Biosynthesis of Austdiol and Synthesis of a Deuterium Labelled Biogenetic Precursor

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Incorporation studies on austdiol (1), a metabolite from *Aspergillus ustus* were carried out with [1,2-$^{13}C_2$]-acetate, [*Me*-^{13}C]-, and [*Me*-$^{2}H_3$]-methionine. Specific incorporation of 4,6-dihydroxy-3,5-dimethyl-2-(2-oxopropyl)benzaldehyde (2) into austdiol (1) was demonstrated using ^{2}H n.m.r. spectroscopy. The total synthesis of the ketoaldehyde (2), allowing the specific insertion of a label, is described.

Austdiol (1), the main toxic metabolite from *Aspergillus ustus* (Bainier) Thom and Church, first isolated in 1973 by Steyn,[1] was assigned the structure (7*R*,8*S*)-7,8-dihydro-7,8-dihydroxy-3,7-dimethyl-6-oxo-6*H*-2-benzopyran-5-carbaldehyde by chemical and spectroscopic investigations.[2,3] Initial incorporation experiments with [1,2-$^{13}C_2$]acetate and [*Me*-^{13}C]methionine were reported in a preliminary communication.[4]

Before any incorporation studies with expensive ^{13}C-precursors could be carried out, austdiol production was optimised. Initially surface stationary cultures of *Aspergillus ustus* were carried out using synthetic and semisynthetic media, but the yield of austdiol was unacceptably low. This problem was overcome using malt extract medium. The proton noise decoupled (p.n.d.) ^{13}C n.m.r. spectrum of austdiol from [1,2-$^{13}C_2$]acetate shows that seven of the carbon signals (C-1, -3, -4, -5, -10, and -11) have two satellite signals resulting from the presence of ^{13}C at an adjacent position whereas the signals corresponding to carbons 6,7,8, and 9 have two pairs of satellite signals (Table 1). The spectrum is consistent with the labelled austdiol being a 50 : 50 mixture of the two labelled molecules shown in Scheme 1. Thus the two pairs of satellite signals corresponding to the signals from carbons 6,7,8, and 9 arise from the coupling to either of their adjacent carbons. Although the signal from C-5 exhibits only one pair of satellite signals it is consistent with a coupling with either carbon 6 or 12 which happen to have the same coupling constants. The p.n.d. ^{13}C n.m.r. spectrum of the austdiol derived from [*Me*-^{13}C]methionine shows that the extent of enrichment at C-13 (12.1%) is approximately twice that (4.8%) at C-12 and C-1. This labelling pattern is also consistent with the labelling patterns of the two austdiol molecules in Scheme 1, and supports the role of the symmetrical dialdehyde (3) or dicarboxylic acid (4) as biosynthetic precursors of (1) (Scheme 2).[4] On the basis of the analogy with the well established biosynthesis of similar compounds such as citrinin [5] and ascochitine,[6] we could assume that the first biosynthetic step is the reduction of the enzyme-bound thioester, followed by oxidation of the methyl group arising from methionine.

Actually, the ^{2}H n.m.r. spectrum of [*Me*-$^{2}H_3$]methionine-derived austdiol showed three broad signals at 1.47, 7.73, and 10.34 p.p.m. The comparison of these chemical shifts with the chemical shifts of the ^{1}H n.m.r. signals indicates the presence of labelling in the C-13 methyl group and in both positions C-1 and C-12. The quantitative determination of the isotope distribution, deduced from the signal integrals, corresponded to a 46 : 9 : 6 relative ratio. However the results from [*Me*-^{13}C]methionine incorporations show ^{13}C enrichments at C-13, C-1 and C-12 to be 12.1 : 4.8 : 4.8. Clearly some discrepancy in these results may be due to experimental error in evaluating the integrals of the signal at 7.73 p.p.m. in the ^{2}H n.m.r. spectrum, because it is very close to that of the natural abundance $CDCl_3$ used as solvent. Consequently the relative ratio 46 : 27, calculated taking into account the numbers of hydrogen atoms at C-13 and C-1, must be increased. The ratio 46 : 18 for C-13 and C-12 is in good agreement with that observed for ^{13}C enrichments.

Table 1. ^{13}C Chemical shifts (p.p.m.) and ^{13}C–^{13}C coupling constants (Hz) of austdiol diacetate (1a)

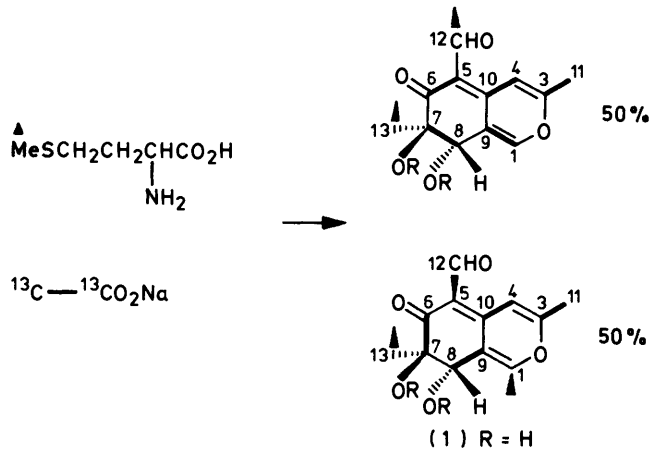
Carbon	Chemical shift	% Enrichment [c]	J (^{13}C–^{13}C) [a]
1	151.7 [b]	5.6	75.5
3	148.2	3.5	50.9
4	106.1	7.4	50.9
5	108.0	2.5	58.7
6	190.1	2.0; 1.8	58.6; 41.9
7	80.3	0.2; 2.7	87.9; 41.5
8	67.2	0.7; 2.6	87.1; 48.1
9	116.9	1.2; 1.6	76.0; 48.9
10	165.8	0.5	51.0
11	19.8	15.7	51.0
12	189.0 [b]	8.0	59.1
13	17.4 [b]		

[a] ^{13}C–^{13}C Coupling constants in the proton noise decoupled ^{13}C n.m.r. spectrum of austdiol derived from [1,2-$^{13}C_2$]acetate. [b] Enhanced in intensity after incorporation of [*Me*-^{13}C]methionine. [c] Enrichments, due to incorporation of [1,2-^{13}C]acetate, were calculated by means of the formula reported by Vining *et al.* (*J. Chem. Soc., Chem. Commun.*, 1974, 282).

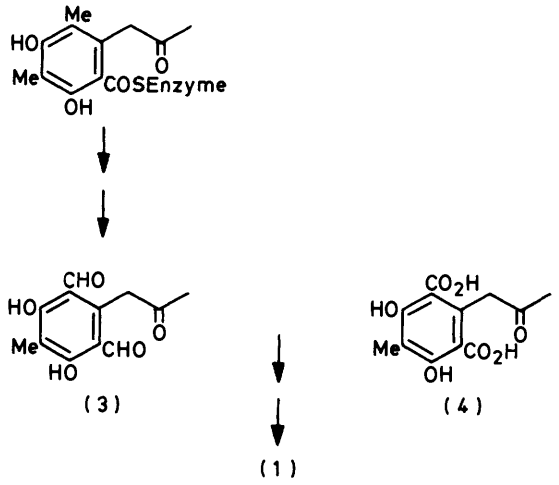
Complementary information on the correct identification of the labelled positions was obtained by difference spectroscopy. The difference spectrum of the normalized non-deuteriated and deuteriated ^{1}H n.m.r. spectra showed three signals ascribable to the methyl group at C-13 (δ 1.39) and to the protons at C-1 and C-12 (δ 7.64 and 10.18).

Consequently the symmetrical dialdehyde (3) plays a central role as an intermediate in the biosynthetic pathway, and the most probable sequence of enzymic reactions leading to (3) involves the reduction of the enzyme-bound thioester and the eventual oxidation of the methyl group.

However, doubt remains concerning the timing of these two steps. Although the reduction may precede the oxidation, as suggested by our cited evidence, the reverse cannot be excluded. To settle this question, we carried out incorporation experiments with the possible advanced precursor (2), labelled with deuterium at the methyl group at C-13; the latter compound was added to an intact surface culture of *A. ustus* (4-day



Scheme 1.



Scheme 2.

culture broths). The contact between the mycelium and the precursor-containing medium was facilitated by mild magnetic stirring. Six days after the addition, compound (1) was isolated and transformed into its diacetate derivative in order to obtain a more stable and soluble product, which gave rise to a peak at δ 1.43 in the ^2H n.m.r. spectrum.

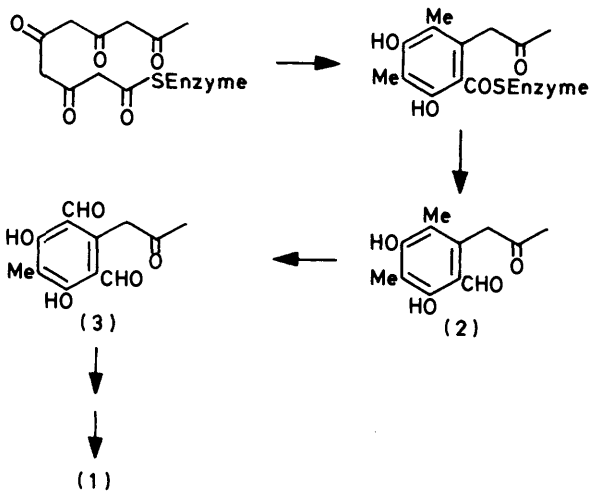
By analogy with the chemical shift in the ^1H n.m.r. spectrum of austdiol, this signal can be ascribed to the presence of deuterium in the C-13 methyl group. Comparison of the intensity of this peak with that of natural abundance [$^2H_6$]-dimethyl sulphoxide, used as solvent, showed that 10.1% of the administered ketoaldehyde was specifically incorporated into austdiol. An isotopic trap experiment was carried out to establish whether the aldehyde (2) is an obligatory intermediate in the biosynthetic pathway. Introduction of [*Me*-^{14}C]-methionine into the culture together with a large amount of unlabelled aldehyde (2), and interruption of the fermentation when only part of (2) had been transformed, allowed the recovery of the label in (2) and in austdiol (1) (Table 2).

We can conclude therefore that the ketoaldehyde (2) is a biosynthetic precursor to austdiol or, in other words, that the oxidation of the C-12 methyl group follows the reduction of the enzyme-bound thioester.

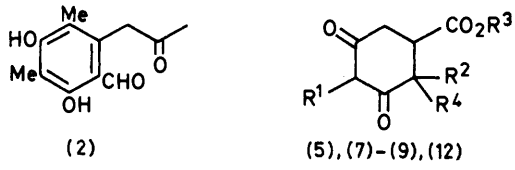
The biosynthetic pathway can be summarized as shown in Scheme 3. The central role of compounds similar to the keto-

Table 2. Incorporation of [*Me*-^{14}C]methionine into compounds (2) and (1)

Fermentation time (h)	Total incorporation (%)	Compound
24	0.05	(2)
	1.12	(1)



Scheme 3.



(5) $R^1 = R^2 = R^3 = H$, $R^4 = Me$
(7) $R^1 = H$, $R^2 = CO_2Bu^t$, $R^3 = Et$, $R^4 = Me$
(8) $R^1 = H$, $R^2 = CO_2Bu^t$, $R^3 = H$, $R^4 = Me$
(9) $R^1 = H$, $R^2 = CO_2H$, $R^3 = H$, $R^4 = Me$
(12) $R^1 = CD_3$, $R^2 = R^3 = H$, $R^4 = Me$

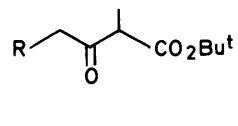
aldehyde (2) had already been observed in the biosynthesis of ascochitine and citrinin. The attempted synthesis of 4,6-dihydroxy-3,5-dimethyl-2-(2-oxopropyl)benzaldehyde (2) in a convenient way to introduce labelling is briefly reported.

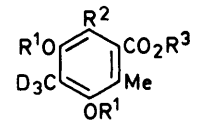
Our efforts were first directed to the synthesis of 2-methyl-3,5-dioxocyclohexanecarboxylic acid (5), which would allow *C*-methylation of the β-dicarbonyl system and its subsequent aromatization to 2,4-dimethyl-3,5-dihydroxybenzoic acid. t-Butyl 2-methyl-3-oxobutanoate (6), after treatment with sodium ethoxide in ethanol, reacted with diethyl fumarate affording ethyl 2-methyl-2-t-butoxycarbonyl-3,5-dioxocyclohexane carboxylate (7).

During the purification of the crude product by extraction with aqueous sodium hydroxide, hydrolysis of the ethyl ester occurred with formation of the acid (8).

Treatment of this crude compound with trifluoroacetic acid yielded the diacid (9), which was successively decarboxylated by boiling in benzene for two hours.

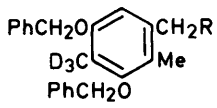
The resulting β-dicarbonyl compound (5) was tentatively *C*-alkylated with methyl iodide under various reaction conditions, *i.e.* aqueous sodium hydroxide in dioxane [7] or methanol solution.[8] Alkylation was performed in phase-transfer



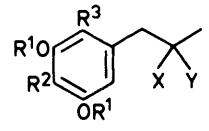


(6) R = H
(10) R = CD_3

(11) $R^1 = R^2 = R^3 = H$
(13) $R^1 = R^2 = H$, $R^3 = Me$
(14) $R^1 = CH_2Ph$, $R^2 = H$, $R^3 = Me$



(15), (16)



(17) - (19)

(15) R = OH
(16) R = Br

(17) $R^1 = CH_2Ph$, $R^2 = CD_3$, $R^3 = Me$, X = CN, Y = OCHMeOEt
(18) $R^1 = H$, $R^2 = CD_3$, $R^3 = Me$, X,Y = O
(19) $R^1 = CH_2Ph$, $R^2 = CD_3$, $R^3 = Me$, X,Y = O

conditions (aqueous Bu^n_4NOH–toluene) and using Amberlite IRA 900[9] with methyl 2-methyl-3,5-dioxocyclohexanecarboxylate as starting material. This intermediate was synthesised by treatment of compound (5) with diazomethane and subsequent hydrolysis by hydrochloric acid of the resulting methyl 2-methyl-3-methoxy-5-oxocyclohex-3-enecarboxylate. Nevertheless poor yields of the *C*-alkylated product were obtained.

We therefore modified the above synthetic scheme by using t-butyl 2-methyl-4-[$^2H_3$]methyl-3-oxobutanoate (10) as starting material. This compound was obtained by treatment of t-butyl 2-methyl-3-oxobutanoate (6) with sodium hydride and subsequently with n-butyl-lithium, followed by γ-alkylation with [$^2H_3$]methyl iodide.

The aromatic compound (11) was obtained by bromination of the acid (12) and subsequent catalytic hydrogenation. Treatment of the acid(11) with diazomethane in diethyl ether yielded almost quantitatively the ester (13). After protecting the two phenolic groups as dibenzyl ethers, the ester (14) was reduced with $LiAlH_4$ and the primary alcohol (15) converted into the benzyl bromide (16) by treatment with phosphorous tribromide.

The lithium (1-ethoxyethoxy)acetaldehyde cyanohydrin was employed as an acetyl carbanion equivalent in the condensation with the benzyl bromide (16).

Hydrolysis of the *O*-protected cyanohydrin (17) and subsequent debenzylation by catalytic hydrogenation provided the dihydroxyketone (18), converted in turn into the target molecule (2) with triethyl orthoformate and hydrogen chloride, followed by treatment with aqueous sodium hydroxide.

Experimental

M.p.s were determined on a Buchi hot-stage apparatus and are uncorrected. I.r. spectra were taken on a Perkin-Elmer 681 or Nicolet MX-1 spectrometer. ^1H N.m.r. spectra were run on a Varian EM 360 or Varian XL 100 spectrometer, and ^2H n.m.r. spectra on a Varian KL 200 spectrometer. Mass spectra were taken on a Varian MAT 112 mass spectrometer. Kieselgel 60 F_{254} (Merck) was used for t.l.c.; 70—230 mesh silica gel (Merck) was used for column chromatography and 40—60 mesh silica gel (Merck) for flash chromatography.

Culture of Aspergillus ustus.—Surface stationary cultures of *Aspergillus ustus* MRC 1232 were carried out at 28 °C in 750-ml flasks containing malt extract medium (100 ml). Each flask was innoculated with a suspension of *A. ustus* spores in Tween 20 (0.002 g ml^{-1}). [^{13}C]Methionine, [1,2-$^{13}C_2$]acetate, and [*Me*-$^2H_3$]methionine were introduced after 2, 3, and 4 days in aqueous solution. The final concentration of the ^{13}C precursors was *ca.* 0.001 g ml^{-1} and the concentration of the deuteriated precursor was *ca.* 0.0018 g ml^{-1}. The aldehyde (2) was administered in acetone solution after 4 days. Its final concentration was *ca.* 0.55 mg ml^{-1}. The solution was gently stirred and austdiol was harvested 6 days after addition of the precursors.

Harvesting and Isolation of Austdiol.—The mycelium was removed by gravity filtration of the medium through a glass-wool plug, treated with ethyl acetate, and blended. The medium was extracted with ethyl acetate. The organic extracts were collected, washed with water, and evaporated under reduced pressure. The crude residue was purified by column chromatography (chloroform–methanol) to yield austdiol (0.8 g l^{-1}). Crystallization from methanol gave pure austdiol as bright yellow needles.

'Isotopic Trap' Experiment.—[*Me*-^{14}C]Methionine (50 μCi) was added to a 750-ml flask of culture (100 ml) after 72 h fermentation. After a further 24 h fermentation, unlabelled aldehyde (2) (100 mg) was added to the culture. After a further 24 h fermentation, the usual work-up gave a residue which was chromatographed over silica gel. Benzene–ethyl acetate (8 : 2) eluted the aldehyde (2) (18 mg) which was crystallized from diethyl ether to constant radioactivity (2 980 disint. min^{-1} mg^{-1}). With chloroform–methanol as eluant, austdiol (1) (8 mg) was obtained and crystallized from methanol to constant activity (1.56×10^5 disint. min^{-1} mg^{-1}).

t-Butyl 2-Methyl-3-oxobutanoate (6).—*Method A.* t-Butyl 3-oxobutanoate (30 g) was added at room temperature to a solution of sodium ethoxide, prepared by treatment of sodium (4.36 g) with dry ethanol (125 ml). The stirred mixture was heated at reflux and methyl iodide (12.5 ml) was added dropwise during 1 h. After 30 min the solution was reduced by evaporation to a small volume and treated with saturated aqueous NH_4Cl. The organic layer was separated and the aqueous phase extracted with diethyl ether. The organic extracts were collected, dried (Na_2SO_4), and evaporated. The crude mixture was distilled under reduced pressure (73 °C/10 mmHg) to give compound (6) (60%); δ($CDCl_3$) 3.40 (1 H, q, CHMe, *J* 8 Hz), 2.20 (3 H, s, MeCO), 1.50 (9 H, s, $OCMe_3$), and 1.30 (3 H, d, MeCH, *J* 8 Hz).

Method B. A mixture of NaOH (8 g) and tetrabutylammonium hydrogen sulphate (40.3 g) was added to a stirred solution of t-butyl 3-oxobutanoate (15.8 g) and methyl iodide in dichloromethane (75 ml). The reaction was complete in a few minutes. The organic layer was separated and evaporated, giving a residue which was taken up in diethyl ether, filtered from tetrabutylammonium iodide, dried (Na_2SO_4) and evaporated. The crude product was purified as in method A affording pure compound (10) (13.4 g, 78%).

t-Butyl 2-Methyl-4-[$^2H_3$]methyl-2-oxobutanoate (10).—t-Butyl 2-methyl-3-oxobutanoate (6) (28.380 g) was added dropwise at 0 °C to a stirred slurry of NaH (50% in oil, 8.160 g) in tetrahydrofuran (THF) (200 ml) under nitrogen. The mixture was stirred at room temperature during 30 min until generation of hydrogen ceased; to the resulting suspension, cooled to 0 °C, 1.5M-Bu^nLi (113 ml) was added. After 10 min at 0 °C [$^2H_3$]methyl iodide (25 g) was added, the temperature was raised to 25 °C, and after 15 min the mixture was treated with saturated aqueous NH_4Cl (200 ml). The aqueous phase was

extracted with diethyl ether and the organic extracts were dried (Na_2SO_4) and evaporated. The crude mixture was distilled (93—96 °C/20 mmHg) giving pure compound (10) (19.800 g, 63.5%); δ($CDCl_3$) 3.25 (1 H, q, CHMe), 2.20—2.70 (2 H, m, CH_2CO), 1.47 (9 H, s, Me_3C), and 1.22 (3 H, d, MeCH) (Found: C, 63.3; H, 11.2. $C_{10}H_{15}D_3O_3$ requires C, 63.46; H, 11.18%).

2-*Methyl*-4-[$^2H_3$]*methyl*-3,5-*dioxocyclohexanecarboxylic Acid* (12).—To a solution of sodium ethoxide prepared with sodium (2.500 g) and ethanol (38 ml) at 25 °C was added compound (14) (19.700 g). After 10 min the mixture was cooled to 0 °C and treated with diethyl fumarate (18.670 g). The temperature was raised to 25 °C and the dark red solution was stirred for 30 min. The solvent was reduced by evaporation to a small volume and the residue was taken up with aqueous 10% NaOH and extracted with diethyl ether. The aqueous phase was heated at 80 °C for 30 min, acidified (dilute HCl) with cooling until it reached pH 2, and extracted three times with ethyl acetate. The organic extracts were collected, dried (Na_2SO_4), and evaporated. The crude mixture was then treated with trifluoroacetic acid (150 ml) and stirred for 30 min.

The solution was evaporated and the residue heated in boiling benzene for 1 h. The solvent was distilled off under reduced pressure. Compound (12) (5.700 g) separated as a white solid by treatment of the residue with a small volume of diethyl ether. The mixture was filtered and the mother-liquors, purified by chromatography [ethyl acetate–acetic acid (99 : 1)], gave a further amount of (12) (8.3 g; total yield 72%); m.p. 176—178 °C (diethyl ether) (Found C, 57.6; H, 8.1. $C_9H_9D_3O_4$ requires C, 57.74; H, 8.08%); $\nu_{max.}$ (Nujol) 1 702 (CO_2H), 1 635 (CO), and 1 555 (C=C); δ[$CDCl_3$–10%$(CD_3)_2SO$] 9.2 (2 H, bs, CO_2H and OH), 2.25—3.40 (4 H, m, CH_2 and CH), and 1.05 (3 H, d, $CHCH_3$). The spectrum of the corresponding nondeuteriated compound showed also a peak at δ 1.60 (3 H, s, CH=CMe); *m/z* 187 (M^+, 15%), 142 (90), 114 (63), and 101 (100).

3,5-*Dihydroxy*-2-*methyl*-4-[$^2H_3$]*methylbenzoic Acid* (11).—A solution of bromine (26.16 g) in acetic acid (300 ml) was added dropwise during 15 min to a stirred suspension of the acid (12) (13.900 g) in acetic acid (60 ml). During the addition of bromine the starting material dissolved. The solution was stirred at 25 °C overnight and then the solvent was distilled off under reduced pressure. The residue in aqueous 2M-NaOH (200 ml) and methanol (50 ml) was treated with hydrogen at 25 °C in the presence of 10% palladium on charcoal (3.00 g) for 4 h. The reaction mixture was filtered on Celite, washed with aqueous 2M-NaOH, acidified with diluted HCl, and extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated, giving a crude product which was purified by column chromatography [ethyl acetate–acetic acid (99 : 1)] to yield pure compound (11) (7.150 g, 52%); m.p. 160—162 °C (decomp.); $\nu_{max.}$ (Nujol) 3 450(OH) and 1 690 cm^{-1} (CO); δ[$(CD_3)_2SO$] 12.40 (1 H, bs, CO_2H), 9.15 and 8.15 (2 H, 2s, OH), 6.95 (1 H, s, ArH), and 2.20 (3 H, s, ArMe). The spectrum of the corresponding non-deuteriated compound also showed a peak at δ 2.06 (3 H, s, ArMe); *m/z* 185 (M^+, 59%), 141 (66), and 140 (100) (Found: C, 58.2; H, 7.1. $C_9H_7D_3O_4$ requires C, 58.37; H, 7.08%).

Methyl 3,5-*Dihydroxy*-2-*methyl*-4-[$^2H_3$]*methylbenzoate* (13).—A solution of the acid (11) (7.100 g) in diethyl ether (100 ml) was treated at 0 °C with ethereal diazomethane. The mixture was then evaporated under reduced pressure and the residue purified by flash chromatography [n-hexane–ethyl acetate (6 : 4)] and crystallized from dichloromethane–n-hexane to give compound (13) (5.370 g, 75%), m.p. 119—120 °C; $\nu_{max.}$ (Nujol) 1 701 cm^{-1} (CO); δ[$CDCl_3$–20% $(CD_3)_2SO$] 7.83 and 6.20 (2 H, 2 bs, 2 OH), 6.97 (1 H, s, ArH), 3.82 (3 H, s, OMe), and 2.36 (3 H, s, ArMe); *m/z* 199 (M^+, 82%), 184 (21), 168 (70), 167 (56), 140 (56), and 139 (100) (Found: C, 6.3; H, 7.5. $C_{10}H_9D_3O_4$ requires C, 60.29; H, 7.59%).

Methyl 3,5-*Bis*(*benzyloxy*)-2-*methyl*-4-[$^2H_3$]*methylbenzoate* (14).—A mixture of compound (13) (5.630 g) in dry butanone (50 ml) with freshly distilled benzyl chloride (13 ml) and anhydrous potassium carbonate (24.300 g) was heated at reflux with stirring for 6 h. The solvent was reduced to a small volume, water (50 ml) and diethyl ether (50 ml) were added, and the organic layer was separated and washed with 10% sodium hydroxide solution (2 × 20 ml), dried (Na_2SO_4) and evaporated.

The residue was purified by flash chromatography [n-hexane –ethyl acetate (9 : 1)] and crystallized from n-hexane–diethyl ether to give pure compound (14) (6.760 g, 63%), m.p. 70—72 °C (Found: C, 75.8; H, 7.2. $C_{24}H_{21}D_3O_4$ requires C, 75.96; H, 7.17%); $\nu_{max.}$ (Nujol) 1 720 cm^{-1} (CO); δ($CDCl_3$) 7.46 (10 H, m, Ph), 7.16 (1 H, s, ArH), 5.10 and 4.75 (4 H, 2 s, $2CH_2$), 3.90 (3 H, s, OMe), and 2.50 (3 H, s, ArMe); *m/z* 379 (M^+, 64%), 348 (35), 181 (32), and 91 (100).

3,5-*Bis*(*benzyloxy*)-2-*methyl*-4-[$^2H_3$]*methylbenzyl Alcohol* (15).—The methyl ester (14) (6.660 g) in dry THF (250 ml) was added dropwise to a slurry of lithium aluminium hydride (0.580 g) in THF (125 ml) with stirring. After 15 min saturated aqueous NH_4Cl was added cautiously. The aqueous phase was extracted three times with diethyl ether and the collected extracts were dried (Na_2SO_4) and evaporated to give the *alcohol* (15) which was crystallized from diethyl ether (4.705 g, 77%), m.p. 100—102 °C (Found: C, 78.5; H, 7.8. $C_{23}H_{21}D_3O_3$ requires C, 78.60; H, 7.74%); $\nu_{max.}$ (Nujol) 3 300 cm^{-1} (OH); δ($CDCl_3$) 7.48 (10 H, m, Ph), 6.87 (1 H, s, ArH), 5.06 and 4.80 (4 H, 2 s, $2CH_2$), 4.63 (2 H, s, CH_2OH), and 2.26 (3 H, s, ArMe); *m/z* 351 (M^+, 26%), 181(100), and 91(63).

3,5-*Bis*(*benzyloxy*)-2-*methyl*-4-[$^2H_3$]*methylbenzyl Bromide* (16).—A stirred suspension of the alcohol (15) (4.600 g) in diethyl ether (80 ml) was treated with phosphorous tribromide (0.655 ml). After 5 min the mixture was poured into crushed ice and the aqueous phase extracted three times with ethyl acetate. The organic extracts were collected, washed with saturated aqueous K_2CO_3 and water, then dried (Na_2SO_4) and evaporated, to give the pure bromide (16) (4.390 g, 81%) which was crystallized from n-hexane–diethyl ether, m.p. 78—80 °C (Found: C, 66.6; H, 6.3. $C_{23}H_{20}BrD_3O_2$ requires C, 66.67; H, 6.33%); δ($CDCl_3$) 7.48 (10 H, m, Ph), 6.78 (1 H, s, ArH), 5.06 and 4.78 (4 H, 2 s, $2CH_2$), 4.50 (2 H, s, CH_2Br), and 2.30 (3 H, s, ArMe); *m/z* 413 and 415 (M^+, 23 and 16%), 334 (7), 181 (24), and 91 (100).

2-*Cyano*-2-(1-*ethoxyethoxy*)-1-{3,5-*bis*(*benzyloxy*)-2-*methyl*-4-[$^2H_3$]*methylphenyl*}*propane* (17).—A solution of 1-cyano-1-(1-ethoxyethoxy)ethane (1.375 g) in dry THF (5.000 ml) was added dropwise to a solution of 1M-LDA in THF (9.360 ml) at −78 °C. After 5 min a solution of the bromide (16) (4.300 g) in THF (20 ml) was added during 10 min. The reaction mixture was stirred at −78 °C for 1 h, then treated with saturated aqueous NaCl. The aqueous phase was extracted three times with diethyl ether and the organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography [n-hexane–ethyl acetate (85 : 15)] to give compound (17) (3.440 g, 70%) as an oil and the starting compound (16) (0.515 g, 12%); δ($CDCl_3$) 7.40 (10 H, m, Ph), 6.78 (1 H, s, ArH), 5.10 and 4.75 (4 H, 2 s, $2CH_2$), 4.85—5.15 (1 H, m, CHO), 3.20—3.80 (2 H, m, CH_2O), 2.90—3.15 (2 H,

m, $ArCH_2$), 2.26 (3 H, s, ArMe), 1.52 (3 H, d, MeCH), 1.47 (3 H, s, Me_3CCN), and 1.10—1.40 (3 H, m, $MeCH_2$).

(3,5-*Dihydroxy*-2-*methyl*-4-[$^2H_3$]*methylphenyl*)*propanone* (18).—A solution of (19) (1.710 g) in ethanol (200 ml) was treated with hydrogen in the presence of 10% Pd–C (0.500 g), for 4 h at room temperature and pressure. The mixture was then filtered on Celite, washed with ethanol, and evaporated off under reduced pressure. The residue was purified by flash chromatography [n-hexane–ethyl acetate (65 : 45)] and crystallized from ethanol–n-hexane (0.635 g, 71%), m.p. 128—130 °C (Found: C, 66.9; H, 8.6. $C_{11}H_{11}D_3O_3$ requires C, 66.98; H, 8.69%); $\nu_{max.}$ (Nujol) 3 480 and 3 240(OH), and 1 690—(CO); δ(C_5D_5N) 6.68 (1 H, s, ArH), 3.65 (2 H, s, CH_2CO), 2.55 (3 H, s, ArMe), and 2.04 (3 H, s, COMe); *m/z* 197 (M^+, 35%), 154 (180), 123 (10), 91 (100), and 43 (50).

(3,5-*Dibenzyloxy*-2-*methyl*-4-[$^2H_3$]*methylphenyl*)*propanone* (19).—A solution of the *O*-protected cyanohydrin (17) (3.340 g) in methanol (6 ml) was treated with aqueous 5% H_2SO_4 (2 ml). More methanol was added until the solution was homogeneous (*ca.* 10 ml). After being stirred at 25 °C for 10 min the solvent was reduced to a small volume and the residue treated with saturated brine and diethyl ether (15 ml). The organic layer was separated and treated with aqueous 2M-NaOH (30 ml) with vigorous stirring. The ethereal phase was separated, dried (Na_2SO_4), and evaporated to give a crude product which was chromatographed (n-hexane–ethyl acetate), affording the ketone (19) as a yellow oil (1.72 g, 65%); $\nu_{max.}$ (Nujol) 1 710 cm^{-1} (CO); δ($CDCl_3$) 7.37 (10 H, m, Ph), 6.68 (1 H, s, ArH), 5.03 and 4.72 (4 H, 2s, $2CH_2O$), 3.00 (2 H, s, CH_2CO), 2.24 (3 H, s, ArMe), and 2.15 (3 H, s, COMe); *m/z* 377 (M^+, 8%), 334 (58), 91 (100), and 43 (70).

4,6-*Dihydroxy*-3-*methyl*-5-[$^2H_3$]*methyl*-2-(2-*oxopropyl*)*benzaldehyde* [5-Me-$^2H_3$]-(2).—A solution of compound (18) (0.550 g) in triethyl orthoformate (2 ml) was treated with hydrogen chloride at 0 °C until a yellow solid precipitated. The oxonium chloride was filtered, dried *in vacuo* and dissolved in aqueous 5% NaOH. The cooled mixture was acidified with aqueous 6M-HCl (pH 2) and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and evaporated giving a residue which was purified by flash chromatography [benzene–ethyl acetate (8 : 2)] and crystallized from diethyl ether (0.220 g, 35%); m.p. 125—127 °C (Found: C, 64.0; H, 7.6. $C_{12}H_{11}D_3O_4$ requires C, 63.98; H, 7.61%); $\nu_{max.}$ (Nujol) 3 420 (OH), 1 710 and 1 705 cm^{-1} (CO); δ(C_5D_5N) 10.34 (1 H, s, CHO), 4.31 (2 H, s, ArMe), and 2.30 (3 H, s, MeCO). The ^2H n.m.r. spectrum run in C_5H_5N showed a single peak at δ 2.45; *m/z* 225 (M^+, 40%), 207 (36), 182 (65), and 43 (100).

2-*Methyl*-3,5-*dioxocyclohexanecarboxylic Acid* (9).—The title compound was synthesised following the same procedure as that used for the synthesis of the homologous compound (12). The total yield from t-butyl 2-methyl-3-oxobutanoate was 50—60% depending on the reaction scale.

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Received 22*nd February* 1983; ***Paper*** 3/285