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MOULD METABOLITES—IV*

THE ISOLATION AND CONSTITUTION OF SOME ERGOT PIGMENTS

D. J. ABERHART, Y. S. CHEN, P. DE MAYO and J. B. STOTHERS
Department of Chemistry, University of Western Ontario, London, Canada

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Abstract—The mixture of ergot pigments from a Portuguese ergot drug has been separated. Aside from ergoflavin five other pigments have been characterized and structural proposals for four of these made.

THE study of the pigments produced in the sclerotia of the fungus *Claviceps purpurea* when grown on rye has a history of nearly one hundred years. Until recently the subject has been characterized by little informative chemistry and a plethora of unjustifiable nomenclature.† Part of the confusion, which has not been entirely eliminated,[2–4] derives from the difficulty of separation of closely related substances.

When the present study was undertaken only one pigment, ergoflavin, had been adequately characterized and the subject of informative chemical degradation.[1] In the intervening period the development of thin layer chromatography (TLC) had made available a tool adequate to meet the demands of the problem. We shall first describe the systematic separation of the pigments, and then discuss the individual substances in the light of the presently available knowledge concerning them.

The crude pigment mixture from a Portuguese ergot drug was supplied by Sandoz through the good offices of Dr. H. Schwartz (Sandoz) to whom we are glad to express our warm appreciation. As received the material had been defatted and freed from alkaloids by precipitation from methanolic solution with aqueous tartaric acid. After preliminary removal of some insoluble material the crude mixture showed, on TLC, 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 chloroform–benzene led to the separation of a pigment designated Pigment 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

* Part 3. M. C. Fallona, P. de Mayo and A. Stoessl, *Can. J. Chem.* **42**, 394 (1964). This paper also represents Pt. VII in the series, NMR Studies [Pt. VI. J. B. Stothers, J. D. Talman and R. R. Fraser, *Can. J. Chem.* **42**, 1530 (1964)]

† For a concise summary of the early work, see Ref. 1.

[1] G. Eglinton, F. E. King, G. Lloyd, J. W. Loder, J. R. Marshall, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 1833 (1958).

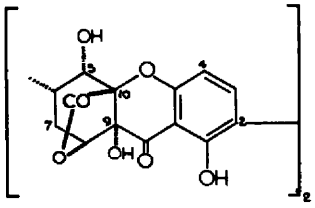
[2] B. Franck and E. M. Gottschalk, *Angew. Chem.* **76**, 438 (1964).

[3] J. W. ApSimon, J. A. Corran, N. G. Creasey, W. Marlow, W. B. Whalley and K. Y. Sim, *Proc. Chem. Soc.* 313 (1963).

[4] B. Franck, O. W. Thiele and T. Reschke, *Chem. Ber.* **95**, 1328 (1962).

eventually isolated. Approximately 28% of the crude pigment weight was eventually obtained 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) was identified as ergoflavin by analysis, UV and IR absorption spectra, and by conversion into ergoflavinic acid,[1,5] the hexa-acetate,[1,5,6] and the tetramethyl ether.[1] Whilst evidence had been accumulated regarding the structure of ergoflavin, before these permitted definitive conclusions to be drawn we were very kindly informed by Professor W. B. Whalley of his chemical findings,[7] and also by Professor Whalley and Dr. G. A. Sim of the results of the X-ray investigation[8] leading to the representation (I) as the stereostructure of this substance. We will, therefore, only discuss the results we have obtained by NMR spectroscopic examination of this substance since these have a direct relevance to the structural elucidation of the other pigments, and were essential to our studies.



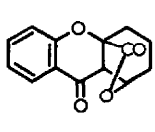
I

The 60 Mc/s spectrum in pyridine (Fig. 1a) 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) ppm. (All peak positions are given relative to internal TMS.) The highest field band ($W_{1/2} = 9$ c/s) is to be attributed to two identical $\underline{CH_3}CH$ moieties. Clearly defined doublets ($J \sim 6$ c/s) are observed for these protons in the spectra of the tetramethyl ether and the hexaacetate, both in $CDCl_3$ solution. The two lower field multiplets arise from methine protons on carbon bearing oxygen, and have been assigned to the C_5 and C_8 protons, respectively. Double irradiation showed that each of these nuclei were spin-coupled to protons absorbing at 2·15 ppm, being therefore the protons at C_6 and C_7. The signals from the remaining protons were lost in the solvent bands. The resonance positions of these protons could be measured in deuterated dimethyl sulphoxide. The presence of a simple AB pattern (Fig. 2c) due to two identical pairs of ortho protons (δ_A 6·82, δ_B 7·66 ppm, $J_{AB} = 8·6$ c/s) attests to the symmetrical structure of ergoflavin. A similar pattern is observed in acetone solution. In addition to the four bands at 1·09, 2·02, 4·12 and 5·25 ppm, there is a sharp singlet at 11·77 ppm presumably due to the hydrogen-bonded phenolic protons.

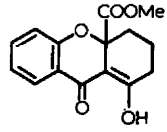
The fastest moving pigment had m.p. 254° and resembled, but differed from, the secalonic acid isolation by Kraft,[9] Stoll[10] and Franck[4] and also from that later reported

[5] W. Bergmann, *Ber. Dtsch. Chem. Ges.* **65**, 1486, 1489 (1932).
[6] A. Freeborn, *Pharm. J.* **88**, 568 (1912).
[7] J. W. ApSimon, J. A. Corran, N. G. Creasey, K. Y. Sim and W. B. Whalley, *Proc. Chem. Soc.* 209 (1963).
[8] J. D. M. Asher, A. T. McPhail, J. M. Robertson, J. V. Silverton and G. A. Sim, *Proc. Chem. Soc.* 210 (1963).
[9] F. Kraft, *Arch. Pharm.* **244**, 336 (1906).
[10] A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta* **35**, 2022 (1952).

by Whalley.[11] Subsequently Franck has revealed[2] that their 'secalonic acid' is a mixture of two closely related substances which they have termed secalonic acid A and B. Our Pigment I appears to be identical with their secalonic acid B, and their nomenclature will be accepted.*

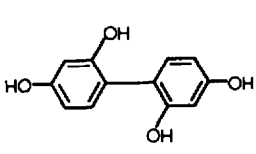


(i)

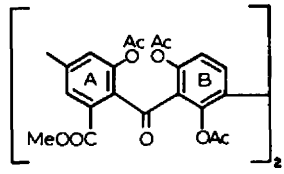


(ii)

Secalonic acid B has the empirical formula $C_{32}H_{30}O_{14}$. It contains two methoxyl groups which the infrared spectrum suggests are present as esters. On fusion with alkali it gave the tetrahydroxybiphenyl (II), succinic and methyl succinic acids,[10] whilst on acetylation with acetic anhydride and pyridine[10] or with acetic anhydride and sodium acetate an optically inactive hexaacetate, m.p. 203–205° was obtained. This had retained the carbomethoxy groups and was entirely aromatic. Bearing in mind



II



III

the previous obtention of the biphenyl (II) it was attributed the structure (III) based on the following observations.†

The empirical formula is compatible with the formulation whilst the presence of the aromatic methyl groups is revealed by the NMR spectrum. A benzophenone-like structure was indicated by a comparison of the UV absorption of the acetate with that of benzoylbenzoic acid methyl ester (Fig. 3). The NMR spectrum (in $CDCl_3$) indicated that there was a pair of identical AB quartets (δ_A 7·10, δ_B 7·45 ppm, $J_{AB} = 8{\cdot}3$ c/s) indicating the presence of two pairs of ortho aromatic protons. In addition to these signals, the NMR spectrum showed two multiplets at 7·19 and 7·56 ppm each with a half-band width of ca. 3 c/s. The latter observation suggests that these signals arise from two pairs of aromatic protons in a *meta* orientation. Because the UV spectra and general properties of the pigment (see below) require that the acetoxyl group and the carbomethoxyl group in ring A be *ortho* to the ketonic bridge the absence of

* A number of pigments having variations on the bis-(hexahydroxanthonyl)-system have now been isolated and more may be expected. The following system has been adopted by Professor Whalley and ourselves following discussion of the former with the Editor of the Journal of the Chemical Society (London).

Pigments which contain two units having the essential features of (i) irrespective of the position of the biphenyl coupling are termed ergoflavin A, B, C etc. As yet there is but one member of this group. Those which contain a unit of (i) and of (ii) are termed ergochrysins, whilst those containing two (ii) units are termed secalonic acids.

† The same compound, obtained from his secalonic acid, has been briefly reported by Whalley.[7]

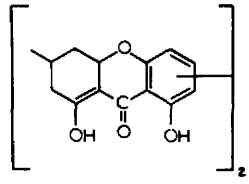
[11] W. B. Whalley, *private communication;* see also Ref. 7.

vicinal aromatic protons requires that the methyl group in this ring be *para* to the bridge with the carbomethoxyl group *ortho* thereto.

Secalonic acid B gives a brown ferric chloride reaction in contrast to the green colour exhibited with ergoflavin. The acidic function was therefore probably enolic. That this is true was indicated by the following sequence. Treatment of the pigment with hydrazine gave an amorphous, but essentially homogeneous product. Oxidation of this with potassium permanganate gave pyrazole-3,4,5-tricarboxylic acid identical with an authentic specimen obtained by the oxidation of 3,4,5-trimethylpyrazole.[12]

It was concluded that secalonic acid B was an enolised β-diketone in contrast to the earlier report of Franck[4] that "secalonic acid" was an enolic α-diketone. Since the second ketonic function appears as the acetate in ring A of the benzophenone its position in formula III is justified.

From these facts the part-formula (IV) for secalonic acid B follows, leaving the location of the remaining hydroxyl function uncertain. In pyridine solution the NMR spectrum of secalonic acid B (Fig. 1c) showed a doublet, centred at 1·17 ppm (6H) with a spacing of 6 c/s. There is sharp singlet, also due to six protons, at 3·57 ppm, a signal at 4·55 ppm (2H) whose width at half-height was 3·5 c/s, and a complex series of bands in the region 120–190 c/s.



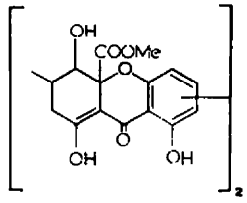
IV

The relative simplicity of the spectrum indicated the symmetry of the structure. The high field doublet is attributed to two identical $\underline{CH_3}$·CH groupings 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 ppm, since simultaneous irradiation 122 c/s up-field from the methine absorption serves to sharpen this peak (at 4·55 ppm), whilst irradiation 80 c/s to lower field from the methyl doublet produces a sharp singlet at 1·17 ppm. These results require that the methyl and hydroxyl groups be vicinal; that is, that the hydroxyl group be at C_6 or C_7. In either case the obtention of methylsuccinic acid by brief treatment of secalonic acid with 50% alkali[10] may be readily rationalized. Of these two formulations, whilst conclusive evidence is not available, we prefer that with the hydroxyl at C_6 since (a) the methylene protons of the secalonic acid units appear at significantly lower field (as expected in an allylic position) than those in ergoflavin and its derivatives, and (b) the IR spectra shows the shift to longer wavelength in secalonic acid of the same allylic methylene[13] (near 1435 cm^{-1}) as compared with that (near 1475 cm^{-1}) of ergoflavin derivatives (KBr).

The absence of a signal for a proton at C_{10} requires that this position be filled by

[12] R. v. Rothenburg, *J. Prakt. Chem.* [2], **52**, 23 (1895).
[13] P. de. Mayo and H. Takeshita, *Canad. J. Chem.* **41**, 440 (1963).

the remaining functional group—the carbomethoxyl group—as was concluded from the bibenzophenone acetate spectrum. The part-structure may thus be expanded to V.



V

Support for this view was derived from the UV absorption of the ultimate product of mild alkaline hydrolysis. The quantitative results are given in Table 1 but inspection of Fig. 4 shows that after 80 hr secalonic acid B has essentially the same absorption as ergoflavinic acid which is that of a dichromanone, and may be compared with that of 2-hydroxy-6-methoxyacetophenone. The same results and conclusions are recorded for other pigments reported in this paper.

The extra two acidic functions revealed by this alkaline treatment are phenolic, as shown by titration, and are to be attributed to the carboxyl produced by hydrolysis of the carbomethoxyl group and that produced by cleavage of the β-diketone.

In an effort to ascertain the nature of the biphenyl coupling we have attempted to distinguish *ortho* from *para* coupling by application of the Gibbs test as modified by King *et al.*[14] We find, contrary to other reports,[3,4] that all pigments give a positive test, measured spectroscopically, in the same time and under the same conditions (Experimental). Furthermore, the intensities achieved are comparable. However, we do not regard the undoubtedly positive nature of the test as indicative of a free *para* position. It is quite possible that under the conditions of the reaction particularly where there is an acidic proton as in the secalonic acids, β-elimination may readily occur with the liberation of the second phenolic group.

In this context the known mutarotation of secalonic acid[10] in pyridine is relevant. We have confirmed this mutarotation, but the transformation is complex since TLC reveals a number of products. As judged by the change in the NMR spectrum such isomerism is an important factor, at least in the early stages. This isomerism was originally tentatively attributed to C_9,[3] but this, in view of the enolic form of the carbonyl is improbable, and is more properly attributed to inversion at C_{10} following β-elimination and readdition.[15] Under such circumstances the phenoxyl 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 by no means certain that transformation products of these pigments (for example, acetates or ethers not made with diazomethane) need have the same biphenyl junction as the original pigment, or need provide structural evidence regarding them. The signals for the aromatic protons in the NMR spectra were also studied since there was a possibility that the nature of the biphenyl coupling might be reflected here. In each case an AB pattern was to be expected, 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.

[14] F. E. King, T. J. King and L. C. Manning, *J. Chem. Soc.* 563 (1957).

[15] G. M. Badger and J. W. Clark-Lewis, *Molecular Rearrangements* (Edited by P. de Mayo) pp. 652–653. Interscience (1963).

In ergoflavin and in all pigments believed to contain an ergoflavin unit (Table 2) very good agreement was obtained for the chemical shifts. This strongly suggests that the biphenyl junction is at C_2 in all cases.

Secalonic acid B was too insoluble for determination of the spectrum in a suitable solvent, but in secalonic acid C (see later) two separate AB patterns are found. Since the orientation of the C_5—OH, in itself, would hardly be likely to affect the aryl proton shifts and conformational inversion of the ring does not affect the position of the carbomethoxyl group the differences may be due either to alternative biphenyl coupling or to a difference in preferred conformation of the otherwise freely rotating carbomethoxyl group because of possible hydrogen bonding. However, ergochrysin A (Pigment V) and ergochrysin B (Pigment III) each contain a diastereoisomeric secalonic acid unit: one of each of the two contained in secalonic acid C. The aromatic proton patterns are nevertheless very close, and are also very close to one of the AB quartets contained in secalonic acid C. Since it seems improbable that inversion of the carbomethoxyl group would leave unaffected the chemical shift of the C_5 proton it may be tentatively be concluded that a difference in biphenyl coupling may be involved between the *same* unit as contained in secalonic acid C and in ergochrysins A and B.

It is interesting that the ergoflavin unit gives a constant pattern since it differs from the secalonic acids in that there is no ready mechanism for elimination—addition which could result in the formation of isomers in biphenyl union or stereochemistry.

In agreement with the general views here expressed it should be noted that the positions of the hydroxyl protons in the secalonic acid and ergoflavin units (Table 1) do not change in the various combinations as might be expected if stereochemical changes were involved in these combinations other than diphenyl fusion.

The enolic nature of secalonic acid B was confirmed by its reaction with diazomethane to give, rapidly, a dimethyl ether, giving a green ferric chloride reaction, which under very mild acidic conditions could be reconverted to the original acid.

The structure arrived at above, insofar as its features are firmly established, corresponds to that suggested by Whalley[3] in a preliminary communication for chrysergonic acid (but see Ref. 16).

Pigment II, now termed secalonic acid C, resembles B in general properties, but is different from substances previously reported.[2-4,11,16] It gives approximately the same yield of pyrazole-3,4,5-tricarboxylic acid as does secalonic acid B, and likewise forms a dimethyl ether which can be readily hydrolysed to the starting material. The UV spectra of acids B and C are identical, and the IR spectra suggest a close relationship. In agreement with this, vigorous acetylation gives a mixture from which the benzophenone (III), obtained from acid B, could be isolated.

The proton resonance spectrum of secalonic acid C in pyridine solution indicates that the pigment contains one acid B unit since signals appear at 1·16, 3·53 and 4·54 ppm (in relative intensities of 3:6:1) together with a complex pattern at 120–190 c/s. As before, double irradiation experiments showed that the signals at 1·16 and 4·54 ppm were coupled to the same proton at 2·54 ppm.

The environment of the second carbomethoxyl group in the other unit appears to be identical with, or very similar to, that of the acid B unit, since a single methoxyl signal due to six protons appears at 3·53 ppm. The methine and methyl protons of the second unit are, however, in somewhat different environments since the signals

[16] B. Franck, E. M. Gottschalk, U. Ohnsorge and G. Baumann, *Angew. Chem.* **76**, 438 (1964).

appear at 4·12 and 1·22 ppm respectively. In addition the C_6 proton is now a doublet ($J \sim 10$ c/s). Double irradiation experiments were performed to show that these protons were mutually coupled to a proton at 2·78 ppm. Again, therefore, the hydroxyl and methyl groups are vicinal, but in this unit they are *trans*-diequatorial to permit the large coupling of the now *trans*-diaxial protons at C_5 and C_6.

The second unit in secalonic acid C, therefore, differs from that in secalonic acid B in the stereochemical relationship of the hydroxyl and methyl group. From the present data no statement can be made with regards the carbomethoxyl group.

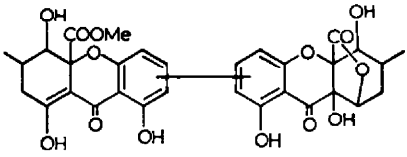
The aromatic protons, visible in the NMR spectrum in dimethylsulphoxide-d_6 (Fig. 2a) give rise to two overlapping AB patterns (Table 2) showing that the two units for this acid are different. Additional evidence is furnished by the appearance of *two* sharp signals at 11·60 and 11·72 ppm attributable to two, slightly different, phenolic protons.

A second product, formed together with the benzophenone, was also obtained on milder acetylation. This substance apparently still retained its optical activity and was completely acetylated. It gave no colour with ferric chloride.

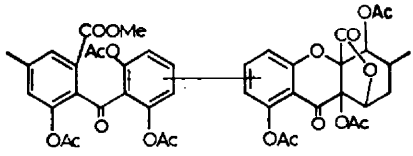
Pigments III and V are closely related. From the proton resonance spectrum it was apparent that they both contained one ergoflavin unit. The observed signals for the spectrum of pigment III, shown in Fig. 1b, (pyridine solution) appear at 5·53 (1H), 4·54 (2H), 3·57 (3H), 2·54 (1H), 2·21 (1H) and 1·17 (6H) ppm together with a broad pattern 130–170 c/s. The chemical shifts for the methine protons at 2·54 and 2·21 were determined by double irradiation. From a comparison of the spectra in Fig. 1a, b, c the second unit in pigment III corresponds to that in secalonic acid B. Further support for these conclusions was obtained from the NMR spectrum in DMSO-d_6 solution, in which the four bands characteristic of the aliphatic protons in an ergoflavin unit are found at 1·08, 2·03, 4·18 and 5·26 ppm. The aromatic protons appear as two separate AB patterns (see Fig. 2b and 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 and, as additional evidence for this, a singlet due to two phenolic protons is found at 11·76 ppm.

Pigment V (in C_5H_5N) exhibits absorption at 5·56 (1H), 4·60 (1H), 4·18 (1H, doublet $J \sim 9{\cdot}6$ c/s), 3·57 (3H), 2·85 (1H), 2·27 (1H), 1·17 ppm (6H) and the usual complex absorption in the range 130–175 c/s. Again, the C_6 methine chemical shifts of 2·85 and 2·24 were measured by double irradiation experiments. Four of these bands are characteristic of one ergoflavin unit while the remaining signals correspond to those for that unit in secalonic acid C which is different from that found in Pigment III. These conclusions are borne out by the spectrum obtained in DMSO-d_6 solution, for which two overlapping AB patterns are found (Table 2) and whose parameters agree closely with the appropriate patterns in the ergoflavin and secalonic acid C spectra (see above).

From this, pigments III and V could tentatively be ascribed the structure VI, without stereochemical implication. In agreement both pigments gave a brown ferric chloride colour (β-diketonic system) and after treatment with hydrazine and oxidation gave the pyrazole-3,4,5-tricarboxylic acid obtained from the secalonic acids, but in approximately half the yield, indicating the presence of but one such unit. Finally, vigorous acetylation[5] of each gave the same benzophenone (VII). The NMR spectrum (in $CDCl_3$) of VII exhibited the expected absorption bands for the aliphatic protons,



VI



VII

1·00 (J ~ 6 c/s) $\underline{CH_3}CH$—; 1·55, 1·97, 2·02, 2·14, 2·18 (CH_3COO—); 2·39 ($\underline{CH_3}$—Ar); 3·69 (CH_3OCO—); 5·03, 5·77 ppm (J ~ 5 c/s) (CH—OAc). The absorption in the aromatic region is particularly informative since the pattern observed for the bibenzophenone acetate from secalonic acid B and C is duplicated together with one additional AB quartet which must arise from the aromatic protons of the ergoflavin portion of this molecule.

In a comparison of the pigments isolated by us and by Professor Whalley's group it became evident that pigment V was the substance termed ergochrysin[5] by the U.K. group and identity was established in both laboratories. The structure (VI) for ergochrysin, now termed ergochrysin A, has since been briefly reported.[3] Pigment III is now designated ergochysin B. It seems probable that the secalonic acid unit in ergochrysin A is that of secalonic acid C.

The remaining pigment, the fourth, has, for the present, been termed ergoxanthin.* It has the empirical formula $C_{31}H_{28}O_{14}$ and contains an ergoflavin moiety for which it shows the appropriate signals in its NMR spectrum (in DMSO-d_6 and C_5H_5N). The second unit is not of the secalonic acid type, however. Further work with this compound will be reported in due course.

EXPERIMENTAL

NMR measurements were determined on a Varian DP-60 instrument in the solvents noted (5–10% w/v). Both $CDCl_3$ and DMSO-d_6 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 an H–P 522B frequency counter. All peak positions are given relative to internal TMS. The double irradiation experiments were accomplished with an NMR Specialties, Inc., Model PD-60 homonuclear spin-decoupling unit.

The solvents for rotations are indicated parenthetically. M.ps were determined on the Kofler hot stage and are uncorrected. Kieselgel was used for TLC.

Preliminary separation of the mixed pigments

The mixed pigment concentrate (40 g) was exhaustively extracted with cold $CHCl_3$ (500 ml) and 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 (ca. 5 l) $CHCl_3$, until the eluant became nearly colourless. Thin layer chromatography (TLC) of a small portion of this extract [eluant: $CHCl_3$–acetic acid (9:1)] showed six spots of R_f and $FeCl_3$ 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 original material to which this name was applied was too ill-defined to be recognisable if reencountered.[17]

[17] W. T. Wenzell, *Am. J. Pharm.* **82**, 410 (1910).

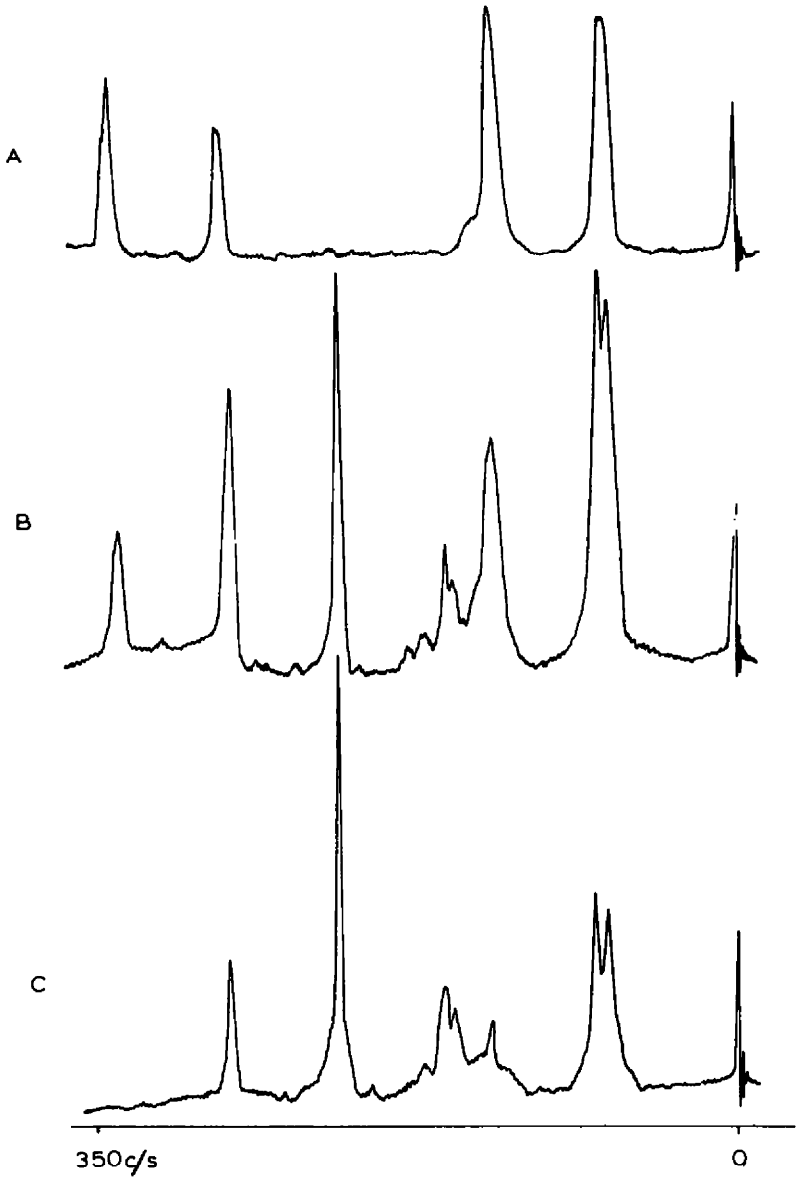


FIG. 1 Typical spectra obtained in C_5H_5N solution showing the region 0–340 c.p.s. from TMS. (A) Ergoflavin, (B) Ergochrysin B (equivalent to A + C), (C) Secalonic Acid B.

The $CHCl_3$ eluate was concentrated to 100 ml, benzene (100 ml) added, and the solution cooled. A precipitate of crude pigment VI (1·0 g)* was collected and washed with $CHCl_3$. The filtrate was concentrated and added to a column of silicic acid (750 g, B.A. reagent sieved < 100 mesh and activated for 3 days at 100°) in $CHCl_3$–benzene (1:1). The column was then eluted with $CHCl_3$–benzene (1:1), (ca. 100 l, recycling the solvent) over 2 weeks. The eluting and developing solvent was then changed to $CHCl_3$–benzene (7:3) when 3 red bands were eluted and were discarded. Solvent was passed until the first yellow band reached the bottom of the column (ca. 50 l). The solvent was

* The six pigments were designated I–VI in order of decreasing R_f value on TLC.

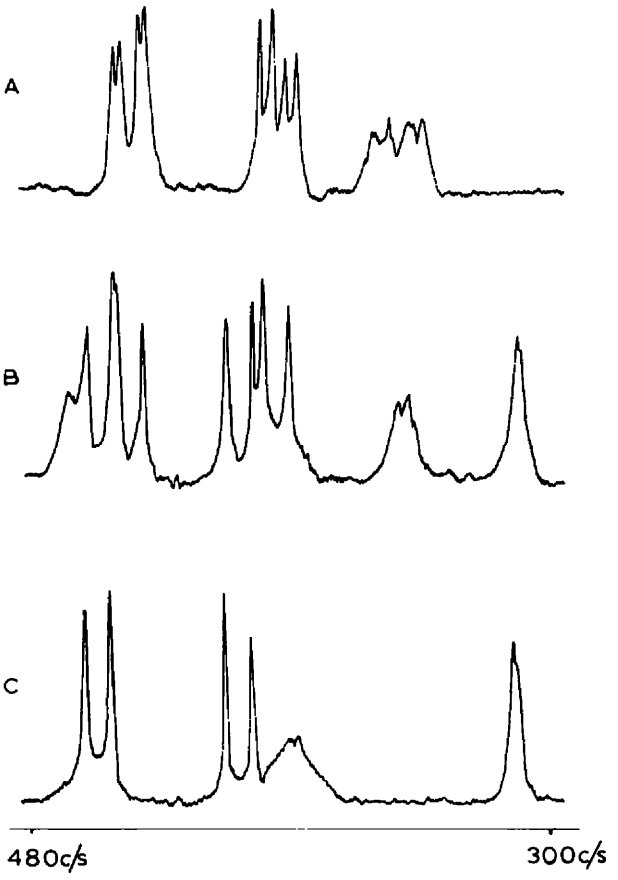


FIG. 2 Low field region (300–480 c.p.s. from TMS) of some typical spectra obtained in DMSO-d_6 solution. (A) Secalonic acid C, (B) Ergochrysin B, (C) Ergoflavin.

then drained and the column extruded by air press. The column was divided into 7 equal fractions from which the pigments were extracted with a mixture of $CHCl_3$ 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 $CHCl_3$ and concentrated to 25 ml. A yellow precipitate of pigment V slowly separated. This was collected and washed with $CHCl_3$. 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 was concentrated to 25 ml and cooled giving crystalline pigment IV (940 mg).

The mother liquors were again evaporated to dryness, taken up in $CHCl_3$. Addition of CCl_4 then gave crude pigment III (300 mg).

Fraction 4 was dissolved in $CHCl_3$, and from this pigment II was obtained (see below) after seeding. This, combined with the material from Fractions 5, 6 and 7 gave a total of 5·50 g of pigment II.

The mother liquors from this crystallization were chromatographed on silicic acid (70 g) and eluted with $CHCl_3$–benzene (7:3). After extrusion of the column, extraction of the slowest-moving one-third of the column gave, on evaporation and crystallization from $CHCl_3$–CCl_4 crude pigment III (800 mg combined with the material from fractions 1–3).

Fraction 5 consisted of nearly pure pigment II (2·90 g).

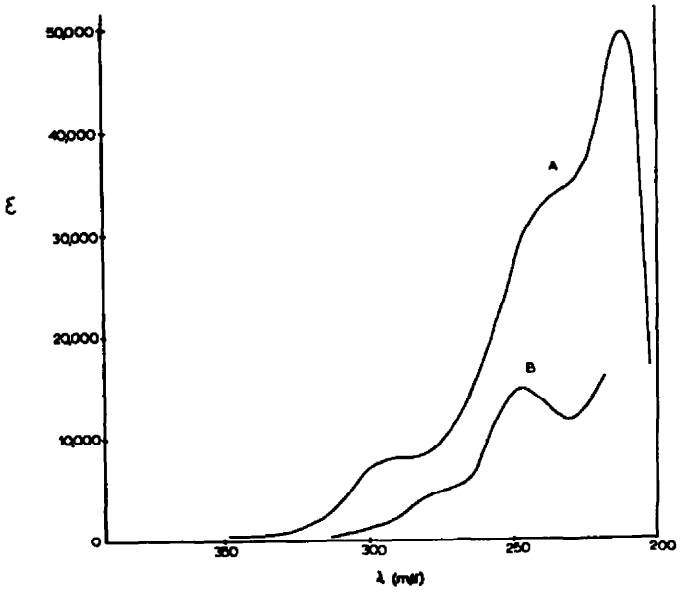


FIG. 3 Ultraviolet spectra of (A) bibenzophenone acetate and (B) *o*-benzoylbenzoic acid, methyl ester in EtOH.

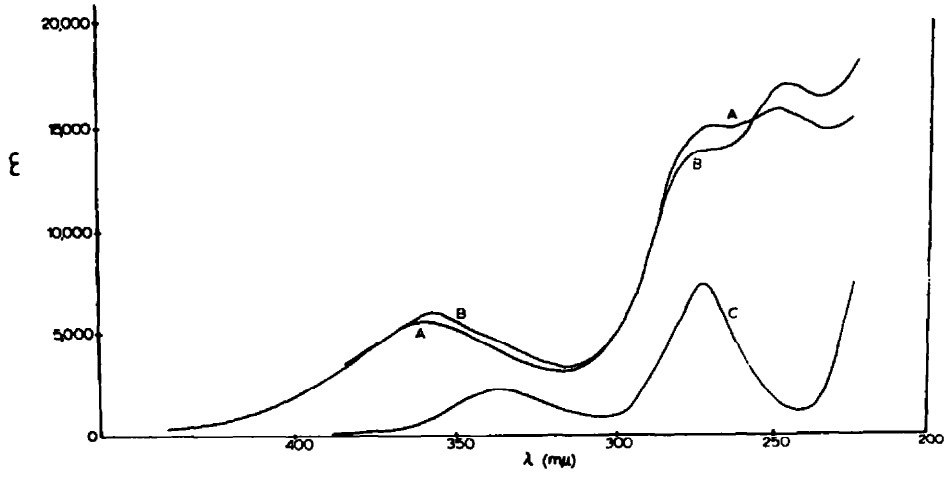


FIG. 4 Ultraviolet spectra of (A) alkaline degradation product of pigment I (Secalonic acid B), (B) ergoflavinic acid and (C) 2-hydroxy,6-methoxy acetophenone in water.

Fractions 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 which were removed from the viscous solution by centrifugation: washing with $CHCl_3$ and acetone gave pigment I (510 mg).

The mother liquors were evaporated and dissolved in $CHCl_3$. Addition of CCl_4 gave crystalline pigment II (2·10 g).

Pigment I (*Secalonic acid* B)

The pigment was crystallized from dioxane–water to give prisms. The physical constants are

TABLE 1. ULTRAVIOLET SPECTRA*

Pigment	Neutral (95% EtOH)	Alkaline[a] (immediate)	Alkali[b] (standing)[hr.]	Acidified[c]	Alkali[d] consumption (eq.)
I, Secalonic Acid B	214 (18900) 242 (16000) 262 (infl.) (14000) 340 (30400) 377 (infl.) (7550)	247 (22300) 364 (29100)	274 (17700) 392 (9000) [80 hr.]	254 (16300) 268 (15400) 357 (6500)	—
II, Secalonic acid C	210 (20000) 242 (18500) 261 (infl.) (15300) 341 (33500) 375 (infl.) (7800)	246 (20300) 362 (29800)	274 (18800) 394 (10300) [80 hr.]	253 (16400) 268 (14900) 356 (6300)	5·30 (phenolphthalein) 3·74 (methyl red)
III, Ergochrysin B	208 (20000) 241 (16500) 266 (17000) 336 (17000) 374 (8000)	227 (19300) 362 (15800) 400 (infl.) (9800)	274 (14700) 393 (8600) [80 hr.]	252 (15400) 268 (13200) 359 (6100)	4·60 (phenolphthalein) 3·16 (methyl red)
V, Ergochrysin A	208 (19900) 243 (16400) 270 (16700) 336 (16300) 374 (7300)	229 (19200) 361 (14000) 400 (infl.) (9450)	273 (14800) 394 (8800) [80 hr.]	251 (14700) 268 (13000) 360 (5600)	—
VI, Ergoflavin	207 (25000) 241 (19300) 279 (20000) 380 (7300)	237 (20200) 286 (13600) 414 (11800)	273 (17200) 391 (9400) 2 hr.]	246 (19500) 270 (15000) 360 (6600)	—
Ergoflavinic acid	247 (17300) 271 (14000) 362 (5400) (H_2O)	273 (14900) 392 (7600)	—	—	—

* [a] Samples dissolved in 0·1N NaOH.
[b] Same solutions after standing at room temp for the number of hr indicated in parentheses, after which time no spectral changes occured
[c] Solutions acidified with 6N HCl
[d] Samples of the pigments on 0·1N NaOH were titrated with 0·1N HCl, after standing at room temp for 80 hr.

TABLE 2. CHEMICAL SHIFTS FOR HYDROXYL AND AROMATIC PROTONS IN ERGOFLAVIN PIGMENTS
(ppm from TMS; DMSO-d_6 solution)

Pigment	Aromatic protons			Hydroxylic protons	
	δ_A	δ_B	J_{AB} c/s	Aliph.	Phenolic
Ergoflavin	6·82	7·66	8·7	6·52 7·67	11·77
Ergochrysin A	6·81	7·63	8·5	6·04	11·69
	6·66	7·50	8·0	6·66 7·67	11·79
Ergochrysin B	6·82	7·63	8·9	5·89	11·76
	6·62	7·47	8·6	6·61 7·75	
Secalonic acid C	6·64	7·47	8·4	5·83	11·60
	6·58	7·43	8·2	6·03	11·72

recorded below and compared with those for Secalonic Acid B.[3,4] (Found: C, 59·65; H, 5·30; O, 35·00; OMe 9·61. Calc. for $C_{33}H_{30}O_{14}$: C, 60·19; H, 4·73; O, 35·08; OMe (2) 9·71%).

	Pigment I	*Secalonic acid* B
m.p.	254–255°	254–256° (dec.)
$[\alpha]_D$ ($CHCl_3$)	+ 156° (*c*, 0·5)	+ 196°
$[\alpha]_D$ (pyridine)	+ 183° (*c*, 0·91)	+ 194°
ν_{max} (KBr)	1742, 1613	1738, 1607 (for "Secalonic acid")
λ_{max}^{EtOH}	214 (ε = 18,900)	220 (α 34·8)
	242 (16,000)	242 (α 33·5)
	262 (14,000)	338 (α 54·3)
	340 (30,400)	
	377 (7,550)	
$FeCl_3$ colour	red-brown	red-brown

Pigment II (*Secalonic acid* C)

This was crystallized from $CHCl_3$–CCl_4 as prisms, m.p. 159–161°, $[\alpha]_D$ +23° (*c*, 1·31, acetone), $[\alpha]_D$ +14° (*c*, 2·58, $CHCl_3$), $[\alpha]_D$ −14° (*c*, 0·99, pyridine), ν_{max} 1740, 1615, 1592, 1567 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 210 (20,000), 242 (18,500), 261 (15,300), 340 (33,500), 375 (7,800) mμ. The pigment gave a red-brown colour with $FeCl_3$. (Found: C, 59·86; H, 4·51; OMe, 9·55. Calc. for $C_{33}H_{30}O_{14}$: C, 60·19; H, 4·73; OMe (2) 9·71%).

Pigment III (*Ergochrysin* B)

This was crystallized as prisms from $CHCl_3$–CCl_4 m.p. 196–199°, $[\alpha]_D$ +82° (*c*, 1·36, acetone), $[\alpha]_D$ +127° (*c*, 1·05, pyridine), ν_{max} 1798, 1737, 1613 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 208 (20,000), 241 (16,500) 266 (17,000), 336 (17,000), 374 (8,000) mμ. It gave a red-brown $FeCl_3$ 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; OMe (1) 4·97%).

Pigment IV (*Ergoxanthin*)

This was crystallized from 95% EtOH to give needles, m.p. 185–188°, $[\alpha]_D$ +124° (*c*, 1·40, $CHCl_3$), $[\alpha]_D$ +138° (*c*, 0·91, pyridine), ν_{max} 1793, 1739, 1644, 1622 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 209 (25,400), 268 (23,600), 373 (8,000) mμ. It gave a green $FeCl_3$ colour. (Found: C, 58·19; H, 4·31; O, 37·51; OCH_3, 5·45. M. wt. determined mass spectrometrically: 624. Calc. for $C_{31}H_{28}O_{14} \cdot H_2O$; C, 57·95; H, 4·71; O, 37·35; OCH_3, 4·83%. M. wt: 624·6).

Pigment V (*Ergochrysin* A)

This crystallized from $CHCl_3$ in plates, m.p. 198–200° and 260–265° $[\alpha]_D$ −37° (*c*, 1·47, acetone), $[\alpha]_D$ −52° (*c*, 0·96, pyridine), ν_{max} 1805, 1750, 1615 cm^{-1} (KBr), λ_{max}^{EtOH} 208 (19,900), 243 (16,400), 270 (16,700), 336 (16,300), 374 (7,300) mμ. The compound gave a red-brown colour with $FeCl_3$. (Found: C, 59·76; H, 5·15; O, 35·12; OMe, 4·87. M. wt. 624 (mass spec.). Calc. for $C_{31}H_{28}O_{14}$: C, 59·62; H, 4·52; O, 35·86; OMe (1), 4·97%. M. wt. 624·6). Pigment V was identical with the ergochrysin (Ergochrysin A) isolated by Whalley[3] in m.p., mixed m.p. UV and IR spectra, specific rotation and R_F value.

Pigment VI (*Ergoflavin*)

This crystallized from MeOH in yellow needles, m.p. > 360° (dec), $[\alpha]_D$ +37° (*c*, 1·87, acetone), $[\alpha]_D$ +103° (*c*, 0·97, pyridine), ν_{max} 1795, 1645, 1620 cm^{-1} (KBr), λ_{max}^{EtOH} 207 (25,000), 241 (19,300), 279 (20,000), 380 (7,300) mμ. The compound gave an olive green colour $FeCl_3$. (Found: C, 59·53; H, 4·56; O, 35·79, M. wt: 610 (mass spec.). Calc. for $C_{30}H_{26}O_{14}$: 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[1].

Pigment II *dimethyl ether* (*Secalonic acid* C, *dimethyl ether*)

The pigment (653 mg) in MeOH was treated with ethereal diazomethane for 5 min, and the resultant mixture (5 components) separated by TLC on silicic acid developing with $CHCl_3$–acetic

acid (9:1). The product of lowest R_F (0·25) was removed from the plates and crystallized from $CHCl_3$–CCl_4 as orange plates (210 mg), m.p. 257–259°, $[\alpha]_D$ +4° (*c*, 2·83, $CHCl_3$), ν_{max} 1745, 1635 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 265 (12,800), 327 (27,900), 374 (infl). (6,650) mμ. NMR spectrum ($CDCl_3$): δ 1·14 (6H, doublet, J ~ 6 c/s), 3·69 (3H, OCH_3) 3·73 (3H, OCH_3) 3·90 (3H, OCH_3) 3·95 (3H, OCH_3), two AB patterns, δ_A 6·42, δ_B 7·29 ppm, J_{AB} = 8·4 c/s and δ_A 6·51, δ_B 7·37 ppm J_{AB} = 8·4 c/s, 13·12 (1H, singlet), 13·29 (1H, singlet). Apart from chemical shift differences a similar spectrum was obtained in DMSO-d_6. The compound gave a green $FeCl_3$ colour. (Found: C, 61·47; H, 5·04; O, 34·11; OMe, 17·89. Calc. for $C_{34}H_{34}O_{14}$: C, 61·26; H, 5·14; O, 33·60; OMe (4), 18·62%).

The ether (5 mg) was dissolved in 0·5 ml $CHCl_3$, MeOH (5 ml) added and 6N HCl (1 ml) and the homogeneous mixture shaken at room temp for 24 hr. The product, on isolation, was identical with secalonic acid C in IR spectrum, UV spectrum and chromatographic behaviour.

Pigment I *dimethyl ether* (*Secalonic acid* B, *dimethyl ether*)

The pigment (76 mg) in ethyl acetate (20 ml) was treated with ethereal diazomethane and the mixture allowed to stand 3 hr. After evaporation of solvent the product was separated by TLC. The material of lowest R_F (0·14) (eluant: $CHCl_3$–acetic acid (9:1)) (35 mg) crystallized from $CHCl_3$ as prisms of m.p. 237–239°, $[\alpha]_D$ +59° (*c*, 0·56, $CHCl_3$), ν_{max} 1740, 1635 cm^{-1} (KBr), λ_{max}^{EtOH} 210 (21,400), 265 (14,000), 328 (26,400), 376 (infl.) (6,400) mμ. The compound gave a green $FeCl_3$ reaction. (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; OMe (4) 18·62%).

Franck *et al.*[4] record the following data for his dimethyl secalonic acid: m.p. 236–238° (dec), ν_{max} 1737, 1631, 1588 cm^{-1} (KBr), olive-green $FeCl_3$ reaction (see also curve (b) in Fig. 4 Ref. 4).

The ether (5 mg) was treated under hydrolytic conditions as described for the ether of secalonic acid C. The recovered product was identical with secalonic acid B in spectra, $FeCl_3$ reaction and chromatographic behaviour.

Acetylation of pigment V (*Ergochrysin* A)

Pigment V (60 mg), sodium acetate (500 mg) and acetic anhydride (2 ml) were heated under reflux for ½ hr. The product was isolated with $CHCl_3$, the $CHCl_3$ layer washed with water. After evaporation of solvent the residue (3 components) was separated by TLC. The fastest moving component (eluant: $CHCl_3$–acetic acid [9:1]) (R_F 0·59) was isolated and crystallized from $CHCl_3$–CCl_4, and from MeOH to give VII (23 mg) as white needles, m.p. 241–243°, $[\alpha]_D$ +34° (*c*, 1·0, $CHCl_3$), $[\alpha]_D$ +15 (*c*, 0·92, pyridine), ν_{max} 1810, 1775, 1765, 1735, 1695, 1620, 1190 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 210 (39,200), 238 (32,900), 264 (18,700), 294 (5,450), 333 (2,000) mμ. (Found: C, 59·46; H, 4·30; O, 36·17; OMe, 3·70. Calc. for $C_{43}H_{38}O_{19}$: C, 60·14; H, 4·46; O, 35·40; OMe (1) 3·61%).

For this compound Whalley[5] records m.p. 245° (dec), $[\alpha]_D$ +15° (pyridine), NMR signals include τ 8·40 (3H, singlet), 8·96 (3H, doublet), J ~ 6c/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 $CHCl_3$-soluble portion of the product was separated by TLC, and the fastest moving component (R_F 0·59; eluant: $CHCl_3$–acetic acid (9:1)) isolated. It was crystallized from $CHCl_3$–CCl_4, and from MeOH, in needles (15 mg) m.p. 241–243° undepressed on admixture with the benzophenone derivative obtained from Pigment V. Their IR spectra were identical.

Pyrazole-3,4,5-tricarboxylic acid

3-Methyl-2,4-pentanedione was converted into 3,4,5-trimethylpyrazole by the method of Rothenburg.[18] It had m.p. 137–138·5° and its IR spectrum was identical with that reported.[17]

The pyrazole (1·0 g), 0·5 N KOH (50 ml) and sat $KMnO_4$aq (85 ml) were heated at 80° for 4 hr. The excess oxidant was destroyed with $NaHSO_3$, and the solution filtered and evaporated. The product was dissolved in 3N HCl (50 ml) and exhaustively extracted with ether.

The crystalline product (1·18 g) was crystallized from ether or acetone (sublimed at ~130°) or from water (m.p. 230° dec). Reported m.p. (from water) 233° (dec).[12]

A sample (5·2 mg) sublimed at 80° and 10 mm was titrated with 0·10 N NaOH. It consumed 3·05 equivs.

[18] R. Hüttel, H. Wagner and P. Jochum, *Liebigs Ann.* **593**, 179 (1955).

Formation of pigment pyrazoles and their oxidation

The following is illustrative: Pigment II (204 mg), hydrazine hydrate (85%; 0·14 ml), MeOH (7·5 ml), and $CHCl_3$ (15 ml) were heated under reflux for 24 hr. The product was insoluble in $CHCl_3$ but appeared homogeneous on TLC (eluant: MeOH). It was basic to litmus (hydrazide), reacted very rapidly with acetone and gave an intense green colour with $FeCl_3$ in MeOH.

The crude pyrazole, 1N KOH (50 ml) and sat. $KMnO_4$aq (100 ml) were heated at 80° for 24 hr. The excess permanganate was destroyed with $NaHSO_3$, the solution filtered and evaporated. The product was redissolved in 3N HCl (50 ml) and the solution extracted with ether (15 × 100 ml). After drying (Na_2SO_4) 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:

Pigment	*Yield (%) based on* 1 *or* 2 *β-diketones*	
I (Secalonic acid B)	42	2
II (Secalonic acid C)	44	2
III (Ergochrysin B)	52	1
IV (Ergoxanthin)	—	–
V (Ergochrysin A)	54	1
VI (Ergoflavin)	0	–

Gibbs tests

The tests were performed as follows.[14] 2,6-Dichloroquinone chloroimide was crystallized 3 times from MeOH–water before use (Borden Chemical Co.). 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). The absorption near 680 mμ changed with time and reached its maximum in 20 min. The resultant extinctions are recorded below:

Pigment	λ_{max} (mμ)	ε
I (Secalonic acid B)	686	23,800
II (Secalonic acid C)	685	24,000
III (Ergochrysin B)	676	17,500
IV (Ergoxanthin)	680	18,400
V (Ergochrysin A)	675	17,000
VI (Ergoflavin)	680	25,000
"Chrysergonic acid"	688	21,800

Rotation changes in pyridine

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

Time (hr)	$([\alpha]_D)$		
	I	*II*	*V*
0	+183	−14	−52
2	+108	+27	−26
17	+78	+42	+ 4

In all cases more than one product was formed.

Acetylation of pigment I (*Secalonic acid* B)

The pigment (92 mg) was heated under reflux under N_2 in acetic anhydride (15 ml) and pyridine (1 ml) for 2½ hr. The solvent was evaporated and the products separated by TLC (eluant: 7% acetic acid in $CHCl_3$). The fastest moving component (40 mg) was crystallized from MeOH to give colourless needles, m.p. 203–205°, $[\alpha]_D$ 0°, $[\alpha]_{5461}$ 0°, ν_{max} 1770, 1723, 1185 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 211 (52,800), 234 (36,000), 289 (8,350) mμ. (Found: C, 61·36; H, 4·53; OMe, 7·16. Calc. for $C_{44}H_{28}O_{18}$: C, 61·83; H, 4·48; OMe 7·26%).

Acetylation of pigment II (*Secalonic acid* C)

The pigment (260 mg) in pyridine (5 ml) was heated on the steam bath with acetic anhydride (50 ml) for 4 hr. After evaporation of the solvents the product (3 components) was separated by TLC (eluant: 7% acetic acid in $CHCl_3$).

The fastest moving component (106 mg) gave crystals (from MeOH) m.p. 203–205°. This was identical with the benzophenone in m.p., mixed m.p., IR spectrum and R_F value.

This compound could also be obtained more conveniently by refluxing the pigment (497 mg) in acetic anhydride (10 ml) and sodium acetate (4·5 g) for 2 hr, yielding, after chromatography and crystallization 92 mg of the benzophenone.

The slowest moving component crystallized from MeOH (needles) with m.p. 237–239°, $[\alpha]_D$ 0° $[\alpha]_{546}$ +4° (*c*, 1·20, $CHCl_3$), ν_{max} 1780, 1760 cm^{-1} $CHCl_3$), λ_{max}^{EtOH} 211 (20,400), 254 (37,200), 298 (33,100), 305 (32,000), 333 (18,700). The NMR spectrum (in $CDCl_3$) exhibits the following distinctive features: two $\underline{CH_3}$—CH doublets, 1·10 and 1·13 ppm (J ~ 7 c/s, in each), six $\underline{CH_3}COO$ groups 2·12 and 2·21 ppm, two identical $COO\underline{CH_3}$ groups, 3·74 ppm, a complex multiplet due to two protons in the region 320–370 c/s, and an AB pattern (δ_A 6·93, δ_B 7·05 ppm, J_{AB} = 8·2 c/s) arising from 4 aromatic protons. The compound gave no colour with $FeCl_3$. (Found: C, 59·74; H, 4·53; OMe 5·85. Calc. for $C_{44}H_{42}O_{20}$: C, 59·33; H, 4·75; OMe (2) 6·97%).

Ergoxanthin tetra-acetate

Ergoxanthin (32·1 mg), acetic anhydride (10 ml) and pyridine (3 ml) were heated on the steam bath for 15 min. The product was purified by TLC yielding 28·4 mg of a white amorphous product, $[\alpha]_D$ +75·5° (*c*, 1·09, $CHCl_3$), ν_{max} 1810, 1780, 1760, 1745, 1698, 1615, 1465 cm^{-1} ($CHCl_3$), λ_{max}^{EtOH} 214 (20,300), 242 (26,400), 330 (4,600) mμ.

In its NMR spectrum ($CDCl_3$), characteristic signals for acetyl methyls and a lone methoxyl group were found at 2·05, 2·08, 2·12 and 3.72 ppm, respectively. Other signals were observed at 4·46 (d), 5·07, 5·80 (d), each of which is due to 1H, and a 2-proton signal at 3·03 ppm, together with C—CH_3 bands, 57–80 c/s and aromatic signals in the region, 415–450 c/s. The compound gave no colour with $FeCl_3$. (Found: C, 58·92; H, 4·77. Calc. for $C_{39}H_{36}O_{18}$: C, 59·09; H, 4·58%).

Acknowledgements—We would like to thank Professor W. B. Whalley for exchange of information and for a view of their manuscript before publication. It is our understanding that their work is being presented for publication at this time also.