Studies in Mycological Chemistry. Part XX.[1] Synthesis of (±)-Tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran, a Racemic Form of a Laevorotatory Degradation Product of Dihydrosterigmatocystin

By J. A. Knight, John C. Roberts, and (in part) Patrick Roffey

A synthesis of (±)-2,3,3a,8a-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran is described. Spectroscopic comparison of this material with a laevorotatory compound formed by degradation of the dihydro-derivative of sterigmatocystin (a metabolite of *Aspergillus versicolor*) has confirmed the structure of the degradation product.

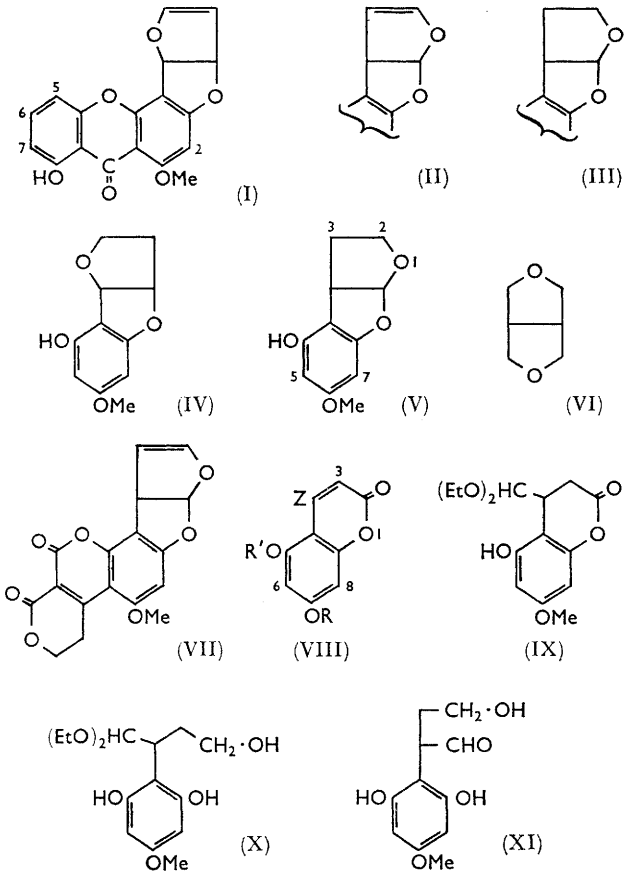
OUR initial [2] investigations into the chemistry of sterigmatocystin [a metabolite of some strains of *Aspergillus versicolor* (Vuillemin) Tiraboschi] led us to suggest that it had structure (I). Further, we proposed that a degradation product of dihydrosterigmatocystin had structure (IV). The structure of the metabolite was later [3] amended to (II). This amendment carries with it the corollary that dihydrosterigmatocystin has structure (III) and that the degradation product, which is laevorotatory, is to be represented by structure (V). We now describe the synthesis of a racemate corresponding to structure (V) and a spectroscopic comparison of this racemate with the degradation product.

The occurrence of two different asymmetric centres in 2,3,3a,8a-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]-benzofuran (V) implies that it might exist as four optically active stereoisomers. We believe, however, that the laevorotatory degradation product is a *cis*-form for the following reasons. First, construction of the *cis*- and *trans*-fused systems with the aid of scale-models shows that the latter forms are relatively very much more strained. Secondly, 3,7-dioxabicyclo[3,3,0]octane (VI) appears to exist [4] solely in the *cis* form. Thirdly, sterigmatocystin (II) probably has the *cis*-configuration [3] and it is noteworthy that the *cis*-configuration has been unequivocally established,[5] by *X*-ray analysis, for a closely related mould metabolite, aflatoxin G_1 (VII); if, therefore, sterigmatocystin has the *cis*-configuration, then dihydrosterigmatocystin (III) is a *cis*-form and we suppose that the degradation product (V) also has this configuration. In attempting a synthesis of a compound of structure (V), we therefore suspected that only one racemate, namely the (±)-*cis*-fused form, would be produced. In the event (see below), we obtained a single crystalline product of sharp m. p. and of the required analysis.

In order to test our projected synthesis, we first carried out the easier task (see Experimental section) of synthesising the (±)-*O*-methyl derivative of compound (V). A single product was obtained with the expected analysis and spectroscopic properties. The synthesis of the required racemate (V) was then achieved in the following way. Selective methylation [6] of 5,7-dihydroxy-4-methylcoumarin (VIII; R = R′ = H; Z = Me) yielded 5-hydroxy-7-methoxy-4-methylcoumarin (VIII; R = Me; R′ = H; Z = Me) which was then benzylated to give 5-benzyloxy-7-methoxy-4-methylcoumarin (VIII; R = Me; R′ = Ph·CH_2; Z = Me). Oxidation of this latter compound with selenium dioxide [7] gave the aldehyde (VIII; R = Me; R′ = Ph·CH_2; Z = CHO) which was converted into its acetal [VIII; R = Me; R′ = Ph·CH_2; Z = $CH(OEt)_2$]. Hydrogenation of this acetal, using Adams catalyst, removed the benzyl

[1] Part XIX, J. H. Birkinshaw, J. C. Roberts, and P. Roffey, *J. Chem. Soc.* (*C*), 1966, 855.
[2] J. E. Davies, D. Kirkaldy, and J. C. Roberts, *J. Chem. Soc.*, 1960, 2169.
[3] E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1962, 4179.
[4] K. Weinges and R. Spänig, *Chem. Comm.*, 1966, 9.
[5] K. K. Cheung and G. A. Sim, *Nature*, 1964, **201**, 1185.
[6] P. L. Sawhney and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 1953, **37***A*, 592.
[7] A. Schiavello and E. Cingolani, *Gazzetta*, 1951, **81**, 717.

protecting group and reduced the coumarin to yield the dihydrocoumarin (IX). Reduction of the acetate of this dihydrocoumarin with lithium aluminium hydride gave the benzene derivative (X) which was not isolated. Hydrolysis of this acetal (X) with aqueous mineral acid yielded the aldehyde (XI) (not isolated) which, under the reaction conditions, cyclised to give a product (in the



form of colourless prisms, m. p. 152°) with the correct analysis for tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]-benzofuran (V). This synthetic product and the degradation product had the same chemical properties (see ref. 2) and virtually identical ultraviolet (u.v.), infrared (i.r.), proton magnetic resonance (p.m.r.), and mass spectra (see below). This confirmation of the structure of the degradation product (V) forms additional evidence for the correctness of structure (II) for sterigmatocystin.

For reasons given above, the synthetic product is classed as the (±)-*cis* form. The m. p. of a mixture of the synthetic product (m. p. 152°) and the degradation product (m. p. 153—154°) was *ca.* 142°. Hence the former product is a racemic *compound*.

The work described above discloses a new synthesis for the *cis*-tetrahydrofuro[2,3-*b*]benzofuran system.* We are attempting to adapt this synthesis to a synthesis of some of the aflatoxins.

* Other syntheses for this system have been reported [8, 9] but, for various reasons, were not applicable to our particular problem.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Optical rotations were measured with an Ericsson E.T.L.–N.P.L. automatic polarimeter, type 143A. U.v. spectra were recorded, for ethanolic solutions, with a Unicam S.P. 700, or (in cases where no ε value is given) with a Perkin-Elmer (model 137 U.V.), spectrophotometer. (For several compounds described below, there is recorded only one $\lambda_{max.}$ value. This measurement is the longest wavelength, in the 260—400 mμ region, at which a peak absorption occurs for the individual compound concerned. A knowledge of these values was often useful in following and interpreting successive stages in the synthesis.)

I.r. spectra were determined, for compounds in potassium bromide discs, with a Unicam spectrophotometer (S.P. 200). P.m.r. spectra (on substances in $CDCl_3$ solution) were recorded on a Perkin-Elmer model R.10, 60 Mc./sec., spectrometer, tetramethylsilane being used as an internal reference; in the sequel, figures in parentheses, following the statement of the nature of the signal, indicate intensities.

4-Diethoxymethyl-5,7-dimethoxycoumarin [VIII; R = R′ = Me; Z = $(EtO)_2CH$].—A stream of dry hydrogen chloride was passed for 1 min. through a solution of 4-formyl-5,7-dimethoxycoumarin [7] (10 g.) in dry ethanol (500 ml.). After addition of triethyl orthoformate (10 ml.), the mixture was warmed to 50°, kept at room temperature overnight, and then added to a solution of sodium hydrogen carbonate (5 g.) in water (250 ml.). The *coumarin*, isolated by ether extraction, crystallised from methanol in colourless needles (11 g.), m. p. 123—124° (Found: C, 62·4; H, 6·5. $C_{16}H_{20}O_6$ requires C, 62·3; H, 6·5%), $\lambda_{max.}$ 328 mμ.

4-Diethoxymethyl-3,4-dihydro-5,7-dimethoxycoumarin (IX; OMe for OH).—A solution of the foregoing coumarin (10 g.) in ethyl acetate (150 ml.) was shaken with Adams catalyst (250 mg.) in an atmosphere of hydrogen (at room temperature and pressure) until absorption (0·98 mol.) ceased. The mixture was filtered and the solvent removed from the filtrate by evaporation. Crystallisation of the residue from light petroleum (b. p. 40—60°) gave the *dihydrocoumarin* (9·2 g.) as colourless prisms, m. p. 89—90° (Found: C, 61·9; H, 7·2. $C_{16}H_{22}O_6$ requires C, 61·9; H, 7·2%), $\lambda_{max.}$ 280 mμ.

(±)-*Tetrahydro-4,6-dimethoxyfuro*[2,3-b]*benzofuran* (V; OMe for OH).—A solution of the foregoing dihydrocoumarin (9·0 g.) in dry ether (150 ml.) was added dropwise to lithium aluminium hydride (3 g.) in dry ether (100 ml.). The mixture was heated under reflux for 4 hr. and the excess of hydride was decomposed with 2N-hydrochloric acid (250 ml.). The ether layer was separated and the aqueous portion was extracted with ether (2 × 100 ml.). The combined ether extracts were washed with water and dried ($MgSO_4$). Removal of the solvent gave a crude product (5 g.) which crystallised from ether to give (±)-*tetrahydro-4,6-dimethoxyfuro*[2,3-b]*benzofuran* as colourless prisms (2·9 g.), m. p. 102—103° [Found (on a sample which had been sublimed at 90°/0·2 mm.): C, 64·9; H, 6·5%; *M* (thermistor drop), 217; *M* (mass spec.), 222. $C_{12}H_{14}O_4$ requires C, 64·9; H, 6·4%; *M*, 222], $\lambda_{max.}$ 207, 333(sh.), 271, and 278 mμ (ε 42,100, 7200,

[8] C. H. Eugster and P. Kuser, *Chimia* (*Switz.*), 1964, **18**, 358.
[9] H. E. Zaugg, F. E. Chadde, and R. J. Michaels, *J. Amer. Chem. Soc.*, 1962, **84**, 4567.

840, and 750, respectively). The i.r. spectrum showed no band above 3120 cm.$^{-1}$ (absence of OH). The p.m.r. spectrum showed (i) a doublet (1) at τ 3·75 (J = 6 c./sec.) (O–C*H*–O), (ii) a triplet (two partially overlapping doublets) (2) at τ 4·0 (J = 2 c./sec.) (2 × Ar·*H*), (iii) a multiplet (3) between τ 5·8 and 6·6 (Ar·C*H* and O·CH_2), (iv) two singlets (each 3) at τ 6·22 and 6·26 (2 × O·CH_3), and (v) a multiplet (2) at τ 7·85 [CH_2 at position 3—see (V; OMe for OH)].

5-Hydroxy-7-methoxy-4-methylcoumarin (VIII; R = Me; R′ = H; Z = Me).—Dimethyl sulphate (75 ml.) was added dropwise, during ½ hr., to a solution of 5,7-dihydroxy-4-methylcoumarin (100 g.) in 10% aqueous sodium carbonate (1 l.), the temperature of the reaction mixture being kept throughout at 80°. The mixture was cooled and the crude 7-*O*-methyl derivative collected by filtration. This material was mixed with 5% aqueous sodium hydroxide (1 l.) and the insoluble portion (the 5,7-di-*O*-methyl derivative) was filtered off. Acidification of the filtrate, and crystallisation of the washed and dried precipitate from ethanol, yielded the required coumarin (35 g.) as colourless needles, m. p. 252—254° (lit.,[6] m. p. 256—257°).

5-Benzyloxy-7-methoxy-4-methylcoumarin (VIII; R = Me; R′ = Ph·CH_2; Z = Me).—A solution of the foregoing coumarin (10 g.) in dry acetone (750 ml.) together with benzyl chloride (8 g.), sodium iodide (8 g.), and anhydrous sodium carbonate (13 g.) was heated under reflux for 8 hr. The solids were filtered off and the filtrate was taken to dryness *in vacuo*. Crystallisation of the residue from ethanol gave the required *coumarin* (11·5 g.) as colourless needles, m. p. 138—141° (Found: C, 72·7; H, 5·2. $C_{18}H_{16}O_4$ requires C, 73·0; H, 5·4%), $\lambda_{max.}$ 320 mμ.

5-Benzyloxy-4-formyl-7-methoxycoumarin (VIII; R =Me; R′ = Ph·CH_2; Z = CHO).—A solution of the foregoing benzyl ether (10 g.) in xylene (500 ml.), together with recently sublimed selenium dioxide (10 g.), was heated under reflux with stirring for 6 hr. and the hot mixture was filtered. The filtrate, when cooled to 0°, gave a precipitate which was collected and was crystallised from acetone to give the *aldehyde* (6·2 g.) as pale yellow needles, m. p. 189—191° (Found: C, 69·5; H, 4·2. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·6%), $\lambda_{max.}$ 340 mμ.

5-Benzyloxy-4-diethoxymethyl-7-methoxycoumarin [VIII; R = Me; R′ = Ph·CH_2; Z = (EtO)$_2$CH].—This *acetal*, prepared from the foregoing aldehyde (6 g.) by a method similar to that used for the 5,7-dimethoxy analogue (above), crystallised from ethyl acetate–light petroleum (b. p. 40—60°) as colourless prisms (6·5 g.), m. p. 98—99° (Found: C, 69·0; H, 5·9. $C_{22}H_{24}O_6$ requires C, 68·7; H, 6·3%), $\lambda_{max.}$ 325 mμ.

4-Diethoxymethyl-3,4-dihydro-5-hydroxy-7-methoxycoumarin (IX).—The foregoing acetal (6 g.) was hydrogenated [ethyl acetate (150 ml.), Adams catalyst (750 mg.)] to give the *dihydrocoumarin*, which crystallised from methanol as colourless prisms (4·2 g.), m. p. 124—125° [Found (on a sample recrystallised from ethyl acetate–light petroleum, b. p. 40—60°): C, 60·8; H, 6·8. $C_{15}H_{20}O_6$ requires C, 60·8; H, 6·8%], $\lambda_{max.}$ 280 mμ.

(±)-*Tetrahydro-4-hydroxy-6-methoxyfuro*[2,3-b]*benzofuran* (V).—The foregoing dihydrocoumarin (4 g.) was converted (pyridine and acetic anhydride) into the acetate (4·1 g., pale yellow oil). A solution of this crude acetate (4·0 g.) in dry ether (50 ml.) was added dropwise to lithium aluminium hydride (2 g.) in dry ether (100 ml.). The mixture was heated under reflux for 4 hr., the excess hydride was decomposed with 2N-hydrochloric acid (125 ml.), and the product was isolated as described above for the dimethoxy analogue. The crude product (2·5 g.) was crystallised from chloroform to give (±)-*tetrahydro-4-hydroxy-6-methoxyfuro*-[2,3-b]*benzofuran* (1·2 g.) as colourless prisms, m. p. 150—151°, or, after sublimation at 110°/0·2 mm., m. p. 152° (Found: C, 63·5; H, 5·7. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8%), $\lambda_{max.}$ 208, 225, 270, and 278 mμ (ε 42,400, 8950, 700, and 550, respectively); $\nu_{max.}$ 3350, 1634 cm.$^{-1}$ (strong), and other bands virtually identical with those in the spectrum [2] of the (−)-degradation product. The p.m.r. spectrum was the same as that described below for the laevorotatory form. The mass spectrum showed prominent peaks at *m/e* 208, 193, 179, 151, 91, 69, 39, 32, and 28, duplicating those in the spectrum of the (−)-isomer.

(−)-*Tetrahydro-4-hydroxy-6-methoxyfuro*[2,3-b]*benzofuran* (V).—To a solution of dihydro-5-hydroxysterigmatocystin [2] (0·75 g.) in 1% aqueous sodium hydroxide (200 ml.) was added 3% hydrogen peroxide solution (160 ml.). The light absorption of the solution was measured at 15 min. intervals and, when the peak at 326 mμ had virtually disappeared (*ca.* 4 hr.), the product was isolated as previously described.[2] The crude product (80 mg.) gave, on sublimation at 105°/0·1 mm., the tetrahydrobenzofuran (35 mg.) as colourless prisms, m. p. 153—154° (Found: M, 208·0740. Calc. for $C_{11}H_{12}O_4$: M, 208·0736), $[\alpha]_D^{28}$ = −155° (*c* 0·246 in $CHCl_3$), $\lambda_{max.}$ 208, 227(sh), 271, and 278 mμ (ε, 35,600, 8400, 580, and 520, respectively). [The peak at 326 mμ previously recorded [2] in the u.v. spectrum of this compound was probably due to the presence of a very small amount of impurity (possibly dihydro-5-hydroxysterigmatocystin) with intense absorption at about this wavelength. No peak is shown in this region when the *sublimed* material is used.] The p.m.r. spectrum showed (i) a doublet (1) at τ 3·70 (J = 6 c./sec.) (O–C*H*–O–), (ii) two doublets (1 each) at τ 3·97 and 4·06 (J = 2 c./sec. in each case) (2 × Ar·*H*), (iii) a multiplet (4) between τ 5·7 and 6·5 (Ar·C*H*, O·CH_2, and O*H*), (iv) a singlet (3) at τ 6·30 (O·CH_3), and (v) a multiplet (2) at τ 7·8 [CH_2 at position 3—see (V)].

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