Constitution and Absolute Configuration of Austdiol, the Main Toxic Metabolite from *Aspergillus ustus*

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The fungal metabolite austdiol, elaborated by *Aspergillus ustus*, has been identified as (7*R*,8*S*)-7,8-dihydro-7,8-dihydroxy-3,7-dimethyl-6-oxo-6*H*-2-benzopyran-5-carbaldehyde. The absolute configuration was deduced by the ' partial resolution ' method of Horeau.

STRAINS of *Aspergillus ustus* (Bainier) Thom. and Church were isolated from stored foodstuffs in the course of our continuing search for toxigenic fungi. Maize meal cultures of *A. ustus* (C.S.I.R. 1128) were found to cause acute toxicoses in day-old ducklings. The toxic principles were quantitatively extracted from the mouldy maize meal and systematic fractionation, guided by bioassay, led to the isolation of five biogenetically related dioxopiperazines and the major active component.[1] We now report the detailed structural and stereochemical characterization of the major toxin, designated austdiol.

Austdiol (I), a gastro-intestinal toxin [2] was obtained as optically active yellow needles. Mass spectrometry defined the molecular formula as $C_{12}H_{12}O_5$, in agreement with the elemental analysis. The u.v. spectrum [$\lambda_{max.}$ (EtOH) 256 and 376 nm (log ε 4·18 and 4·38)] indicated the presence of an extended conjugated system, and was unaffected by addition of base; however prolonged treatment with base led to extensive degradation. The i.r. spectrum showed $\nu_{max.}$ 3370 (OH), 3460 (OH), 3100 (C=C), 1670w (aldehyde CO), 1620 (conj. CO), and 1600 cm^{-1} (C=C). The n.m.r. spectrum (Table 1) is in good agreement with the proposed structure (I). The chemical shifts correspond closely with those reported for similar compounds, *e.g.* mitorubrin (II),[3] with the exception of that of the 4-proton. The low-field position this of signal may be attributed to the deshielding effect of the aldehyde carbonyl group which, for steric and electronic reasons, is apparently aligned in the plane of the ring system and oriented towards the 4-proton.

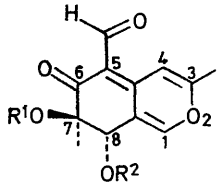
The presence of an aldehyde function was confirmed by the ready formation of a dark red 2,4-dinitrophenylhydrazone [τ 1·00 (CH=N)].

Chemical evidence for the presence of two hydroxy-groups was provided by acetylation, to yield either a monoacetate (III) or a diacetate (IV). Austdiol did not react with ethereal diazomethane, and since the chromophore is unaffected by acetylation, the hydroxy-groups must be non-phenolic. The n.m.r. signal for the 8-proton (see Table 1) suffers a marked downfield shift upon each acetylation. This shift seems to indicate that the two hydroxy-groups are in a *trans*-vicinal arrangement, in agreement with the finding that austdiol is inert towards periodate. The *trans* disposition of the *vic*-diol would also explain the fact that no acetonide derivative of austdiol could be prepared. Instead when austdiol was treated with acetone in the presence of perchloric acid, a condensation product, $C_{15}H_{16}O_5$, was isolated. This was assigned structure (V) on the basis of spectroscopic evidence. The n.m.r. spectrum showed the presence of D_2O-exchangeable protons at τ *ca.* 4·8 and the absence of the aldehyde proton. The singlet at τ 7·78 (3H) was assigned to the methyl group of the acetyl residue and that at τ 2·26 (2H) to the olefinic protons of the newly-formed double bond. Upon addition of D_2O the *trans* olefinic proton signals appeared as an AB system [τ 2·22 (1H) and 2·30 (1H), J_{AB} 16·0 Hz].

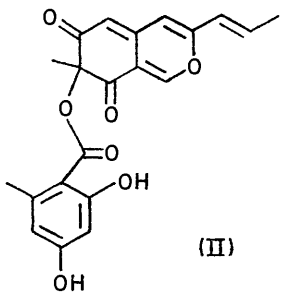
Hydrogenation of austdiol diacetate (IV) in methanol over palladium–charcoal gave the trimethyl compound (VI), $C_{16}H_{18}O_6$. The n.m.r. spectrum showed the absence of the aldehyde proton signal, and the singlet at τ 8·23 (3H) was assigned to the newly-formed 5-methyl

[1] P. S. Steyn, *Tetrahedron*, 1973, **29**, 107.
[2] S. J. van Rensburg, personal communication.
[3] G. Büchi, J. D. White, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1965, **87**, 3484.

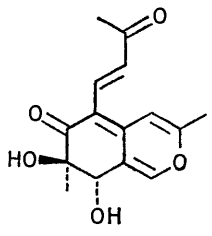
group. The 4-proton signal appeared at τ 4·02; *cf.* τ 1·66 in austdiol diacetate (IV); this marked upfield shift is readily explained by the absence of deshielding due to the diamagnetic anisotropy of the aldehyde group.



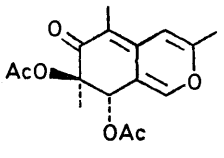
(I) $R^1 = R^2 = H$
(III) $R^1 = H$, $R^2 = Ac$
(IV) $R^1 = R^2 = Ac$



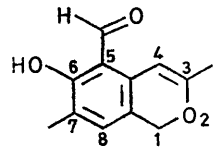
(II)



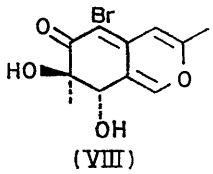
(V)



(VI)



(VII)

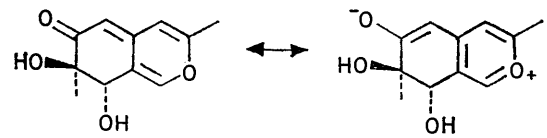


(VIII)

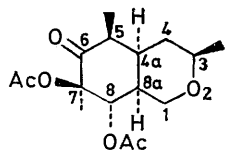
The change in conjugation is evident from the bathochromic shift of the long wavelength u.v. band from 376 in (IV) to 354 nm in the hydrogenation product (VI).

At this stage of the structural elucidation it was not clear whether the aldehyde group was at C-3 or C-5. [The location of a vinylic methyl group at C-3 was established through hydrogenation of (IV) to yield (X), which contained a secondary methyl group on an oxygen-bearing carbon atom (see later).] Hydrogenation of austdiol diacetate (IV) in ethyl acetate over platinum oxide yielded, as one of the products, a yellow optically inactive compound. Mass spectrometry indicated a molecular formula of $C_{12}H_{12}O_3$ and structure (VII) was assigned, with which the n.m.r. spectrum is fully compatible. The singlet at τ −0·26 was assigned to the aldehyde proton, and that at −1·98 (1H, exchangeable) is characteristic of an intramolecular hydrogen-bonded phenolic proton. [The i.r. spectrum showed a band at 1620 cm^{-1} (chelated CO) and a green colour was obtained with ethanolic iron(III) chloride.] The two-proton singlet at τ 5·06 is ascribed to the equivalent benzylic protons, and the two singlets at τ 3·02 (1H) and τ 3·86 (1H), which exhibit a slight splitting as a result of long-range coupling ($J_{4,8}$ 0·5 Hz), are assigned to the 8- and 4-protons, respectively. The two high-field singlets at τ 7·84 (3H) and 8·02 (3H) are assigned to the 3- and 7-methyl groups. The formation of compound (VII) can be envisaged as a reductive aromatization with the simultaneous expulsion of two molecules of acetic acid.[4]

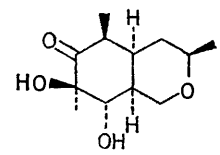
The reaction of austdiol with either pyridinium hydrobromide perbromide or bromine (evidence from t.l.c. on silica) in acetic acid gave a monobromo-derivative (VIII), $C_{11}H_{11}BrO_4$. The i.r. spectrum lacked an aldehyde CO band. The n.m.r. spectrum was similar to that of austdiol and assignments are summarized in Table 1. Hydrogenation of (VIII) over platinum dioxide in acetic acid (uptake *ca.* 2·5 mmol) yielded a complex mixture from which some starting material and a debromination product were isolated. The latter, $C_{11}H_{12}O_4$, was assigned structure (IX). Comparison of the n.m.r. spectrum (Table 1) with that of mitorubrin (II) (Table 1)



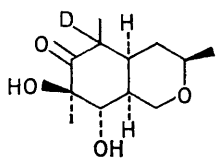
(IX) (IXa)



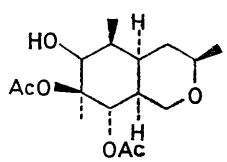
(X)



(XI)



(XII)



(XIII)

provides support for its interpretation. The large difference in chemical shifts of the 5-proton (τ 4·66) and the 1-proton (τ 2·58) reflects their different molecular environments; the latter is deshielded by the adjacent oxygen atom whereas the former can be shielded by delocalization of the non-bonding electrons of the pyrone oxygen atom. This difference will be maximal in the charge-separated resonance form (IXa), in which the 1-proton lies in the plane of the pyrylium ring.

The foregoing results firmly established the position of the aldehyde group at C-5 in austdiol. The positions of the *vic*-diol system and the tertiary methyl group were proved as follows. Hydrogenation of austdiol diacetate (IV) in acetic acid over platinum dioxide gave mainly two

[4] E. J. Haws, J. S. E. Holker, A. Kelly, A. D. G. Powell, and A. Robertson, *J. Chem. Soc.*, 1959, 3598.

compounds. The major compound, $C_{16}H_{24}O_4$, was assigned structure (X) [$\nu_{max.}$ 1745 (acetate CO) and 1730 cm^{-1} (six-membered ring CO)]. The c.d. spectrum showed a ketone Cotton effect at 286 nm ($\Delta\varepsilon$ + 1·57). The n.m.r. spectrum (Table 2) shows two *O*-acetyl singlets [τ 7·93 (3H) and 8·00 (3H)] and the singlet at τ 8·60 (3H) is assigned to the 7-methyl group. The 5-methyl signal part of the ABX system, is obscured at τ *ca.* 7·8. The foregoing assignments were confirmed by spin–spin decoupling experiments.

Saponification of the *O*-acetate functions in (X) gave the diol (XI), M^+ 228. The n.m.r. spectrum of this compound (Table 2) showed a singlet at τ 8·64 (3H), assigned to the tertiary 7-methyl group. The methine proton at

TABLE 1

Chemical shifts (τ) and multiplicity * in the n.m.r. spectral of austdiol and derivatives

	1-H	3-Me	4-H	5-CHO	5-Me	5-H	7-Me	7-OAc	7-OH	8-OH	8-OAc	8-H
Austdiol (I)†	1·76(d)‡	7·93	1·58	−0·67			8·54		5·28	3·66		5·05(d)‡
Mitorubrin (II)	1·75(d)§		3·43			4·39(d)§	8·33					
Austdiol monoacetate (III)	2·40(d)‡	7·63	1·62	−0·16			8·78		6·45		7·78	4·08(d)‡
Austdiol diacetate (IV)	2·34(d)‡	7·66	1·66	−0·19			8·63	7·99			7·83	3·32(d)‡
(VI)	3·16(d)‡	7·89	4·02		8·23		8·70	7·99			7·84	3·29(d)‡
(VIII)	2·51(d)‡	7·74	3·50				8·80		5·92	5·62		5·32(d)‡
(IX)	2·58(q)‡§	7·82	4·00			4·66(d)§	8·78		6·39 (2H)			5·37(d)‡

* Unless otherwise indicated all signals are singlets. † In [$^2H_5$]pyridine. ‡ $J_{1,8}$ 2·0 Hz. § $J_{1,5}$ 1·0 Hz.

TABLE 2

Chemical shifts (τ) and multiplicity (J in Hz) in the n.m.r. spectra of the hydrogenation products of austdiol

	1*ax*-H	1*eq*-H	3-Me	3-H	4-H	5-Me	5-H
(X)	6·51(dd) J_{gem} 12·0 $J_{1ax,8a}$ 2·1	6·14(dd) J_{gem} 12·0 $J_{1eq,8a}$ 1·5	8·82(d) J 6·5	*ca.* 6·6(m)	Obscured	8·92(d) J 6·5	7·28(m) J 6·5 $J_{4a,5}$ 5·5
(XI)	6·52(dd) J_{gem} 11·5 $J_{1ax,8a}$ 2·5	5·66(dd) J_{gem} 11·5 $J_{1eq,8a}$ 1·5	8·86(d) J 6·5	6·60(m) J 6·5 $J_{3,4ax}$ 12·0 $J_{34,eq}$ 2·5	*ca.* 8·6(m) obscured	8·97(d) J 6·5	6·97(m) J 6·5 $J_{4a,5}$ 5·5
(XII)	6·52(dd) J_{gem} 11·5 $J_{1ax,8a}$ 2·5	5·66(dd) J_{gem} 11·5 $J_{1eq,8a}$ 1·5	8·86(d) J 6·5	6·60(m) J 6·5 $J_{3,4ax}$ 12·0 $J_{3,4eq}$ 2·5	*ca.* 8·6(m) obscured	8·97(s)	
(XIII)	6·62(dd) J_{gem} 12·0 $J_{1ax,8a}$ 2·5	6·08(dd) J_{gem} 12·0 $J_{1eq,8a}$ 1·5	8·83(d) J 6·5	*ca.* 6·7(m)	Obscured	9·11(d) J 6·5	Obscured

	6-H	7-Me	7-OAc	8-OAc	8-H	8a-H	4a-H
(X)		8·60(s)	7·93(s)	8·00(s)	3·77(d) $J_{8,8a}$ 11·5	*ca.* 7·78(m) obscured	Obscured
(XI)		8·64(s)			6·07(d) $J_{8,8a}$ 11·0	8·20(m) $J_{8,8a}$ 11·0 $J_{4a,8a}$ 5·0 $J_{1ax,8a}$ 2·5 $J_{1eq,8a}$ 1·5	7·74(m) $J_{4ax,4a}$ 14·0 $J_{4a,5}$ 5·5 $J_{4a,8a}$ 5·0 $J_{4eq,4a}$ 5·0
(XII)		8·64(s)			6·07(d) $J_{8,8a}$ 11·0	8·20(m) $J_{8,8a}$ 11·0 $J_{4a,8a}$ 5·0 $J_{1ax,8a}$ 2·5 $J_{1eq,8a}$ 1·5	7·74(m) $J_{4ax,4a}$ 14·0 $J_{4a,8a}$ 5·0 $J_{4eq,4a}$ 5·0
(XIII)	5·00(d) $J_{5,6}$ 3·0	8·76(s)	7·87(s)	7·91(s)	4·59(d) $J_{8,8a}$ 11·5	Obscured	Obscured

appears as a doublet at τ 8·92 (J 6·5 Hz) owing to coupling with the 5-proton. The signal for the latter appears as a quintet, τ 7·28 (1H, J 6·5, $J_{4a,5}$ 5·5). The 3-methyl signal appears as a doublet, τ 8·82 (J 6·5 Hz), owing to coupling with the C-3 methine proton, which appears as a partly obscured multiplet at τ 6·6. The 8-proton appears as a doublet at τ 3·77 ($J_{8,8a}$ 11·5 Hz). The magnitude of $J_{8,8a}$ indicates a *trans* diaxial configuration for the 8- and 8a-protons. The methylene protons at C-1 appear as the AB part of an ABX system at τ 6·14 (1*eq*-H, J_{gem} 12·0, $J_{1eq,8a}$ 1·5 Hz) and 6·51 (1*ax*-H, J_{gem} 12·0, $J_{1ax,8a}$ 2·1 Hz). The signal of the 8a-proton, the X C-8 appeared as a doublet, τ 6·07 ($J_{8,8a}$ 11·0 Hz) and the 8a-proton as a broad complex doublet at τ 8·20 ($J_{8,8a}$ 11·0, $J_{4a,8a}$ 5·0, $J_{1ax,8a}$ 2·5, $J_{1eq,8a}$ 1·5 Hz). The methylene protons at C-1 appeared as the AB part of an ABX system, τ 5·66 (1*eq*-H, J_{gem} 11·5, $J_{1eq,8a}$ 1·5 Hz) and 6·52 (1*ax*-H, J_{gem} 11·5, $J_{1ax,8a}$ 2·5 Hz). A pair of quartets at τ 7·74 ($J_{4ax,4a}$ 14·0, $J_{4a,5}$ 5·5, $J_{4a,8a}$ 5·0, $J_{4eq,4a}$ 5·0 Hz) was assigned to the 4a-proton. The 5-methyl signal appeared as a doublet at τ 8·97 (J 6·5 Hz) and that of the 5-proton as a quintet, τ 6·97 (J 6·5, $J_{4a,5}$ 5·5 Hz). The doublet at τ 8·86 (3H, J 6·5 Hz) was assigned to the 3-methyl group. The 3-proton signal appeared as a multiplet, τ 6·60

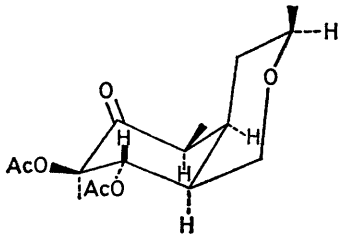
($J_{3,4ax}$ 12·0, J 6·5, $J_{3,4eq}$ 2·5 Hz), partly obscured by the signal assigned to one of the C-1 methylene protons. The C-4 methylene signal appeared as a complex obscured multiplet at τ *ca.* 8·6.

Irradiation at the centre of the resonance frequency of the 8-proton (τ 6·07) resulted in the collapse of the broad complex doublet at τ 8·20 (8a-H) to a broad multiplet ($J_{4a.8a}$ 5·0, $J_{1ax.8a}$ 2·5, $J_{1eq.8a}$ 1·5 Hz). Similar irradiation at the frequency of the 8a-proton resulted in the collapse of the 8-H doublet to a singlet and the disappearance of the fine splitting in the signals assigned to the 1-protons.

Base-catalysed deuterium exchange of compound (XI) gave the deuterio-compound (XII), M^+ 229, indicating incorporation of one deuterium atom. This result agrees with the structures assigned to compounds (XI) and (X) and thus with the structure (I) for austdiol. The n.m.r spectrum of (XII) (see Table 2) lacks the quintet at τ 6·97 (1H) which was assigned to the 5-proton. The 5-methyl signal appears as a singlet at τ 8·97 and the 4a-proton signal as a pair of triplets, τ 7·74 ($J_{4ax.4a}$ 14·0, $J_{4a.8a}$ 5·0, $J_{4eq.4a}$ 5·0 Hz).

The minor compound isolated from the hydrogenation reaction ($C_{16}H_{26}O_6$) was assigned structure (XIII).

The relative configurations of compounds (X) and (XI) could be deduced from the torsion angles obtained from the coupling constants through application of the Karplus equation.[5] As the absolute configuration of austdiol has been deduced (see later), the structures represent the absolute configurations. The conformation of (X) is shown in the Figure.



Proposed conformation of compound (X)

Absolute Configuration of Austdiol.—The absolute configuration of the 8-hydroxy-group was determined by the 'partial resolution' method of Horeau.[6] Esterification of austdiol with racemic α-phenylbutyric acid anhydride proceeded smoothly, leading quantitatively to the 8-*O*-α-phenylbutyrate. The mass spectrum of the ester (M^+ 382) indicated the absence of diester. The recovered α-phenylbutyric acid had $[\alpha]_D^{23}$ −7·3° (*c* 5·94 in benzene). Austdiol must therefore have the 8*S*-configuration.[6-8] As it has previously been established that the *vic*-diol system in austdiol is *trans*, the chiral centre at C-7 must have the *R*-configuration. Verification of these assignments by *X*-ray crystallography of compound (VIII) is in progresss.

Austdiol is structurally related to a class of compounds known generically as azaphilones, *e.g.* mitorubrin (II). The name azaphilone is derived from the ready reaction of these metabolites with ammonia to yield vinylogous γ-pyridones. Treatment of austdiol with ammonia, however, leads to extensive decomposition. Attempts to oxidize the 8-hydroxy-group in austdiol with a variety of oxidizing reagents in the hope of obtaining a azaphilone structure all proved abortive.

The assigned structure (I) for austdiol is biogenetically feasible; the molecule can be considered as a straight chain pentaketide formally alkylated at two methylene positions to give the aldehyde group at C-5 and the methyl group at C-7.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured for solutions in ethanol (Unicam SP 800 spectrometer). I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer. Mass spectra were taken on an A.E.I. MS9 double-focussing spectrometer. N.m.r. spectra were recorded for solutions in $CDCl_3$ on a Varian HA-100 spectrometer with tetramethylsilane as internal standard. T.l.c. was carried out on Merck precoated silica plates (thicknesses 0·25 and 2 mm for analytical and preparative separations, respectively).

Isolation of Austdiol.—*A. ustus* was grown in bulk on wet sterilized maize meal for 20 days. The dried, milled, mouldy maize (6·3 kg) was extracted with chloroform–methanol (1 : 1 v/v) for 2 days and the solvent was evaporated off under reduced pressure. The solid residue was treated with chloroform (2 l) and the mixture was filtered to separate soluble material (470 g) and an insoluble homogeneous crystalline material (270 g).

The latter, *austdiol* (7,8-*dihydro*-7,8-*dihydroxy*-3,7-*dimethyl*-6-*oxo*-6H-2-*benzopyran*-5-*carbaldehyde*)(I), represented the main toxic component of the fungal culture and had m.p. 255° (decomp); $[\alpha]_D^{26}$ +160·3° (*c* 1·25 in pyridine); $\lambda_{max.}$ 256 and 376 nm (log ε 4·18 and 4·38); $\nu_{max.}$ (KBr) 3370 (OH), 3460 (OH), 3100 (C=C), 1670w (aldehyde CO), 1620 (conjugated CO), and 1600 cm^{-1} (C=C) (Found: C, 60·85; H, 4·95%; M^+, 236·0660. $C_{12}H_{12}O_5$ requires C, 61·0; H, 5·1; *M*, 236·0685).

Austdiol 2,4-*dinitrophenylhydrazone* had m.p. 310° (decomp.) (from methanol); $\lambda_{max.}$ 270, 298, 372, and 458 nm (log ε 4·06, 3·82, 4·25, and 4·36); $\nu_{max.}$ (KBr) 1610 cm^{-1}; τ ([$^2H_5$]pyridine) 8·42 (3H, s, 7-Me), 7·68 (3H, s, 3-Me), 4·94 (1H, d, $J_{1.8}$ 2·0 Hz, 8-H), 2·01 (1H, s, 4-H), 1·86 (1H, d, $J_{1.8}$ 2·0 Hz, 1-H), 1·00 (1H, s, HC=N), 3·2br (D_2O-exchangeable), and 2·15 (1H, d, J_{ortho} 9·0 Hz), 1·63 (1H, dd, J_{ortho} 9·0, J_{meta} 3·0 Hz), and 0·93 (1H, d, J_{meta} 3·0 Hz) (aromatic protons) (Found: N, 13·45. $C_{18}H_{16}N_4O_8$ requires N, 13·2%).

Acetylation of Austdiol.—(*a*) A mixture of austdiol (100 mg) and anhydrous sodium acetate (50 mg) in acetic anhydride (10 ml) was stirred at room temperature for 15 min to give the unstable monoacetate (III) (90 mg), M^+ 278 ($C_{14}H_{14}O_6$).

(*b*) Austdiol (500 mg) in acetic anhydride (125 ml) at −70° was treated with perchloric acid (70%; 0·1 ml). The solution was allowed to attain room temperature and poured on ice. Standard work-up and crystallization from benzene–n-hexane gave *austdiol diacetate* (IV) (415 mg) as

[5] M. Karplus, *J. Amer. Chem. Soc.*, 1963, **85**, 2870.
[6] A. Horeau, *Tetrahedron Letters*, 1961, 506.
[7] A. Horeau and J. K. Sutherland, *J. Chem. Soc.* (*C*), 1966, 247.
[8] W. Herz and H. B. Kagan, *J. Org. Chem.*, 1967, **32**, 216.

small yellow needles, m.p. 238—239° (decomp.); $[\alpha]_D^{22}$ +31° (*c* 1·20 in $CHCl_3$); $\lambda_{max.}$ 254 and 375 nm (log ε 4·18 and 4·37); $\nu_{max.}$ (KBr) 1745 (acetate CO), 1680w (aldehyde CO), and 1630 cm^{-1} (Found: C, 59·8; H, 5·1. $C_{16}H_{16}O_7$ requires C, 60·0; H, 5·05%).

Reaction of Austdiol with Acetone.—Austdiol (500 mg) in acetone (300 ml) was treated with perchloric acid (70%; 0·2 ml). The acetone was evaporated off after 24 h and the solid residue was dissolved in chloroform. The solution was washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on alumina with chloroform–methanol (9 : 1 v/v) as eluant and crystallized from benzene to give 7,8-*dihydro*-7,8-*dihydroxy*-3,7-*dimethyl*-5-(3-*oxobut*-1-*enyl*)-2-*benzopyran*-6-*one* (V) (450 mg), m.p. 141—142°, $[\alpha]_D^{22}$ +272° (*c* 1·15 in pyridine); $\lambda_{max.}$ 254 and 400 nm (log ε 4·02 and 4·27); $\nu_{max.}$ ($CHCl_3$) 3580 (OH), 3380br (OH), 1665 (conj. CO), and 1620 cm^{-1}; τ ([$^2H_5$]-pyridine) 8·53 (3H, s, 7-Me), 7·97 (3H, s, 3-Me), 7·78 (3H, s, Ac), 5·12 (1H, d, $J_{1.8}$ 2·0 Hz, 8-H), *ca.* 4·8br (2H, D_2O-exchangeable), 3·28 (1H, s, 4-H), 2·26 (2H, s, HC=CH–CO), and 2·12 (1H, d, $J_{1.8}$ 2·0 Hz, 1-H) (Found: C, 65·4; H, 5·9. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·85%).

Hydrogenation of Austdiol Diacetate (IV).—(*a*) Compound (IV) (380 mg) in methanol (50 ml) was hydrogenated over 10% palladium–charcoal (40 mg). After 30 min (when *ca.* 2 mol. equiv. of H_2 had been taken up), the mixture was filtered and evaporated to give a complex mixture. Preparative t.l.c. with chloroform–methanol (97 : 3 v/v) gave a pure fraction. Crystallization from benzene–n-hexane gave 7,8-*dihydro*-7,8-*dihydroxy*-3,5,7-*trimethyl*-2-*benzopyran*-6-*one* as small needles, m.p. 209—211°, $[\alpha]_D^{24}$ −13° (*c* 1·59 in $CHCl_3$); $\lambda_{max.}$ 235 and 354 nm (log ε 3·65 and 4·11); $\nu_{max.}$ (KBr) 1750 (acetate CO) and 1630 cm^{-1} (Found: C, 62·9; H, 6·1. $C_{16}H_{18}O_6$ requires C, 62·75; H, 5·9%).

(*b*) Austdiol diacetate (IV) (480 mg) in ethyl acetate (25 ml) was hydrogenated over platinum oxide (15 mg) for 30 min (uptake *ca.* 2 mol. equiv.). Work-up as in (*a*) gave a yellow solid. T.l.c. in chloroform indicated the presence of a single yellow non-polar compound and a complex mixture of polar compounds. Column chromatography of the mixture on silica with chloroform as eluant gave 6-*hydroxy*-3,7-*dimethyl*-1H-2-*benzopyran*-5-*carbaldehyde* (VII) (64 mg) as a yellow glass, $\lambda_{max.}$ 251, 288sh, 298, and 402 nm (log ε 3·92, 3·98, 3·98, and 3·68); $\nu_{max.}$ ($CHCl_3$) 1620 cm^{-1}; τ 8·02 (3H, s, 7-Me), 7·84 (3H, s, 3-Me), 5·06 (2H, s, 1-H), 3·86 (1H, d, $J_{4.8}$ 0·5 Hz, 4-H), 3·02 (1H, d, $J_{4.8}$ 0·5 Hz, 8-H), −0·26 (1H, s, CHO), and −1·98 (1H, s, D_2O-exchangeable, OH) (Found: M^+, 204·0777. $C_{12}H_{12}O_3$ requires *M*, 204·0786).

Bromination of Austdiol.—Austdiol (I) (236 mg) in acetic acid (10 ml) was treated with pyridinium hydrobromide perbromide (320 mg). After 30 min the mixture was diluted with water (30 ml) and extracted with chloroform to yield a yellow solid, which was purified by preparative t.l.c. with chloroform–methanol–acetone (18 : 1 : 1 v/v/v). Crystallization from benzene–n-hexane gave 5-*bromo*-7,8-*dihydro*-7,8-*dihydroxy*-3,7-*dimethyl*-2-*benzopyran*-6-*one* (VIII) (130 mg) as small needles, m.p. 144—145°, $[\alpha]_D^{23}$ +324° (*c* 1·18 in $CHCl_3$); $\lambda_{max.}$ 229 and 362 nm (log ε 3·70 and 4·24); $\nu_{max.}$ ($CHCl_3$) 1625 cm^{-1} (Found: C, 46·05; H, 4·0; Br, 27·9. $C_{11}H_{11}BrO_4$ requires C, 46·0; H, 3·85; Br, 27·85%).

Hydrogenation of the Bromide (VIII).—Compound (VIII) (500 mg) in acetic acid (50 ml) was hydrogenated over platinum dioxide (50 mg). After *ca.* 2·5 mol. equiv. of H_2 had been absorbed, the mixture was filtered, diluted with water (50 ml), and extracted with chloroform to give a solid. Preparative t.l.c. with chloroform–methanol–acetone (18 : 1 : 1 v/v/v) showed two clearly defined bands which upon work-up gave starting material (60 mg) and 7,8-*dihydro*-7,8-*dihydroxy*-3,7-*dimethyl*-2-*benzopyran*-6-*one* (IX) (37 mg), m.p. 165—166° (from benzene), $[\alpha]_D^{20}$ +190° (*c* 0·26 in $CHCl_3$); $\lambda_{max.}$ 229, 244, and 349 nm (log ε 3·68, 3·63, and 4·27); $\nu_{max.}$ ($CHCl_3$) 1610 cm^{-1} (Found: C, 63·65; H, 5·9. $C_{11}H_{12}O_4$ requires C, 63·45; H, 5·8%).

Exhaustive Hydrogenation of Austdiol Diacetate (IV).—Compound (IV) (1·60 g) in acetic acid (50 ml) containing platinum dioxide (320 mg) was stirred in hydrogen for 36 h (uptake *ca.* 6 mol. equiv.). Work-up as before yielded an oil. T.l.c. with chloroform–methanol (98 : 2 v/v) indicated the presence of two major and numerous minor products. The two major products, (X) and (XIII) were isolated and purified by column chromatography with chloroform as eluant.

3,5,7-*Trimethyl*-6-*oxoperhydro*-2-*benzopyran*-7,8-*diyl diacetate* (X) (420 mg) had m.p. 133—135° (from benzene–n-hexane), $[\alpha]_D^{23}$ −33° (*c* 1·66 in $CHCl_3$); c.d. λ 248, 286, and 330 nm (Δε 0, +1·57, and 0); $\nu_{max.}$ ($CHCl_3$) 1745 (acetate CO) and 1730 cm^{-1} (CO) (Found: C, 61·6; H, 7·75. $C_{16}H_{24}O_6$ requires C, 61·5; H, 7·75%).

6-*Hydroxy*-3,5,7-*trimethylperhydro*-2-*benzopyran*-7,8-*diyl diacetate* (XIII) (75 mg) had m.p. 236—237° (from benzene–n-hexane), $[\alpha]_D^{24}$ −30° (*c* 1·04 in $CHCl_3$); $\nu_{max.}$ (KBr) 3440 (OH) and 1740 cm^{-1} (acetate CO) (Found: C, 61·05; H, 8·3. $C_{16}H_{26}O_6$ requires C, 61·15; H, 8·35%).

Saponification of the Diacetate (X).—Compound (X) (140 mg) in methanolic 0·1N-potassium hydroxide was stirred for 1 h at room temperature. The crude product was purified by preparative t.l.c. with chloroform–methanol (97 : 3 v/v) to give the *diol* (XI) (60 mg) as an oil, $\nu_{max.}$ ($CHCl_3$) 3600 (OH), 3480 (OH), and 1720 cm^{-1} (CO) (Found: M^+, 228·1366. $C_{12}H_{20}O_4$ requires *M*, 228·1361).

Deuteriation of the Diol (XI).—Compound (XI) (40 mg) in methan[^2H]ol (5 ml) was treated with sodium deuteroxide in D_2O (40%; 0·5 ml) and stirred at room temperature for 12 h. The mixture was acidified (6N-HCl), diluted with water (10 ml), and extracted with chloroform to give the 5-deuterio-derivative (XII) (35 mg) as an oil, M^+ 229 ($C_{12}H_{19}DO_4$).

Absolute Configuration of Austdiol (I).—A solution of α-phenylbutyric acid anhydride (391 mg, 1·26 mmol) and austdiol (124 mg, 0·525 mmol) in anhydrous pyridine (3 ml) was stirred at room temperature for 8 h. The excess of anhydride was destroyed by adding water and leaving the mixture at room temperature for 4 h. Chloroform was added (15 ml) and the solution was extracted with 6N-sodium hydrogen carbonate (3 × 10 ml), washed (6N-HCl), dried (Na_2SO_4), and evaporated. The residual ester (198 mg) contained no starting material and had M^+ 382 ($C_{22}H_{22}O_6$).

The combined sodium hydrogen carbonate extracts were acidified (6N-HCl) and extracted with chloroform to yield α-phenylbutyric acid (297 mg), $[\alpha]_D^{23}$ −7·2° (*c* 5·94 in benzene) (theoretical $[\alpha]_D$ −25·4° [7]). The optical yield therefore was 28·4% (−), based on an esterification yield of 100%.

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