The Structure of Dihydrodeoxy-8-*epi*-austdiol and the Absolute Configuration of the Azaphilones

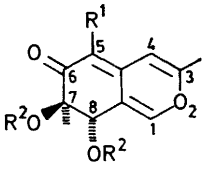
By **Pieter S. Steyn** * and **Robert Vleggaar**, National Chemical Research Laboratory, Council for Scientific and Industrial Research, Pretoria, Republic of South Africa

The fungal metabolite dihydrodeoxy-8-*epi*-austdiol, elaborated by *Aspergillus ustus*, has been identified as (7*R*,8*R*)-7,8-dihydro-7,8-dihydroxy-3,5,7-trimethyl-2-benzopyran-6-one. The absolute configuration of the azaphilones has been deduced.

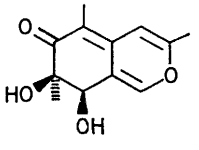
WE have previously described[1] the elucidation of the structure and absolute configuration of austdiol (1), the main toxic metabolite from *Aspergillus ustus* (Bainier) Thom and Church. The conformation and absolute configuration of austdiol have subsequently been confirmed by *X*-ray crystallography of the bromo-derivative (2).[2]

In the present investigation, toxic material, obtained from maize meal (10 kg) infected with *A. ustus*, yielded, in addition to austdiol, a new metabolite, isolated after chromatography on formamide-impregnated cellulose powder.

The new metabolite, dihydrodeoxy-8-*epi*-austdiol (3), $C_{12}H_{14}O_4$, showed $[\alpha]_D^{23}$ +250° (*c* 0.64 in $CHCl_3$). The u.v. spectrum [$\lambda_{max.}$ 231 and 352 nm (log ε 3.84 and 4.30)] indicated the presence of an austdiol-type conjugated



(1) R^1=CHO, R^2=H
(2) R^1=Br, R^2=H
(5) R^1=CHO, R^2=Ac
(6) R^1=Me, R^2=Ac
(7) R^1=Me, R^2=H



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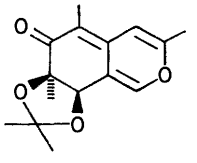
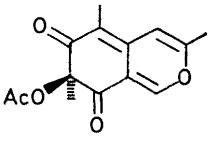


(4)

chromophore.[1] The i.r. spectrum showed strong OH absorption at 3530 and 3460 cm^{-1}. The n.m.r. spectrum agrees well with the structure (3) assignments are summarized in Table 1. The appearance of the 8- and 1-proton signals as singlets at τ 5.72 and 2.79, respectively, is of particular interest as the corresponding protons in



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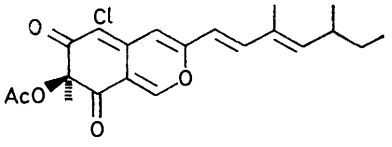

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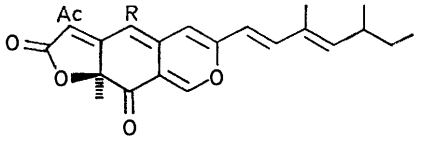


(10) R=H
(11) R=Cl

austdiol (1), which has the 8*S*-configuration, appear as doublets ($J_{1,8}$ 2.0 Hz).[1] The 8*R*-configuration is therefore indicated for the metabolite (3), as this configuration has the requisite ' allylic ' angle of *ca.* 0°, *i.e.* $J_{1,8} \simeq 0$.[3]

The above argument points to a *cis*-disposition of the *vic*-diol system in (3). The *cis*-vicinal arrangement of the two hydroxy-groups was confirmed by treatment of (3) with acetone in the presence of perchloric acid to give the isopropylidene derivative (4).

Chemical evidence for structure (3) was obtained as follows. Hydrogenation of austdiol diacetate (5) in methanol over palladium–charcoal gave the trimethyl derivative (6). Removal of the acetyl residues with sodium methoxide proceeded smoothly to give the

[1] R. Vleggaar, P. S. Steyn, and D. W. Nagel, *J.C.S. Perkin I*, 1974, 45.
[2] G. J. Kruger, personal communication.
[3] L. M. Jackman and S. Sternhell, ' Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon, Oxford, 2nd edn., p. 316.

required (7*R*,8*S*)-diol (7), $[\alpha]_D^{23}$ +243° (*c* 1.50 in $CHCl_3$). The fact that no isopropylidene derivative of (7) could be formed is in keeping with the *trans*-diequatorial conformation of the *vic*-diol system in austdiol (1) and its derivatives.[1,2] The diastereoisomeric relationship of the diols (3) and (7) was evident from their n.m.r. spectra (Table 1). The 7*R*,8*R*-configuration is therefore assigned to compound (3).

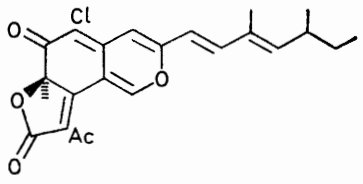
TABLE 1

N.m.r. data (τ values)

Compd.	1-H	3-Me	4-H	5-Me	7-Me	7-OR	8-OR	8-H	*gem*-Me_2
(3)	2.79	7.83	3.92	8.18	8.74			5.72	
(4)	2.85	7.90	3.97	8.25	8.59			5.68	8.72
									8.74
						R = H			
(7)	2.67 †	7.80	3.93	8.19	8.83	6.0		5.45 †	
						R = Ac			
(8)	2.24	7.83	3.83	8.16	8.51	7.85			

† d, $J_{1,8}$ 2.0 Hz

Oxidation of the diol (7) with *N*-chlorosuccinimide and dimethyl sulphide [4] and acetylation of the oxidation product gave the unstable azaphilone (8), $[\alpha]_D^{23}$ +114° (*c* 0.30 in $CHCl_3$). The poor yield obtained in this conversion together with the small amount of (3) at our disposal precluded the oxidation of (3) to the common product (8).



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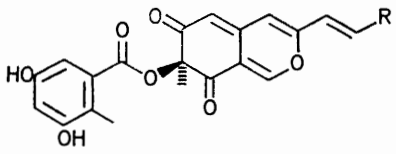
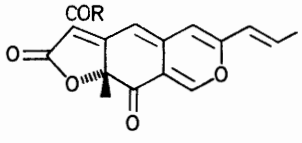


(13) R = Me
(14) R = CH_2OH
(15) R = CO_2H



(16) R = n-C_5H_{11}
(17) R = n-C_7H_{15}

The availability of the azaphilone (8), however, enabled us to deduce the absolute configuration at C-7 of the azaphilones. Whalley has shown that the sign of the specific rotation of these metabolites is apparently controlled by the absolute configuration at C-7.[5] Accordingly the c.d. spectra of (8) and (+)-sclerotiorin (9), both belonging to the (+)-series, were compared (Figures 1 and 2). On the basis of the c.d. spectra and the known 7*S*-configuration of (8), the 7*R*-configuration is assigned to (+)-sclerotiorin (9). The definition of the absolute configuration at C-7 of (+)-sclerotiorin establishes [5] the absolute stereochemistry of the azaphilones as shown in Table 2.

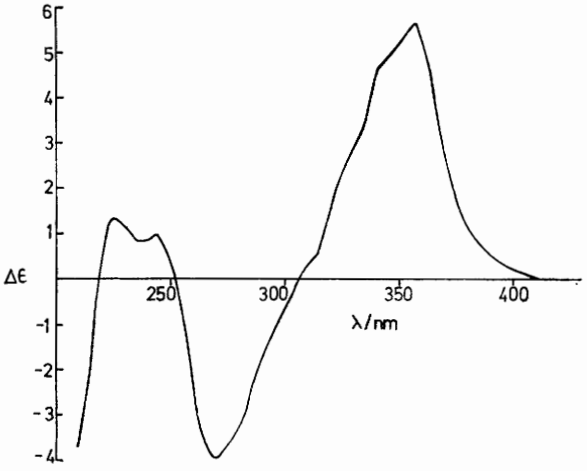


FIGURE 1 C.d. spectrum of compound (8)

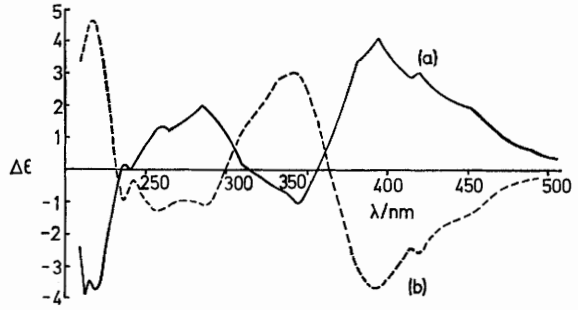


FIGURE 2 C.d. spectra of (a) (+)-sclerotiorin and (*b*) (−)-sclerotiorin

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. absorptions were measured (for solutions in ethanol) with a Unicam SP 800 spectrometer, i.r. spectra (solvent $CHCl_3$) with a Perkin-Elmer 237 spectrometer, mass spectra

[4] E. J. Corey and C. U. Kim, *Tetrahedron Letters*, 1974, 287.
[5] F. C. Chen, P. S. Manchard, and W. B. Whalley, *J. Chem. Soc.* (*C*), 1971, 3577.

with an A.E.I. MS9 double-focusing spectrometer, and n.m.r. spectra (for solutions in $CDCl_3$) with a Varian HA-100 spectrometer (with tetramethylsilane as internal standard). C.d. spectra were measured for solutions in methanol (JASCO J-20 spectropolarimeter). T.l.c. was carried out on Merck pre-coated silica plates (thicknesses 0.25 and 2 mm for analytical and preparative purposes, respectively).

TABLE 2

Absolute configuration of the azaphilones

Compound	Configuration at C7
(+)-Sclerotiorin (9)	*R*
(+)-Rotiorin (10)	*S*
(+)-5-Chlororotiorin (11)	*S*
(+)-5-Chloroisorotiorin (12)	*R*
(+)-Mitorubrin [a] (13)	*S*
(+)-Mitorubrinol [a] (14)	*S*
(+)-Mitorubrinic acid [a] (15)	*S*
(−)-Sclerotiorin	*S*
(−)-Rubropunctatin (16)	*R*
(−)-Monascorubrin (17)	*R*
(−)-Rubrorotiorin †	*S*
(−)-Mitorubrin [b]	*R*
(−)-Mitorubrinol [b]	*R*
(−)-Mitorubrinic acid [b]	*R*

† 7-*epi*-5-Chloroisorotiorin.

[a] W. Steglich, M. Klaar, and W. Furtner, *Phytochemistry*, 1974, **13**, 2874. [b] G. Büchi, J. D. White, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1965, **87**, 3484.

Isolation of the Metabolites.—*A. ustus* was grown in bulk on wet sterilized maize meal for 20 days. The dried, milled, mouldy maize (10.0 kg) was processed as previously described [6] to yield austdiol (1) (480 g) and toxic material (120 g). The toxic material was separated by chromatography on formamide-impregnated cellulose powder (2 kg). The cellulose column was developed consecutively with hexane, hexane–benzene, and benzene; 2 500 fractions (each 30 ml) were collected.

Fractions A—E were collected as previously described.[6] Fraction F (tubes 1 900—2 200) contained austocystins G, H, and I [7] as well as dihydrodeoxy-8-*epi*-austdiol (3).

Purification of Dihydrodeoxy-8-epi-*austdiol* [(7R,8R)-7,8-*Dihydro*-7,8-*dihydroxy*-3,5,7-*trimethyl*-2-*benzopyran*-6-*one*] (3).—Fraction F (2.6 g) was separated by preparative t.l.c. with benzene–acetone (4 : 1 v/v) to give autocystins G (6 mg), H (6 mg), and I (12 mg),[7] and dihydrodeoxy-8-*epi*-austdiol (3) (29 mg). *Dihydrodeoxy*-8-epi-*austdiol* (3) had m.p. 208—210° (from acetone–n-hexane), $[\alpha]_D^{23}$ +250° (*c* 0.64 in $CHCl_3$); $\lambda_{max.}$ 231 and 352 nm (log ε 3.84 and 4.30); c.d. $\Delta\varepsilon_{410}$ 0, $\Delta\varepsilon_{360}$ +7.8, $\Delta\varepsilon_{366}$ 0, $\Delta\varepsilon_{313}$ −6.5, $\Delta\varepsilon_{255}$ −0.2, $\Delta\varepsilon_{235}$ −1.6, and $\Delta\varepsilon_{217}$ 0; $\nu_{max.}$ 3 530 (OH), 3 460 (OH), and 1 620 cm^{-1} (Found: C, 64.8; H, 6.4. $C_{12}H_{14}O_4$ requires C, 64.85; H, 6.35%).

Reaction of Dihydrodeoxy-8-epi-*austdiol* (3) *with Acetone.*—Compound (3) (7 mg) in acetone (1 ml) was treated with perchloric acid (70%; 0.05 ml) to give 7,8-*dihydro*-7,8-*isopropylidenedioxy*-3,5,7-*trimethyl*-2-*benzopyran*-6-*one* (4) (7 mg) as a glass (Found: M^+, 262.1219. $C_{15}H_{18}O_4$ requires *M*, 262.1204).

Hydrogenation of Austdiol Diacetate (5).—Compound (5) [1] (960 mg) in methanol (50 ml) was hydrogenated over 10% palladium–charcoal (40 mg). After 1 h (when *ca.* 2 mol. equiv. had been taken up), the mixture was filtered and evaporated. Column chromatography on silica with benzene–acetone (4 : 1 v/v) gave (7*R*,8*S*)-7,8-diacetoxy-7,8-dihydro-3,5,7-trimethyl-2-benzopyran-6-one (6) (366 mg), m.p. 209—211° (from benzene–n-hexane) (lit.,[1] 209—211°).

Saponification of the Diacetate (6).—The diacetate (6) (120 mg) was dissolved in a solution of sodium methoxide (45 mg) in methanol (5 ml) and stirred at room temperature for 10 min. The mixture was acidified (6N-HCl), diluted with water (30 ml), and extracted with chloroform to give (7R,8S)-7,8-*dihydro*-7,8-*dihydroxy*-3,5,7-*trimethyl*-2-*benzopyran*-6-*one* (7) (83 mg) as a glass, $[\alpha]_D^{23}$ +243° (*c* 1.50 in $CHCl_3$); $\lambda_{max.}$ 234 and 356 nm (log ε 3.81 and 4.27); c.d. $\Delta\varepsilon_{410}$ 0, $\Delta\varepsilon_{372}$ +2.7, $\Delta\varepsilon_{346}$ +1.3, $\Delta\varepsilon_{305}$ +3.3, $\Delta\varepsilon_{264}$ 0, $\Delta\varepsilon_{246}$ −4.5, $\Delta\varepsilon_{233}$ 0, $\Delta\varepsilon_{226}$ +1.5, and $\Delta\varepsilon_{216}$ 0; $\nu_{max.}$ 3 580 (OH), 3 440 (OH), and 1 620 cm^{-1} (Found: M^+, 222.0893. $C_{12}H_{14}O_4$ requires *M*, 222.0892).

Oxidation of the Diol (7).—Dimethyl sulphide (0.12 ml) was added to a solution of *N*-chlorosuccinimide (176 mg) in toluene (5 ml) at 0 °C under nitrogen. The mixture was cooled to −25 °C and a solution of the diol (7) (66 mg) in toluene (3 ml) was added with stirring. After 3 h a solution of triethylamine (0.2 ml) in toluene (1 ml) was added dropwise. The mixture was allowed to attain room temperature, diluted with chloroform (15 ml), and washed with ice-cold 1% hydrochloric acid. The organic layer yielded the crude oxidation product, which was stirred with toluene-*p*-sulphonic acid (5 mg) in benzene (10 ml) (to hydrolyse the methylthiomethyl ether present) for 6 h. The benzene solution was washed with water, dried (Na_2SO_4), and evaporated to give the crude ketol. The ketol was acetylated with acetic anhydride (1 ml) and pyridine (0.5 ml) at room temperature for 1 h and the product was purified by preparative t.l.c. with benzene–acetone (9 : 1 v/v) to give the unstable azaphilone [(7S)-7-*acetoxy*-3,5,7-*trimethyl*-2-*benzopyran*-6,8-*dione*] (8) (7 mg) as a glass, $[\alpha]_D^{23}$ +114° (*c* 0.30 in $CHCl_3$), $\lambda_{max.}$ 221 and 338 nm (log ε 4.06 and 4.09); c.d. $\Delta\varepsilon_{410}$ 0, $\Delta\varepsilon_{357}$ +5.6, $\Delta\varepsilon_{306}$ 0, $\Delta\varepsilon_{270}$ −4.0, $\Delta\varepsilon_{252}$ 0, $\Delta\varepsilon_{244}$ +0.9, $\Delta\varepsilon_{225}$ +1.3, $\Delta\varepsilon_{219}$ 0, and $\Delta\varepsilon_{210}$ −3.7; $\nu_{max.}$ 1 730 (CO) and 1 635 cm^{-1} (Found: M^+, 262.0853. $C_{14}H_{14}O_5$ requires *M*, 262.0841).

We thank Professor W. B. Whalley for samples of (+)- and (−)-sclerotiorin.

[5/1320 *Received*, 4th *July*, 1975

[6] P. S. Steyn and R. Vleggaar, *J.C.S. Perkin I*, 1974, 2250.
[7] P. S. Steyn and R. Vleggaar, *J.S. African Chem. Inst.*, in the press.