155. *Studies in Mycological Chemistry. Part XII.* Two New Metabolites from a Variant Strain of* Aspergillus versicolor (*Vuillemin*) *Tiraboschi.*

By E. Bullock, D. Kirkaldy, John C. Roberts, and J. G. Underwood.

The isolation of two new metabolites from the mycelium of a variant strain of *A. versicolor* is described. One of these is closely related to the normal metabolite, sterigmatocystin, and is very probably 6-methoxysterigmatocystin (I; R = OMe). The second, now called aversin, is an anthraquinone derivative to which is assigned structure (V or VI; R = H).

Some strains of *Aspergillus versicolor* (Vuillemin) Tiraboschi produce a yellow metabolite, sterigmatocystin,[1-3] for which we have previously established [4,5] structure (I; R = H). We have investigated two strains of the mould [4] for their ability to produce this metabolite. One of these, kindly provided by Professor Birkinshaw, behaved initially in the usual way and gave an acceptable yield of sterigmatocystin. Later, we found that this strain, having been repeatedly sub-cultured for about three months, formed a variant. This variant, although macroscopically indistinguishable from the original strain, produced two new metabolites (A and B, see below) but no sterigmatocystin. This paper records our work on the structures of these two new compounds. Because of the small amounts available, our investigations have depended mainly on comparative physical measurements.

Substance A.—This substance crystallised in yellow needles, m. p. 222—223°, and gave analytical figures in close agreement with the formula $C_{17}H_8O_5(OMe)_2$ differing from that of sterigmatocystin, $C_{17}H_9O_5{\cdot}OMe$, by CH_2O. It was strongly lævorotatory and almost insoluble in aqueous sodium hydroxide. It gave a green-brown ferric reaction (in ethanol) and a positive Gibbs test.[6] It yielded a monoacetate and was catalytically hydrogenated to a dihydro-derivative.

The infrared absorption spectrum of the metabolite, which closely resembled that of sterigmatocystin [4] (except in the region associated with the aromatic substitution pattern), indicated the presence of a hydrogen-bonded hydroxyl group (3447 cm.$^{-1}$), a carbonyl group (1662 cm.$^{-1}$), and a vinyl ether system (3096, 1618, 1060, and 724 cm.$^{-1}$). The bands due to the vinyl ether system were absent from the spectrum of the dihydro-derivative.

The ultraviolet absorption spectra of substance A and its dihydro-derivative are similar to the spectrum of dihydro-5-hydroxysterigmatocystin,[4] all these spectra possessing an extra peak at *ca.* 280 mμ which is not shown in the spectrum of sterigmatocystin (see

* Part XI, *J.*, 1962, 4179.

[1] Hatsuda and Kuyama, *J. Agric. Chem. Soc. Japan*, 1954, **28**, 989.
[2] Davies, Roberts, and Wallwork, *Chem. and Ind.*, 1956, 178.
[3] Birkinshaw and Hammady, *Biochem. J.*, 1957, **65**, 162.
[4] Davies, Kirkaldy, and Roberts, *J.*, 1960, 2169.
[5] Bullock, Roberts, and Underwood, *J.*, 1962, 4179.
[6] King, King, and Manning, *J.*, 1957, 563.

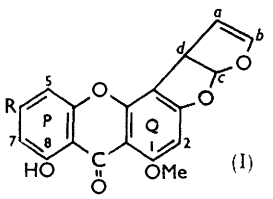
TABLE 1.

Ultraviolet absorption spectra (in ethanol).

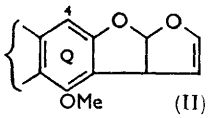
Compound	$\lambda_{max.}$ in mμ; ($10^{-3}\varepsilon$ in parentheses)
Sterigmatocystin	235 (24·8), 249 (27·6), — , 329 (13·1)
Substance A	232 (24·1), 248 (26·8), 279 (11·2), 331 (12·1)
Dihydro-5-hydroxysterigmatocystin	— , 248 (25·6), 280 (10·6), 329 (10·2)
Dihydro-derivative of A	232 (24·0), 249 (27·0), 278 (12·0), 330 (12·0)
O-Acetyl derivative of A	240 (34·3), 246 (32·6), — , 313 (13·1)
O-Acetyl-dihydro-derivative of A	240 (34·6), 247 (35·0), — , 314 (15·8)

Table 1). The spectra of the dihydro- and acetyldihydro-derivative of A are sufficiently similar to those of the respective unsaturated materials to indicate that the double bond of the vinyl ether system is not in conjugation with the main chromophore. It thus seemed probable that substance A was a methoxy-derivative of sterigmatocystin.

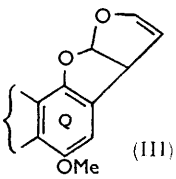
Proton magnetic resonance studies of the acetate of the metabolite, in methylene dichloride solution, gave results in accord with structure (I; R = OMe) for substance A. The proton, H-2, as in sterigmatocystin,[5] gives a singlet at relatively high field (3·68 τ). The low field triplet and other signals due to the AXY system in the spectrum of sterigmatocystin are replaced (in the spectrum of the metabolite acetate) by two doublets (at 3·34 and 3·24 τ) which show splitting ($J \approx 2$ c./sec.) characteristic of two aromatic



(I)



(II)



(III)

protons in *meta*-positions. In addition to the xanthone nuclear protons, the spectrum shows a singlet of intensity 6 at 6·08 τ (O-CH_3)$_2$, a singlet of intensity 3 at 7·60 τ (acetyl-CH_3), and signals due to the bicyclic side chain as in sterigmatocystin [5] (see Table 2). On addition of trifluoroacetic acid to the solution of the acetate in methylene dichloride,

TABLE 2.

Magnetic resonance absorption of bicyclic side-chain protons.*

	τ Scale (J in c./sec.)					
Compound	H_a	J_{ab}	H_b	H_c	J_{cd}	H_d
Acetate of A	4·50	2·5	3·55	2·95	6·7	5·14
Sterigmatocystin	4·53	2·5	3·48	3·16	6·9	5·19

* Cf. structures (I; R = OMe; OAc for OH) and (I; R = H).

shifts (of *ca.* 0·2 p.p.m. to higher field) are observed in the positions of the two doublets (corresponding to H-5 and H-7) and of the singlet (corresponding to acetyl-CH_3), whereas no shift is observed for the singlet due to H-2. On the assumption that these shifts result from trans-esterification, this observation places the hydroxyl group of substance A in the 8- rather than in the 1-position.

The similarity of the ultraviolet absorption spectrum of substance A to the spectra of sterigmatocystin and dihydro-5-hydroxysterigmatocystin probably excludes structures for substance A which involve 1,2-attachment of the bicyclic side chain.[7] The proton magnetic resonance observations exclude structures such as (III) because of the high τ value for the single proton in ring Q. Structure (II), however, is not excluded by this evidence since the τ value for H-4 would be expected to be about equal to that of H-2 in (I; R = OMe). In view of the obviously close chemical and biochemical relation of the

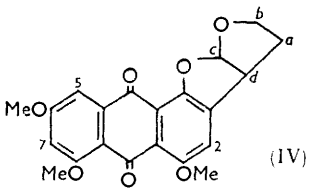
[7] Cf. Scheinmann and Suschitzky, *Tetrahedron*, 1959, **7**, 31.

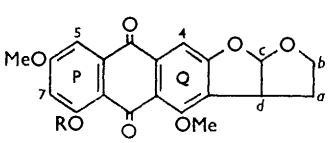
metabolite to sterigmatocystin, it seems very probable that substance A is 6-methoxysterigmatocystin (I; R = OMe).

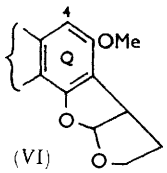
Substance B.—This compound, $C_{18}H_{10}O_5(OMe)_2$, for which we propose the name, aversin, forms slender golden needles, m. p. 217°. It is strongly lævorotatory and gives a reddish-brown ferric reaction in ethanol, a positive Gibbs test,[6] and an intense purple colour with concentrated sulphuric acid. It is almost insoluble in aqueous alkali but dissolves in aqueous-ethanolic alkali to give a red colour, the solution so formed giving a positive test (with zinc dust) for a hydroxyquinone. Aversin yields a monoacetate and a monomethyl ether.

Its formula and general properties indicated that aversin might be an α-hydroxydimethoxyanthraquinone carrying a dihydrosterigmatocystin-type of bicyclic side chain. Spectroscopy (proton magnetic resonance, infrared, and ultraviolet) has confirmed the nature of the nucleus and of the side-chain.

The proton magnetic resonance spectrum of *O*-methylaversin is interpretable on the basis of structure (IV), (V; R = Me), or (VI; R = Me). The proton H-2 or H-4 gives a singlet at 2·96 τ, while the protons H-5 and H-7 give two doublets at 2·98 and 3·47 τ ($J \approx 3$ c./sec.), respectively. The closeness of the values for H-5 and the singlet favours structure (V or VI; R = Me), owing to the similarity of the environments of H-4 and H-5. This conclusion is supported by a consideration of the values for the nuclear protons in 1,3,6,8-tetramethoxyanthraquinone which lie at 2·97 (H-4 and H-5) and 3·44 τ (H-2 and H-7) (J = 2·3 c./sec.). In addition to the anthraquinone proton signals, the spectrum of *O*-methylaversin shows a singlet of intensity 6 at 6·13 τ, together with a singlet of intensity 3 at 6·07 τ (3 × O-CH_3 groups), and signals due to a bicyclic side chain as in dihydrosterig-







matocystin [5] (see Table 3). The lack of separation within the groups attributed to H_a and H_b in the spectrum of *O*-methylaversin is presumably due to a smaller influence of ring-currents on these protons than on the corresponding protons in dihydrosterigmatocystin. The spectrum of acetylaversin showed similar absorptions, but addition of trifluoroacetic acid to the solution caused signals due to H-5 and H-7 to move to higher field whilst that due to H-4 was unaffected. We conclude that in aversin the hydroxyl group occupies position 8.

TABLE 3.

Magnetic resonance absorption of bicyclic side-chain protons.*

	τ Scale (J in c./sec.)				
Compound	H_a	H_b	H_c	J_{cd}	H_d
O-Methylaversin	7·74	6·40	3·78	5·5	6·0
Dihydrosterigmatocystin	8·76 7·32	6·35 5·80	3·58	5·8	6·35

* Cf. structures (V; R = Me) and (I; R = H; no double bond in side-chain).

Evidence from the infrared absorption spectra of aversin and of its derivatives supports these ideas. The presence of a hydroxyl group α to one of the carbonyl groups is shown by a comparison of the spectrum of aversin with that of *O*-methylaversin. The former has bands at 1665 (>C=O) and 1628 cm.$^{-1}$ (hydrogen-bonded >C=O), whilst the latter has bands at 1673 and 1667 cm.$^{-1}$ (cf. ref. 8). A comparison of the infrared absorption spectrum

[8] Bloom, Briggs, and Cleverley, *J.*, 1959, 178.

of *O*-methylaversin with the spectra of 1,3,6,8-tetramethoxy- and 1,3,6,8-tetramethoxy-2-methyl-anthraquinone reveals the presence of extra bands in the first-mentioned spectrum due to the bicyclic side chain. In the C–H stretching region, extra bands are present at 2937 ($>CH_2$) and 2880 cm.$^{-1}$ ($\geq CH$) with corresponding C–H deformation bands at 1440 and 1419 cm.$^{-1}$. Extra bands are also present (at 1103 and 1035 cm.$^{-1}$) in the C–O–C (cyclic ether) stretching absorption range.[9,10] Bands due to a vinyl ether system are not present in the spectrum of the metabolite.

Dealkylation of aversin (with hydrogen bromide in acetic acid) yielded a minute quantity of material which was too small to be purified and characterised but showed

TABLE 4.

Ultraviolet absorption spectra (in ethanol).

Compound	$\lambda_{max.}$ in mμ; ($10^{-3}\varepsilon$ in parentheses)
O-Methylaversin	224 (33·1), 285 (36·8), 350 (4·58), 409 (4·20)
1,3,6,8-Tetramethoxy-2-methylanthraquinone	222 (32·5), 281 (37·3), 350 (5·24), 405 (3·88)
1,3,6,8-Tetramethoxyanthraquinone	223 (44·2), 281 (31·1), — , 412 (5·82)
4,5,7-Trimethoxy-2-methylanthraquinone (*O*-trimethylemodin)	223 (40·5), 277 (25·5), — , 405 (7·7)

ultraviolet absorption bands at 226, 268, 285, and 448 mμ. It has been established [11–13] that, in the hydroxyanthraquinone field, the wavelength of the principal maximum above 350 mμ is mainly dependent on the number of α-hydroxyl groups. The value of 448 mμ indicates the presence of two α-hydroxyl groups in the degradation product. The carbonyl absorption ($\nu_{max.}$ 1683 and 1628 cm.$^{-1}$) of the degradation product excludes the possibility of a 1,3,5,7-oxygenation pattern for aversin. A comparison of the ultraviolet absorption spectrum of *O*-methylaversin with the spectra of 1,3,6,8-tetramethoxy-2-methylanthraquinone and of related compounds (see Table 4) provided excellent confirmation of structure (V or VI; R = Me) for the first-mentioned compound.

The concurrence of the evidence from the various spectroscopic investigations leaves virtually no doubt that aversin has structure (V or VI; R = H).

The nature of these two metabolites, produced by a variant strain of the mould, is obviously important in connexion with the biogenesis of sterigmatocystin. This problem is under investigation.

EXPERIMENTAL

M. p.s were determined on the Kofler block. Optical rotations were measured on an Ericsson E.T.L-N.P.L. automatic polarimeter, type 143A. Ultraviolet spectra (of ethanolic solutions) were determined on a Perkin-Elmer 137 recording specrtophotometer or on a Unicam S.P. 500 instrument. Infrared spectra (of substances in potassium bromide discs) were determined on a Unicam S.P. 100 instrument. Proton magnetic resonance spectra (of substances in methylene dichloride solution, except where otherwise stated) were recorded on an A. E. I. (RS 2) spectrometer; these spectra were calibrated by the side-band technique, tetramethylsilane being used as internal reference.

For convenience, substance A is referred to in this section as 6-methoxysterigmatocystin (I; R = OMe) although other structures, *e.g.*, (II; R = OMe), have not been entirely excluded (see above).

Isolation of Metabolites.—Aspergillus versicolor (Vuillemin) Tiraboschi (L. S. H. T. M., Cat. No. Ac. 59) was kept in sub-culture on Czapek–Dox agar slopes. For production of the metabolites, the mould was grown at 25° for 3 weeks in surface culture (in about 80 flasks) on a solution of glucose (5%) and inorganic salts (Czapek–Dox formula [3]). The washed, dried, and powdered mycelium was extracted (Soxhlet) with light petroleum (b. p. 40—60°) for 1

[9] Cf. Barr and Rose, *J.*, 1954, 3766.
[10] Bellamy, " Infra-red Spectra of Complex Molecules," Methuen, London, 2nd edn., 1958, p. 119.
[11] Briggs, Nicholls, and Paterson, *J.*, 1952, 1718.
[12] Birkinshaw, *Biochem. J.*, 1955, **59**, 485.
[13] Birkinshaw and Gourlay, *Biochem. J.*, 1961, **81**, 618.

week. The first fermentation yielded [4] sterigmatocystin. The light petroleum extract from the mycelium produced in a later fermentation deposited a brown-orange solid, m. p. 186—194°. A solution of this material in chloroform was chromatographed on a column (30 × 5 cm.) of alumina (Spence's type H). Continued percolation of the column with chloroform gave a yellow eluate. Evaporation of the solvent *in vacuo* and crystallisation of the residue from acetone gave 6-methoxysterigmatocystin (*ca.* 10 mg.).

The light petroleum extract from the mycelium produced in the next fermentation deposited an orange solid, m. p. 170—180°. This material was collected and washed with a little light petroleum. The residue separated from hot acetone as an amorphous orange powder, M (4·25 g.), much of which was consumed in numerous attempts to achieve a separation of the mixture. Methods involving fractional crystallisation from acetone and chromatography in benzene solution on acid-washed alumina or magnesium trisilicate were only partially successful and yielded small quantities of aversin.

A more successful separation of the materials in the mixture, M, proceeded *via* the acetates. A solution of material M (0·5 g.) in pyridine (5 ml.) and acetic anhydride (2 ml.) was kept for 5 days at room temperature. The solvents were removed *in vacuo* and the residue was crystallised from ethanol to give *O*-acetylaversin (see below) as a first crop (230 mg.). The second crop (140 mg.) from the ethanol solution consisted of *O*-acetyl-6-methoxysterigmatocystin (see below).

The *O*-acetylaversin was hydrolysed with 2% ethanolic potassium hydroxide (5 ml.) at room temperature for 12 hr. The solvent was removed *in vacuo* and a solution of the residue in water (25 ml.) was acidified with dilute acetic acid. The solid was collected, washed, and dried, and then crystallised from acetone to give aversin (115 mg.) as golden needles. Similarly, 6-methoxysterigmatocystin (*ca.* 70 mg.) was obtained from its acetate as yellow needles.

General Properties of 6-Methoxysterigmatocystin.—The sublimed (200°/0·05 mm.) *compound* formed pale-yellow needles, m. p. 223° (decomp.), $[\alpha]_D^{20}$ −360° (*c* 0·238 in $CHCl_3$) [Found: C, 64·4; H, 4·2; OMe, 17·4; *C*-Me, 0. $C_{17}H_8O_5(OMe)_2$ requires C, 64·4; H, 4·0; OMe, 17·5%], $\nu_{max.}$ 3447, 3383, 3121, 3096, 3016, 2971, 2927, 2853, 1662, 1634, 1618, 1595, 1559, 1498, 1462, 1444, 1419, 1398, 1360, 1345, 1339, 1302, 1285, 1262, 1248, 1233, 1223, 1199, 1187, 1168, 1148, 1140, 1099, 1078, 1060, 1042, 1017, 979, 960, 930, 881, 853, 825, 788, 767, 744, 737, and 724 cm.$^{-1}$. It is almost insoluble in aqueous alkali and gives an orange colour with ethanolic alkali. It yields a dark brown colour with concentrated sulphuric acid and a green-brown colour with ferric chloride (in ethanol). It gives a positive Gibbs reaction [6] ($\lambda_{max.}$ 675 mμ).

O-*Acetyl-6-methoxysterigmatocystin.*—6-Methoxysterigmatocystin (30 mg.), pyridine (5 ml.) and acetic anhydride (1 ml.) were kept at room temperature for 5 days. The solvents were removed *in vacuo* and the residue was crystallised from ethanol to give the *product* as colourless needles (20 mg.), m. p. 228° [Found: C, 63·5; H, 4·3; OMe, 14·1; Ac, 13·8. $C_{17}H_7O_5(OMe)_2(CO{\cdot}CH_3)$ requires C, 63·6; H, 4·0; OMe, 15·6; Ac, 10·9%]; $\nu_{max.}$ included 1760 (aryl acetate >C=O), 1660 (xanthone >C=O) and 3088, 1615, and 725 cm.$^{-1}$ (vinyl ether). This substance gave no ferric reaction. The proton magnetic resonance spectrum showed (i) a doublet at 2·95 τ, J = 6·7 c./sec. (H_c); (ii) doublets at 3·24 and 3·34 τ, $J \approx 2$ c./sec. (H-5 and H-7); (iii) complex absorption at 3·55 τ (H_b); (iv) a singlet at 3·68 τ (H-2); (v) complex absorption at 4·5 τ (in chloroform) (H_a); (vi) two triplets at 5·14 τ (H_d); (vii) a singlet, intensity 6, at 6·08 τ (2 × O-CH_3); (viii) a singlet, intensity 3, at 7·60 τ (acetyl-CH_3). On addition of trifluoroacetic acid to this solution, the spectrum remained essentially the same except for the doublets at 3·24 and 3·34 and the singlet at 7·60 τ which were moved, during ½ hr., to 3·48, 3·58, and 7·77 τ, respectively. This compound was also obtained during the isolation of the parent compound and of aversin from the mixture, M.

Dihydro-6-methoxysterigmatocystin.—6-Methoxysterigmatocystin (10 mg.), ethyl acetate (5 ml.), and 10% palladised charcoal (5 mg.) were shaken in an atmosphere of hydrogen. Separation of the catalyst and evaporation of the solvent yielded a residue which crystallised from ethanol to give the *dihydro-derivative* as pale yellow needles (5·2 mg.), m. p. 248° (Found: C, 63·3; H, 5·0. $C_{19}H_{16}O_7,\frac{1}{2}C_2H_5{\cdot}OH$ requires C, 63·3; H, 5·0%); $\nu_{max.}$ included 3450 (OH), 1650 (C=O) cm.$^{-1}$, but bands due to the vinyl ether system in the starting material were absent. This compound was insoluble in alkali and gave a red colour with concentrated sulphuric acid.

O-*Acetyldihydro-6-methoxysterigmatocystin.*—*O*-Acetyl-6-methoxysterigmatocystin (30 mg.) was hydrogenated as above, to give the *derivative* which separated from ethanol as colourless rhombs (20 mg.), m. p. 231° (Found: C, 63·3; H, 4·7. $C_{21}H_{18}O_8$ requires C, 63·3; H, 4·6%);

$\nu_{max.}$ included 1765 (aryl acetate C=O) and 1660 (xanthone C=O), but bands due to a vinyl ether were absent.

General Properties of Aversin.—*Aversin* crystallises from acetone in slender golden needles, m. p. 217°, $[\alpha]_D^{20}$ −222° (*c* 0·248 in $CHCl_3$) [Found: C, 65·2; H, 4·3; OMe, 15·3. $C_{18}H_{10}O_5(OMe)_2$ requires C, 65·2; H, 4·4; OMe, 16·8%], $\lambda_{max.}$ 224, 251, 285, 313, 363, and 440 mμ (10^{-3} ε 36·7, 13·4, 33·6, 8·90, 4·96, and 7·83, respectively) in neutral solution, and, after the addition of alkali, $\lambda_{max.}$ 240, 263, 307, 355, 498, and 501 mμ (10^{-3}ε 21·3, 34·8, 13·8, 5·04, 6·89, and 6·89, respectively), $\nu_{max.}$ 3470, 3090, 2970, 2935, 2880, 2840, 1725, 1665, 1628, 1595, 1558, 1479, 1436, 1423, 1375, 1354, 1329, 1298, 1243, 1210, 1174, 1160, 1118, 1104, 1084, 1076, 1044, 990, 953, 911, 864, 821, 798, 771, 758, and 747 cm.$^{-1}$. The metabolite gave a positive Gibbs reaction [6] ($\lambda_{max.}$ 680—685 mμ). It gave a red-brown ferric reaction (in ethanol), an intense purple colour with concentrated sulphuric acid, and did not fluoresce in glacial acetic acid. It was almost insoluble in aqueous alkali. Aversin dissolved in aqueous-ethanolic alkali to give a red solution which was decolorised when warmed with zinc dust, the colour being restored on contact of the solution with air. The substance was recovered unchanged after an attempt to hydrogenate it under the conditions used for the hydrogenation of 6-methoxysterigmatocystin (see above).

O-*Acetylaversin.*—This *compound* was obtained during the isolation of aversin (see above). It formed yellow needles, m. p. (after two crystallisations from ethanol) 243—246° [Found: C, 64·4, 64·6; H, 4·6, 4·6; OMe, 14·4. 15·1. $C_{20}H_{12}O_6(OMe)_2$ requires C, 64·4; H, 4·4; OMe, 15·1%], $\lambda_{max.}$ 219, 283, and 407 mμ (10^{-3} ε 27·0, 36·6, and 4·40, respectively); $\nu_{max.}$ included 1760 (aryl acetate) and 1665 cm.$^{-1}$ (anthraquinone C=O). This compound gave a deep blue colour with concentrated sulphuric acid. It gave no ferric reaction. The proton magnetic resonance spectrum of acetylaversin in methylene dichloride–trifluoroacetic acid solution showed initially (i) a singlet at 2·62 τ (H-4); (ii) two doublets at 2·78 and 3·30 τ, J = 3 c./sec. (H-5 and H-7); (iii) a doublet at 3·52 τ, J = 6 c./sec. (H_c); (iv) two poorly resolved triplets at 5·71 τ (H_d); (v) two singlets of intensity 3 at 5·99 and 6·05 τ (2 × O-CH_3); (vi) complex absorption at *ca.* 6·1 τ (H_b); (vii) a singlet of intensity 3 at 7·43 τ (acetyl-CH_3), and (viii) complex absorption at 7·65 τ (H_a). The doublets, at 2·78 and 3·30, and the singlet at 7·43 τ had moved, after ½ hr., to 2·84, 3·38, and 7·59 τ, respectively, the other absorptions remaining in the same positions.

O-*Methylaversin.*—Aversin (90 mg.), freshly prepared silver oxide (2 g.), methyl iodide (5 ml.), and dry acetone (20 ml.) were stirred at room temperature for 3 days. Separation of the solid and removal of the solvents *in vacuo* gave a residue which crystallised from methanol to give the *ether* as golden-yellow needles (80 mg.), m. p. 212—213° [Found (on a sublimed sample, m. p. 229—230°): C, 66·0; H, 4·7. $C_{21}H_{18}O_7$ requires C, 65·9; H, 4·8%], $\nu_{max.}$ 3460, 3100, 3089, 3053, 3019, 3004, 2986, 2947, 2937, 2880, 2845, 2664, 1673, 1667, 1602, 1572, 1510, 1463, 1440, 1431, 1419, 1357, 1349, 1332, 1323, 1307, 1281, 1255, 1238, 1209, 1201, 1188, 1178, 1163, 1114, 1103, 1085, 1060, 1035, 990, 958, 942, 925, 914, 897, 871, 850, 841, 836, 787, 778, and 753 cm.$^{-1}$. The compound gave no ferric reaction but gave a red-violet colour with concentrated sulphuric acid. The proton magnetic resonance spectrum showed (i) a singlet, intensity 1, at 2·96 τ (H-4); (ii) two doublets at 2·98 and 3·47 τ, J = 3 c./sec. (H-5 and H-7); (iii) a doublet at 3·78 τ, J = 5·5 c./sec. (H_c); (iv) complex absorption at 6·0 τ (H_d); (v) three singlets each of intensity 3 at 5·92, 5·99, and 6·01 τ (in $CHCl_3$) (3 × O-CH_3); (vi) complex absorption at 6·4 τ (H_b); (vii) complex absorption at 7·74 τ (H_a).

Demethylation of Aversin.—Aversin (5 mg.) was heated with a 25% solution of hydrogen bromide in glacial acetic acid (15 ml.) at 100° for 6 hr. Evaporation of the solvent *in vacuo* gave a residue which was extracted into aqueous alkali. The solution was acidified and the precipitate was collected, washed with water, and dried; it had $\lambda_{max.}$ 226, 268, 285, and 448 mμ, and $\nu_{max.}$ included ($CHCl_3$ solution) 1683 and 1628 cm.$^{-1}$.

1,3,6,8-*Tetrahydroxyanthraquinone.*—A donated sample consisted of orange needles, m. p. >360° [lit.,[14,15] >360°,[16] 353° (decomp.)], $\lambda_{max.}$ 223, 254, 261(sh), 292, 319(sh), and 450 mμ (10^{-3} ε 28·3, 13·2, 12·9, 23·6. 8·24, and 9·40, respectively).

1,3,6,8-*Tetramethoxyanthraquinone.*—The hydroxyquinone (140 mg.), methyl iodide (25 ml.), and freshly prepared silver oxide (2 g.) were stirred at room temperature for 15 hr. Removal of the solids and evaporation of the solvent gave a residue which separated from methanol as yellow needles (120 mg.), m. p. 203—205° (lit.,[14] 221°,[15] 241—242°). Sublimation of this

[14] Sutherland and Wells, *Chem. and Ind.*, 1959, 291.
[15] Koller and Russ, *Monatsh.*, 1937, **70**, 54.
[16] Shibata, *J. Pharm. Soc. Japan*, 1941, **61**, 103; *Chem. Abs.*, 1950, **44**, 9396.

material, at 200°/0·05 mm., gave yellow rhombs, m. p. 225—226° [Found: C, 65·9; H, 4·6; OMe, 39·6. Calc. for $C_{14}H_4O_2(OMe)_4$: C, 65·8; H, 4·9; OMe, 37·8%], $\nu_{max.}$ 3472, 3108, 3095, 3073, 3013, 2998, 2985, 2947, 2887, 2843, 2667, 1677, 1665, 1600, 1572, 1467, 1458, 1433, 1356, 1335, 1322, 1257, 1242, 1226, 1202, 1174, 1163, 1143, 1125, 1073, 1055, 1007, 953, 943, 910, 893, 883, 855, 840, 829, 820, 758, and 745 cm.$^{-1}$. The proton magnetic resonance spectrum showed (i) doublets at 2·97 and 3·44 τ, J = 2·3 c./sec., and (ii) a singlet at 6·17 τ.

1,3,6,8-*Tetramethoxy-2-methylanthraquinone*.—A donated sample had m. p. 193—194°, and $\nu_{max.}$ 3465, 3096, 3015, 2995, 2971, 2942, 2932, 2842, 2669, 1667, 1602, 1583, 1463, 1429, 1400, 1324, 1312, 1265, 1237, 1216, 1203, 1193, 1158, 1137, 1116, 1063, 1012, 997, 944, 926, 891, 879, 856, 823, 797, 767, and 739 cm.$^{-1}$.

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