>(methyl red)</td>
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<tr>
<td><strong>II, Secalonic acid C</strong></td>
<td>336(17000)</td>
<td>374(8000)</td>
<td>208(19900)</td>
<td>229(19200)</td>
<td>273(14800)</td>
</tr>
<tr>
<td></td>
<td>361(14000)</td>
<td>394(8800)</td>
<td>251(14700)</td>
<td>268(13000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>374(7300)</td>
<td>400(infl.) (9450)</td>
<td>[80 hr.]</td>
<td>360(5600)</td>
<td></td>
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<tr>
<td><strong>III, Ergochrysin B</strong></td>
<td>207(25000)</td>
<td>237(20200)</td>
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<td>246(19500)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>241(19300)</td>
<td>286(13600)</td>
<td>270(15000)</td>
<td>380(7300)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>279(20000)</td>
<td>414(11800)</td>
<td>391(9400)</td>
<td>360(6600)</td>
<td></td>
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<tr>
<td><strong>V, Ergochrysin A</strong></td>
<td>380(7300)</td>
<td>247(17300)</td>
<td>273(14900)</td>
<td>246(19500)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>271(14000)</td>
<td>362(5400)</td>
<td>392(7600)</td>
<td>360(6600)</td>
<td></td>
</tr>
<tr>
<td><strong>Ergoflavinic acid</strong></td>
<td>247(17300)</td>
<td>273(14900)</td>
<td></td>
<td>246(19500)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>271(14000)</td>
<td>362(5400)</td>
<td></td>
<td>360(6600)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(H2O)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
a Samples dissolved in 0.1N. Sodium hydroxide.

b Same solution after standing at room temperature for the number of hours indicated in parentheses, after which time no spectral changes occurred.

c Solution acidified with 6N. hydrochloric acid.

d Samples of the pigments in 0.1N. sodium hydroxide were titrated with 0.1N. hydrochloric acid, after standing at room temperature for 80 hours.
separated by TLC. The material of lowest \( R_F \) (0.14) (eluant: CHCl\(_3\) - acetic acid (9:1) ) (35 mg) crystallized from chloroform as prisms of m.p. 237-239\(^\circ\), \([\alpha]_D^0 + 59^\circ\) (C, 0.56, CHCl\(_3\)), \( \nu_{\text{max}} \) 1740, 1635 cm\(^{-1}\) (KBr). \( \text{E+OH} \) \( \nu_{\text{max}} \) 210 (21,400), 265 (14,000), 328 (26,400), 376 (inf1.) (6,400) m\( \mu \). The compound gave a green ferric chloride colour. (Found: C, 61.34; H, 5.01; OCH\(_3\), 16.02. Calc. for \( C_{34}H_{34}O_{14} \): C, 61.26; H, 5.14; OCH\(_3\) (4), 18.62%). Franck et al (42) record the following data for their dimethyl secalonic acid: m.p. 236-238\(^\circ\) (dec.), \( \nu_{\text{max}} \) 1737, 1631, 1588 cm\(^{-1}\) (KBr), olive green ferric chloride reaction (see also curve (b) in Fig. 4, Ref. 42).

The ether (5 mg) was treated under hydrolytic conditions as described below for the ether of secalonic acid C. The recovered product was identical with secalonic acid B in spectra, ferric chloride colour, and \( R_F \) value.

**Acetylation of Pigment I (Secalonic acid B)**

The pigment (92 mg) was heated under reflux under nitrogen in acetic anhydride (15 ml) and pyridine (1 ml) for 2 1/2 hours. The solvent was evaporated and the products separated by TLC (eluant: 7% acetic acid in CHCl\(_3\)). The fastest moving component (40 mg) gave needles from methanol, m.p. 203-205\(^\circ\), \([\alpha]_D^0 \), \([\alpha]_{546}^0 \), \( \nu_{\text{max}} \) 1770, 1723, 1185 cm\(^{-1}\) (CHCl\(_3\)), \( \text{E+OH} \) \( \nu_{\text{max}} \) 211 (52,800), 234 (36,000), 289 (8,350) m\( \mu \). (Found: C, 61.36; H, 4.53; OCH\(_3\), 7.16. Calc. for \( C_{44}H_{38}O_{18} \): C, 61.83;
H, 4.48; OCH₃ (2), 7.26%.

Pigment II dimethyl ether (Secalonic acid C dimethyl ether)

The pigment (653 mg) in methanol was treated with ethereal diazomethane for 5 minutes, and the resultant mixture (5 components) separated by TLC (eluant: CHCl₃ - acetic acid (9:1)). The product of lowest Rₖ (0.25) was isolated (210 mg) and gave orange plates from chloroform - carbon tetrachloride, m.p. 257-259°, [α]D₂₀ + 4° (C, 2.83, CHCl₃), νmax 1745, 1635 cm⁻¹ (CHCl₃), νmax 265 (12,800), 327 (27,900), 374 (inf1.) (6,650) mμμ. NMR spectrum (CDCl₃): δ, 1.14 (6H, doublet, J~6 c.p.s.), 3.69 (3H, OCH₃), 3.73 (3H, OCH₃), 3.90 (3H, OCH₃), 3.95 (3H, OCH₃), two AB patterns for 4 aromatic protons: see Table 2; 13.12 (1H, singlet), 13.29 (1H, singlet). Apart from chemical shift differences, a similar spectrum was obtained in DMSO-d₆. The compound gave a green ferric chloride colour. (Found: C, 61.47; H, 5.04; O, 34.11; OCH₃, 17.89. Calc. for C₃₄H₃₄O₁₄: C, 61.26; H, 5.14; O, 33.60; OCH₃ (4), 18.62%.)

The ether (5 mg) was dissolved in chloroform (1/2 ml), methanol (5 ml) was added, and hydrochloric acid (6N., 1 ml) and the homogeneous mixture shaken at room temperature for 24 hours. The product, on isolation, was identical with secalonic acid C in IR and UV spectra, and Rₖ value.
Acetylation of Pigment II (Secalonic acid C)

The pigment (260 mg) in pyridine (5 ml) and acetic anhydride (50 ml) was heated at 80° for 4 hours. After evaporation of the solvent, the product (3 components) was separated by TLC (eluant: 7% acetic acid in CHCl₃).

The fastest moving component (106 mg) gave needles from methanol, m.p. 203-205°. This was identical with the benzophenone XLI obtained from pigment I in all respects.

This compound could also be obtained more conveniently by refluxing the pigment (500 mg) in acetic anhydride (10 ml) with sodium acetate (41/2 g) for 2 hours, yielding after chromatography and crystallization 92 mg. of the benzophenone.

The slowest moving component crystallized from methanol in needles, m.p. 237-239°, [α]D 0°, [α]5461 14° (C, 1.20, CHCl₃), νmax 1780, 1760 cm⁻¹ (CHCl₃), νmax 211 (20,400), 254 (37,200), 298 (33,100), 305 (32,000), 333 (18,700) mμ.

The NMR spectrum (CDCl₃) exhibits the following distinctive features: two CH₃-CH doublets, 1.10 and 1.13 p.p.m. (J ~ 7 c.p.s.), six CH₃COO groups, 2.12 and 2.21 p.p.m., two identical COOCH₃ groups, 3.74 p.p.m., a complex multiplet due to two protons in the region 320-370 c.p.s., and an AB pattern for two equivalent pairs of aromatic protons - see Table 2. The compound gave no colour with ferric chloride. (Found: C, 59.74; H, 4.53; OCH₃, 5.85. Calc. for C₄₄H₄₂O₂₀: C, 59.33; H, 4.75; OCH₃ (2), 6.97%).
Treatment of this compound with dilute hydrochloric acid in methanol overnight at room temperature produced an essentially homogeneous (TLC), but non-crystalline, colourless product which gave a red-brown colour with ferric chloride, and exhibited an intense band in the IR spectrum at 1610 cm\(^{-1}\). On account of its instability, it could not be purified.

**Pyrazole-3,4,5-tricarboxylic acid**

3-Methyl-2,4-pentanedione was converted into 3,4,5-trimethylpyrazole by the method of Rothenburg (55). It had m.p. 137-138.5° and its IR spectrum was identical with that reported (55a).

The pyrazole (1 g), potassium hydroxide (0.5N., 50 ml) and saturated aqueous potassium permanganate (85 ml) were heated at 80° for 4 hours. The excess oxidant was destroyed with sodium bisulphite, and the solution filtered and evaporated. The product was dissolved in hydrochloric acid (3N., 50 ml) and exhaustively extracted with ether.

The crystalline product (1.18 g) was crystallized from ether or acetone (sublimed at \(\sim 130^\circ\)) or from water, m.p. 230° (dec). Reported m.p. (from water) 233° (dec) (55).

A sample (5.2 mg) sublimed at 80° and 10 mm Hg was titrated with sodium hydroxide (0.10 N); it consumed 3.05 equivalents.
Formation of pigment pyrazoles and their oxidation

The following is illustrative: Pigment II (204 mg), hydrazine hydrate (85%, 0.14 ml), methanol (7.5 ml) and chloroform (15 ml) were heated under reflux for 24 hours. The product was insoluble in chloroform, but appeared homogeneous on TLC (eluant: MeOH). It was basic to litmus (hydrazide), reacted very rapidly with acetone, and gave a green colour with ferric chloride.

The crude pyrazole, potassium hydroxide (1 N., 50 ml) and saturated potassium permanganate (100 ml) were heated at 80°C for 24 hours. The excess permanganate was destroyed with sodium bisulphite, the solution filtered and evaporated. The product was redissolved in hydrochloric acid (3 N., 50 ml) and extracted with ether (15 x 100 ml). After drying (Na₂SO₄) and evaporation of the solvent the product was crystallized twice from ether giving needles (57.4 mg, 44%) identical in every respect with pyrazole-3,4,5-tricarboxylic acid.

The yields obtained are tabulated below:

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Yield (%) based on 1 or 2 β-diketones</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Secalonic acid B)</td>
<td>42</td>
</tr>
<tr>
<td>II (Secalonic acid C)</td>
<td>44</td>
</tr>
<tr>
<td>III (Ergochrysin B)</td>
<td>52</td>
</tr>
<tr>
<td>V (Ergochrysin A)</td>
<td>54</td>
</tr>
<tr>
<td>VI (Ergoflavin)</td>
<td>0</td>
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</table>
Acetylation of pigment V (Ergochrysin A)

Pigment V (60 mg), sodium acetate (1/2 g) and acetic anhydride (2 ml) were refluxed for 1/2 hour. The product was isolated with chloroform, the chloroform layer washed with water. After evaporation of solvent the residue (3 components) was separated by TLC. The fastest moving component (eluant: CHCl₃-acetic acid (9:1)) (Rₚ 0.59) was isolated and crystallized from chloroform-carbon tetrachloride, then methanol to give LXXVII as white needles, m.p. 241-243°, \( [\alpha]_D^{+} + 34^\circ \) (C, 1.0, CHCl₃), \( [\alpha]_D^{+} + 15^\circ \) (C, 0.92, pyridine), \( \gamma_{\text{max}}^{\text{CHCl₃}} 1810, 1775, 1765, 1735, 1695, 1620, 1190 \text{ cm}^{-1} \) (CHCl₃), \( \lambda_{\text{E}^0 \text{OH}}^{\text{max}} 210 \) (39,200), 238 (32,900), 264 (18,700), 294 (5,450), 333(2,000) m\( \mu \). (Found: C, 59.46; H, 4.30; O, 36.17; OCH₃, 3.70.

Calc. for C₄₃H₃₈O₁₉: C, 60.14; H, 4.46; O, 35.40; OCH₃ (1), 3.61%).

For this compound, Whalley (49) records m.p. 245° (dec), \( [\alpha]_D^{+} + 15^\circ \) (pyridine), NMR signals including \( ^1H 8.40 \) (3H, singlet), 8.96 (3H, doublet, J= 6 c.p.s.) and peaks for 6 aromatic protons.

Acetylation of Pigment III (Ergochrysin B)

The pigment (80 mg) was acetylated as described for pigment V. The chloroform-soluble product mixture was separated by TLC, and the fastest moving component (Rₚ 0.59; eluant: CHCl₃-acetic acid (9:1)) isolated. It was crystallized from chloroform-carbon tetrachloride, and from methanol
in needles (15 mg), m.p. 241-243°, undepressed on admixture with the acetate obtained from pigment V. Their IR spectra were superimposable.

**Acid treatment of ergoxanthin**

Ergoxanthin (147 mg), 95% ethanol (70 ml), and hydrochloric acid (6N., 5 ml) were refluxed for 6 days, and the solvent evaporated. The resultant mixture (3 components) was separated by TLC (eluant: CHCl₃-acetic acid (4:1)). The slowest moving component (R_F = 0.2) crystallized from chloroform in round aggregates, m.p. 205-208° (dec). This compound was identical with demethylergoxanthin (described below).

The compound of intermediate R_F (0.65) formed yellow needles from 95% ethanol. It was identical in all respects with ergoxanthin.

The final product (R_F = 0.72) was not obtained crystalline, but was chromatographically pure: [α]_D + 85° (C, 1.49, CHCl₃), ν_max 1796, 1745, 1649, 1626 cm⁻¹ (CHCl₃), λ max 222 (19,000), 261 (20,000), 373 (6,600) m/μ. NMR spectrum (CDCl₃): δ 1.22 (9H, multiplet), 2.13 (4H, multiplet), 2.83 (2H, multiplet), 3.14 (2H, broad singlet), 4.21 (2H, quartet, J ~ 7 c.p.s.) - a 1 H signal buried beneath this quartet; 4.43 (1H, broad singlet), 5.29 (1H, broad singlet), two AB patterns for 4 aromatic protons-see Table 2; 11.87 (2H, singlet). (Found: C, 59.57; H, 4.56; OC₂H₅, 4.47. Calc. for C₃₂H₃₀O₁₄: C, 60.19; H, 4.73; OC₂H₅ (1), 7.05%). The compound gave a green colour with ferric chloride.
Demethyl ergoxanthin

Ergoxanthin (114 mg) was treated with sodium hydroxide (0.1 N., 50 ml) overnight at room temperature. The solution was acidified and extracted with ethyl acetate. The product was refluxed for 2 hours in glacial acetic acid (30 ml), and the solvent evaporated. The resultant product, demethyl ergoxanthin, which was homogeneous on TLC, gave round crystalline aggregates from chloroform, m.p. 205-208° (dec), \([\alpha]_D^\circ \times 41^\circ (C, 0.98, \text{MeOH}), \gamma_{\text{max}} 1790, 1755, 1652, 1621 \text{ cm}^{-1} (\text{KBr}), \lambda_{\text{max}}^{E_{\text{OH}}} 258 (19,200), 369 (5,500) \text{ m} \mu. \ \text{NMR spectrum (pyridine):} \delta 1.25 (6\text{H, doublet, } J \sim 5 \text{ c.p.s.}), 2.20 (4\text{H, multiplet}), 3.00 (2\text{H, multiplet}), 3.51 (2\text{H, multiplet}), 4.59 (1\text{H, broad singlet, } W_{1/2} \sim 4 \text{ c.p.s.}), 4.94 (1\text{H, broad singlet, } W_{1/2} \sim 4 \text{ c.p.s.}), 5.55 (1\text{H, doublet, } J \sim 3 \text{ c.p.s.}); \ \text{the remaining peaks were lost under the solvent bands. (Found: C, 57.94; H, 4.56. Calc. for } C_{30}H_{26}O_{14}\cdot H_2O: \text{C, 57.33, H, 4.48%). The compound gave a green ferric chloride colour.}

Methylation of demethyl ergoxanthin

The above compound (10 mg) in ethyl acetate was methylated with excess ethereal diazomethane for 30 seconds, and the excess diazomethane and solvents were evaporated. The major component of the resultant mixture was isolated by TLC (eluant: CHCl₃-acetic acid [9:1]) and crystallized from 95% ethanol in needles, m.p. 185-188°. It was identical with authentic ergoxanthin in all respects.
Ergoxanthin tetraacetate

Ergoxanthin (32.1 mg), acetic anhydride (10 ml), and pyridine (3 ml) were heated on the steam bath (75°c for 15 minutes. The product was purified by TLC yielding 28.4 mg of a white amorphous, but chromatographically pure, product; \([\alpha]_D^1 + 75.5^\circ \text{ (C, 1.09, CHCl}_3)\), \(\nu_{max}^\text{max} \ 1810, 1780, 1760, 1745, 1698, 1615, 1465 \text{ cm}^{-1} \text{ (CHCl}_3)\), \(\nu_{E=OH}^\text{max} 214 (20,300), 242 (26,400), 330(4,600) \text{ m}^\nu\). The NMR spectrum\(^{\text{a}} \text{ (CDCl}_3)\) showed characteristic signals for acetyl groups and one methoxyl at 2.05, 2.08, 2.12, and 3.72 p.p.m., respectively. Other signals were observed at 4.46 (d), 5.07, 5.80 (d), each due to 1 H, and a 2-proton signal at 3.03 p.p.m., together with C-CH\(_3\) bands, 57-60 c.p.s. and aromatic signals (Table 2). (Found: C, 58.92; H, 4.77. Calc. for \(\text{C}_{39}\text{H}_{36}\text{O}_18\): C, 59.09; H, 4.58%). The compound gave no colour with ferric chloride.

\(^{\text{a}}\) A-60 spectrum

Hydrolysis of ergoxanthin tetraacetate

Ergoxanthin tetraacetate (5 mg) in methanol (5 ml) was treated with sodium hydroxide (0.1N., 5 ml) at room temperature for 24 hours. The mixture was then acidified, extracted with ethyl acetate, and the product refluxed in acetic acid for 2 hours. Evaporation gave a product which crystallized from chloroform, m.p. 205-208° (dec), identical to demethylergoxanthin in all respects.

Ergoxanthin trimethyl ether

Ergoxanthin (160 mg) in chloroform (5 ml) was treated
with excess ethereal diazomethane at 0° for 24 hours. The resultant mixture was separated by TLC (eluant: CHCl₃-acetic acid (9:1)) and the major component (R₉ 0.50), 89 mg was isolated. It crystallized from chloroform-pet. ether (b.p. 60-80°) in small, colourless prisms, m.p. 120-125° (dec), [α]D - 4° (C, 1.00, CHCl₃), γ max 1798, 1770 (weak), 1747, 1691, 1596, 1462 cm⁻¹ (CHCl₃), λ max 216 (20,000), 250 (24,500), 337 (4,400) m/µ. NMR spectrum (CDCl₃): § 1.23 (6H, doublet, J ~ 6 c.p.s.), a complex multiplet due to 7 protons in the region 100-180 c.p.s., 3.08 (2H, singlet), four OCH₃ groups 3.43, 3.56, 3.73; 4.13 (1H, multiplet), 4.48 (1H, doublet, J ~ 3 c.p.s.), 5.42 (1H, doublet, J ~ 3 c.p.s.) and two AB patterns arising from 4 aromatic protons: see Table 2. (Found: C, 61.30; H, 5.44; OCH₃, 17.10. Calc. for C₃₄H₃₄O₁₄: C, 61.26; H, 5.14; OCH₃ (4), 18.62%).

**Ergoxanthin trimethyl ether monoacetate**

Ergoxanthin trimethyl ether (41.4 mg), acetic anhydride (16 ml) and pyridine (4 ml) were heated at 75° under nitrogen for 15 minutes. The product was isolated by TLC (eluant: 7% acetic acid in CHCl₃) yielding 38.3 mg pure acetate which crystallized from 95% ethanol in white prisms, m.p. 238-240°, [α]D - 21° (C, 0.95, CHCl₃), γ max 1804, 1752, 1696, 1598 cm⁻¹ (CHCl₃), λ max 230 (24,500), 254 (30,000), 339 (5,350) m/µ. NMR spectrum (CDCl₃): § 1.05 (3H, doublet, J ~ 5 c.p.s.), 1.30 (3H, doublet, J ~ 5 c.p.s.), 2.21 (CH₃COO), 120-170 c.p.s. (6H, multiplet), 3.07 (2H, singlet), methoxyls (4) at 3.39, 3.55, 3.58, 3.73; 4.48 (1H, broad singlet, W₁/₂ ~ 4 c.p.s.),
5.69 (1H, doublet, J ~ 3 c.p.s.) and aromatic protons (4) - see Table 2. (Found: C, 61.44; H, 5.15; Calc. for C_{36}H_{36}O_{15}: C, 61.01; H, 5.11%). Ferric chloride: negative.

**Ergoxanthin pentaacetate**

Ergoxanthin (40.4 mg), acetic anhydride (12 ml), and pyridine (3 ml) were heated under nitrogen at 75° for 15 hours. The product was isolated by TLC (eluant: 7% acetic acid in CHCl₃), giving a white, amorphous product, 36.0 mg, homogeneous on TLC: [α]_{D}^20 = -60° (C, 0.97, CHCl₃), ν_{max} 1810, 1780, 1750, 1745, 1698, 1625, 1460 cm⁻¹ (CHCl₃); ν_{max} E有一个OH

218 (26,000), 243 (37,800), 265 (infl., 21,800), 325 (4,500).^NMR spectrum (CDCl₃): δ 1.02 (3H, doublet, J ~ 6 c.p.s.), 1.20 (3H, doublet, J ~ 6 c.p.s.), 1.87 (3H, singlet), 2.11 and 2.18 (12H ~ 4 CH₃CO), complex multiplet 120-180 c.p.s. (6H), 3.78 (3H, singlet), 4.53 (1H, doublet, J ~ 3 c.p.s.), 5.07 (1H, broad singlet, W₁/₂ ~ 5 c.p.s.), 5.45 (1H, singlet) 5.79 (1H, doublet, J ~ 5 c.p.s.), and signals for 4 aromatic protons (Table 2). (Found: C, 59.31; H, 5.09; OCH₃: 3.08. Calc. for C_{41}H_{38}O_{19}: C, 58.99; H, 4.59; OCH₃ (1), 3.72%).

**Hydrolysis of ergoxanthin pentaacetate**

The pentaacetate (5 mg) in chloroform (2 ml) containing hydrochloric acid (3N., 2 ml) and methanol (4 ml) was allowed to stand for 24 hours at room temperature. After isolation, the product was purified by TLC (eluant: 7% acetic acid in

*A ~ 60 spectrum.*
CHCl₃). Its Rₚ value and IR spectrum were identical with those of ergoxanthin tetraacetate.

**Ergoxanthin-2,4-dinitrophenylhydrazone**

2,4-Dinitrophenylhydrazone (60 mg) was dissolved in methanol (6 ml) with hydrochloric acid (3N., 0.30 ml). To this was added ergoxanthin (62 mg) and the solution was refluxed for 3 hours (milder conditions furnished little or no derivative). The product was isolated by TLC (eluants: ether-benzene; 1:1, then CHCl₃-acetic acid; 9:1) yielding 41 mg of orange product which formed prisms from methanol, m.p. 212-215°, [α]D²⁺ ≈ 70° (C, 0.36, CHCl₃), νmax 1798, 1745, 1625, 1600, 1505, 1438, 1340 cm⁻¹ (CHCl₃), λmax E=OH 215 (32,300), 240 (28,500), 380 (26,600) mμ. NMR spectrum (CDCl₃): $\delta$ 1.25 (6H, doublet, J ≈ 6 c.p.s.), 2.12 (8H, multiplet), 2.87 (1H, multiplet), 3.13 (1H, multiplet), 3.76 (OCH₃), 4.35 (1H, broad singlet, W₁/₂ ≈ 4 c.p.s.), 4.55 (1H, broad singlet, W₁/₂ ≈ 4 c.p.s.), 5.26 (1H, broad singlet, W₁/₂ ≈ 4 c.p.s.); an AB pattern $\delta_A$ 6.60, $\delta_B$ 7.50, J₁₂ = 9 c.p.s., due to two equivalent pairs of aromatic protons; an AB pattern (2H), $\delta_A$ 7.33, $\delta_B$ 8.37, J₁₂ = 8 c.p.s., the low field pair being further split with J ≈ 2 c.p.s; 9.15 (1H, doublet, J ≈ 2 c.p.s.), 11.40 (1H broad singlet), 11.63 (1H, singlet), 11.89 (1H, singlet). (Found: N, 6.75. Calc. for C₃₇H₃₂O₁₇N₄N₂, 6.96%). Carbon and hydrogen analyses were not reproducible nor satisfactory.
Conversion of ergochrysin A into Ψ-ergoxanthin

Ergochrysin A (331 mg) was treated with sodium hydroxide (0.1N, 26.5 ml, 5 equivalents) for 21/2 hours at room temperature. Then the mixture was heated on the steam bath for 11/2 hours after which time all the alkali had been consumed. The solution was acidified and extracted with ethyl acetate, the extract dried (Na₂SO₄) and evaporated. The product was refluxed 1 hour in glacial acetic acid (25 ml). Evaporation and methylation with diazomethane yielded a mixture of products, the major component having an Rₚ value close to that of ergoxanthin. This was isolated by TLC (eluant: CHCl₃-acetic acid (85:15)), yielding 38.5 mg of chromatographically pure, but amorphous, product. This had [α]D + 77° (C, 1.59, CHCl₃). The IR spectrum is shown in Fig. 12. \( E^{+}OH_{\text{max}} \) 272 (21,400), 370 (7,300). NMR spectrum (CDCl₃): 1.22 (6H, doublet, J = 6 c.p.s.), 2.10 (3H, multiplet), a complex multiplet between 150 and 250 c.p.s. (7H), 3.18 (2H, broad singlet), 3.72 (OCH₃), 4.32 (1H, broad singlet), 4.72 (1H, doublet, J = 6 c.p.s.), 5.24 (1H, broad singlet), 11.86 (2H, singlet), and peaks for 4 aromatic protons (Table 2). (Found: C, 59.08; H, 4.40; O, 36.39. Calc. for C₃₁H₂₈O₁₄: C, 59.62; H, 4.52; O, 35.86%). The compound gave a green colour with ferric chloride.
Gibbs tests

The tests were performed as follows (45). 2,6-Dichloroquinone chloroimide (Borden Chemical Co.) was crystallized 3 times from methanol-water before use. 8 mg was dissolved in pyridine (5 ml), and 2 ml aliquots pipetted into a solution of ~250 μg of pigment in 0.50 ml pyridine. The solution was made up to 10 ml with borate buffer (pH 9.2). A blank was prepared in the same way. The absorption near 680 μm changed with time and reached its maximum in 20 minutes. The resultant extinctions are recorded below:

<table>
<thead>
<tr>
<th>Pigment</th>
<th>λ_{max} (μm)</th>
<th>ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Secalonic acid B)</td>
<td>686</td>
<td>23,800</td>
</tr>
<tr>
<td>II (Secalonic acid C)</td>
<td>685</td>
<td>24,000</td>
</tr>
<tr>
<td>III (Ergochrysin B)</td>
<td>676</td>
<td>17,500</td>
</tr>
<tr>
<td>IV (Ergoxanthin)</td>
<td>680</td>
<td>18,400</td>
</tr>
<tr>
<td>V (Ergochrysin A)</td>
<td>675</td>
<td>17,000</td>
</tr>
<tr>
<td>VI (Ergoflavin)</td>
<td>680</td>
<td>25,000</td>
</tr>
<tr>
<td>&quot;Chrysergonic acid&quot;</td>
<td>688</td>
<td>21,800</td>
</tr>
</tbody>
</table>

Rotation changes in pyridine

The solutions of the pigments were heated in sealed tubes at 70° ± 1°. Rotations were measured at room temperature. Pigments IV and VI did not undergo change. Pigment III underwent chemical change, as indicated by TLC, but rotation changes were erratic.

* Authentic specimen supplied by Prof. W.B. Whalley.
Time (hours) \[ [\alpha^\prime]_D \]

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>I</th>
<th>II</th>
<th>V</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>+183°</td>
<td>-14°</td>
<td>-52°</td>
</tr>
<tr>
<td>2</td>
<td>+108°</td>
<td>+27°</td>
<td>-26°</td>
</tr>
<tr>
<td>17</td>
<td>+78°</td>
<td>+42°</td>
<td>+4°</td>
</tr>
</tbody>
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In all cases more than one product was formed.
PART II

PRIMYCIN
Primycin was first isolated in 1954 from cultures of a species of Actinomycetes denoted as Streptomyces primyceini obtained from the intestinal tract and faeces of a strain of the larvae of the wax moth Galeria melonella (78). It was found active against various mycobacteria and Gram-positive bacteria (79), and forms complexes with polydeoxyribonucleotides and polynucleotides (80), a property also possessed by streptomycin.

The antibiotic was found to have an empirical formula C_{18}H_{37}O_{7}N, later revised to C_{18}H_{37}NO_{8}, and did not reduce Fehling's solution or react with aldehyde reagents, but exhibited a positive Sakaguchi reaction (81, 82, 83), indicating that the compound, like streptomycin CXII, possesses a mono-substituted guanidine moiety. The infrared spectrum of primycin (84, 85) (Fig. 15) was rather uninformative on account of its lack of character, but it possessed intense absorption at ca. 3300 cm\(^{-1}\) due to strongly bonded hydroxyls. Absorption centered about 1675 cm\(^{-1}\) was attributed in part to the guanido function (86), although the presence of carbonyl functions could not be ruled out from this spectrum. In the ultraviolet spectrum, primycin exhibited only end absorption, which has been attributed entirely to the guanido function (87).

Szilagyi (88) has found that under acidic conditions, primycin gives a biologically inactive, but Sakaguchi positive, aglycone, and a carbohydrate considered to be arabinose, isolated only as the p-nitrophenylhydrazone.
Fig 15
Infrared Spectrum of Primycin (KBr)
The foregoing discussion represents the complete knowledge of the chemistry of primycin until January, 1965, when the present work was undertaken. We were fortunate in being supplied with generous quantities of the pure compound by the Hungarian group.\textsuperscript{a}

The first objective was a determination of the empirical formula and molecular weight of primycin. That this was no mean feat will become overwhelmingly obvious from the following discussion.

Primycin could be converted into a crystalline picrate. The visible spectrum of this compound allowed an estimation of the equivalent weight of primycin with respect to picric acid, inasmuch as the picrate anion may be considered to absorb in the visible independent of its associated cation. By comparison with model compounds, an equivalent weight of 1440 was derived for the antibiotic. This was intuitively satisfying since the guanidine unit possesses three nitrogen atoms, and the previous formula of $C_{18}H_{37}NO_8$, molecular weight 395, was obviously untenable.

Another approach to the determination of the equivalent weight was made through potentiometric titration of the basic function in the molecule. However, as primycin itself did not show basic properties, it must have been isolated as a salt. The associated anion was removed by the use of a

\textsuperscript{a} to whom we express our appreciation.
strongly basic anion exchange resin, the effluent solution containing the free base (or hydroxide) acidified with excess sulphuric acid, and the excess back-titrated potentiometrically with alkali. The difference in the titre between the sample and a blank gives the equivalent weight, and the pH at a position halfway between the sample equivalence point and that of the blank gives the pKₐ, in theory. (Fig. 16) In fact, however, primycin was quite insoluble as the hydroxide and began to precipitate out at ca. pH 10. The average equivalent weight found was 1320 g., with pKₐ ca. 11, although this latter figure must be considered inexact.

The problem of the anion associated with primycin was solved when it was discovered that primycin gave a precipitate with barium chloride. The infrared spectrum of the filtered precipitate was identical with that of barium sulphate (89). The presence of sulphur in the molecule was confirmed by elemental analysis, and indicated an equivalent weight with respect to sulphate anion of ca. 1300, assuming that all the sulphur was present in primycin as the anion. This was later confirmed. The fact that the sulphur in primycin eluded detection for some ten years is reminiscent of the chemistry of penicillin (3), and senegenin (90), and points out the importance of a complete elemental analysis of new complex natural products.

The accurate determination of the sulphate content of
Fig. 16  Potentiometric Titration of Primycin
primycin by a titrimetric method (91) was undertaken as another route to the equivalent weight. The method was checked against sodium sulphate decahydrate and guanidine sulphate (92), and found to be accurate and reproducible. By this method the equivalent weight with respect to sulphate was found to be 2285 g., differing thus from the value based on sulphur analysis by a factor of two. No explanation of this discrepancy can be offered at present.

Primycin was converted to the free base (hydroxide) by ion exchange, and thence to the hydrobromide. This compound was not obtained crystalline, but was homogeneous on t.l.c. Elemental analysis showed 10.60% bromine, giving an equivalent weight with respect to bromine of 754 and suggesting the presence of two bromide ions per molecule. A crystalline perchlorate, prepared in the same way, showed analogous behavior. Moreover, a complete analysis of this salt showed the absence of sulphur, proving that all the sulphur in primycin is contained in its anion. That primycin was stable to the ion exchange procedure was indicated by the regeneration of apparently unchanged starting material upon addition of sulphuric acid to the effluent from the column.

Thus primycin appeared to have an equivalent weight in the region of 1300 and formed a dihydrobromide, a diperchlorate, and a monopicrate, with the nature of the sulphate being uncertain.

An attempt was made to confirm chemically the presence of the guanido function in the molecule. The approach was
that used in the study of streptomycin CXII (93), and
tetrodotoxin CXIII (95, 96), namely vigorous oxidation with
permanganate, through which guanidine has been obtained from
the above compounds, isolated as the picrate. From primycin,
the quantity of guanidine produced was too small for isolation,
but its presence was shown by t.l.c. (96). This evidence in
conjunction with the Sakaguchi reaction and the basicity of
primycin provides a strong case for the presence of a mono-
substituted guanido function in the antibiotic.

Although insoluble in most media, primycin was extremely
soluble in dimethyl sulphoxide, and the n.m.r. spectrum was
determined in this solvent (Fig. 17). Although devoid of
characteristic patterns, C-methyl absorption appeared at
1.22 p.p.m., methylene bands at ca. 1.63 and 1.92 p.p.m., and
weak absorption was visible at 7.95 and 8.62 p.p.m., which
may be attributed to the guanido function. These bands may be
compared with the corresponding absorption observed for
cyclohexylguanidine hydrochloride (97), which absorbed at
7.30 (3H, singlet) and 7.90 (1H, doublet, J ~ 8 c.p.s.) p.p.m.
in this solvent. The spectrum did not allow the detection of
methoxyl functions because of the interfering water peak
present, but a Ziesel determination confirmed their absence.

Alkaline fusion of primycin gave a low yield of n-caproic
acid as the only major product. This is indicative of a part
structure CXIV for primycin, although the nature of the oxygen
substituent cannot be stated. n-Caproic acid has been obtained
CXII
Streptomycin

CXIII
Tetrodotoxin

CXIV

CXV
Fungichromin
Fig 17  N.M.R. Spectrum of Primycin in DMSO-d$_6$
from the macrolide fungichromin CXV (98), under oxidative conditions. Attempts at isolating larger fragments from the alkaline fusion mixture, or via nitric acid oxidation, met with no success.

Attention was then turned to a study of the acidic hydrolysis of primycin, which had been reported (88) to yield arabinose and an aglycone. The method previously used involved neutralization of the hydrolysis mixture with sodium hydroxide, followed by separation of the basic product (hydrochloride) and the sugar by cellulose chromatography. However this had the disadvantage that the sugar obtained was accompanied by sodium chloride and hence could only be isolated as a derivative.

Clearly, here was a situation where the techniques of ion exchange could be used to advantage. Primycin was treated with refluxing dilute hydrochloric acid in methanol/water under Szilagyi's conditions, and then the excess acid was removed by passing the solution through a column of Dowex 3, a weakly basic anion exchange resin. The neutral product mixture showed two spots on t.l.c., one having a positive Sakaguchi reaction, the other exhibiting a red aniline phthalate colour. The solution was then passed through a column of Dowex 50W-X2, a strongly acidic cation exchange resin. This column exchanged protons for guanidinium ion whereas the neutral sugar passed through unaffected. The hydrochloric acid now present in the solution was eliminated by a second
passage through a Dowex 3 column, giving a solution containing only the sugar.

The sugar was obtained in crystalline form, and was identical with D(-)arabinose CXVI in all respects. Moreover, it was converted into a p-nitrophenylhydrazone which was indistinguishable from that prepared from an authentic specimen of D(-)arabinose. This sugar, while much less common than its enantiomer, is known in nature (8), and occurs especially as the aloeanthraquinone glycoside in tubercle bacilli (99).

The other product of the acid hydrolysis, namely the aglycone, now termed norprimycin, was obtained most simply by the following method. The hydrolysate, containing excess hydrochloric acid, was passed very slowly through a column of Dowex 1-X2, OH⁻ form, a strongly basic anion exchanger. During this procedure, the arabinose present was destroyed and the decomposition products were absorbed by the column, allowing norprimycin hydroxide alone to be eluted. This was immediately acidified to ca. pH 4 with hydrochloric acid, and evaporated to dryness. The product was not crystalline, but was best handled as a froth obtained by evaporating in vacuo a solution of norprimycin hydrochloride in methanol/chloroform or methanol/acetone. A crystalline perchlorate could be obtained by addition of perchloric acid to the hydroxide. However, norprimycin did not give a crystalline picrate.

The infrared spectra of the norprimycin salts were practically identical with those of the primycin salts, and revealed
Fig 18

NMR Spectrum of Norprimycin Hydrochloride
no new information. In contrast to primycin, norprimycin hydrochloride was quite soluble in several solvents including pyridine, and the n.m.r. spectrum of the compound was determined in this medium (Fig. 18). The most obvious feature of the spectrum is a large water peak at 5.72 p.p.m., although the solvent was quite dry. However, it was found that the water could be removed by drying the material in high vacuum at room temperature over a two week period. As was the case for primycin, the spectrum of norprimycin hydrochloride was devoid of character, but revealed immense methylene absorption as well as absorption due to C-methyl groups, and, significantly, there were apparently no quaternary methyls as no singlets appeared in the requisite region of the spectrum.

Analysis of intensively dried norprimycin hydrochloride gave data fitting best the formula C_{53}H_{102}N_{3}O_{14}Cl, which has an equivalent weight of 1028. In addition, a Kuhn-Roth determination indicated the presence of 6.52% CH$_3$. As such determinations by themselves are seldom reliable, the accuracy of the analyst was checked by simultaneous submission of a sample of erythromycin$^a$ X (6), which has ten C-methyls, all of which in theory can be determined by the Kuhn-Roth method. However the results obtained for this compound were low by ca. 25%. Correcting the data on norprimycin hydrochloride for the "analytical error" found for erythromycin gave 8.8% CH$_3$, and for a molecular weight of 1000, this is equivalent to 6-7

$^a$ I should like to thank the Eli Lilly Co. (Canada) for a gift of erythromycin.
C-methyl groups. This figure may need to be adjusted somewhat, however. It should be noted that norprimycin forms a monohydrochloride, and also a monoperchlorate, in contrast to primycin.

The empirical formula derived for norprimycin hydrochloride is almost that of a saturated compound. Replacement of the guanidine hydrochloride function, -CH$_5$N$_3$Cl, by hydrogen gives C$_{52}$H$_{98}$O$_{14}$, which has four sites of unsaturation. In fact, for a long time it was believed that primycin (and norprimycin) possessed no carbon-carbon double bonds. However, a critical examination of the ultraviolet spectra of these compounds indicated that this conclusion required revision. Part of the confusion was due to Szilagyi's claim (84, 85) that the ultraviolet spectrum of primycin was due entirely to the guanidine function.

Primycin exhibits only end absorption in the ultraviolet spectrum. However, the extinction coefficient of ca. 22,000 at 200 m$\mu$ is far too high to be due only to the guanidine group. For example, cyclohexylguanidine hydrochloride has an extinction coefficient of 5,000 at 200 m$\mu$. Similarly, norprimycin hydrochloride has an extinction coefficient of 22,000 at 200 m$\mu$. However, it also exhibits weaker absorption at longer wavelengths, and although this spectrum has not been interpreted, it indicates that the transformation of primycin into norprimycin involves more than the hydrolysis of a glycosidic linkage. From the ultraviolet spectra, it was obvious that primycin contained carbon-carbon double
bonds. This was supported by the following observations.

Primycin rapidly consumed bromine, as did norprimycin. Calculation of the equivalent weights of these compounds with respect to bromine gave equivalents of ca. 750 and 700, respectively, although with primycin, contact of the material with excess bromine over a period of several minutes led to further uptake, presumably through oxidation of the arabinose function. These figures indicate the presence of two double bonds in these molecules, since the equivalent weights observed are roughly half the values obtained by analytical means.

Primycin could not be dissolved in a suitable hydrogenation solvent, but norprimycin hydrochloride was very soluble in methanol, and over platinum rapidly consumed ca. two moles of hydrogen. The product, tetrahydronorprimycin hydrochloride was not crystalline, and its homogeneity could not be established because in all solvent systems, it behaved exactly the same as norprimycin hydrochloride on t.l.c. In addition, its infrared spectrum was identical with that of norprimycin hydrochloride. However, it now had an ultraviolet spectrum as expected for a saturated alkyl guanidine derivative. No information is available about the nature of the substitution about the carbon-carbon double bonds in primycin or norprimycin. Although vinyl protons could not be detected in the n.m.r. spectra, these could easily have been missed in view of the large number of protons in the compounds.
In view of the strongly bonded hydroxyl absorption visible in the infrared spectrum of primycin and norprimycin, it was not unlikely that vicinal hydroxyls could be present. However, primycin was very resistant to oxidation by periodate, and could be recovered unchanged after several days at room temperature. The fact that primycin contains arabinose makes this observation surprising, but the nature of the sugar linkage is not known, and it is possible that the sugar is linked as the furanoside CXVII, wherein the vicinal hydroxyls are trans. This might explain their lack of reactivity. A second possibility was that one of the arabinose hydroxyls was esterified with n-caproic acid, as in CXVIII, a situation which has analogy in magnamycin CXIX (100) and other related compounds (6). This possibility was eliminated, however, when it was found that norprimycin also gave n-caproic acid on alkaline fusion.

In contrast to primycin, norprimycin consumed periodate and the uptake depended markedly on the pH of the medium. Using norprimycin hydroxide, the uptake was more rapid, being ca. two moles in 24 hours, and ca. four moles in a week, whereas with the hydrochloride, the uptake after 48 hours was ca. 1.4 moles, with no acidity being produced in the solution over 120 hours (1.9 moles consumed). Two tentative conclusions may be drawn from these observations. First, hydrolysis of primycin liberates a hydroxyl function adjacent to at least
CXVI
D (-) Arabinose

CXVII

CXVIII

n-C₅H₁₁

CXIX
Magnamycin
one other hydroxyl group. An alternative to this is the possibility that primycin possesses an epoxide linkage which is opened under the acidic conditions to a vicinal glycol. Secondly, the dependence on pH may indicate that a second site of attack of periodate is being liberated in a base catalyzed reaction, for example lactone opening as in CXX \rightarrow CXXI. Such behavior has been observed for fungichromin CXV (98), and it provides the first hint that primycin may be a macrolide. The products of periodate treatment have not been extensively investigated.

With acetic anhydride and pyridine at room temperature, norprimycin gave a mixture of two acetates, separable by preparative t.l.c. Treatment of the mixture with hot acetic anhydride alone gave purely the faster running compound, termed acetate 1. The other, acetate 2, could be obtained as the sole product with refluxing acetic anhydride in acetic acid. Hot acetic anhydride in pyridine gave other products which were unstable in air.

Acetate 2 was a glass which could not be crystallized, and since it ran as a long zone rather than a single spot on t.l.c., it is difficult to ascertain its homogeneity. It possesses an intense band in the infrared spectrum at 1725 cm\(^{-1}\), a weaker band at 1675 cm\(^{-1}\), and absorption at ca. 3300 cm\(^{-1}\), much reduced in intensity from that in the starting material. The ultraviolet spectrum was the same as that of the starting material. The n.m.r. spectrum included an intense band at
2.02 p.p.m., which integrated approximately for 27 protons, assuming that the CH₃C-absorption represents 18 protons. This figure may be too large however, and must be considered tentative only.

Acetate 1 was also an amorphous glass, although it gave a single spot on t.l.c. The absorption in the infrared near 3300 cm⁻¹ was much reduced, and the band at 1675 cm⁻¹ was replaced by a band at 1630 cm⁻¹. Moreover, the compound possessed an ultraviolet spectrum markedly different from that of norprimycin. However, it has been observed (101) in a study of simple guanidine derivatives that conversion of a monoacetylated guanidine into its diacetate results in a marked change in the ultraviolet spectrum, and the behavior of acetates 1 and 2 paralleled the reported results very closely. The n.m.r. spectrum (Fig. 19) was essentially the same as that of acetate 2. It should be noted, however, that a small shoulder at ca. 5.3 p.p.m. was visible on the large absorption centered at 4.9 p.p.m. and this may be indicative of vinylic protons in this compound. Integration indicated the presence of ca. 22 acetyl protons, but again because of overlapping bands, the method is untrustworthy. However it seems likely that the acetates possess ca. 7-9 acetyl groups, with two of these being N-acetyl in acetate 1, one in acetate 2. The molecular weight of acetate 1, determined by osmometry, was found to be 1210.
Attention was then turned to methylation studies in an effort to obtain a more reliable estimate of the number of hydroxyl groups in norprimycin. The compound did not react with diazomethane. Methylation with dimethyl sulphate and potassium carbonate in acetone gave a product having variable amounts of hydroxyl absorption in the infrared spectrum, although it gave a single spot on t.l.c., of the same $R_F$ value as the starting material, in all cases. Attempts at completion of the methylation with silver oxide and methyl iodide in dimethylformamide did not meet with success. However, a significant result was obtained from the methylation studies. In the infrared spectrum of the product, independent on the degree of methylation of the material, appeared an intense band 1740 cm$^{-1}$, under which a second band at slightly lower frequency ($\sim$ 1720 cm$^{-1}$) appeared to be buried.

This observation indicated that norprimycin might contain an ester or lactone function (as earlier signalled by the periodate titrations), and possibly a keto group. Aldehydic functions had been ruled out by Szilagyi's group (78).

Under very mild conditions, primycin formed a 2,4-dinitrophenylhydrazone, having $\lambda_{max}$ 365 nm. The derivative could not be crystallized, and although it ran as a single spot on t.l.c., its $R_F$ value was the same as that of norprimycin. It was becoming obvious that t.l.c. is no criterion of purity in this problem. Comparison of the
intensity of absorption at 365 mλ with that of model compounds gave an equivalent weight of 2110 g. for the derivative. As this is roughly twice as large as any previously obtained equivalent weight, it was suspected that the compound was contaminated with unreacted starting material, which could not be detected by t.l.c. However, the spectra of derivatives prepared by reaction periods of one hour and 12 hours at room temperature were the same, and thus, the C₅₃ formula for norprimycin may need to be doubled. Other evidence to support this theory will be presented later.

Experiments were undertaken to strip norprimycin of all functional groups and obtain saturated hydrocarbon containing all the carbon atoms of the main skeleton. The approach was that developed by Cope, and which has been used to determine the carbon skeletons of a number of macrolides, including fungichromin CXV (98), pimamicin (102), (the structure of which has recently been revised (103)), filipin (104), lucensomycin (105), and rimocidin (106, 107). Another approach to the same goal by high pressure hydrogenation has been investigated by Cope, (107, 108) but was not applied to primycin. The present method involves lithium aluminum hydride reduction to a polylol, treatment of this with hydriodic acid and red phosphorus, and then a second lithium aluminum hydride reduction. The resultant hydrocarbon is reduced and chromatographed yielding, in the above examples, mainly one saturated hydrocarbon whose structure is determined with the aid of mass
spectrometry.

This sequence was carried out on norprimycin hydrochloride. However, a single hydrocarbon was not obtained, but instead a very complex mixture of products without any major component. The sequence, repeated on tetrahydronorprimycin hydrochloride, also gave a complex mixture, although not the same as that previously obtained. The approach was therefore abandoned.

Considering the presence of two isolated double bonds in norprimycin, and the large number of hydroxyl groups, it was biogenetically reasonable that one or more of the latter might be situated in an allylic position. However, the failure of active manganese dioxide (109) to produce any change in the ultraviolet spectrum of norprimycin indicated the absence of such an arrangement.

Norprimycin hydrochloride does not exhibit a positive reaction with Fehling's or Tollens reagent, and therefore the presence of an acyloin function is obviated. However, an \( \alpha \)-alkoxyketo group could not be ruled out. That this was not present was shown by the failure of zinc in acetic acid to effect an elimination from norprimycin. Instead, acetate 2 was formed quantitatively, and this product could also be formed with refluxing zinc acetate in acetic acid.

The presently known functional groups of primycin are summarized in Fig. 20.

At this point, alkaline treatment was pursued as a means
as $\text{SO}_4^-$ salt

$-\text{OH} \sim 5-8$, not including arabinose

$-\text{D}(-)$ arabinose as glycoside

two $\text{C} = \text{C}$

$\text{n-} \text{C}_5 \text{H}_{11}$

ca. 6 $\text{CH}_3 \text{CH}$

in norprimycin

possible in norprimycin

Absence of:

$\text{C} = \text{C}$ and $\text{C} = \text{C}$

Fig 20 Primycin: Functional Groups
of degrading primycin, since this very large molecule could not be effectively studied as a unit. Because of its favourable solubility characteristics, the studies were carried out on norprimycin hydrochloride. Initially the reaction was followed titrimetrically. With N/10 sodium hydroxide in ethanol/water at 100° (sealed flask), the uptake of alkali was complete in eight hours with the apparent uptake of ca. three equivalents of alkali (for M = 1000). Examination on the products by t.l.c. indicated a large number of products, and for preparative work, these were separated by an ion exchange technique as follows (Fig. 21).

The basic products were absorbed on a column of Dowex 50W-X2, and the neutral and acidic products eluted out. The absorbed bases were then obtained by converting the column into its sodium form, and eluting with sodium hydroxide. The basic product consisted of a number of products, and these were further separated on the basis of the distribution of their hydrochlorides between n-butanol and water.

Those in the water layer could be separated into a chloroform soluble and a chloroform insoluble portion. That which was insoluble in chloroform was shown to be ammonium chloride by its infrared spectrum (110). The other component was crystalline, and gave a crystalline picrate, but was not identified. It was isolated only in trace amounts from the hydrolysis mixture.
The butanol soluble base hydrochlorides were fractionated by extraction from an alkaline solution with chloroform. This gave an apparently homogeneous, but non-crystalline base, designated $B_1$, which will be described later. The aqueous phase from this extraction, on acidification and extraction with butanol, gave a mixture of two other bases (hydrochlorides), separable by t.l.c. and both possessing positive Sakaguchi reactions. This mixture was, in fact, the major component of the hydrolysate, and this is remarkable in view of the known instability of guanidine derivatives to alkaline conditions (III), wherein ordinarily hydrolysis occurs with the formation of a urea, or, under forcing conditions, an amine. Under the same conditions, tetrahydronorprimycin hydrochloride gave no Sakaguchi positive products.* This fact must mean that the double bonds are intimately involved with the guanidine function in norprimycin, but the nature of the interaction is not known. Unknown also is anything about the chemistry of these two Sakaguchi positive products.

The neutral and acidic product mixture was separated by absorption of the acids on a column of Dowex 1-X2, OH⁻ form, the neutral products passing through unaffected. The acidic materials could then be obtained by conversion of the column into the chloride form, and elution with hydrochloric acid.

* I should like to thank Mr. R.C. Jain for this observation.
FIGURE 21
ALKALINE HYDROLYSIS SCHEME

Nor-Primycin Hydrochloride
2.0 g.

1) wash, OH⁻
2) Cation exchange → Acidic + Neutral

Basic (+ NaCl)

2) HCl

BuOH/H₂O
H₂O
BuOH
BuOH/H₂O
蒸馏进入 HCl

B₃.HCl + NH₄Cl

CHCl₃

Insoluble 70.6 mg
Soluble 5.3 mg

(NH₄Cl) B₃.HCl

H₂O/OH⁻
蒸馏进入 picric acid until alkaline

B₃.picrate

B₃.HCl + NH₄Cl

OH⁻/H₂O/CHCl₃

Aqueous

CHCl₃

B₁ 11.8 mg

H⁺ BuOH

B₂ 1.15 g.

H₂O/OH⁻

Anion Exchange (Dowex 1-X2)

1) Wash, H⁺
2) Anion Exchange (Dowex 3)

Neutral 300 mg
Acidic

Silicic acid Chromatography

64 mg.

βD(−)Arabinopyranoside

1) Evap.
2) Collect distillate 13 mg
3) OH⁻
4) Evap. mainly n-caproic acid
5) H⁺
No information is available about the nature of the acidic or neutral products except that n-caproic acid was obtained in low yield, as described in the experimental. Also a very small amount of methyl $\beta$ D(-)-arabinopyranoside (112) was isolated from the neutral product mixture. This was apparently present in the starting material as an impurity, and a control experiment showed that this compound was unaffected by the alkaline treatment and isolation procedure, whereas arabinose was completely destroyed.

The basic product designated $B_1$ appeared to be of the most interest. It was a strong base yet did not exhibit a Sakaguchi reaction. The ultraviolet spectrum was essentially that of norprimycin, and the infrared spectrum was very similar, except that absorption at $1675 \text{ cm}^{-1}$ was essentially eliminated. In addition the n.m.r. spectrum was closely similar to that of norprimycin. Potentiometric titration of the free base, by adding excess hydrochloric acid and back-titrating the excess with sodium hydroxide gave an equivalent weight for $B_1$ of 1890, and $pK_a$ 9.2. It therefore appears that the compound is an amine (113) and that norprimycin possesses two guanidine functions, one of which is lost in the present transformation, the other converted into a primary amine. This observation also provides evidence that the empirical formula of norprimycin requires to be doubled, giving an equivalent weight of ca. 2000, a conclusion already signalled by the
visible spectrum of the 2,4-dinitrophenylhydrazone.

Although no attempt can be made at this time to present structural proposals for primycin, some comments can be made. It seems possible that primycin is a member of the macrolide family of antibiotics (6), but in its huge molecular weight it dwarfs the others of known structure. Woodward (100) has commented on the difficulties of working with such large molecules, and the state of our knowledge of the formula of primycin bears this out. The usual spectroscopic techniques have been of only limited applicability, and the difficulties of obtaining crystalline or homogeneous reaction products are evident. Similarly the use of elemental analysis for the determination of empirical formulae was hopeless because of the insensitivity of the composition to small changes in formula, and the correct formula will not likely be known until the complete structure is determined. However, the C_{53} (or C_{106}) formula obtained for norprimycin is probably not far wrong.

Such large compounds as primycin seldom are found in nature, aside from proteins etc., which may be considered polymeric materials, with the exception of some fatty acids produced by the tubercle bacilli (23) e.g. the mycolic acid CXXII (114), but even these are comparatively simple in their lack of diverse functional groups. It may be that primycin is composed of several simpler fragments, but it has not yet
CXX

CXXI

CH₃(CH₂)₁₇CH-CH-CH(CH₂)₃₃CH-CH-COOCH₃

CH₃OCH₃

OH C₂₄H₄₉

CXXII

CXXIII

CXXIV

CXXV
been possible to break the molecule down under a variety of conditions.

The list of naturally occurring guanidine derivatives is rapidly increasing. Since Roch's review in 1960 (115), a number of interesting structures have appeared, including tetrodotoxin CXII (95), blasticidin S CXXII (116, 117), hordatine A and B CXXIII (118), and the cipridina luciferin CXXIV (119). The Pfizer handbook (23) lists several Sakaguchi positive metabolites of unknown structure: rhizobacidin (120), seligocidin (121), and virtosin (122), as well as primycin. Spinamycin, (123) a recently reported antibiotic of empirical formula $C_{16}H_{16}N_2O_2$, exhibited a Sakaguchi reaction, and is probably a monosubstituted guanidine derivative.
EXPERIMENTAL

As received from Dr. Szilagyi, Debrecen, Hungary, Primycin had m.p. 192-195° (dec), (microcrystalline), $\gamma_{\text{max}} = 3400$ (intense), 2950, 2880, 1710(sh), 1675, 1640(sh), 1462, 1385, 1117 cm$^{-1}$ (KBr). U.V. EtOH: end absorption, $\varepsilon_{205} = 14,000$, $\varepsilon_{200} = 22,000$ (based on M = 1500). NMR spectrum: see Fig. 16.

Colour reactions:  Sakaguchi +
Dragendorff +
FeCl$_3$ -
Beilstein -

Sodium fusion of Primycin revealed the presence of nitrogen and sulphur (weak test), and the absence of halogens.

Primycin gave a precipitate with aqueous barium chloride - see sulphate titration experiment.

(Found: C, 56.71; H, 9.63; O, 27.16; N, 3.81; S, 2.65, 2.31; ash, 0; OCH$_3$, $< 0.05\%$).

Potentiometric Titration of Primycin

Primycin (17.12 mg) in methanol (25 ml) was passed through a 5x1 cm. column of Dowex 1-X2, 50-100 mesh, OH$^-$ form, and washed through with 75 ml. solvent. To the alkaline effluent was added sulphuric acid (0.02N., 2.00 ml), and the solution was evaporated in vacuo at room temperature to $\sim$6 ml.
Methanol (5 ml) was added and the solution was titrated with 0.02 N sodium hydroxide, following the pH with an E.I.L. Model 23A pH meter. The solution became cloudy at ~ pH10. The $pK_a$ is given by the pH at a point in the titration halfway between the sample and blank equivalence points—see Fig. 15.

Equivalence point: 1.32 ml. 0.02N NaOH.

$\therefore$ 0.68 ml. consumed by Primycin.

$0.01712 \text{ mg. Primycin } = 0.68 \text{ ml. 0.02N NaOH or } 1.36 \times 10^{-5}$
equiv.

$1 \text{ equivalent } = \frac{0.01712}{1.36 \times 10^{-5}} = 1260 \text{ g.}$

A second determination gave an equivalent weight of 1380 g.

**Primycin Picrate**

Primycin (20.5 mg) and picric acid (7.9 mg) were dissolved in methanol and the solution was concentrated to 1/2 ml. On addition of water, the microcrystalline salt separated out. After 6 recrystallizations it had the constant m.p. 166-168°, $\gamma_{\text{max}}$ 3380, 1660, 1620, 1560, 1320, and 1080 cm$^{-1}$ (KBr), $\lambda_{\text{MeOH max}}$ 244, 354, 384 m$\mu$.

Triphenylguanidine picrate had $\lambda_{\text{MeOH max}} 356(\varepsilon = 15,100)$ and 384($\varepsilon = 11,500$), in the visible spectrum. Also pyridine picrate had $\lambda_{\text{MeOH max}} 356(\varepsilon = 14,300)$ and 385($\varepsilon = 11,500$). Hence, assuming an $\varepsilon_{356} = 14,500$ and $\varepsilon_{384} = 11,500$, the equivalent weight of primycin picrate could be calculated:
i.e. Sample = 0.5723 mg/10 ml MeOH (1 cm cell)

Absorbance at 354 μm = 0.587

\[ \text{Conc.} = 4.05 \times 10^{-5} \text{ M/1.} \]

Similarly from A_{384}, conc. = 3.90 \times 10^{-5} \text{ M/1.}

Average conc. = 3.97 \times 10^{-5} \text{ M/1.}

Equivalent weight = \frac{0.0005723 \times 10^2}{3.97 \times 10^{-5}} = 1440 \text{ g.}

(Found: C, 54.47; H, 8.10; N, 6.71%)

Sulphate Titration of Primycin

Standard 0.005M barium perchlorate in methanol/water (4/1) was prepared by direct weighing and made to pH 3.5 with perchloric acid.

Primycin (28.76 mg) in methanol was passed through a 5x1cm column of Dowex 50W-X2, 200-400 mesh, H\(^+\) form, eluting with methanol until neutral. The effluent was made acidic to pH 3 with perchloric acid, and titrated with barium chloride, using Thorin (2(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid) as indicator. Titre = 2.153 ml.

There are 0.002155x0.005M = 1.255 \times 10^{-5} \text{ gm-ions SO}_4^{2-} \text{ in the sample. } \therefore 0.02876 \text{ gm primycin} = 1.255 \times 10^{-5} \text{ mole.}

1 mole = 2290 g.

The average of 3 determinations was 2285 g.

The precipitate from the titrations was filtered, washed, and dried. Its infrared spectrum (KBr) was identical with that published for barium sulphate (89).
Primycin Hydrobromide

Primycin (39 mg) in methanol was passed through a 5x1 cm. column of Dowex 1-X2, 50-100 mesh, OH⁻ form. The alkaline solution was evaporated, hydrobromic acid was added to neutralize, and the solution evaporated. The product was precipitated from methanol with water, and centrifuged giving a non-crystalline powder: Beilstein positive, infrared spectrum identical with primycin, \([\alpha]_D^{\text{MeOH}} + 13^\circ (c, 0.91, \text{MeOH})\). (Found: C, 56.5%; H, 9.40; N, 3.94; Br, 10.60%).

Primycin Perchlorate

Primycin (102.5 mg) was converted to the perchlorate by anion exchanger (OH⁻ form) followed by addition of perchloric acid. The product crystallized from methanol, a microcrystalline salt with no definite melting point due to decomposition. Its infrared spectrum was identical with that of primycin. (Found: C, 54.41; H, 8.61; N, 3.63; O, 28.09; Cl, 4.99%).

Degradation of Primycin to Guanidine

Primycin (100 mg), saturated aqueous potassium permanganate (100 ml) and potassium hydroxide (1/2 g) were heated at 60° for 1 hour. The excess potassium permanganate was destroyed with sodium bisulphite, and the manganese dioxide was filtered. The solution was acidified with hydrochloric acid.
The solution was evaporated and triturated with 5 ml 95% ethanol, and the extract examined by t.l.c. on cellulose (eluant: n-BuOH/HOAc/H₂O-4/1/5). A small spot of the same Rₐ as guanidine hydrochloride could be detected with alkaline ninhydrin (96). However, the quantity was too small for isolation.

Isolation of Arabinose

Primycin (1.719 g), hydrochloric acid (0.2N, 250 ml) and methanol (250 ml) were refluxed for 12 hours. The cooled solution was passed through a 2 1/2 x 30 cm column of Dowex 3, 20-50 mesh, free base form. The neutral effluent was then passed through a 3 x 25 cm column of Dowex 50W-X2, 50-100 mesh, H⁺ form, and the acidic effluent was again passed through the (regenerated)Dowex 3 column. The neutral effluent was evaporated, giving 166.5 mg of product.

Characterization of the Sugar Component

TLC on cellulose of the product in 3 solvent systems showed one spot of Rₐ value identical with that of Arabinose:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuOH 4</td>
<td>0.24</td>
</tr>
<tr>
<td>AcOH 1</td>
<td>0.2</td>
</tr>
<tr>
<td>H₂O 5</td>
<td>0.33</td>
</tr>
<tr>
<td>BuOH 2</td>
<td></td>
</tr>
<tr>
<td>Acetone 7</td>
<td></td>
</tr>
<tr>
<td>H₂O 1</td>
<td></td>
</tr>
<tr>
<td>Pyridine 1</td>
<td></td>
</tr>
<tr>
<td>H₂O 1</td>
<td></td>
</tr>
</tbody>
</table>

(detected with Aniline Phthalate- red-brown spots)

The sugar crystallized from 95% ethanol in prisms, having m.p. 158-159° after 4 recrystallizations, mixed m.p. with D(-)

Arabinose (recrystallized from 95% EtOH) undepressed, mixed
m.p. with L(+) Arabinose 133-148°; the infrared spectrum was superimposable on the of D(-) Arabinose; \( [\alpha]_D^{113^\circ} \) (lit. -104°) (c, 0.30, \( \text{H}_2\text{O} \)).

**p-Nitrophenylhydrazones**

The sugar component (25 mg) and p-nitrophenylhydrazine (38 mg) were heated in methanol (25 ml) in the steam bath for 1 hour. The product was isolated by t.l.c. on Kieselgel (eluant: \( \text{CHCl}_3/\text{MeOH}-4/1 \)), giving the p-nitrophenylhydrazone (14 mg), crystallized from methanol to constant m.p. 186-188°, mixed m.p. with authentic D(-)Arabinose p-nitrophenylhydrazone undepressed, mixed m.p. with L(+)Arabinose p-nitrophenylhydrazone 163-171°. Their infrared and ultraviolet spectra were identical.

**Norprimycin Hydrochloride**

Primycin (5.97 g), hydrochloric acid (0.2N, 750 ml) and methanol (750 ml) were refluxed for 12 hours. The cooled solution was passed through a 5 x 28 cm column of Dowex 1-X2, 50-100 mesh, \( \text{OH}^- \) form (\( \sim \) 500 ml) at a rate of \( \sim \) 1 ml/minute. The column was washed with 500 ml MeOH/\( \text{H}_2\text{O} \) (1:1). The effluent was made acidic to \( \sim \) pH 4 with hydrochloric acid and evaporated to dryness. The product could not be crystallized, but was obtained solid by evaporating in vacuo a solution in MeOH/\( \text{CHCl}_3 \) (1:1), which led to frothing after the solvents had been removed. **Yield**: 5.58 g.
TLC of the product on cellulose showed no trace of arabinose. Norprimycin hydrochloride had $\nu_{\text{max}}^{\text{KBr}}$ 3330 (intense), 2930, 2850, 1710 (sh), 1670, 1640 (sh), 1460, 1380, 1090 cm$^{-1}$ (KBr), $\Lambda$ MeOH 232 (3220), 275 (745), 285 (sh, 685) m$\mu$, as well as end absorption $\varepsilon_{200} = 22,000$, $\varepsilon_{205} = 13,000$ (for $M = 1300$). NMR spectrum, see Fig. 17 - Analysis of intensively dried sample (14 days at room temperature in high vacuum) of material frothed from methanol/acetone: Found: C, 61.26; H, 9.79; N, 4.00; O, 22.27; Cl, 3.30. Calc. for $C_{55}H_{102}N_{14}O_{14}Cl$: C, 61.16; H, 9.88; N, 4.04; O, 21.52; Cl, 3.41%. Also - Found: CH$_3$, 6.52%. Found for erythromycin (free base): CH$_3$, 15.27. Calc. for $C_{37}H_{67}NO_{3}(10\text{CH}_3)$: 20.5%.

Number of CH$_3$ groups in norprimycin hydrochloride:

$$\# = \frac{6.52 \times 1000}{15.035 \times 10^2} = 4.34 \text{ CH}_3$$

Correcting for "analytical error" found in erythromycin:

$$\# \text{ CH}_3 = 4.34 \times \frac{20.5}{15.3} = 5.8 \text{ CH}_3$$

Norprimycin perchlorate, prepared by addition of perchloric acid to norprimycin hydroxide (from ion exchange), crystallized from methanol as a microcrystalline powder, m.p. 154-155° (dec), infrared and ultraviolet spectra identical with those of norprimycin hydrochloride, $[\alpha]_D^0 + 14°$ (c, 1.74, MeOH). (Found: C, 60.57; H, 8.92; N, 4.52; O, 22.32; Cl, 3.77%).

Norprimycin did not react with diazomethane.
Alkaline Fusion

Primycin (500 mg), sodium hydroxide (1/2 g) and potassium hydroxide (1/2 gm) were fused at ~360° for 30 minutes. The product was acidified (pH 2-3) with 2N sulphuric acid, and extracted continuously with ether for 20 hours. After separation into acidic and neutral by extraction, the acids were converted to the methyl esters with diazomethane. VPC of the volatile esters on a 5' x 1/4" column of 20% Carbowax 6000 at 95° and flow rate of 120 ml/min. showed one major peak of retention time 5 minutes (identical to methyl n-caproate). This was isolated by preparative VPC and was identical with methyl n-caproate in the spectra. Higher temperature VPC of the less volatile esters from primycin showed a multitude of peaks with no major components.

A crystalline amide was prepared from the caproic acid, m.p., m.m.p. 99°.

Identical results were obtained from norprimycin hydrochloride.
Bromine Titrations

(a) Primycin

Primycin (13.5 mg) was dissolved in methanol (5 ml). Bromine in carbon tetrachloride (0.0387 N., 2.00 ml) was added. After a measured interval (5 min), potassium iodide (20%, 1 ml) was added and the solution titrated with 0.020 N. sodium thiosulphate.

Titre: 1.285 ml. The equivalent wt. of Primycin with respect to bromine was calculated:

\[ \text{Br}_2 \text{ solution consumed} = 2.00 - \left( \frac{1.285 \times 0.020}{0.0387} \right) = 1.33 \text{ ml.} \]

This contains \( \frac{0.00133 \times 0.0387}{2} = 2.57 \times 10^{-5} \) moles \( \text{Br}_2 \).

Equivalent weight of Primycin = \( \frac{0.0135}{2.57 \times 10^{-5}} \) gm = 525 gm.

The titration was repeated, varying the reaction time of \( \text{Br}_2 \) with Primycin:

<table>
<thead>
<tr>
<th>Sample (mg)</th>
<th>Reaction time (sec)</th>
<th>Titre (ml ( S_2O_3^{2-} ))</th>
<th>Equivalent wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>300</td>
<td>1.285</td>
<td>525</td>
</tr>
<tr>
<td>16.6</td>
<td>300</td>
<td>0.721</td>
<td>529</td>
</tr>
<tr>
<td>19.4</td>
<td>30</td>
<td>0.840</td>
<td>640</td>
</tr>
<tr>
<td>16.8</td>
<td>10</td>
<td>1.488</td>
<td>705</td>
</tr>
<tr>
<td>14.8</td>
<td>5</td>
<td>1.940</td>
<td>765</td>
</tr>
<tr>
<td>20.0</td>
<td>5</td>
<td>1.197</td>
<td>746</td>
</tr>
<tr>
<td>20.1</td>
<td>5</td>
<td>1.309</td>
<td>786</td>
</tr>
<tr>
<td>17.0</td>
<td>2</td>
<td>1.590</td>
<td>746</td>
</tr>
<tr>
<td>16.9</td>
<td>2</td>
<td>1.761</td>
<td>803</td>
</tr>
</tbody>
</table>

(b) Norprimycin Hydrochloride

The titration was performed exactly as with Primycin:
<table>
<thead>
<tr>
<th>Sample (mg)</th>
<th>Reaction time (sec)</th>
<th>Titre (ml $S_2O_3^{2-}$)</th>
<th>Equivalent wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.1</td>
<td>180</td>
<td>1.044</td>
<td>610</td>
</tr>
<tr>
<td>14.4</td>
<td>30</td>
<td>1.640</td>
<td>650</td>
</tr>
<tr>
<td>16.5</td>
<td>10</td>
<td>1.505</td>
<td>700</td>
</tr>
<tr>
<td>15.1</td>
<td>5</td>
<td>1.723</td>
<td>708</td>
</tr>
<tr>
<td>14.3</td>
<td>2</td>
<td>1.860</td>
<td>712</td>
</tr>
<tr>
<td>12.0</td>
<td>2</td>
<td>2.060</td>
<td>660</td>
</tr>
</tbody>
</table>

The bromo compounds were unstable, and could not be purified.

**Tetrahydronorprimycin Hydrochloride**

Norprimycin hydrochloride (121 mg) was hydrogenated in methanol (5 ml) containing platinum oxide (Engelhard, 33 mg) for 3 hours. It consumed two moles of hydrogen. The catalyst was filtered off and the solvents evaporated, giving the tetrahydro compound, as a non-crystalline froth from methanol/chloroform. It was indistinguishable from norprimycin hydrochloride by t.l.c. The compound had end absorption only in the ultraviolet spectrum, with $\varepsilon_{205} = 2,400$, $\varepsilon_{200} = 5,000$. Cyclohexylguanidine hydrochloride had $\varepsilon_{205} = 2,620$, $\varepsilon_{200} = 5,000$ (in 95% EtOH).

**Periodate Titrations**

Norprimycin hydroxide (205.4 mg) was dissolved in methanol (10 ml), 10 ml of a sodium metaperiodate solution (~800 mg/50 ml $H_2O$) was added and the mixture adjusted to 50 ml with methanol. The reaction mixture was allowed to stand in the dark at room temperature; 5 ml aliquots were taken, diluted with water (10 ml) and sodium bicarbonate (2 ml, 10%), added
sodium arsenite (2 ml, 0.10N.), and potassium iodide (2 ml, 25%). The mixture was titrated after 5 minutes with aqueous iodine (0.10N.).

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Sample</th>
<th>Blank</th>
<th>Difference</th>
<th>Moles $10^{-4}$ (for M = 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>0.700</td>
<td>0.535</td>
<td>0.165</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>0.750</td>
<td>0.525</td>
<td>0.225</td>
<td>0.71</td>
</tr>
<tr>
<td>5 1/2</td>
<td>0.820</td>
<td>0.520</td>
<td>0.300</td>
<td>0.95</td>
</tr>
<tr>
<td>12</td>
<td>0.900</td>
<td>0.520</td>
<td>0.380</td>
<td>1.20</td>
</tr>
<tr>
<td>24</td>
<td>1.130</td>
<td>0.520</td>
<td>0.590</td>
<td>1.85</td>
</tr>
<tr>
<td>152</td>
<td>1.930</td>
<td>0.546</td>
<td>1.384</td>
<td>4.35</td>
</tr>
</tbody>
</table>

With norprimycin hydrochloride, uptake of periodate was slower, being $\approx 1.4$ moles after 48 hours. No acidity was observed in the solution after 120 hours ($\approx 1.9$ moles $10^{-4}$ consumed).

Primycin consumed no periodate under the same conditions.

**Acetylation of Norprimycin**

Norprimycin hydrochloride (174 mg), dry pyridine (50 ml) and acetic anhydride (25 ml) were kept at room temperature for 1 hour. The solution was evaporated in vacuo at room temperature. The product showed two spots on t.l.c. (eluant: CHCl$_3$: methanol; 4:1) of $R_F \approx 0.8$ (Acetate 1) and $R_F \approx 0.4$ (Acetate 2). These were separated by t.l.c. giving 55.5 mg Acetate 1 and 90.8 mg Acetate 2.

Acetate 1 could be produced as the only product by redissolving the evaporated mixture above in acetic anhydride (50 ml) and heating at 70° for 5 hours.
Heating norprimycin hydrochloride in acetic anhydride/pyridine produced a complex mixture of products.

Acetate 2 could be produced as the only product by refluxing norprimycin hydrochloride (25 mg) in acetic acid (3 ml) and acetic anhydride (6 ml) for 24 hours.

**Acetate 1**

This was an amorphous glass, homogeneous on t.l.c., having $\nu_{\text{max}}$ 3470 (weak), 1725, 1630, 1600, 1380, 1250, 1220, 1026 cm$^{-1}$ (CHCl$_3$), $\lambda_{\text{MeOH max}}$ 222 (10,600 for M = 1210), 253 (12,300) m$\mu$. NMR spectrum (CDCl$_3$): see Fig. 18. Molecular weight, determined by osmometry: 1210 g. Beilstein: negative; Sakaguchi: negative.

**Acetate 2**

This compound was an amorphous glass, homogeneous on t.l.c., having $\nu_{\text{max}}$ 3320, 3150, 1725, 1675, 1380, 1250, 1220, 1026 cm$^{-1}$ (CHCl$_3$), $\lambda_{\text{MeOH max}}$ 226 (sh, 3900, for M = 1200), 273 (sh, 630) as well as end absorption, $\varepsilon_{200} = 20,000$. Sakaguchi: negative.

**Methylation of Norprimycin**

Norprimycin hydrochloride (106 mg), dimethyl sulphate (10 ml), acetone (50 ml) and anhydrous potassium carbonate (10 g) were refluxed for 48 hours. The filtered solution was evaporated and the product treated overnight with water. Extraction with chloroform gave 131 mg of a gum, which exhibited mainly
one spot on t.l.c. (eluant: CHCl₃: MeOH 1:1), and had \( \gamma_{\text{max}} \)
3400, 1740, 1720, 1600, 1570, 1270 cm\(^{-1}\) (CHCl₃).

2,4-Dinitrophenylhydrazone of Norprimycin

Norprimycin hydrochloride (85.9 mg), 2,4-dinitrophenyl-
ydrazine (40 mg), methanol (100 ml), and 3N hydrochloric
acid (1/2 ml) were allowed to stand at room temperature for
1 hour. The product was isolated by t.l.c. (eluant: CHCl₃:
MeOH 2:1) giving 57.2 mg of the amorphous product, having \( \gamma_{\text{max}} \)
3300 (intense), 1670, 1620, 1600, 1335, 1308 cm\(^{-1}\), \( \gamma_{\text{EtOH max}} \)
227 (15,200 for M = 1500), 252 (12,100), 367 (16,000) m\(^\lambda\).
The compound had the same \( R_F \) value as norprimycin in all solvent systems.

The DNP prepared at room temperature for 1 hour was
examined by ultraviolet spectroscopy. Assuming \( \varepsilon \) at 367 m\(^\lambda\)
= 22,000, the equivalent weight found in 2110 g.

When the reaction period was extended to 12 hours, the
isolated DNP had an equivalent weight of 2020 g, thus indi-
cating that the high equivalent weight was not due to dilution
with unreacted starting material.

Lithium Aluminum Hydride Reduction of Norprimycin

Norprimycin hydrochloride (208 mg) was refluxed in tetra-
hydrofuran (50 ml) with excess lithium aluminum hydride added
in portions over 48 hours. The excess hydride was destroyed
with ethyl acetate, the solvents evaporated, and the residue
was dissolved in 2N. sulphuric acid (250 ml). This solution was extracted continuously with ether for 12 hours. The ether extract was dried (Na$_2$SO$_4$), concentrated and examined by VPC on a 20% Carbowax column at 120°, previously calibrated with n-hexanol. No trace of n-hexanol was detected in the ether extracts.

Norprimycin hydrochloride (523 mg) was treated with lithium aluminum hydride as above. The excess hydride was destroyed. The solvent evaporated and the residue dissolved in sulphuric acid (6N., 100 ml). The solution was extracted with n-butanol (3 x 75 ml), and the butanol washed with sodium carbonate and water. Evaporation gave 531 mg. of a gummy product.

Reduction with Hydriodic acid and Red Phosphorus

The lithium aluminum hydride product in constant boiling hydriodic acid (30 ml) was refluxed for 36 hours with red phosphorus (500 mg). The solution was diluted with 150 ml. water and extracted with chloroform (3 x 100 ml).

The chloroform extract was washed with sodium thiosulphate (2%, 150 ml), and water, dried over magnesium sulphate, and evaporated, giving 238 mg of product.

Lithium Aluminum Hydride Reduction

The above product in tetrahydrofuran (50 ml) was refluxed for 12 hours with excess lithium aluminum hydride. The
excess was destroyed with ethyl acetate, and the product dissolved in sulphuric acid (6N, 100 ml). This solution was extracted with petrol (b.p. 60-80, 3 x 100 ml). The petrol washed with 10% sodium carbonate and dried over sodium carbonate. Evaporation gave 53.7 mg of an oily product. This was chromatographed on a column of aluminum (Woelm, neutral, activity grade I, 3 g). The first pentane fraction (25 ml) weighed 13.2 mg and was examined by VPC, using a 1/8" x 5' column of 5% SE-30 on chromosorb W, programming the temperature between 123° and 250°. Numerous peaks were observed, but no major component was present.

The same sequence, repeated on tetrahydronorprimycin hydrochloride gave an analogous result.

Treatment of Norprimycin with Manganese Dioxide

Norprimycin hydrochloride (25.4 mg) and 'active' manganese dioxide (60 mg) were shaken at room temperature in tetrahydrofuran, and samples were taken, filtered and their ultraviolet spectra examined. After 8 hours, the ultraviolet was still identical with that of starting material.

Treatment of Norprimycin with Zinc Acetate and Acetic Acid

Norprimycin hydrochloride (59 mg) and zinc acetate dihydrate (440 mg) were refluxed in acetic acid (25 ml) for 10 hours. The product was extracted into n-butanol, giving 65.0 mg of a glass, having an infrared and n.m.r. spectrum, and
t.l.c. behavior identical with acetate 2.

The same product was obtained with zinc powder instead of zinc acetate.

**Sodium Hydroxide Titrations**

Norprimycin hydrochloride (497.3 mg), sodium hydroxide (0.10 N., 20 ml) and 95% ethanol (20 ml) were heated in a sealed flask at 100°. At intervals, 4.00 ml aliquots were titrated with 0.10 N. hydrochloric acid. Cyclohexylguanidine was used as a control.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Titre (Sample, ml)</th>
<th>Control (ml)</th>
<th>Moles OH⁻ consumed (M = 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4</td>
<td>0.84</td>
<td>2.01</td>
<td>2.5</td>
</tr>
<tr>
<td>1</td>
<td>0.67</td>
<td>2.02</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>0.61</td>
<td>2.01</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>0.51</td>
<td>2.01</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>0.39</td>
<td>2.00</td>
<td>3.2</td>
</tr>
<tr>
<td>18</td>
<td>0.39</td>
<td>2.01</td>
<td>3.2</td>
</tr>
<tr>
<td>43</td>
<td>0.39</td>
<td>2.00</td>
<td>3.2</td>
</tr>
</tbody>
</table>

In another experiment at 70°, and using 0.01 N. sodium hydroxide, the uptake was also ~ 3 moles of OH⁻, but the reaction required ~ 120 hours to come to apparent completion.

**Large Scale Alkaline Hydrolysis of Norprimycin** (see Fig. 20)

Norprimycin hydrochloride (1.972 g), sodium hydroxide solution (0.10 N., 200 ml) and 95% ethanol (200 ml) were heated in a sealed flask at 100° for 24 hours. An amine odour was apparent in the solution after reaction (but not before).

The cooled solution was passed through a 2 1/2 x 13 cm
column of Dowex 50W-X2, 50-100 mesh, H⁺ form at a rate of
≈ 2 ml/min. The column was washed with 200 ml methanol/water
(1:1) and the effluents ("Acidic + Neutral") evaporated,
giving 0.73 gm product. The solvents were collected during
the evaporation and had the distinct odour of n-caproic acid.
This was isolated as follows: The solution was made alkaline,
evaporated to 1/4 of the original volume, made acidic, and
steam distilled. The steam distillate was extracted with
chloroform; evaporation of the chloroform extract gave 13 mg
of a product giving mainly one peak on VPC corresponding to
n-caproic acid.

The "Acidic + Neutral" fraction in methanol/water (1:1)
was passed through a 2 1/4 x 16 cm column of Dowex 1-X2,
50-100 mesh, OH⁻ form, and washed through with 300 ml solvent.
Evaporation of the effluent gave 300 mg of "Neutral" product.
This showed on t.l.c. a Dragendorff negative spot, Rᶠ ≈ 0.5,
and several faster running Dragendorff positive spots (streak-
ing) (eluant: CHCl₃;MeOH 2:1). The acidic products were ob-
tained by washing this column with 0.1N hydrochloric acid in
methanol/water (1:1) until the effluent solution became acidic
+ 200 ml extra.

**Basic Fraction**

This was obtained by washing the absorbed bases off the
Dowex 50W-X2 column with 0.2N. sodium hydroxide in methanol/
water (1:1), until alkaline + 200 ml. The solution was made
slightly acidic (HCl) and evaporated to dryness. The product was extracted with 200 ml n-Butanol and 200 ml H₂O. Each phase was back-washed with 50 ml solvent. The butanol extract was evaporated to dryness giving ca. 1.30 g. of product (B₁ + B₂). The aqueous phase also was evaporated to dryness, and then taken up in 100 ml H₂O. The solution was made alkaline (pH ~10) with sodium hydroxide and distilled directly into ~ 25 ml 0.1N HCl. Approximately 75 ml of distillate was collected, and the solution evaporated, giving a white solid. The infrared spectrum of the product resembled that of ammonium chloride, but other small bands were visible. Thus, the product was triturated with 10 ml warm chloroform, the solution filtered and evaporated, giving 5.3 mg of chloroform-soluble product. The chloroform-insoluble ammonium chloride weighed 70.6 mg.

The chloroform-soluble crystalline hydrochloride had m.p. 200-220°C, ν max 3350, 2900, 2400 (broad), 1475, 1240, 1205 cm⁻¹ (CHCl₃). A picrate was prepared by steam distilling 2 mg of the hydrochloride in 10 ml water (made alkaline) into 1 mg picric acid (less than one equivalent) in methanol, and evaporating. The picrate crystallized nicely from ethyl acetate/petrol (b.p. 60-80) in prisms, m.p. 180-200°C. The butanol soluble Basic material in water (250 ml) made alkaline was extracted twice with 250 ml chloroform, and twice further with 100 ml chloroform. The chloroform extracts were back-
washed with water, and then evaporated giving 118 mg of product, designated B₁. The aqueous phase was acidified, extracted with 2 x 100 ml distilled n-butanol. The extract was washed with water and evaporated, giving 1.145 gm B₂. B₂ exhibited two spots on t.l.c. (eluant: CHCl₃:MeOH 1:1) both having positive Sakaguchi reactions.

**Examination of B₁**

The hydrochloride was separated from a small amount of faster-running impurity by t.l.c. (solvent: CHCl₃/MeOH (6/4)), and was non-crystalline but could be obtained as a powder by precipitating from chloroform with petrol (60-80). It had \( \nu_{\text{max}} \) 3380 (intense), 2950, 2850, 1705, 1635, 1460, 1380 cm\(^{-1}\) (KBr), \( \lambda_{\text{max}} \) 225 m\( \mu \) (sh) (\( E = 3,350 \) for \( M = 1000 \)); also end absorption, \( E_{205} = 12,800 \), \( E_{200} = 20,000 \), i.e. very similar to the ultraviolet norprimycin hydrochloride. Addition to B₁ (free base) of excess 0.01N hydrochloric acid and back-titration of the excess with 0.01 N sodium hydroxide indicated an equivalent weight of 1860 g. (following pH with a pH meter); duplicate: 1920 g., and \( pK_a \) 9.20 (solvent: 50% EtOH/H₂O).

B₁·HCl did not react with diazomethane.

An attempt was made to fractionate B₁ by distribution between chloroform and aqueous acid or alkali. In either case, the majority of the material was to be found in the chloroform layer.
Acidic Fraction

The acidic products in methanol/water (1:1) + HCl (from washing the Dowex 1-X2 column) were passed through a 3 1/2 x 12 cm column of Dowex 3, 20-50 mesh, free base form and washed through with 200 ml solvent. Evaporation gave 63.6 mg of Acidic product.

Neutral Fraction

Part of the neutral fraction (89 mg) was chromatographed on a column of Silicic acid (Fisher, 10 gm) prepared in chloroform/methanol (19/1). A crystalline fraction was eluted with chloroform/methanol (9:1), which still showed two spots, \( R_F \approx 0.9 \) (Dragendorff +) and \( R_F \approx 0.5 \) (Dragendorff -) on t.l.c. (eluant: \( \text{CHCl}_3: \text{MeOH} \ 2:1 \)). This was separated by t.l.c., giving 16.0 mg of the faster-running material (non-crystalline), and 13.0 mg of the slower-running compound which crystallized from chloroform in prisms, having m.p. 160-164°. Its infrared spectrum was identical with a synthetic (112) specimen of \( \beta \)-methyl D(-)-arabinopyranoside. The synthesis of this compound was slightly modified in that the hydrogen chloride was removed by passing the solution through a column of Dowex 3, free base form, prepared with anhydrous methanol. This is a considerable improvement over the barium carbonate method.

A mixed m.p. with synthetic compound was undepressed.
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