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Some Metabolites of *Chaetomium affine* Corda

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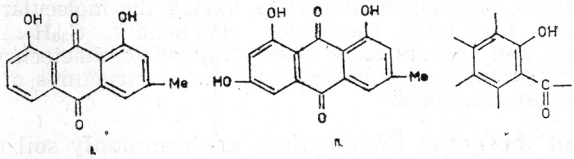
From the fungus *Chaetomium affine* Corda three complex metabolites have been isolated of which two, chaetochrysin and chaetoflavin, are yellow isomerides having the molecular formula $C_{31}H_{26}O_{11}$. The third compound, chaetoalbin, $C_{30}H_{28-30}O_{11}$, is colourless and appears to be closely related to chaetochrysin. The results of preliminary investigations on the structures of the metabolites are described.

The fungi of the genus *Chaetomium* are commonly soil organisms which utilise cellulose as their carbon source and consequently are frequently responsible for the rots and mildews atacking cotton, jute and similar materials[1]. A number of these moulds are unable to grow satisfactorily on media containing more than about 0.2% sugar, a dilution which greatly hampers the isolation of their metabolites. However, *C. affine* Corda[2] (possibly identical with *C. globosum* Kunze apud Kunze and Schmidt[3]) has been found to accept a medium consisting of 4% aqueous glucose and the Czapek-Dox salts together with small quantities of malt or yeast extract[1] and, in addition to succinic acid, mannitol, and ergosterol, to produce a complex mixture of yellow pigments.

From the mycelium of this organism petroleum ether extracted a dark fat, the colouring of which was isolated by chromatography and shown to be chrysophanol (1,8-dihydroxy-3-methylanthraquinone) (I). The chief part of the colouring matter of the mycelium, however, was isolated with acetone and obtained as a dull yellow powder consisting of a mixture of the free pigments and their potassium salts. By selective extractions in conjunction with chromatographic analysis this mixture was resolved into chrysophanol (I), emodin (1,6,8-trihydroxy-3-methylanthraquinone) (II), two new yellow compounds which have been named chaetochrysin and chaetoflavin, a colourless compound, chaetoalbin, and a quantity of amorphous material which has not yet been examined closely but may yield further crystalline metabolites.

Chaetochrysin and chaetoflavin are isomerides with the molecular formula $C_{31}H_{26}O_{11}$ which contain one methoxyl group and have a high optical rotation but which do not crystallise readily and on being heated to 300° decompose without melting, characteristics that extend to their derivatives and necessitate the use of spectroscopic criteria of purity and identity. A further difficulty is that chaetoflavin and its derivatives form solvates of considerable stability which can seldom be freed from solvent without general decomposition setting in. Because of their molecular formulae and their ge-

neral properties in conjunction with their occurrence along with chrysophanol and emodin the new pigments are strongly reminiscent of the fungal dianthraquinones typified by rugulosin $C_{30}H_{22}O_{11}$ and penicillopsin $C_{30}H_{24}O_8$[4] but, in contrast, chaetochrysin was stable to sulphuric acid at 100° and was carbonised at 140° under conditions favouring the smooth dehydration of rugulosin, whilst chaetoflavin did not undergo interaction with dithionite comparable to the ready reductive fission of rugulosin and penicillopsin to simple anthraquinones. Nevertheless, the new pigments must be related in some way to anthraquinone since they could be oxidised by air (in alkaline solutions) to chrysophanol (I). Even when effected by hydrogen peroxide in either acetic acid or dilute aqueous alkali, this reaction gave yields of less than 1%, but the ease with which traces of chrysophanol can be separated and identified chromatographically ensured that the initial materials were not contaminated and gave us confidence in these results.



It is clear that neither pigment contains an unmodified chysophanol nucleus because colour tests for hydroxyanthraquinones were negative and also because their ultra-violet absorption spectra (Figs. 1 and 2) lack the necessary band between 400 and 450 mμ. Further, the spectrum (Fig. 1) of chaetochrysin diacetate (see below), in which the auxochromic effect of hydroxyl

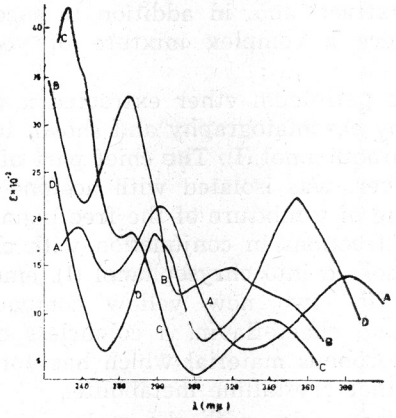


Fig. 1.
A Chaetochrysin (in $CHCl_3$)
B Chaetochrysin diacetate
C Di-O-methylchaetochrysin.
D Tetrahydrochaetochrysin.

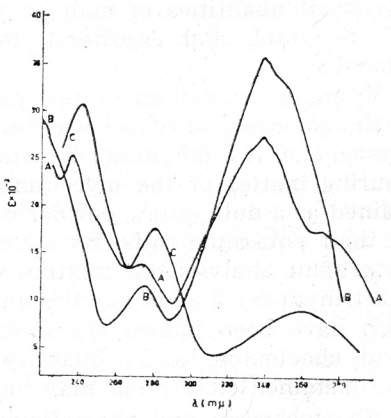


Fig. 2.
A Chaetoflavin
B Tetrahydrochaetoflavin
C Chaetoalbin.

groups is annulled, does not present an obvious correlation with the spectra of anthraquinone or the methoxyanthraquinones. Possible spectroscopic correlations with appropriate benzophenones suggested that a system of the type

exemplified by barbaloin[5] should be considered, but an anthraquinone was not produced by oxidation of chaetochrysin with ferric chloride[5] or sodium bismuthate[6].

Whilst it did not exhibit infra-red absorption bands near 3 μ, chaetochrysin gave a dimethyl ether with methyl iodide and potassium carbonate in boiling acetone and therefore contains two bonded hydroxyl groups which account for the solubility of the pigment in basic media and for its ferric reaction. The corresponding carbonyl groups have so far escaped characterisation since the oxime has not yet been crystallised, but it is probable that there are no further hydroxyl groups to be identified because the dimethyl ether was insoluble in cold aqueous sodium hydroxide, devoid of a ferric reaction, and, in solution in chloroform, transparent at 3 μ. Potentiometric titration of chaetochrysin in aqueous acetone showed that the pigment was a monobasic acid *p*Ka 6.92 (i. e. *p*Ka ~ 4.5 in water), an acidity of this order being usually associated with the carboxyl group. Acetylation of chaetochrysin gave a compound which regenerated the parent on treatment with sodium methoxide but which, on the basis of analytical data alone, might be either a di- or tri-acetate; the evidence supplied by the dimethyl ether appears to favour the former alternative. In consequence of the neutral character of this diacetate, the acidity of chaetochrysin must be ascribed to a system vinylogous with carboxyl as, for example, a triacylmethane or a tetronic acid[7]. The acidity of one of the hydroxyl groups of chaetochrysin is reflected in the fact that with diazomethane there is formed a monomethyl ether which is relatively easily purified and is now the starting-point for more detailed current investigations. This ether retained the ferric reaction of the parent compound and was phenolic rather than acidic since it could not be titrated in aqueous acetone and, curiously enough, exhibited weakly-bonded hydroxyl absorption at 3 μ.

In preliminary investigations, hydrogenation of chaetochrysin was found to be erratic and to give complex amorphous mixtures. With a platinum catalyst, more than 4 mols. of hydrogen were slowly absorbed, but with a palladium catalyst the reaction could be stopped at an arbitrary point permitting isolation of a crystalline tetrahydro-derivative with weak hydroxyl absorption at 3 μ in the infra-red. In potentiometric titrations, this derivative behaved as a weak acid *p*Ka (water) ~ 7.1, indicating that the original highly acidic centre is profoundly modified by hydrogenation and therefore supporting the conclusion that chaetochrysin is a vinylogue of a carboxylic acid.

Chaetochrysin and its derivatives posses infra-red absorption at 1779 to 1795 $cm.^{-1}$ and therefore appear to contain a five-membered lactone ring which may be responsible for the gradual dissolution of the dimethyl ether in warm aqueous sodium hydroxide. The diacetate has much intensified absorption at this point, presumably because of the expected contributions from vinyl or phenyl acetate residues which usually absorb at 1770—1780 $cm.^{-1}$. Another presumptive carbonyl band appears close to 1645 cm^{-1}. in spectra of chaetochrysin, the monomethyl ether and the tetrahydro-derivative, but is absent from spectra of the diacetate and the dimethyl ether. In conjunction with the failure of the last two compounds to give ferric reactions and the production of chrysophanol (I) on oxidation, the spectroscopic data strongly suggest the presence of an *o*-hydroxycarbonyl grouping (III) which could also be respon-

sible for the amorphous oxime. It is significant that this crude oxime retains a band at 1745—1761 cm.$^{-1}$ of the same intensity at that apparent in chaetochrysin and its derivatives which must therefore be attributed to an ester rather than to a carbonyl function. As the methoxyl group of chaetochrysin was removed by alkali (although the product was amorphous) it is not clear whether this ester function is a lactone or a methoxycarbonyl group, but it is of interest that a band near 1750 cm.$^{-1}$, in conjunction with bands near 1690 cm.$^{-1}$ and 1603 cm.$^{-1}$, is typical of acyltetronic acids[8] and that, appropriately modified, this system can account for the acidity, the ultra-violet absorption[9] (at the shorter wave-lengths), and the behaviour of chaetochrysin on hydrogenation. In view of the complexity of the molecule, however, this suggestion is purely tentative in spite of the fact that acyltetronic acids are already well-known fungal metabolites[10].

Although satisfactory methylation, acetylation, or oximation products have not yet been obtained from chaetoflavin, the di- and tetra-hydro derivatives have been characterised. These compounds do not exhibit lactone absorption near 1780 cm.$^{-1}$ although some fractions of the crude methylation products have a band at this point indicating that the lactone system may be involved in the relationship of the two pigments, but it is evident from the differing ultra-violet absorption spectra (Figs. 1 and 2) and from the relatively weak acidity of the pigment that the isomerism is complex. Neither pigment has yet given significant results in reactions with perbenzoic acid, ozone, lithium aluminium hydride, sodium borohydride, zinc dust, nitric acid, or zinc permanganate.

Chaetoalbin, $C_{30}H_{28-30}O_{11}$, contains one methoxyl group and is not acidic although it forms an orange solution with aqueous sodium hydroxide. Chelated systems are absent, however, because the compound, which reduces Fehlings solution, does not give a ferric reaction or a copper salt, and does not appear to react with carbonyl reagents. It is of interest that in sulphuric acid chaetoalbin exhibited colour reactions qualitatively similar to those of rugulosin. The infra-red spectrum included three bands near 3 μ corresponding to hydroxyl absorption, and also bands at 1787, 1748, 1681, and 1631 cm.$^{-1}$ which corresponded closely to those of chaetochrysin. Lack of material has prevented further study.

EXPERIMENTAL

Melting-points are uncorrected. Unless qualified the petroleum ether employed had boiling-point 60—80°. Infra-red absorption spectra were determined, usually in Nujol mulls, on a Grubb-Parsons double-beam spectrophotometer or on a Perkin-Elmer spectrophotometer Model 21, and ultra-violet spectra in alcoholic solutions of concentrations in the range 10^{-3} to 10^{-4} molar.

Metabolites of Chaetomium affine Corda

Supplied by the Central Bureau voor Schimmelcultures, Baarn, specimens of *Chaetomium affine* Corda were maintained on oatmeal-agar solid media from which, after two weeks at 30°, spore-suspensions in sterile water were prepared and used to innoculate flasks (400) containing Czapek-Dox medium (500 ml.) with added Marmite or malt extract (0.5%) and ferrous sulphate heptahydrate (0.01%). Raulin-Thom, Sabaraud, and William Saunders W. D. 5 and W. D. 10 media were less satisfactory. When the flasks were kept at 25°, a dense white mycelial felt developed

in 10 days which gradually became green on top and yellow-brown underneath; after 50 days growth was interrupted. The yellow pigmentation did not develop when the fungus was grown at 30°.

The reddish, viscous, slightly alkaline metabolic liquor was concentrated in a vacuum to approximately 20 l. At this stage, extraction with ether removed negligible qantities of material but after acidification (*p*H 3) continued ethereal extraction supplied a yellow gum from which petroleum ether precipitated succinic acid, m. p. 185°, identical with an authentic specimen. The petroleum solution slowly deposited an orange phenol which has not been examined further.

The dry, powdered mycelium (1160 g.) was continuously extracted (*a*) with ether for 4 days, (*b*) petroleum ether (b. p. 40—60°) for 6 days, and (*c*) methanol for 4 days. Ergosterol separated from the petroleum extract and, on crystallisation from acetone, formed needles (2.8 g.), m. p. and mixed m. p. 163°, giving a blue-green Liebermann-Burchard reaction, and forming the acetate, m. p. and mixed m. p. 174°. When the dark fat left on removal of the petroleum was chromatographed from benzene on silica gel (mesh 200—300) there appeared a dark orange band containing chrysophanol (I) which crystallised from benzene in orange plates (0.12 g.), m. p. and mixed m. p. 195° λ max. 226, 256, 278, 288, 436 mμ ($\varepsilon \times 10^{-3}$ 41, 28, 14, 14, 11.8). This compound was identified with a synthetic specimen[11] by the following infra-red absorption: 1675, 1631, 1609, 1572, 1550, 1482, 1294, 1280, 1215, 1182, 1167, 1085, 1055, 1030, 997, 973, 905, 872, 841, 815, 808, and 758 cm.$^{-1}$.

Anal. (1) 5.648. mg. subst.: 14.65 mg. CO_2, 1.97 mg. H_2O
(2) 5.237 mg. subst.: 13.60 mg. CO_2, 1.82 mg. H_2O
$C_{15}H_{10}O_4$ (254.23) calc'd.: C, 70.86; H, 3.96%
found: (1) C, 70.78, H, 3.90%
(2) C, 70.86, H, 3.88%.

On being concentrated and kept the methanol extract gave mannitol (8 g.), m. p. 165°, on purification, forming the hexa-acetate, m. p. and mixed m. p. 126°.

From the concentrated acetone extract a yellow-brown powder was precipitated by addition of petroleum ether and was partially purified by repeated treatment with boiling carbon tetrachloride. This product left a large residue of potassium salts on ignition and was therefore thoroughtly triturated with 2*N*-hydrochloric acid until this residue was minimised. On one occasion further interaction of the acid-washed material with carbon tetrachloride left a solid which crystallised from methanol giving *Fraction A*. In general the acid-washed material was extracted with methanol and the residue (33 g.) then crystallised from acetone giving *Fraction B*. The methanol-soluble fraction formed a brown oil when the solvent was removed, and, when repeatedly triturated with petroleum ether, this oil supplied a yellowish powder (106 g.) partly soluble in benzene. The benzene solution was poured on a silica column (42×5 cm.) and the chromatogram developed first by benzene and then by mixtures of chloroform and methanol; fractions (each 500 ml.) were taken as follows:

Fractions 1—4. Eluted by benzene, these fractions gave a crimson copper salt and supplied chrysophanol (0.116 g.), purified and identified as described above.

Fractions 5—7. Eluted by chloroform and rejected.

Fractions 8—16. Eluted by chloroform-methanol (30:1), these fractions gave a yellow-green copper salt, and on evaporation a yellow powder (39 g.) designated *Fraction C*.

Fractions 17—18 gave a brown copper salt and a yellow powder (12.9 g.), *Fraction D*.

Fractions 19—27 were eluted by chloroform-methanol (4:1) and supplied a yellow powder (10.5) which has not yet been examined.

Fractions 28—32 were eluted by chloroform-methanol (3:2) and gave a yellow powder (7.8 g.) not investigated further.

Fraction 33 was eluted by chloroform-alcohol (2:3), giving a yellow solid (16 g.).

Fraction 34 was removed from the column by 100% alcohol.

Part of fraction *D* was insoluble in cold chloroform and was crystallised from chloroform giving emodin[13] in orange plates (0.42 g.), m. p. 253—254°, λ max. 224, 252, 266, 291, and 433 mμ ($\varepsilon \times 10^{-3}$ 32.4, 17.4, 17.7, 20.0, 13.0) having infra-red absorption

bands at 3440, 1671, 1631, 1601, 1592, 1572, 1550, 1486, 1427, 1348, 1313, 1285, 1230, 1171, 1138, 1109, 1036, 944, 909, 877, 823, and 765 cm.$^{-1}$.

The analytical specimen was sublimed at 200°/0.05 mm.
Anal. 5.92 mg. subst.: 14.50 mg. CO_2, 2.04 mg. H_2O
$C_{15}H_{10}O_5$ (270.23) calc'd.: C, 66.67; H, 3.73%
found: C, 66.79; H, 3.86%

Chaetochrysin

Franction *B* was crystallised from acetone, chromatographed from benzene-chloroform (4:1) on silica, and recrystallised from methanol-pyridine or acetone to yield chaetochrysin in pale yellow plates (8.5 g.), melting at 304—306° with profound decomposition. In chloroform, this compound had ultra-violet absorption maxima at 238, 281, and 383 mμ ($\varepsilon \times 10^{-3}$ 19.0, 21.2, 14.2), and in dioxan it had $[\alpha]_{21.2}^{D} = +482°$ (c=0.702). Infra-red absorption bands were noted at 1789, 1754, 1686, 1650, 1605, 1572, 1473, 1370, 1346, 1319, 1299, 1258, 1217, 1168, 1144, 1114, 1092, 1075, 1047, 943, 893, 877, 870, 848, and 800 cm.$^{-1}$. Potentiometric titration of chaetochrysin (24.2 mg.) in acetone (85 ml.) and water (50 ml.) against 0.01 *N* aqueous sodium hydroxide showed the presence of an acid *p*Ka 6.92. In these conditions benzoic acid had *p*Ka 6.62 indicating that, in water, chaetochrysin has *p*Ka ~ 4.50.

Thé analytical specimens were dried to constant weight at 100°/1 mm.

Anal. (1) 5.77 mg. subst.: 13.69 mg. CO_2, 2.38 mg. H_2O
6.56 mg. subst.: 5.34 mg. CO_2
4.82 mg. subst.: 2.45 ml. 0.02 *N* $Na_2S_3O_3$
(2) 5.82 mg. subst.: 13.79 mg. CO_2, 5.34 mg. H_2O
7.01 mg. subst.: 5.71 mg. CO_2
5.02 mg. subst.: 2.61 ml. 0.02 *N* $Na_2S_2O_3$.
(3) 5.43 mg. subst.: 12.89 mg. CO_2, 2.19 mg. H_2O
6.12 mg. subst.: 3.23 ml. 0.02 *N* $Na_2S_2O_3$
$C_{31}H_{26}O_{11}$ (574.52) calc'd.: C, 64.80; H, 4.56; O, 30.64; 1 OMe, 5.40%.
found: (1) C, 64.71; H, 4.61; O, 29.93; OMe, 5.27%.
(2) C, 64.65; H, 4.72; O, 30.00; OMe, 5.38%.
(3) C, 64.77; H, 4.51; OMe, 5.46%.

Molecular weight determined ebullioscopically in dioxan (Menzies-Wright) 568, 574.

Chaetochrysin formed an orange-red solution in aqueous sodium carbonate or sodium hydroxide and was recovered by acidification after one but not after twelve hours. In acetone-alcohol the pigment gave a intense red-brown ferric reaction, and a yellow copper salt insoluble in chloroform. It did not reduce Fehling's solution or Tollen's reagent but reduced permanganate slowly in neutral and rapidly in acid solution. Red dyes resulted with diazotized sulphanilic acid or *p*-toluidine, but bromine in acetic acid was unaffected. Zinc dust reduced the alkaline solution to a straw-coloured product but this has not been isolated: the pigment gave no other colour test for a quinone. Colour tests with magnesium powder and hydrochloric acid, with bleaching-powder, with perchloric acid, and with trichloracetic acid were also negative. 2,4-Dinitrophenylhydrazine sulphate, hydroxylamine acetate, *p*-nitrobenzoyl chloride in pyridine, and dihydropyran failed to give crystalline derivatives. Chaetochrysin was recovered from its solution in alcoholic ammonia after 12 hours, and gave only a yellow solution with boracetic anhydride.

Chaetochrysin (0.3 g.) was heated with 10% aqueous potassium hydroxide under nitrogen for one hour. Precipitated by dilute hydrochloric acid, the amorphous yellow product was purified by re-precipitation from a solution in ethyl acetate by means of petroleum ether giving material which decomposed near 200° and could not be crystallised but in the Zeisel determination gave negative results for methoxyl.

Chaetochrysin (0.10 g.) dissolved in sulphuric acid at 100° giving an orange solution. After 2 hours, the mixture was decomposed with ice and the yellow precipitate, when washed with water and purified from acetone, was indistinguishable from chaetochrysin.

Chaetoflavin

Fraction *C* was combined with that part of Fraction *D* soluble in chloroform and re-chromatographed on silica. Yellow material (12.3 g.) eluted by chloroform-benzene (1:1) was kept but brownish material eluted by chloroform-methanol (95:5) was intractable and was rejected. The yellow solid crystallised from hot ethyl acetate after the addition of some benzene and when purified by a repetition of this process supplied chaetoflavin in yellow plates (10.1 g.) which decomposed near 300° after being dried at 150°/1 mm. for 3 hours (the benzene solvate decomposed at about 183—185°). Chaetoflavin, m. p. 300°, had λ max. 233, 275, 337 mμ ($\varepsilon \times 10^{-3}$ 25.5, 14.4, 27.8) with inflections at 333 and 373 mμ, infra-red absorption bands at 1733, 1698, 1614, 1586, ~ 1490, 1486, 1423, 1368, 1304, 1263, 1222, 1205, 1186, 1171, 1151, 1142, 1112, 1086, 1062, 1049, 1024, ~ 1000, 986, 967, 938, 893, 885, 866, 845, 797, 774 cm.$^{-1}$; and $[\alpha]_{22°}^{D} = +700°$ (c = 0.900 in benzene).

An analytical specimen was dried as above.

Anal. (1) 5.71 mg. subst.: 13.54 mg. CO_2, 2.19 mg. H_2O
(2) 5.36 mg. subst.: 12.75 mg. CO_2, 2.06 mg. H_2O
$C_{31}H_{26}O_{11}$ (574.52) calc'd.: C, 64.80; H, 4.56%.
found: (1) C, 64.76; H, 4.29%
(2) C, 64.91; H, 4.30%.

An analytical specimen was crystallised from ethyl acetate-benzene and dried at 100°/1 mm. for 1 hour.

Anal. 5.54 mg. subst.: 13.95 mg. CO_2, 2.39 mg. H_2O
7.72 mg. subst.: 5.51 mg. CO_2
5.70 mg. subst.: 2,66 ml. 0.02 *N* $Na_2S_2O_3$
$C_1H_{26}O_{11} \cdot 1.5\ C_6H_6$ (691.28) calc'd: C, 69.48; H. 5.11; O, 25.46; OMe, 4,48%.
found: C, 68.78; H, 4,89; O, 26.00; OMe, 4.83%.

An analytical specimen was crystallised as above and dried at 130°/1 mm. for 2 hours.

Anal. 6.40 mg. subst.: 15.71 mg. CO_2, 2.84 mg. H_2O
7.44 mg. subst.: 5.45 mg. CO_2
$C_{13}H_{26}O_{11} \cdot C_6H_6$ (652.26) calc'd.: C, 67.10; H, 5.10; O, 27.01%.
found: C, 67.63; H, 4.67; O, 26.93%.

Molecular-weight determined ebulliscopically in benzene on solvent-free chaetoflavin: 562, 556. Chaetoflavin was hardly soluble in petroleum ether and not very soluble in ether; it dissolved in dioxan, chloroform, acetic acid, and acetone. It behaved in the same way as chaetochrysin in the general tests described above, but in potentiometric titration in acetone — water (5:6) it behaved as a dibasic acid (*E*, 277) *p*Ka 7.22 and changes in *p*H during the earlier part of the tiration suggested that a lactone ring was opening or that rapid enolisation was occurring.

Chaetoalbin

Repeatedly purified from methanol, fraction *A* supplied chaetoalbin in small white needles (0.5 g.), m. p. 285°, having λ max. 242, 282, 360 mμ ($\varepsilon \times 10^{-3}$ 30.8, 17.7, 9.03) but devoid of a ferric reaction in alcohol.

The analytical sample was dried at 100°/0.1 mm. for 1 hour.

Anal. (1) 5.91 mg. subst.: 13.89 mg. CO_2, 2.55 mg. H_2O
5.00 mg. subst.: 2.55 ml. 0.02 *N* $Na_2S_2O_3$
(2) 6.27 mg. subst.: 14.69 mg. CO_2, 2.70 mg. H_2O
5.88 mg. subst.: 3.10 ml. 0.02 *N* $Na_2S_2O_3$
$C_{30}H_{26}O_{11}$ (562.21) calc'd.: C, 64.06; H, 4.65; 1 OMe, 5.52%.
found: (1) C, 64.10; H, 4.82; OMe, 5.28%
(2) C, 63.97; H, 4.81; OMe, 5.44%.

This substance was moderately soluble in cold acetone but nearly insoluble in benzene and ether. It did not form a copper salt and was insoluble in aqueous sodium hydrogen carbonate although it gave an orange solution in 2*N*-aqueous

sodium hydroxide. In cold sulphuric acid it formed a yellow solution that became violet when warmed and red when diluted. The substance reduced Fehling's solution but did not react readily with 2,4-dinitrophenylhydrazine sulphate. Selective infra-red absorption was noted at 3400 (three peaks), 1787, 1748, 1681, and 1631 cm.$^{-1}$.

Oxidation of Chaetochrysin and Chaetoflavin to Chrysophanol

For these experiments the pigments were purified by two additional recrystallisations and by two chromatographic analyses on silica .

(i) Either pigment (1.0 g.) in methanol constaining sodium methoxide (from 2 g. of sodium) was kept at 0° during the gradual addition (2 hours) of 30% hydrogen peroxide (5 ml.) in methanol (10 ml.). After 12 hours at 0°, the red products were liberated by dilute sulphuric acid, extracted into ether, and freed from acids by means of aqueous sodium hydrogen carbonate. Recovered by evaporation in a vacuum the neutral product formed a yellow powder which crystallised from alcohol yielding chrysophanol (5.5 mg.) in yellow plates, m. p. 195°, giving crimson solutions in 2 *N*-aqueous sodium hydroxide and in sulphuric acid.

Anal. 6.55 mg. subst.: 16.92 mg. CO_2, 2.38 mg. H_2O
$C_{15}H_{10}O_4$ (254.23) calc'd.: C, 70.86; H, 3.96%
found: C, 70.47; H, 4.07%.

This substance was identified with authentic material[11] spectroscopically and by means of the dimethyl ether[12], m. p. and mixed m. p. 195°.

(ii) Air was drawn through a solution of either pigment (0.50 g.) in 2*N* aqueous sodium hydroxide (50 ml.). During 8 hours at 80° the red colour changed to green and then deepened to dark brown whereupon the cooled mixture was acidified with dilute sulphuric acid and extracted with chloroform. Chrysophanol (22 mg.), m. p. 195°, was isolated and identified as in (i) above.

(iii) When hydrogen peroxide (30%; 5 ml.) was added gradually (2 hours) to a solution of either pigment (1.0 g.) in hot acetic acid (50 ml.), the colour changed from yellow through orange to deep red. After the addition of water (100 ml.), the products were taken up in ether, washed with aqueous sodium hydrogen carbonate, and extracted into aqueous sodium hydroxide. From the crimson solution chrysophanol (6 mg.) was isolated, purified, and identified as before.

Di-O-Methylchaetochrysin

When a mixture of chaetochrysin (1.0 g.), potassium carbonate (10 g.), and methyl iodide (21 ml.; added portionwise) was heated under nitrogen in boiling acetone (500 ml.), the initial yellow colour faded to straw in 48 hours. The residue was extracted with hot acetone and the combined acetone solutions were evaporated leaving an amorphous solid of which the solution in chloroform was washed with 2 *N*-aqueous potassium carbonate, dried ($MgSO_4$), concentrated and diluted with petroleum ether. The cream precipitate was purified by chromatography from chloroform on silica. The earlier eluates, when concentrated and diluted with petroleum ether, gave solids which were combined and crystallised from benzene — petroleum ether (b. p. 80—100°) (9:1) giving the dimethyl ether in pale cream rods (120 mg.), m. p. 265—270° (decomposition), devoid of a ferric reaction.

For analysis a specimen was dried at 150°/1 mm, for 2 hours.

Anal. 6.14 mg. subst.: 14.88 mg. CO_2, 2.59 mg. H_2O
5.99 mg. subst.: 4.76 mg. CO_2
4.18 mg. subst.: 5.66 ml. 0.02 *N* $Na_2S_2O_3$
$C_{33}H_{30}O_{11}$ (572.24) calc'd: C, 65.77; H, 5.02; O, 29.16; 3 OMe, 15.45%
found: C, 66.16; H, 5.27; O, 28.89; OMe, 14.89%.

This ether, which was insoluble in hot sodium carbonate and cold sodium hydroxide solution, but gave a pink and then a deep orange solution in hot aqueous sodium hydroxide, had λ max. 234, 268, 344 mμ ($\varepsilon \times 10^{-3}$ 49.1, 19.0, 8.91) and infra-red absorption bands at 1790 (shoulder), 1770, 1745, 1739 (shoulder), 1709, 1619, 1605, 1567, 1422, 1326, 1290, 1259, 1242, 1232, 1209, 1185, 1163, 1152, 1089, 1072, 1058, 1037, 1016, 968, 969, 951, 858, 828, 820, 769, 710, 702 cm.$^{-1}$ in Nujol and at 3050, 2967, 2867, 1795, 1773, 1739, 1698, 1610, 1565, 1462, 1420, 1381, 1339, and 1318 cm.$^{-1}$ in chloroform.

Chaetochrysin Diacetate

Vacuum evaporation of the volatile materials from a mixture of chaetochrysin (1.0 g.), sodium acetate (1.0 g.), and pyridine (1 ml.) kept in boiling acetic anhydride (15 ml.) for 2 hours left a gum which gave a grey solid when ground with water. Chromatographed on silica from benzene, this solid gave a bright yellow band that, segregated and eluted with ethyl acetate, furnished the diacetate in faintly yellow needles (0.4 g.), m. p. 246°, λ max. 222, 265 (f), 320 mμ ($\varepsilon \times 10^{-3}$ 36.1, 34.1, 15.4), from ethyl acetate.

Anal. 6.02 mg. subst.: 14.03 mg. CO_2, 2.46 mg. H_2O
6.18 mg. subst.: 2.88 ml. 0.02 *N* $Na_2S_2O_3$
$C_{35}H_{30}O_{13}$ (658.24) calc'd.: C, 63.83; H, 4.60; OMe, 4.71%
found: C, 63.57; H, 4.56; OMe, 4.82%.

This compound gave erratic results in acetyl determinations. Insoluble in cold 2*N*-aqueous sodium hydroxide and having a negative ferric reaction in alcohol, this diacetate (0.4 g.) gave, when kept for 12 hours with sodium methoxide (from 0.1 g of sodium) in methanol (30 ml.), a yellow solution from which 2*N*-aqueous hydrochloric acid precipitated chaetochrysin as a yellow powder (0.3 g.) which on crystallisation from acetone formed yellow needles, m. p. 296°, identified spectroscopically.

O-Methylchaetochrysin

Methanol (1 ml.) and an excess of diazomethane in benzene were added to chaetochrysin (0.5 g.) dissolved in the least quantity of chloroform. After 10 hours at 0°, the mixture, when concentrated at 40° and diluted with petroleum ether (b. p. 40—60°), supplied a yellow solid which, in chloroform, was freed from gums by means of a silica column and then crystallised from benzene giving the methyl ether in small needles (0.32 g.), m. p. 175—185° (decomposition), having a greenish-red ferric reaction and dissolving in aqueous sodium carbonate to give an orange colour, but could not be titrated potentiometrically in aqueous acetone. Infra-red absorption bands were noted at 3367 (weak), 1789, 1761, 1695, 1650, 1637, 1590, 1572, 1439, 1431, 1348, 1309, 1285, 1272, 1238, 1195, 1174, 1145, 1115, 1076, 1048, 1026, 1003, 966, 944, 935, 908, 867, 789, 754, 718, and 682 cm.$^{-1}$.

The analyitical specimen was dried at 100°/1 mm. for 4 hours.
Anal. 5.67 mg. subst.: 13.60 mg. CO_2, 2.38 mg. H_2O
4.91 mg. subst.: 3.92 mg. CO_2
5.08 mg. subst.: 4.96 ml. 0.02 *N* $Na_2S_2O_3$.
$C_{32}H_{28}O_{11}$ (588.22) calc'd.: C, 65.31; H, 4.76; O, 29.93; 2 OMe, 10.54%
found: C, 65.45; H, 4.71; O, 29.04; OMe, 10.42%.

This compound did not contain nitrogen but turned dark yellow when kept in air.

Tetrahydrochaetochrysin

Chaetochrysin (1.1 g.) in acetic acid (20 ml.) containing palladinised barium sulphate (5%; 1.0 g.) absorbed hydrogen (110 ml. N. T. P.: 2.6 mols.) during 1.5 hours. The pale yellow solid (1.1 g.) left on removal of the catalyst and vacuum evaporation of the filtrate was chromatographed from chloroform on silica giving a colourless solid (0.75 g.) which furnished tetrahydrochaetochrysin crystallising from chloroform in plates, m. p. 205—206°, retaining solvent of crystallisation, having a deep green ferric reaction and giving an orange-yellow solution in sulphuric acid.

The analytical specimen was dried (i) at 100°/1 mm. for 2 hours, and (ii) at 150°/1 mm for 4 hours.

Anal. (i) 5.88 mg. subst.: 11.82 mg. CO_2, 2.22 mg. H_2O
4.23 mg. subst.: 1.80 ml. 0.01 *N* $AgNO_3$
(ii) 6.42 mg. subst.: 13.17 mg. CO_2, 2.66 mg. H_2O
$C_{31}H_{30}O_{11} \cdot CHCl_3$ (697.74) calc'd.: C, 55.03; H, 4.48; Cl, 15.25%
found: (i) C, 54.88; H, 4.22; Cl, 15.08%
(ii) C, 55.08; H, 4.64%.

The solvate was insoluble in cold aqueous sodium carbonate but gave a yellow solution in 2*N*-aqueous sodium hydroxide and in potentiometric titrations against 0.01*N* alkali in aqueous acetone had *p*Ka 9.20 [i. e. *p*Ka (water) ~ 7.1]. It had λ max. 280, 358 mμ ($\varepsilon \times 10^{-3}$ 19.7, 23.5) with inflections at 287, 352, and 366 mμ; in the infra-red, bands appeared at 3550, 1795, 1757, 1704, 1650, 1616, 1582, 1545, 1520, 1475, 1443, 1417, 1389, 1362, 1350, 1335, 1304, 1280, 1247, 1220, 1192, 1176, 1167, 1146, 1124, 1093, 1075, 1046, 1036, 1004, 984, 957, 944, 924, 901, 895, 853, 845 and 821 cm.$^{-1}$.

Di- and Tetra-hydrochaetoflavin

In 1.3 hours a solution of chaetoflavin (1.0 g.) in acetic acid (20 ml.) containing palladinised barium sulphate (5%; 1.0 g.) absorbed hydrogen (70.1 ml., N. T. P.; 2.3 mols.) and vacuum evaporation of the filtered solution furnished a pale yellow-brown solid (0.95 g.) which was separated into two fractions by chromatography on silica from benzene — chloroform (1:1). The first fraction (0.15 g.) was eluted by the same solvent and could then be crystallised from benzene giving dihydrochaetoflavin in yellow needles, m. p. 164—170° (decomposition), having a red-brown ferric reaction and with λ max. 220, 272, and 333 mμ., inflections at 239 and 370 mμ. The infra-red spectrum included bands at 1742, 1689, 1616, 1582, 1471, 1435, 1372, 1311, 1274, 1252, 1239, 1190, 1171, 1148, 1118, 1093, 1086, 1043, 1027, 1012, 986, 966, 942, 919, 899, 877, 858, 826, 817, 789 and 769 cm.$^{-1}$.

The analytical specimen was dried at 120°/1 mm. for 2 hours.

Anal. 6.55 mg. subst.: 16.25 mg. CO_2, 2.96 mg. H_2O

$C_{21}H_{28}O_{11} \cdot C_6H_6$ (654.25) calc'd.: C, 67.89; H, 5.24%

found: C, 67.70; H, 5.05%

The second fraction (0.35 g.) was eluted by chloroform and crystallised from benzene giving tetrahydrochaetoflavin in yellow needles, m. p. 200—208° (decomposition) with λ max. 220, 276, 338 mμ ($\varepsilon \times 10^{-3}$ 29.0, 11.5, 35.9) having inflections at 224, 230, 342, and 352 mμ, and selective absorption in the infra-red at 3550, 1733, 1616, 1582, 1451, 1420, 1372, 1361, 1285, 1260, 1253, 1185, 1160, 1146, 1116, 1083, 1036, 1024, 985, 961, 940, 895, 881, 862, 826, 791, 784 and 769 cm.$^{-1}$.

An analytical specimen was dried at 120°/1 mm. for 1 hour.

Anal. 5.87 mg. subst.: 14.55 mg. CO_2, 2.82 mg. H_2O

5.99 mg. subst.: 14.87 mg. CO_2, 2.79 mg. H_2O

$C_{31}H_{30}O_{11} \cdot C_6H_6$ (656.28) calc'd: C, 67,69; H, 5.52%

found: C, 67.75; H, 5.20%

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IZVOD

O metabolitima *Chaetomium affine* Corda

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Iz gljive *Chaetomium affine* Corda izolirana su tri kompleksna metabolita, od kojih su dva, hetohrizin (chaetochrysin) i hetoflavin (chaetoflavin) žuti izomeridi sa bruto formulom $C_{31}H_{26}O_{11}$. Treća komponenta, hetoalbin (chaetoalbin), $C_{30}H_{28-30}O_{11}$, je bezbojna i, čini se, da je vrlo srodna hetohrizinu. Opisani su rezultati prethodnih ispitivanja u svrhu određivanja strukture metabolita.

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