Identification and Isotopic Labelling of the Diastereotopic Methyl Groups of the Prenyl-Derived 2-Hydroxyisopropyl Residues of Natural Products: the Rotenoid Dalpanol

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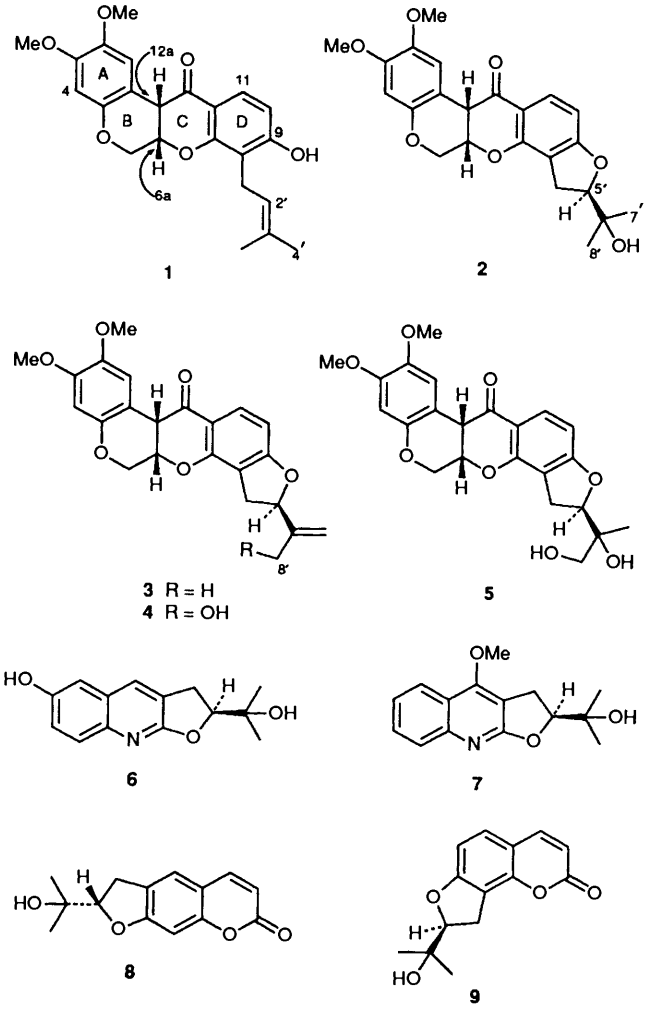
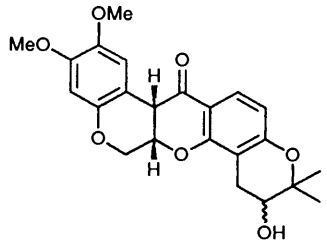
The 2-hydroxyisopropyl (1-hydroxy-1-methylethyl) segment derived from a prenyl residue occurs in a number of natural products, including the rotenoid dalpanol, and the aim of this work is the identification and isotopic labelling of the (*pro-R*) and (*pro-S*)-methyls of the latter compound. The identification sequence involves linkage between (4′-*E*)-labelled rot-2′-enonic acid and the labelled (5′*S*,6′*R*)-stereoisomer of dalpanol (5′-epidalpanol) *via* the (2′*R*,3′*R*)-epoxide of absolute configuration known from X-ray analysis. Dalpanol of the natural (5′*R*)-series is labelled in the (6′*S*)-methyl with deuterium by this sequence and the procedure is applicable to ^{3}H, ^{13}C- and ^{14}C-labelling since such 7′-labelled specimens of rotenone, the precursors of 4′-labelled rot-2′-enonic acids, are available from our earlier work.

A second labelling method employs the separable rotenone (5′*R*,6′*S*)- and (5′*R*,6′*R*)-epoxides, which are reduced with lithium aluminium deuteride, followed by reoxidation at C-12a to give both ^{2}H-labelled (5′*R*,6′*S*)- and (5′*R*,6′*R*)-dalpanols. Oxymercuriation at the 6′,7′-double bond of rotenone is selective, though not specific, for the *si*-face, leading mainly to the (6′*R*)-mercuriated product. Purification of the latter, followed by reduction with sodium borotritide, provides an excellent means for the preparation of specifically radiolabelled (5′*R*,6′*S*)-dalpanol. Monitoring was by ^{3}H NMR spectroscopy, which is more revealing than the deuterium equivalent.

The occurrence of oxidised modifications of the prenyl unit is widespread in natural products and through our interests in rotenoid biosynthesis the relationship between rot-2′-enonic acid **1** and other natural structures, such as those of compounds **2**–**5**, has engaged our attention. Dalpanol **2**, found naturally in *Dalbergia paniculata*,[1] can be made synthetically by epoxidation of rot-2′-enonic acid under basic conditions with concomitant cyclisation of the 2′,3′-epoxide,[2] and the possibility arises that it might have a central role to play in such relationships. This will be considered in a subsequent paper. Dehydration of dalpanol could lead to rotenone **3** and further oxidation could then form amorphigenin **4** and amorphigenol **5**. If such an 'hydroxyisopropyl' structure as dalpanol bears a precursor relationship to rotenone as the product of a stereospecific, enzyme-mediated dehydration, the problem of identifying and labelling the *pro-R*- and *pro-S*-methyls in dalpanol **2** becomes of interest and it is with the solution to this problem that the present paper is concerned. Apart from a number of cases in the flavonoid/isoflavonoid groups, similar relationships are found in other areas of natural product studies such as the quinoline alkaloids (riedelianine **6**[3] and platydesmine **7**[4]) or the coumarins (marmesin **8**[5] and columbianetin **9**[5]) and our methodology is relevant to such cases.

The methyls of the 'hydroxyisopropyl' group of dalpanol are adjacent to the chiral centre at C-5′ and in terms of either their ^{1}H or ^{13}C NMR spectra are distinguishable from each other. In our earlier work[6] we have devised methods for the specific labelling of either the (*E*)- or the (*Z*)-methyl group of rot-2′-enonic acid with isotope, and our method for identifying the stereochemistry of the methyls attached to C-6′ derives from this as is shown in Scheme 1. Since the epoxides of rot-2′-enonoic acid itself are too unstable to handle conveniently, cyclising according to pH to give dalpanol **19** and 5′-epidalpanol **17** or the hydroxydihydrodeguelin epimers **10**,[2] the compounds were stabilised as their acetates. (6a*S*,12a*S*)-9-*O*-Acetylrot-2′-enonic acid **11** was treated with *m*-chloroperbenzoic acid (MCPBA) in the presence of aqueous sodium

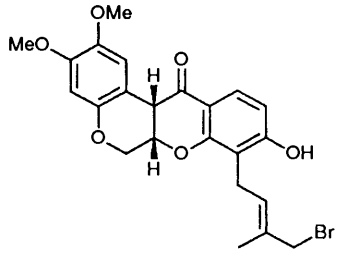




10

hydrogen carbonate to give a mixture of the diastereoisomeric epoxides **13** and **15**. These could be separated chromatographically and the faster eluting epoxide (**A**), having methyl resonances at δ 1.21 and 1.40, formed suitable crystals from diisopropyl ether for single-crystal X-ray analysis by Dr. M. Begley of our laboratory. This showed it to be the (6a*S*,12a*S*,2′*R*) diastereoisomer **13(A)**. After deacetylation with zinc and methanol, rearward attack on the epoxide by the newly formed hydroxy group, followed by cyclisation, gave epidalpanol **17** (5′α) whilst similar treatment of diastereoisomer **B** gave dalpanol **19** (5′β). It is of interest that dalpanol crystallises extremely easily because it forms a benzene solvate. Epidalpanol does not do so presumably on account of the packing of its α-orientated substituent at C-5′. This fortuitous circumstance makes it easy to prepare pure dalpanol from rot-2′-enonic acid by diastereoisomeric separation using crystallisation.

With these relationships at C-5′ established, (*E*)-[4′-^{2}H]rot-2′-enonic acid **12** was made from 4′-bromorot-2-(*E*)-enonic acid **21**[6] by treatment with sodium cyanoborodeuteride which reduces the halide without affecting the 12-carbonyl. The two methyl groups of unlabelled rot-2′-enonic acid give ^{13}C NMR signals at δ_C 17.8 (*Z*) and 25.8 (*E*) whilst the deuterium-labelled specimen **12** also had resonances at δ_E 17.8 and 25.8 but with the latter signal showing typical triplet deuterium splitting. In the proton resonance spectrum the (*Z*)-methyl resonates at δ 1.80 (3 H), the (*E*)-methyl at 1.71 (2 H); the ^{2}H spectrum gave a single resonance at δ_D 1.71. Epoxidation of the [4′-^{2}H]rot-2′(*E*)-



21

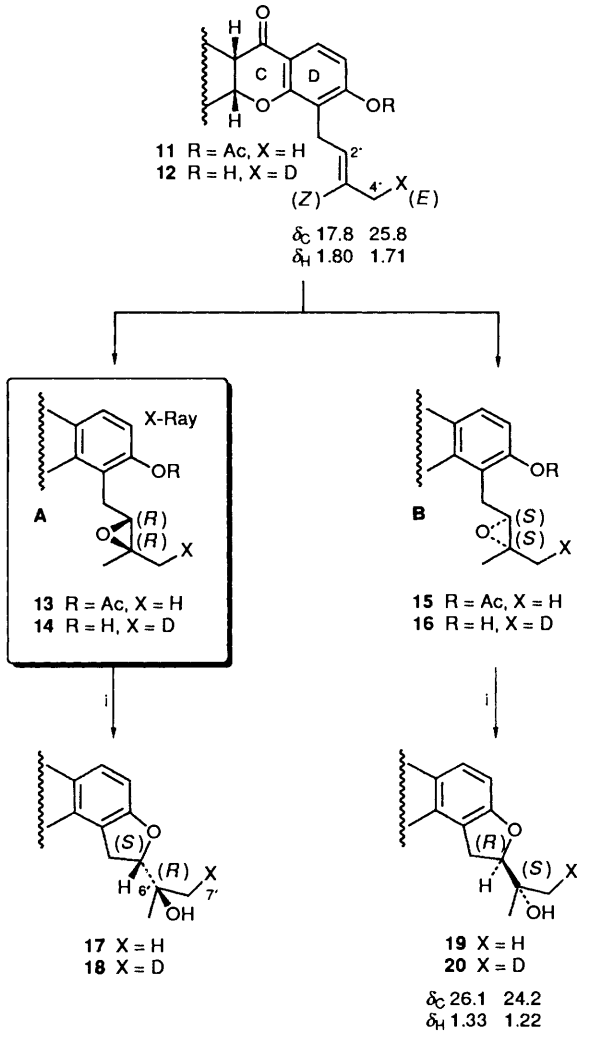
enonic acid in alkaline medium gave, following cyclisation of the epoxides **14** and **16**, a mixture of deuteriodalpanol **20** and deuterioepidalpanol **18** from which the former was recovered by crystallisation as its benzene solvate. The product **20** had δ_H 1.33 (3 H) and 1.22 (2 H) with δ_D 1.22 only, thereby identifying the two methyls as *pro*-(*R*)- and *pro*-(*S*)- respectively (see Scheme 1). The ^{13}C NMR signals for the monodeuterio compound **20** were at δ_C 26.1 (s) and 24.2 (t, deuterium splitting), so the latter relates to the ^{13}C-(*S*)-arrangement.

Aside from the spectroscopic identification of the *pro*-(*R*)- and *pro*-(*S*)-methyls of dalpanol, the sequence (Scheme 1) allows the stereospecific labelling of dalpanol with ^{2}H, ^{3}H, ^{13}C and ^{14}C (since appropriately labelled rot-2′-enonic acids are available, derived from 7′-labelled rotenone), though only in the 7′-(*pro*-*S*)-methyl group. We have therefore developed other methods (below) to make available dalpanol isotopically labelled in either the *pro*-(*R*)- or *pro*-(*S*)-methyl.

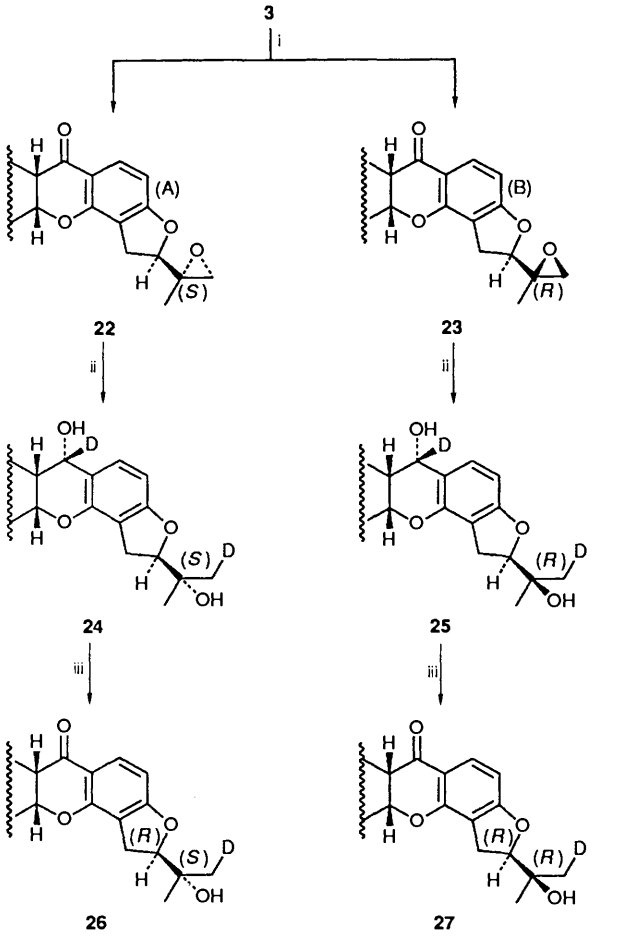
In the first of these, rotenone was converted into the diastereoisomeric pair of 6′,7′-epoxides **22** and **23** by standard methods (Scheme 2).[7] The diastereoisomers could be separated by preparative TLC (PLC) (three sequential developments) on silica gel, and eluting with a mixture of light petroleum (b.p. 40–60 °C), diethyl ether and ethyl acetate (3:3:1). The separated epoxides were now individually reduced with lithium aluminium deuteride which, as well as forming the tertiary alcohol, reduced the 12-carbonyl group. The secondary alcohol was, for each case, reoxidised to the 12-carbonyl by Oppenauer oxidation to give two specimens of dalpanol. One had a ^{2}H NMR signal at δ_D 1.22, the other at δ_D 1.33. From the information above, the former is labelled on the (*S*)-methyl **26**, the latter on the (*R*)-methyl **27**.

A disadvantage of the 6′,7′-epoxide method is the need for lithium aluminium tritide for radiolabelling purposes. This is not now commercially available, though sodium borotritide is. Our next method (Scheme 3) therefore employed the latter.

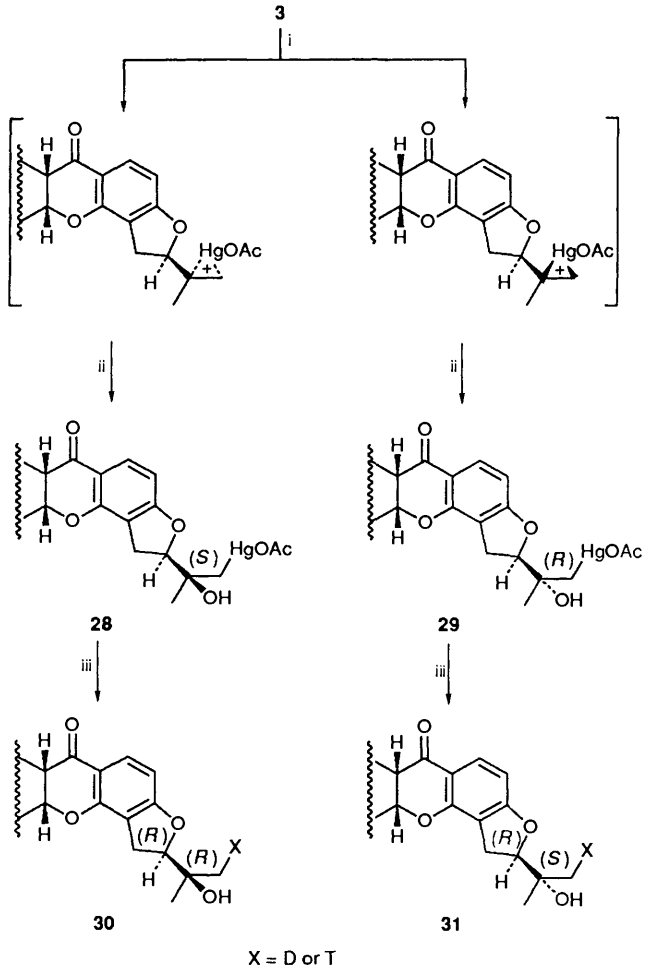
Mercury acetoxylation in aqueous organic medium of the 6′,7′-double bond of rotenone gave a pair of diastereoisomers **28** (7′-Me, δ_H 1.34) and **29** (7′-Me, δ 1.36) in the ratio 1:4. The delivery of the acetoxymercury derivative, whilst being stereofacially selective towards the *si*-face of the 6′,7′-double bond, is thus not stereospecific. However, on reduction with sodium



Scheme 1 Assignment of the prochiral C-7′ and C-8′ methyl groups in dalpanol, and stereospecific labelling of the (*pro*-*S*) position with deuterium. *Reagents:* i, Zn, MeOH for R = Ac.



Scheme 2 Isotopic labelling of the (*pro-R*) and (*pro-S*) methyls of dalpanol *via* rotenone epoxides. *Reagents and conditions:* i, MCPBA, then HPLC; ii, $LiAlD_4$; iii, Oppenauer oxidation.



Scheme 3 Isotopic labelling of the (*pro-R*) and (*pro-S*) methyls of dalpanol *via* acetoxymercuriation. *Reagents:* i, $Hg(OAc)_2$; ii, aq. THF; iii, $NaBX_4$ (X = D or T).

borohydride the chiral 6′-centre was extinguished, giving a product identical with 6′-prochiral natural dalpanol, m.p. 192–194 °C; $[\alpha]_D^{25}$ −131°. Repetition of the experiment using sodium borotritide in the reduction step gave a 4:1 mixture of tritiated dalpanols **31** and **30** having ^3H resonances at $\delta_T([^2H_6]$acetone) 1.21 (*J* 13.5 Hz) and 1.27 (*J* 13.5 Hz), respectively, again demonstrating the inhomogeneity of the mercuriation product. However, two crystallisations of the mercuriated intermediate from ethyl acetate improved the content of the major, (6′*R*)-diastereoisomer **29** to >90% and on reduction of this specimen with sodium borotritide only the ^3H signal at δ_T 1.21 due to [6′*S*-^3H]-**31** could be seen. Similar work was carried out with sodium borodeuteride, leading to [6′*S*-^2H]dalpanol. Because of the broader ^2H NMR signals, it is much easier to overlook stereochemical isotopic inhomogeneity in the final deuteriodalpanol than in the tritiated case with its sharp signals, and purity needs to be established at the mercuriated intermediate stage.

Experimental

Unless indicated otherwise, ^1H and ^3H spectra were recorded in $CDCl_3$ and ^2H in $CHCl_3$. For ^1H and ^{13}C spectra a deuterium lock was employed: ^2H spectra were measured with either a fluorine lock, or an unlocked field. Bruker WP 80 SY, WM 250 and AM 400 spectrometers were employed. *J*-Values are in Hz. UV data were measured in ethanol.

(6aS,12aS)-*Rot*-2′-*enoic Acid Acetate* **11**.—Rotenone (20 g) in pyridine (100 cm^3) was hydrogenated over 5% palladium on barium sulphate catalyst (200 mg) as described earlier.[2] The alkaline extracts were stirred with acetic anhydride (100 cm^3) and the solid which precipitated out was filtered off and crystallised from chloroform–ethanol to give, after removal of rot-3′-enonic acid acetate by further crystallisation, rot-2′-enonic acid acetate **11** (6.5 g, 29%), m.p. 177–178 °C (lit.,[2] 176–177 °C) (Found: M^+, 438. Calc. for $C_{25}H_{26}O_7$: M, 438), ν_{max}(mull)/cm^{-1} 1760, 1675 and 1590; δ_H 1.64 and 1.74 (each 3 H, s, 4′- and 5′-H_3), 2.29 (3 H, s, OAc), 3.23 (2 H, d, *J* 7.5, 1′-H_2), 3.77 and 3.81 (each 3 H, OMe), 3.81 (1 H, d, *J* 3.5, 12a-H), 4.16 (1 H, d, *J* 12, 6-H), 4.60 (1 H, dd, *J* 12, 3.5, 6-H), 4.94 (1 H, t, *J* 3.5, 6a-H), 5.04 (1 H, t, *J* 7.5, 2-H), 6.44 (1 H, s, 4-H), 6.71 (1 H, s, 1-H), 6.72 (1 H, d, *J* 9, 10-H) and 7.82 (1 H, d, *J* 9, 11-H).

Epoxidation of (6aS,12aS)-*Rot*-2′-*enonic Acid Acetate* **11**.—A solution of the acetate (125 mg) in dichloromethane (5 cm^3) was added to a suspension of MCPBA (80 mg) in water (5 cm^3) containing sodium hydrogen carbonate (125 mg). The mixture was stirred at 19 °C for 45 min, when aq. sodium sulphite (25 mg in 2 cm^3) was added, followed by dichloromethane (5 cm^3). The organic phase was separated, washed, dried (anhydrous sodium sulphate) and evaporated. Crystallisation from diethyl ether gave crystals (60 mg), m.p. 152–155 °C, of a mixture of (6aS,12aS,2′R)- and (6aS,12aS,2′S)-*rot*-2′-*enonic acid acetate epoxides* **13** and **15** (Found: M^+, 454.165. $C_{25}H_{26}O_8$ requires M, 454.163); λ_{max}(EtOH)/nm 263 (log ε 4.12), 297 (3.77) and 323 (3.72); ν_{max}(KBr)/cm^{-1} 1755 and 1675. The diastereoisomers

were just resolved by TLC on silica, developed with chloroform–isopropyl alcohol (25:1). The preparation was repeated using the acetate (1.20 g), and the two epoxides were separated by careful chromatography on Woelm silica and elution with chloroform. The first eluted compound, epoxide **A** (**13**), had δ_H 1.21 and 1.40 due to the 4′- and 5′-H_3 groups. The second eluted compound epoxide **B** (**15**), had δ_H 1.25 and 1.36. The two epoxides could also be distinguished by the characteristic shapes of their multiplets near δ_H 2.8 (due to 1′-H_2 and 2′-H). Epoxide **A** (0.36 g) formed prisms, m.p. 162–164 °C, when crystallised from diisopropyl ether, but needles, m.p. 143–143.5 °C, from ethanol. It had *m/z* 454 (M^+, 50%), 412 (3), 221 (3), 192 (100), 191 (42), 177 (16) and 149 (6); δ_H 1.23 and 1.40 (each 3 H, s, 4′- and 5′-H_3), 2.34 (3 H, s, OAc), 2.79 (3 H, m, 1′-H_2 and 2′-H), 3.77 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.90 (1 H, d, *J* 4, 12a-H), 4.19 (1 H, d, *J* 12, 6-H), 4.64 (1 H, dd, *J* 12, 3.5, 6-H), 4.97 (1 H, m, 6a-H), 6.43 (1 H, s, 4-H), 6.70 (1 H, s, 1-H), 6.79 (1 H, d, *J* 9, 10-H) and 7.90 (1 H, d, *J* 9, 11-H).

Epoxide **B** (0.37 g) also showed polymorphism. From ethanol it formed needles, m.p. 147–147.5 °C, but recrystallisation from diisopropyl ether gave mainly fine, amorphous material with a few clusters of prisms, m.p. 132–133 °C. It had *m/z* 454 (M^+, 39%), 412 (6), 221 (4), 192 (100), 191 (41), 177 (9) and 149 (6); δ_H 1.25 and 1.36 (each 3 H, s, 4′- and 5′-H_3), 2.32 (3 H, s, OAc), 2.86 (3 H, m, 1′-H_2 and 2′-H), 3.77 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.91 (1 H, d, *J* 4, 12a-H), 4.20 (1 H, d, *J* 12, 6-H), 4.64 (1 H, dd, *J* 12, 3.5, 6-H), 4.99 (1 H, m, 6a-H), 6.44 (1 H, s, 4-H), 6.70 (1 H, s, 1-H), 6.79 (1 H, d, *J* 9, 10-H) and 7.89 (1 H, d, *J* 9, 11-H). A mixed fraction (0.41 g) contained epoxide **A** and epoxide **B** in an approximate ratio of 4:1 as based on NMR analysis. The single-crystal X-ray structure of epoxide **A** showed it to be the (6a*S*,12a*S*,2′*R*)-stereoisomer **13**.

Hydrolysis of Epoxide **A** (**13**) *to give* (6aS,12aS,5′S)-*Epidalpanol* **17**.—Zinc dust (500 mg) was activated by the method of Gonzalez[8] and stirred at room temperature for 48 h with epoxide **A** (45 mg, 0.1 mmol) in methanol (5 cm^3). After filtration through Celite, the filtrate was concentrated, taken up in chloroform, washed, dried (anhydrous sodium sulphate) and evaporated. The product was purified by HPLC (μ-Porasil, elution with chloroform containing 0.25% methanol) to give *epidalpanol* **17** (36 mg, 88%) as an oil, and recovered epoxide **A** (5 mg).

Epidalpanol had $[\alpha]_D^{24}$ +22.3° (*c* 2, $CHCl_3$) (Found: M^+, 412.152. $C_{23}H_{24}O_7$ requires M, 412.152); *m/z* 412 (5%), 192 (52), 191 (14), 177 (6), 161 (1), 121 (1) and 78 (100); δ_H 1.19 (3 H, s, 7′-H_3), 1.31 (3 H, s, 8′-H_3), 1.87 (1 H, br s, OH), 3.10 (2 H, d, *J* 9.5, 4′-H), 3.77 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.85 (1 H, d, *J* 4, 12a-H), 4.19 (1 H, d, *J* 12.3, 6-H), 4.62 (1 H, dd, *J* 12.3, 3, 6-H), 4.74 (1 H, t, *J* 9.5, 5′-H), 4.93 (1 H, m, 6a-H), 6.46 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.5, 10-H), 6.78 (1 H, s, 1-H) and 7.82 (1 H, d, *J* 8.5, 11-H). The chemical shifts of 7′-H_3, 8′-H_3 and 5′-H show small differences from those of dalpanol and the two C-5′ epimers have different retention times on HPLC under the above conditions.

Hydrolysis of Epoxide **B**(**15**) *to give* (6aS,12aS,5′R)-*Dalpanol* **19**.—The epoxy acetate **15** (30 mg, 0.066 mmol) was hydrolysed by the zinc/methanol method (above). The product was purified by HPLC and crystallised from benzene as the benzene solvate (18 mg, 66%), m.p. 189–191 °C; $[\alpha]_D^{24}$ −122° (*c* 2, $CHCl_3$) (Found: M^+, 412.152. Calc. for $C_{23}H_{24}O_7$: M, 412.152); *m/z* 412 (4%), 192 (55), 191 (14), 177 (7), 161 (2), 121 (3) and 78 (100). The ^1H NMR spectrum was identical with that of dalpanol and the product had the same retention time as authentic, natural dalpanol on HPLC.

(6aS,12aS,5′R)-*Dalpanol* **19**.—(6a*S*,12a*S*)-Rot-2′-enonic acid (400 mg) was treated with a solution of MCPBA (228 mg) in dichloromethane (10 cm^3) in the presence of aq. sodium hydrogen carbonate. Work-up gave a mixture of dalpanol and its (6a*S*,12a*S*,5′*S*)-diastereoisomer **17**. One recrystallisation from benzene gave the (5′*S*)-compound as its benzene solvate (112 mg), m.p. 179–185 °C, raised by one further crystallisation from benzene to give the solvate, m.p. 192–194 °C; $[\alpha]_D^{22}$ −117° (*c* 0.073, $CHCl_3$) (calculated for the unsolvated material; $[\alpha]_D^{22}$ −139.2°) {lit.,[1,2] m.p. 196 °C; $[\alpha]_D^{22}$ −136.3° (*c* 0.62, $CHCl_3$)} (Found: M^+, 412.150. Calc. for $C_{23}H_{24}O_7$: M, 412.152); δ_H 1.22 (3 H, s, 7′-H_3), 1.33 (3 H, s, 8′-H_3), 3.12 (2 H, m, 4′-H_2), 3.77 and 3.81 (each 3 H, s, OMe), 3.85 (1 H, d, *J* 4, 12a-H), 4.19 (1 H, d, *J* 12.3, 6-H), 4.62 (1 H, dd, *J* 12.3, 3, 6-H), 4.68 (1 H, t, 5′-H), 4.93 (1 H, m, 6aH), 6.48 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.5, 10-H), 6.76 (1 H, s, 1-H), 7.37 (6 H, C_6H_6) and 7.83 (1 H, d, *J* 8.5, 11-H).

(6aS,12aS,5′R,6′R)- *and* (6aS,12aS,5′R,6′S)-6′,7′-*Epoxyrotenone* **23** *and* **22**.—A solution of MCPBA (234 mg, 1.17 mmol) in chloroform (5 cm^3) was added to a solution of rotenone (394 mg, 1.0 mmol) in chloroform (5 cm^3) and the mixture was stirred at 20 °C for 48 h, with monitoring of the reaction by TLC. Aq. sodium sulphite (2 cm^3; 10%) was shaken with the product and the chloroform layer was separated, washed, dried (anhydrous sodium sulphate) and evaporated. The epoxide band (R_f 0.51) was isolated by PLC on silica with light petroleum (b.p. 40–60 °C)–diethyl ether–ethyl acetate–methanol (6:6:2:1). The epoxide band was now further separated by PLC [three sequential developments with light petroleum (b.p. 40–60 °C)–diethyl ether–ethyl acetate (3:3:1)]. [An alternative work-up procedure was initial chromatography on flash silica (chloroform) followed by HPLC on μ-Porosil and elution with chloroform–diethyl ether–hexane (1:2:2).] The more rapidly eluted band (A) (75 mg, 18%), had m.p. 183–184 °C (from EtOH) (lit.,[7] 183–185 °C) (Found: C, 66.75; H, 5.35. Calc. for $C_{23}H_{22}O_7$: C, 67.3; H, 5.35%); $[\alpha]_D^{24}$ −136° (*c* 2.0, $CHCl_3$) {lit.,[7] $[\alpha]_D$ −133° (*c* 2, $CHCl_3$)}; *m/z* 410 (M^+, 6%), 395 (5), 351 (2), 208 (2), 193 (25), 192 (100), 191 (31), 177 (30), 149 (9), 121 (6), 110 (5), 105 (7), 69 (7), 65 (5) and 43 (9); λ_{max}/nm 218 (log ε 4.40), 236 (4.20), 245infl (4.00) and 292 (4.20); ν_{max}(KBr)/cm^{-1} 1670; δ_H 7.73 (1 H, d, *J* 9, 11-H), 6.68 (1 H, s, 1-H), 6.41 (1 H, d, *J* 9, 10-H), 6.37 (1 H, s, 4-H), 4.83 (1 H, t, *J* 6, 5′-H), 4.73 (1 H, t, *J* 3, 6a-H), 4.57 (1 H, dd, *J* 12, 3, 6-H^a), 4.14 (1 H, d, *J* 12, 6-H^b), 3.78 (1 H, *J* obscured, 12a-H), 3.76 (3 H, s, OMe), 3.73 (3 H, s, OMe), 3.07 (2 H, m, 4′-H_2), 2.72 (2 H, q, *J* 4, 7′-H_2) and 1.41 (3 H, s, 8′-H_3).

The slower running component (B) was crystallised from ethanol as needles (66 mg, 16%), m.p. 175–176.5 °C (lit.,[7] 178–179 °C); $[\alpha]_D^{24}$ −130° (*c* 2.0, $CHCl_3$) (lit.,[7] $[\alpha]_D$ −133° in $CHCl_3$); *m/z* 410 (M^+, 8%), 397 (7), 353 (5), 351 (5), 208 (7), 193 (28), 192 (100), 191 (31), 177 (31), 149 (12), 121 (8), 115 (5), 110 (7), 105 (7) and 43 (13); λ_{max}/nm 218 (log ε 4.40), 235 (4.20), 245infl (4.00) and 294 (4.20); ν_{max}(KBr)/cm^{-1} 1670; δ_H 7.73 (1 H, d, *J* 9, 11-H), 6.68 (1 H, s, 1-H), 6.39 (1 H, d, *J* 9, 10-H), 6.37 (1 H, s, 4-H), 4.83 (1 H, t, *J* 6, 5′-H), 4.73 (1 H, t, *J* 3, 6a-H), 4.57 (1 H, dd, *J* 12, 3, 6-H^a), 4.14 (1 H, d, *J* 12, 6-H^b), 3.78 (1 H, d, obscured, 12a-H), 3.76 (3 H, s, OMe), 3.73 (3 H, s, OMe), 3.07 (2 H, m, 4′H_2), 2.71 (2 H, dd, *J*, 14, 3, 7′-H_2) and 1.37 (3 H, s, 8′-H_3).

Reduction of the 6′-*Epimeric* 6′-*Epoxyrotenones with Lithium Aluminium Hydride*.—The unseparated epoxy diastereoisomers **22**/**23** (41 mg, 0.1 mmol) were dissolved in dry THF (10 cm^3) and treated with lithium aluminium hydride (~20 mg, 0.52 mmol) and the suspension was stirred (2 h). Aq. ammonium chloride was cautiously added and the product was filtered and extracted with chloroform. Washing, drying (anhydrous sodium sulphate) and evaporation gave the diol (**24**/**25** without deuterium marking) as a foam melting between 90 and 96 °C (Found: M^+, 414.165. $C_{23}H_{26}O_7$ requires M, 414.168); *m/z* 414 (M^+, 4%), 396 (25), 381 (2), 363 (1), 323 (2), 308 (1), 302 (1), 192 (13), 177 (47), 162 (59) and 59 (100); λ_{max}/nm 279infl (log ε 4.00), 287

(4.36) and 295infl (4.06); ν_{max}(KBr)/cm^{-1} 3480, 2920 and 1618; δ_H 7.15 (1 H, d, *J* 9, 11-H), 6.75 (1 H, s, 1-H), 6.47 (1 H, d, *J* 9, 10-H), 6.44 (1 H, s, 4-H), 4.90 (1 H, t, *J* 3, 6a-H), 4.90 (1 H, br s, 12-OH), 4.68 (1 H, t, *J* 9, 5′-H_2), 4.66 (1 H, dd, *J* 12, 3, 6-H^a), 4.50 (1 H, d, *J* 11, 12-H), 4.24 (1 H, m, 12a-H), 4.16 (1 H, d, *J* 12, 6-H^b), 3.80 (3 H, s, OMe), 3.78 (3 H, s, OMe), 3.11 (2 H, d, *J* 9, 4′H), 2.50 (1 H, br s, 6′-OH), 1.32 (3 H, s, 7′-H_3), 1.21 (3 H, s, 8′-H_3). The signals at δ 4.90 and 2.50 disappeared on shaking with D_2O.

Oppenauer Oxidation of Reduced 6′-Epoxyrotenone.—A solution of the reduction product **24**/**25** without D marking (29 mg, 0.07 mmol) in dry acetone (1 cm^3)–dry benzene (1.5 cm^3) was heated to 70 °C and a solution of aluminium isopropoxide (25 mg, 0.12 mmol) in dry benzene (1 cm^3) was added. The mixture was refluxed (2 h), cooled, diluted with water, and extracted with diethyl ether. The product was purified by PLC [developer chloroform–isopropyl alcohol (10:1)] to give dalpanol **19** (16 mg, 55%) as needles, m.p. and mixed with an authentic specimen 192–193 °C, $[\alpha]_D^{24}$ −133° (*c* 2.0, $CHCl_3$). The IR spectrum was superposable on that of an authentic specimen and the two samples co-chromatographed on TLC.

Conversion of (6aS,12aS,5′R,6′S)-*Epoxyrotenone* **22** *into* (6aS,12aS,5′R,6′S)-[7′-^{2}H]*Dalpanol* **26** (≡**20**).—Lithium aluminium deuteride (20 mg, 0.48 mmol) was added to a solution of the epoxide **22** (35 mg, 0.085 mmol) in THF (10 cm^3) and the mixture was stirred at room temperature (2 h). Work-up as above gave the diol **24** as an oil (30 mg, 85%) (Found: M$^+$, 414.1647 Calc. for $C_{23}H_{26}O_7$: M, 414.1678).

Aluminium isopropoxide (25 mg, 0.12 mmol) as a solution in dry benzene (1 cm^3) was added to a solution of the above diol (30 mg, 0.07 mmol) in dry acetone (1 cm^3)–dry benzene (1 cm^3) and the mixture was heated under reflux (2 h). Work-up as above gave (5′*R*,6′*S*)-[7′-^{2}H]dalpanol **26** (≡**20**) (12 mg, 40%), m.p. 190–191 °C (from benzene); $[\alpha]_D^{20}$ −122.6°. The IR spectrum was effectively superposable on that of authentic undeuteriated natural dalpanol and the product co-chromatographed with the latter. It had δ_H 1.22 (2 H, s, 7′-CH_2D), 1.33 (3 H, s, 8′-H_3), 1.61 (1 H, br s, OH), 3.12 (2 H, d, *J* 9, 4′-H_2), 3.76 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.84 (1 H, d, *J* 4.5, 12a-H), 4.18 (1 H, d, *J* 12, 6-H), 4.62 (1 H, dd, *J* 3, 12, 6-H), 4.69 (1 H, t, *J* 9, 5′H), 4.92 (1 H, m, 6a-H), 6.45 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.5, 10-H), 6.76 (1 H, s, 1-H), 7.36 (6 H, s, benzene of solvation) and 7.82 (1 H, d, *J* 8.5, 11-H); δ_D 1.22.

Conversion of (6aS,12aS,5′R,6′R)-*Epoxyrotenone* **23** *into* (6aS,12aS,5′R,6′R)-[7′-^{2}H]*Dalpanol* **27**.—By using the method above, the epoxide **23** (30 mg, 0.073 mmol) was converted into the title labelled dalpanol (10 mg, 33%), m.p. 188–190 °C; $[\alpha]_D^{24}$ −114.8° ($CHCl_3$). A trace of rotenolone, presumably formed by oxidation during work-up, was also formed. The sample of the deuteriodalpanol had δ_H 1.22 (3 H, s, 8′-H_3), 1.33 (2 H, s, 7′-CH_2D), 1.60 (1 H, br s, OH), 3.09 (2 H, d, *J* 9, 4′-H_2), 3.76 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.84 (1 H, d, *J* 4.5, 12a-H), 4.18 (1 H, d, *J* 12, 6-H), 4.62 (1 H, dd, *J* 3, 12, 6-H), 4.67 (1 H, t, *J* 9, 5′-H), 4.92 (1 H, m, 6a-H) 6.45 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.5, 10-H), 6.76 (1 H, s, 1-H), 7.36 (6 H, s, benzene of solvation) and 7.82 (1 H, d, *J* 8.5, 11-H); δ_D 1.33.

(6aS,12aS,5′R)-*Dalpanol* **31** via *the Acetoxymercuriation of Rotenone.*—Mercury(II) acetate (319 mg, 1 mmol) was dissolved in water (1 cm^3)–THF (1 cm^3) and the solution was stirred for 10 min after which rotenone (394 mg, 1 mmol) was added, the progress of the reaction being followed by TLC. After the mixture had been stirred for 10 h at 20 °C all the rotenone had been used up and water (2 cm^3), followed by sodium borohydride (19 mg, 0.5 mmol), was added. Mercury was precipitated out and the mixture was stirred (30 s) and poured into water–chloroform. The product was filtered through a plug of cotton wool and the organic layer was separated and evaporated, and the residue was chromatographed on flash silica (eluent chloroform containing 1% of methanol). Dalpanol **31** (X = H) (168 mg, 41%) was crystallised from benzene, m.p. 192–194 °C, and after being dried had $[\alpha]_D^{25}$ −131° (*c* 2.0, $CHCl_3$). Its IR spectrum and TLC characteristics were identical with those of an authentic specimen.

(6aS,12aS,5′R,6′S)-[7′-^{3}H]*Dalpanol* **31** (*X* = *T*) via *the Acetoxymercuriation of Rotenone.*—Rotenone (150 mg, 0.38 mmol) was oxymercuriated as above except that sodium borotritide (35 mCi, 228 mCi mmol^{-1}) was used in the reduction, followed by sodium borohydride. [^{3}H]Dalpanol (65 mg, 41%) was obtained as needles from benzene, m.p. 192–194 °C (specific activity 130 mCi mmol^{-1}). Its ^{1}H NMR spectrum was effectively identical with that of authentic material but the tritium spectrum [$(CD_3)_2CO$; 266.8 MHz] showed two signals, δ_T 1.22 (t, *J* 13.5, 7′-CH_2T) and 1.27 (t, *J* 13.5, 8′-CH_2T) in an approximate ratio of 4:1.

Mercury(II) acetate (0.83 mg, 2.6 mmol), water (2.5 cm^3), and THF (2.5 cm^3) were stirred together (10 min), rotenone (1 g, 2.54 mmol) was added, and stirring was continued overnight. The mixture was then concentrated to give a solid, which was dried *in vacuo* and twice crystallised from ethyl acetate. The purified mercuriated product **29** had δ_H 1.35 (stereochemical impurity < 10%, s, Me), 1.36 (3 H, s, 8′-H_3), 1.86 (3 H, s, OAc), 2.15 (2 H, AB, 7′-CH_2Hg), 2.89 (1 H, br s, OH), 3.17 (2 H, m, 4′-H_2), 3.64 (3 H, s, OMe), 3.74 (3 H, s, OMe), 3.88 (1 H, d, *J* 3.8, 12a-H), 4.27 (1 H, d, *J* 12, 6-H^a), 4.60 (1 H, dd, *J* 3, 12, 6-H^b), 4.86 (1 H, X of ABX, 5′-H), 5.11 (1 H, m, 6a-H), 6.45 (1 H, s, 4-H), 6.48 (1 H, d, *J* 8.5, 10-H), 6.72 (1 H, s, 1-H) and 7.74 (1 H, d, *J* 8.5, 11-H). Reduction with sodium borotritide as above gave dalpanol showing only one resonance in its ^{3}H spectrum, δ_T[$(CD_3)_2CO$] 1.21.

(6aS,12aS,5′R,6′S)-[7′-^{2}H]*Dalpanol* **31** (*X* = *D*) (≡**20**, ≡**26**) via *the Acetoxymercuriation of Rotenone.*—Mercury(II) acetate (0.83 mg, 2.6 mmol), water (2.5 cm^3), and THF (2.5 cm^3) were stirred together (10 min), rotenone (1 g, 2.6 mmol) was added, and the mixture was stirred overnight. After addition of water (2 cm^3), sodium borodeuteride (55 mg, 1.45 mmol) was added and, after being stirred (20 s), the reaction mixture was poured into chloroform–water (1:1) and worked up as above to give 7′-*deuteriodalpanol* (430 mg, 41%), m.p. 192–194 °C (from benzene); $[\alpha]_D^{25}$ −133° (*c* 0.21, $CHCl_3$) (Found: M$^+$, 413.159. $C_{23}H_{23}O_7D$ requires M, 413.160). The UV and IR spectra were identical with those for authentic dalpanol except for a peak in the IR spectrum of deuteriodalpanol at 860 cm^{-1}. The product had *m*/*z* 413 (M$^+$, 10%), 412 (2), 353 (2), 193 (14), 192 (100), 191 (26), 177 (16), 161 (5), 149 (5), 121 (3), 106 (3), 79 (4), 78 (5) and 60 (6). The ^{1}H spectrum had the expected resonance at δ 1.22 (2 H, s, 7′-CH_2D) replacing the Me signal of the unlabelled material. The product had δ_D 1.22 (br s): despite the lack of evidence it is likely from the tritium work that this sample is only predominantly labelled at C-7′.

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References

1 D. Adinarayana, M. Radhakrishniah, J. Rajesakhara Rao, R. V. Campbell and L. Crombie, *J. Chem. Soc. C*, 1971, 29.
2 L. Crombie, P. W. Freeman and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1277.
3 M. F. Grundon, *Nat. Prod. Rep.*, 1985, 395; L. Jurd and R. Y. Wong, *Aust. J. Chem.*, 1983, **36**, 1615.

4 A. Laguna, *Planta Med.*, 1984, **50**, 112.
5 R. D. H. Murray, J. Mendez and S. A. Brown, *The Natural Coumarins*, Wiley, Chichester, 1982.
6 D. Carson, L. Crombie, G. W. Kilbee, F. Moffatt and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1982, 779 and unpublished results.
7 T. Unai, I. Yamamoto, H.-M. Cheng and J. E. Casida, *Agric. Biol. Chem.*, 1973, **37**, 387.
8 A. G. Gonzalez, Z. D. Jorge and H. Lopez Dorta, *Tetrahedron Lett.*, 1981, **22**, 335.

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