Tetrahedron. Vol. 27, pp. 425 to 431. Pergamon Press 1971 Printed in Great Britain

THE CONVERSION OF STERIGMATOCYSTIN INTO DIHYDROASPERTOXIN

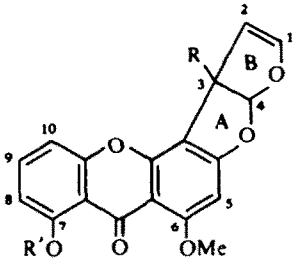
R. D. HUTCHISON and C. W. HOLZAPFEL*

National Chemical Research Laboratory, C.S.I.R., Pretoria, South Africa

(*Received in the UK* 1 *August* 1970; *accepted for publication* 13 *August* 1970)

Abstract—Treatment of O-methyldihydrosterigmatocystin (III) with methanolic alkali under vigorous conditions effected opening of the furan ring to give the compound Va. Reaction of the corresponding acetate with lead tetra-acetate gave dihydroaspertoxin acetate which was converted into dihydroaspertoxin (VIb) by hydrolysis with dilute alkali.

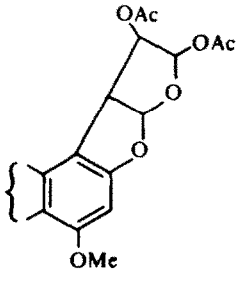
ASPERTOXIN (Ia) a new toxic metabolite, was recently isolated[1,2] from an aflatoxin producing strain of *Aspergillus flavus*. However, the amount of Ia produced by the



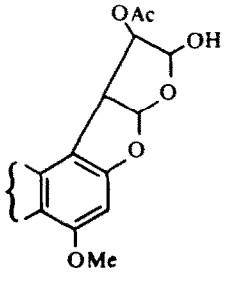
Ia: R = OH, R′ = Me
Ib: R = H, R′ = H
Ic: R = H, R′ = Me
Id: R = H, R′ = Bz

fungus is too small to allow detailed toxicological investigations. Sterigmatocystin (Ib) on the other hand, can be obtained easily on relatively large amounts from a number of fungi.[3] This prompted us to investigate the possibility of converting sterigmatocystin into aspertoxin. This objective has not yet been achieved, but we report here the conversion of sterigmatocystin into dihydroaspertoxin.

Attempts to substitute an acetoxy group directly at the 3-position of O-benzoylsterigmatocystin (Id) by reaction with lead tetra-acetate failed. The reagent



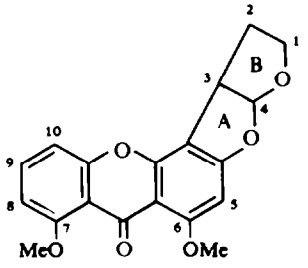
IIa



IIb

* Present address: Department of Chemistry, Rand Afrikaans University, Johannesburg, South Africa.

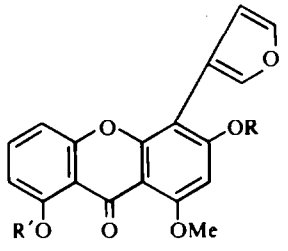
reacted exclusively with the enol ether grouping to give IIa and IIb.[4] Further investigation showed that the 1,2-dihydro-derivative of O-methylsterigmatocystin (III) was completely unreactive towards lead tetra-acetate.



III

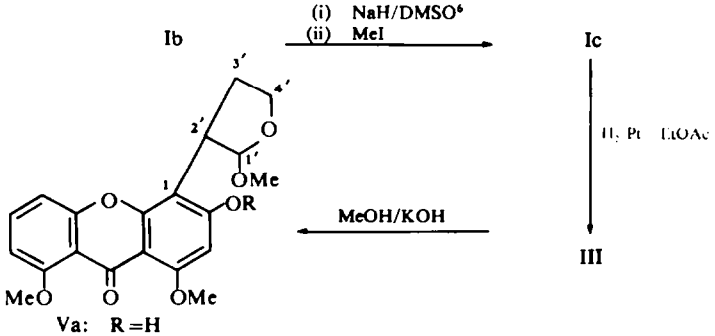
Inspection of Dreiding models suggested that benzylic oxidation (by free radical or ionic mechanism) of this strained system may not be favoured, since a free radical or carbonium ion at the 3-position cannot be effectively stabilized by resonance with the aromatic system. This may, at least in part, account for the inability of lead tetra-acetate to effect benzylic oxidation of III. It was therefore, considered likely that opening of the furan ring A of III would yield a product more susceptible to benzylic oxidation with lead tetra-acetate.

Roberts *et al.*[5] reported the conversion of sterigmatocystin into isosterigmatocystin (IVa) by treatment with alcholic alkali.



IVa: R′=R=H
IVb: R′=Me R=Ac

We found that dihydrosterigmatocystin was completely stable under these conditions. However, O-methyldihydrosterigmatocystin (III) prepared as depicted in Scheme 1,

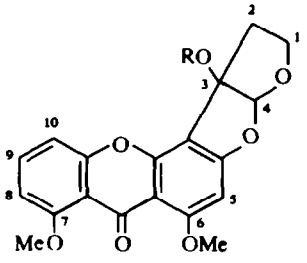


Va: R=H
Vb: R=Ac

SCHEME 1

gave Va in good yield. In this case ring opening probably occurred by nucleophilic attack by a methoxy-ion at the C_4 position of III with subsequent retention of configuration at C_3 and inversion at C_4. These conclusions followed from the following observations. Roberts *et al.*[7,8] synthesised racemic O-methyldihydrosterigmatocystin by a sequence which established that the furan rings are *cis* fused. PMR data of Va showed a doublet at τ 4·74 J (3 c/s) due to the proton situated at C_1. The fact that a clean doublet is observed suggests that one isomer was only formed. Extensive studies[9] of pentafuranose derivatives showed that the coupling constant of vicinal *cis*-hydrogen atoms have a J (4·3 to 6·8 c/s), while the values for vicinal *trans*-hydrogen atoms generally vary from 0·05 to 7·2 c/s. The above data therefore indicated that the protons at $C_{2'}$ and $C_{1'}$ are *trans*, implying an inversion of the configuration of protons on $C_{1'}$, on passing from III to Va. The retention of configuration of the proton at position $C_{2'}$ was confirmed by showing that Va was optically active.

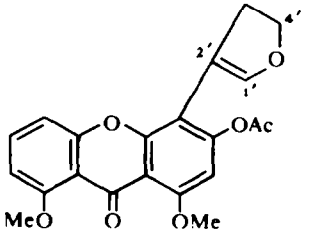
Treatment of 1-[2′-(1′-methoxytetrahydrofurano)]-2-acetoxy-4, 5-dimethoxyxanthone (Vb), with lead tetra-acetate in glacial acetic acid afforded dihydroaspertoxin acetate (VIa).



VIa: R = Ac
VIb: R = H

The PMR spectrum showed a singlet at τ 7·95 due to the acetoxyl protons. Signals due to OMe protons are prominent at τ 6·45 and τ 6·5. One proton singlets at τ 3·61 and τ 3·7 were assigned to protons on C_4 and C_5, respectively. Protons at C_8, C_9 and C_{10} gave rise to the expected signals at low field. Direct comparison of dihydroaspertoxin (VIb; synthesised by hydrolysis of the acetate VIa) with an authentic sample* by mass spectrum and TLC showed the samples to be chemically identical.

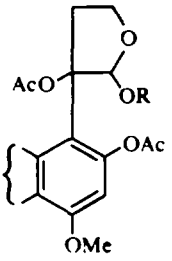
Investigation into the nature of the conversion of Vb into VIa showed that no reaction occurred when Vb was treated with lead tetra-acetate in benzene. It was found that in acetic acid at 80° compound Vb rapidly entered into equilibrium (shown by paper chromatography) with a number of related species in accord with its acetal character. However, the majority of these species proved to be extremely unstable and only one of the major components of the mixture could be characterised fully. It was identified as 1-[2′-(3′,4′-dihydrofurano)]-2-acetoxy-4,5-dimethoxyxanthone (VII). Reaction of VII



VII

* A gift of a sample of aspertoxin from Dr. A. D. Campbell is gratefully acknowledged. Aspertoxin was hydrogenated to dihydroaspertoxin under standard conditions.

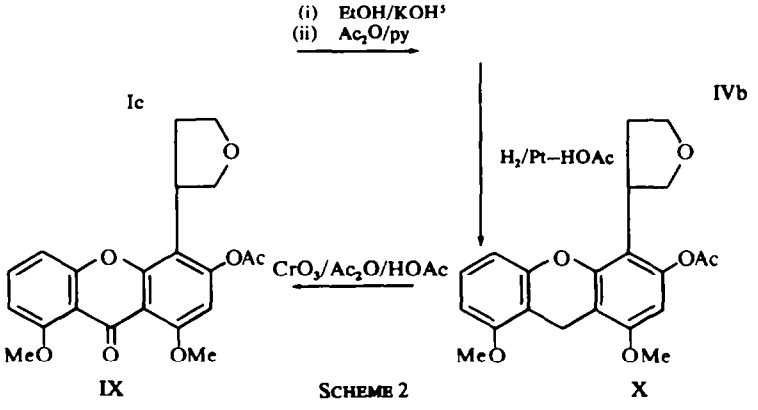
with lead tetra-acetate would be expected to yield the diacetate (VIIIa) and hydroxy acetate (VIIIb) as the immediate products (*cf.* reaction of O-benzoylsterigmatocystin



VIIIa R = Ac

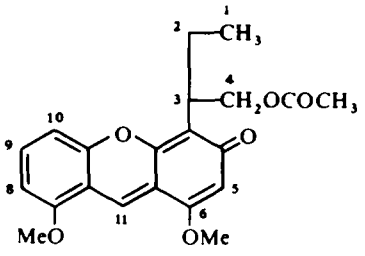
VIIIb R = H

with lead tetra-acetate). Subsequent loss of the elements of acetic anhydride or acetic acid, respectively would yield VIa. However, the alternative possibility that reaction of lead tetra-acetate with VII to yield VIa in a concerted manner must also be considered. Reactions of VII with lead tetra-acetate in acetic acid at 80° did indeed give VIa in good yield. This result does not constitute unequivocal evidence that VII is an intermediate in the conversion of Vb into VIa since VII in acetic acid at 80° rapidly gave rise to the same species (chromatographic evidence) as Vb. In order to rule out the possibility that dihydroaspertoxin acetate resulted from benzylic oxidation of Vb or related species, followed by cyclization, 1-[2′-(tetrahydrofurano)]-2-acetoxy-4,5-dimethoxyxanthone (IX) was synthesised (as depicted in Scheme 2) and its reactions with lead tetra-acetate in acetic acid studied. This scheme involves, among others the hydrogenation of IVb in the presence of Adams catalyst in acetic acid. The best yield of X was obtained on



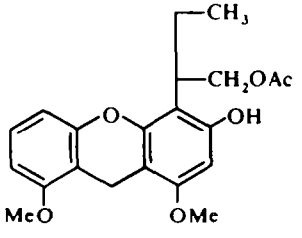
SCHEME 2

allowing 4 equivalents of hydrogen to be taken up. However, compound X did not represent the only product of hydrogenation. In fact, a complicated mixture was generally obtained. During the work up the reaction mixtures become bright red. The colour was shown to be due to the presence of the quinone methide (XI) which often represented the major product of the reaction.



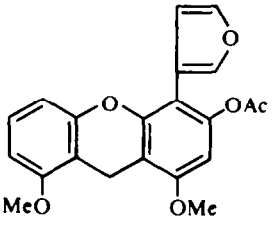
XI

The structure of this compound was deduced on the basis of spectroscopic evidence (Experimental). This compound probably arises by the aerial oxidation of the phenol XII.



XII

Formation of the phenol XII must involve reductive removal of the xanthone CO group followed by 1,4 addition of hydrogen over the furan system and hydrogenolysis of the resulting allylic ether to give a primary alcohol group and finally intramolecular transesterification. Another compound isolated from the reaction mixture after the uptake of 4 equivalents of hydrogen was identified as the xanthen XIII.



XIII

Compound IX was recovered unchanged on treatment with lead tetra-acetate in acetic acid under vigorous conditions. This result provides indirect evidence that VII is the intermediate in the reaction of Vb with lead tetra-acetate to give dihydroaspertoxin acetate.

EXPERIMENTAL

Unless specified to the contrary, UV refers to EtOH and IR absorption to $CHCl_3$ solns. UV spectra (Unicam Model S.P. 800 Spectrometer) and IR spectra (Perkin–Elmer Model 237 Spectrometer). Mass spectra were taken on a MS-9 double focusing mass spectrometer, PMR spectra (HA-100 spectrometer in $CDCl_3$ soln) and α_D values (Bendix N. P. L. Automatic Polarimeter Type 143). Silica for chromatography refers to a supply of E. Merck (0·05–0·2 mm). For preparative TLC chromatoplates were coated with Mercks silica Gel G. (2 mm thickness of silica gel layer). M.ps were determined on a Kofler Hot-stage

microscope and are uncorrected. In PMR spectrum data (s) = singlet, (d) = doublet, (t) = triplet, (m) = multiplet.

O-Methylsterigmatocystin (Ic). To DMSO (5 ml) was added a 50% emulsion of NaH (620 mg), the soln was stirred in a N_2 atmosphere for 45 min. Sterigmatocystin (Ib; 4 g) in DMSO (15 ml) added, and the mixture stirred for 40 min. MeI (10 ml) was added and the mixture stirred for 25 min. The resultant soln was washed with ice H_2O and extracted into $CHCl_3$. The $CHCl_3$ extract gave a crystalline product (Ic; 3·3 g, 79%), m.p. 264–267°, from MeOH (Lit. m.p. 265–267°);[6] λ_{max} 312 and 237 nm (ε 13,800 and 34,850, respectively), ν_{max} 3000, 1660 (xanthone CO), 1640, 1600 and 1480 cm^{-1}. (Found: M$^+$, 338 (M.S.). $C_{19}H_{14}O_6$ calculated for M, 338).

O-Methyldihydrosterigmatocystin (III). Compound Ic (250 mg), EtOAc (125 ml) and Adams catalyst (100 mg) were shaken in an atmosphere of H_2. Separation of the catalyst and evaporation of the solvent yielded an off-white residue (III; 160 mg, 65%), m.p. 283–285° from MeOH (Lit. m.p. 282–283°);[6] λ_{max} 311 and 237 nm (ε 12,380 and 27,900, respectively), ν_{max} 1660 (xanthone CO), 1640, 1600 and 1480 cm^{-1}. (Found: M$^+$, 340 (M.S.). $C_{19}H_{16}O_6$ calculated for M, 340), $[\alpha]_D^{20}$ $CHCl_3$ −243°.

1-[2′-(1′-*methoxytetrahydrofurano*)]-2-*hydroxy*-4,5-*dimethoxyxanthone* (Va). Compound III (140 mg) and 50% KOH/MeOH (15 ml) were heated under reflux in an atmosphere of N_2 for 12 hr. The solvent was removed by evaporation *in vacuo* and H_2O (50 ml) added. Work up by acidification with 1N HCl at 0° and extraction with $CHCl_3$ (3 × 50 ml) followed by crystallization from $CHCl_3$ yielded a crystalline solid Va (107 mg, 70%), m.p. 198–200°; λ_{max} 312 and 239 nm (ε 10,900 and 14,170, respectively). On addition of alkali a bathochromic shift was observed; λ_{max} 340 and 236 nm, ν_{max} 3200 (phenol OH), 3000, 1660 (xanthone CO), 1650, 1600, 1580, 1470, 1320, 1290, 1270, 1380, 1310, 1100 and 905 cm^{-1}. (Found: M$^+$, 372 (M.S.). $C_{20}H_{20}O_7$ requires: M, 372). $[\alpha]_D^{20}$ $CHCl_3$ −32·4°.

1-[2′-(1′-*methoxytetrahydrofurano*)]-2-*acetoxy*-4,5-*dimethoxyxanthone* (Vb). Compound (Va) (100 mg) in 1:1 Ac_2O/py (4 ml) was kept at 20° for 12 hr. The reagents were removed under reduced pressure. The amorphous residue was poured onto ice H_2O, extraction with $CHCl_3$ gave Vb. (90 mg, 81%), m.p. 205–206° from methanol; λ_{max} 335, 308 and 239 nm (ε 7900, 18,100 and 46,600 respectively). On addition of alkali a bathochromic shift was observed, λ_{max} 341 and 238 nm; ν_{max} 3000, 2940, 1780, (acetate CO), 1664 (xanthone CO), 1610, 1600, 1490, 1270, 1190, 1130, 1100 and 890 cm^{-1}. PMR spectrum showed (i) (s) (3H) at τ 7·66 (CH_3 acetyl), (ii) 3(s) (each 3H) at τ 6·66 (OCH_3 aliphatic) τ 6·04 and τ 6·11 (2 × OCH_3 aromatic) (iii) 2(m) (*ca* 5H) at τ 5·6–6·4 and τ 7·7–7·9 (tetrahydrofurano protons), (iv) (d) (1H) at τ 4·83 (J = 3 c/s) (C_1–H) (v) (s) (1H), 2(d) (2 × 1H) and a (t) (1H) at τ 3·54, τ 3·26 (J = 8 c/s), τ 3·0 (J = 8 c/s) and τ 2·5 (J = 8 c/s), respectively (aromatic protons). (Found: M$^+$, 414 (M.S.). $C_{22}H_{22}O_8$ requires: M, 414).

Dihydroaspertoxin acetate (VIa). Compound (Vb (67 mg), Pb $(OAc)_4$ (95 mg) in HOAc (2 ml) were heated at 85° for 1 hr. Work up by addition of ice water (50 ml) and extraction with $CHCl_3$ (3 × 50 ml) followed by chromatography on Whatman No. 3 MM filter paper impregnated with formamide (3:1, benzene/hexane) gave an off white residue. This residue was separated on a preparative chromatoplate (4% MeOH/$CHCl_3$) and after crystallization from (2:1) (Et_2O/$CHCl_3$) yielded VIa. (20 mg, 20%), m.p. 265–268°; λ_{max} 308 and 240 nm (ε 16,830 and 42,900, respectively), ν_{max} 1740 (acetate CO) and 1660 (xanthone CO) cm^{-1}. (Accurate mass M$^+$, 398·101. $C_{21}H_{18}O_8$ calculated for 398·100).

Dihydroaspertoxin VIb. Compound VIa (2 mg) was stirred at 80° for ½ hr with 5% NaOH/MeOH. After standard work up, sample submitted for mass spectrum. (Found: M$^+$, 356. $C_{19}H_{16}O_7$ requires: M, 356). R_f 0·47 (silica, developing system[10] ϕ/MeOH/HOAc 90:5:5).

1-[2′-(3′, 4′-*dihydrofurano*)]-2-*acetoxy*-4,5-*dimethoxyxanthone* (VII). Compound Vb (150 mg) in HOAc (70 ml) was heated at 80° for ½ hr. Ice H_2O (100 ml) was added. Standard work up gave a brown residue (140 mg) which was chromatographed on silica ($CHCl_3$). One crystallization from ether furnished the yellow crystalline product. (70 mg, 55%), m.p. 208–210°; λ_{max} 311 and 240 nm (ε 8600 and 28,600, respectively), ν_{max} 1780 (acetate CO) and 1660 (xanthone CO) cm^{-1}. PMR spectrum showed (i) (s) (3H) at τ 7·71 (CH_3 acetyl), (ii) (2s) (each 3H) at τ 6·1 and τ 6·4 (2 × OCH_3), (iii) (2t) (each 2H) at τ 6·95 and τ 5·51. (J = 10 c/s), (C_3–H and C_4–H), (iv) (s) (1H) at τ 3·37. (C_1–H), (v) (s) (1H), (2d) (2 × 1H), and a (t) (1H) at τ 3·52, τ 3·27 (J = 8 c/s), τ 3·09 (J = 8 c/s) and τ 2·52 (J = 8 c/s), respectively (aromatic protons). (Accurate mass M$^+$, 382·105. $C_{21}H_{18}O_7$ calculate for 382·105).

2-*O-acetyl*-5-*O-methylisosterigmatocystin* (IVb). O-methylsterigmatocystin (Ic; 800 mg) in 15% EtOH/KOH (90 ml) was heated in an atmosphere of N_2 under reflux for 12 hr. The solvent was removed under reduced pressure, to the residue 2:1 Ac_2O/py (6 ml) was added and the soln kept at 20° for 12 hr. The reagents were removed under reduced pressure. Standard work up yielded a white crystalline solid (730 mg, 81%), m.p. 221–224° from MeOH; λ_{max} 242, 312 and 346(s) nm (ε 35,900, 8200 and 4100, respectively).

On addition of alkali a bathochromic shift was observed ν_{max} 1770 (acetate CO), 1660 (xanthone CO) and 870 (furan) cm^{-1}. (Found: M$^+$, 380 (M.S.). $C_{21}H_{16}O_7$ requires: M, 380).

1-[2′-(*tetrahydrofurano*)]-2-*acetoxy*-4,6-*dimethoxyxanthen* (X). Compound IVb (843 mg), HOAc (100 ml) and Adams catalyst (100 mg) were shaken in an atmosphere of H_2. Separation of the catalyst and extraction into $CHCl_3$ afforded a bright red residue. The residue was separated on silica (1:200 MeOH/$CHCl_3$). The three main absorbing bands, 1,2,3 (R_f 0·71, 0·5 and 0·20, respectively) were eluted with 1:1 MeOH/$CHCl_3$. Band 1 was rechromatographed on a silica column, which on elution with $CHCl_3$ yielded after crystallization from ether compound X, (350 mg, 50%), m.p. 131–132°; λ_{max} 219, 275 and 283 nm. (ε 65,700, 5750 and 5340, respectively), ν_{max} 1760 (acetate CO) cm^{-1}. PMR spectrum showed (i) (s) (3H) at τ 7·75 (CH_3 acetyl), (li) (2s) (each 3H) at τ 6·2 and τ 6·24 (2 × OCH_3), (iii) (2m) (*ca* 9H) at τ 7·5–8 and τ 5·8–6·6 (tetrahydrofurano and methene H's), (iv) (s) (1H) (2d) (2 × 1H) and (t) (1H) at τ 3·78, τ 3·47 (J = 8c/s), τ 3·38 (J = 8 c/s) and τ 2·91 (J = 8 c/s), respectively (aromatic protons). (Found: M$^+$, 370 (M.S.), $C_{21}H_{22}O_6$ calculated for M, 370).

Quinone methide (XI). Band 3 (*cf* above separation) yielded a red residue (95 mg). The residue was separated on silica (2% MeOH/$CHCl_3$), and yielded after crystallization from ether XI (40 mg), m.p. 145–155° (d), λ_{max} 217, 269, 285, 298, 384 and 450(s) nm (ε 24,600, 15,100, 7400, 9140, 13,300 and 6800, respectively), ν_{max} 2990, 2960, 2940, 2870, 2840, 1725 (quinone methide CO) cm^{-1}. PMR spectrum showed, (i) (t) (3H) at τ 9·15 (J = 8 c/s) (CH_3 aliphatic), (ii) (m) (2H) at τ 8·6 (H's at C_2), (iii) (m) (H) τ 6·17–6·07 (H at C_3), (iv) (m) (2H) τ 5·5 (H's at C_4), (v) (s) (3H) at τ 8·06 (CH_3 acetyl), (vi) (2s) (each 3H) at τ 6·17 and 6·07 (2 × OCH_3), (vii) (s) (H) τ 4·08 (H at C_5), (viii) (s) (H) τ 1·72 (H at C_{11}). Positions of signals due to protons at C_8, C_9 and C_{10} similar to those observed for starting material. (Accurate mass M$^+$, 370·143. $C_{21}H_{22}O_6$ calculated for 370·142).

Xanthen (XIII). Band 2 (*cf* above separation) gave a yellow residue (100 mg) which on separation on silica (benzene) yielded after crystallization from ether XIII (72 mg), m.p. 193–195°; λ_{max} 278, 273 and 219 nm (ε 3900, 4600 and 10,000, respectively); on addition of alkali a bathochromic shift was observed; λ_{max} 222 and 310 nm; ν_{max} 1760 (acetate CO) and 874 (furan) cm^{-1}. (Accurate mass M$^+$, 366·110. $C_{21}H_{18}O_6$ calculated for 366·110).

1-[2′-(*tetrahydrofurano*)]-2-*acetoxy*-4,5-*dimethoxyxanthone* (IX). Compound X (200 mg), Ac_2O (12 ml), HOAc (6 ml) was treated with CrO_3 (260 mg) in H_2O (6 ml) over a period of 5 hr. The mixture was treated with ice H_2O. Standard work up gave an off-white solid which crystallized from ether to yield the required compound (194 mg, 93%) m.p. 178–180°; λ_{max} 238, 308 and 337(s) nm (ε 32,800, 12,400 and 5600), ν_{max} 1780 (acetate CO) and 1665 (xanthone CO) cm^{-1}. PMR spectrum showed, (i) (s) (3H) at τ 7·66 (CH_3 acetyl), (ii) (2s) (each 3H) at τ 6·07 and 6·11 (2 × OCH_3), (iii) (2m) (7H's) at τ 7·6–8·0 and τ 5·6–6·4 (tetrahydrofurano H's), (iv) (s) (1H), (2d) (2 × 1H) and a (t) (1H) at τ 3·54, τ 3·25 (J = 8 c/s) τ 3·03 (J = 8 c/s) and τ 2·49 (J = 8 c/s), respectively (aromatic protons). (Accurate mass M$^+$, 384·121. $C_{21}H_{20}O_7$ calculated M, 384·121).

REFERENCES

[1] J. V. Rodericks, E. Lustig, A. D. Campbell and L. Stoloff, *Tetrahedron Letters* 2957 (1968)
[2] A. C. Waiss, M. Wiley, D. R. Block and R. Lundin, *Ibid.* 3207 (1968)
[3] C. W. Holzapfel, I. F. H. Purchase, P. S. Steyn and L. Gouws, *African Med. J.*, **40**, 1100 (1966)
[4] P. J. Aucamp, D.Sc. Thesis, Bloemfontein (1969)
[5] J. E. Davies, D. Kirkaldy and J. C. Roberts, *J. Chem. Soc.* 2169 (1960)
[6] S. Hakomori, *Biochem.* 205 (1964)
[7] J. A. Knight, J. C. Roberts and P. Raffey, *J. Chem. Soc.* [*C*] 1308 (1966)
[8] M. J. Rance and J. C. Roberts, *Tetrahedron Letters* 277 (1969)
[9] J. D. Stevens and H. G. Fletcher, *J. Org. Chem.* **33**, 1799 (1968)
[10] Private communication, A. D. Campbell