Isotopically Labelled Geometric Isomers of Vinyl Groups: Reconstructive Synthesis of (*Z*)- and (*E*)-[7′-^{2}H]Rotenone

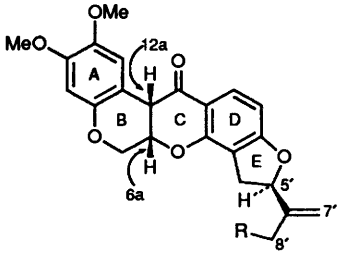
Leslie Crombie, Stephen J. Pegg and Donald A. Whiting
Department of Chemistry, University of Nottingham, Nottingham, NG7 2RD, UK

Rotenone with its 7′-methylene specifically labelled with hydrogen isotope in the (*Z*)- or (*E*)-position offers utility in both biochemical and chemical experiments, and the chemical shifts of the protons in question were identified by NOE experiments. Since an approach to stereospecific synthesis through introduction of a trimethylsilyl-substituted methylene by the Wittig reaction proved unsatisfactory, 12a-β-hydroxyrotenone (rotenolone) trimethylsilyl ether was treated with benzenesulfenyl chloride to give, on desilylation, anti-Markownikov addition products diastereoisomeric at C-6′. On heating, these were converted into the Markownikov diastereoisomers which, by dehydrohalogenation, produced 7′-phenylthiorotenolone as a mixture of (*E*)- and (*Z*)-isomers (~85:15). The latter were separated, and the (*E*)-isomer was desulfurised by deactivated [$^2H_2$]-Raney nickel to give (E)-[7′-$^2H_2$]rotenolone, though deuterium incorporation was incomplete (~60%). To obtain a higher isotopic content, [7′-$^2H_2$]rotenolone trimethylsilyl ether was synthesized and subjected to similar treatment with benzenesulfenyl chloride to give 7′-phenylthio-[7′-^{2}H]rotenolone as (*E*)-/(*Z*)-isomers. Separation and treatment of the (*E*)-isomer with [$^1H_2$]-Raney nickel now produced (*Z*)-[7′-^{2}H]rotenolone having a >95% incorporation of deuterium with >90% positional integrity. Removal of the blocking 12a-hydroxy group with zinc and acetic acid gave the desired (*Z*)-[7′-^{2}H]rotenone.

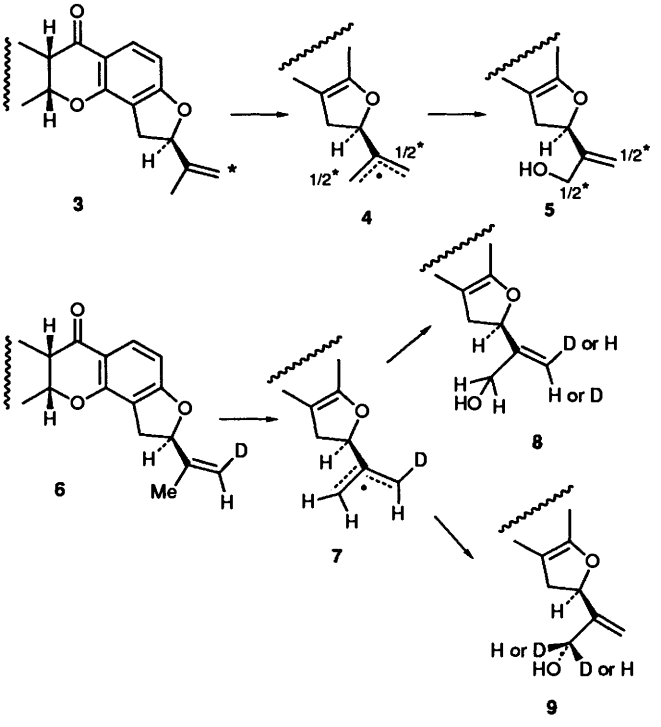
Although 12a-methylrotenone having the unnatural *trans*-B/C fusion formed both the required anti-Markownikov and Markownikov addition products, unwanted removal of ring-E protons by hindered bases from the now more accessible α-face brought about extensive elimination, forming a conjugated diene.

Our studies of the formation of amorphigenin **2** from rotenone **1** by hydroxylation using whole *Amorpha fruticosa* seedlings as the enzyme source show that during the hydroxylation (Scheme 1) a carbon label on the vinyl methylene of rotenone **3** becomes equivalently scrambled between the hydroxymethylene and the vinyl carbon of amorphigenin **5**. An allylic radical **4** is thought to be implicated in the enzyme mechanism. Further stereochemical questions might be probed were the vinyl group of rotenone to be labelled stereospecifically by isotopic hydrogen in either the (*Z*)- or (*E*)-methylene positions **6**. From radical **7** one would expect the alcohols **8** and **9** to be formed in nearly equivalent amounts. The first structure **8** poses the question: is there rotation of the erstwhile double bond in rotenone during the hydroxylation? The second structure **9** poses the question: is the newly formed hydroxylated centre racemic or optically active? These are both questions that should be answerable by NMR techniques, and by the attachment of a suitable chiral probe to the hydroxylic centre.

There are other problems involving the *in vitro* chemistry of rotenone which the availability of labelled substrate **6** could help to solve. Certain reactions of rotenone involve substantial



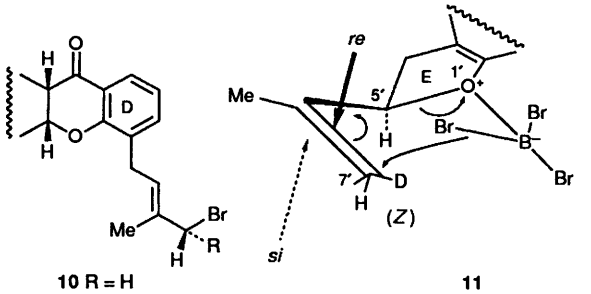
1 R = H
2 R = OH



Scheme 1 Stereochemical and regiochemical relationships from (*Z*)-[^{2}H]rotenone *via* enzymic radical hydroxylation

re-/*si*-selectivity in the attack of reagents on the 6′,7′-double bond (*e.g.*, acetoxymercuriation) whilst others involve selective 1,4-addition to the allylic ether system (*e.g.*, catalytical hydrogenation over palladium in pyridine). Still more striking is the

reaction of rotenone with boron tribromide. This allows specific cleavage of the 1′,5′-carbon–oxygen bond before cleavage of any of the other carbon–oxygen single bonds in the molecule. The reaction is also stereospecific for the formation of the (*E*)-olefin **10**, 4′-bromorot-2′-enonic acid. We have suggested that the mechanism involves a six-membered cyclic transition state **11**[1] brought about by complexation of the boron tribromide to the 1′-oxygen of the dihydrofuran, with delivery of the bromine to one face of the double bond. In transition state **11**, representing (*Z*)-[7′-^{2}H]rotenone, the delivery is to the *re*-face with dissolution of the chirality at C-5′ and the creation of a new chiral centre at C-4′ in the methylbut-2′-enyl group of the rot-2′-enonic acid **12**. Apart from the possibility of testing mechanistic and stereochemical points in the recyclisation of compound **12** to rotenone and its 5′-epimer, compound **12** offers the prospect of conversion into a chirally labelled methyl group. It was for these reasons that we undertook the work described in the present paper on the stereospecific (*Z*)- and (*E*)-labelling of rotenone and related compounds with isotopic hydrogen.

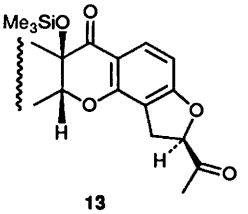


10 R = H
12 R = D

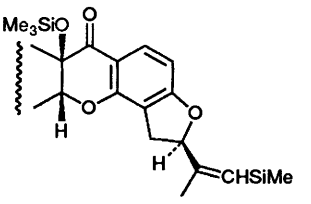
11

The two 7′-vinyl protons in rotenone **1** resonate in $CDCl_3$ as singlets at δ 5.07 and 4.93, and irradiation of the allylic 8′-methyl causes nuclear Overhauser effect (NOE) enhancement at δ 4.93 but not at 5.07; there was a small enhancement at the 5′-methine proton. The matter was investigated in more detail in [$^2H_6$]benzene. In this solvent the two vinyl protons resonate at δ 5.01 and 4.77, and again irradiation of the allylic methyl at δ 1.54 produces NOE enhancement of only the latter signal. Irradiation at δ 4.77 causes enhancement of both the signal at δ 5.01 and the methyl signal. Irradiation at δ 5.01 produces NOE enhancement of the signal at δ 4.77 only. In both solvents, therefore, the upfield signal belongs to the (*E*)-proton, *i.e.* that *cis* to the methyl.

A few synthetic examples having isotopic discrimination between two vinyl protons are known, such as (*E*)- and (*Z*)-[3-^{2}H]methacrylic acids,[2,3] but the methodology used was considered too severe for the present purpose. Indeed, methodology in general for the synthesis of specifically labelled terminal olefins is limited, although vinyl olefins have been made by specific cleavage of a number of substituents. The first method considered was introduction of a vinylsilane function which can be cleaved with DI/D_2O in high yield.[4] Since enolisation at C-12 with attendant β-elimination (which destroys the optical activity of the C-6a, C-12a-system) is brought about in basic medium, the 12a-position was blocked without racemisation at C-6a by a β-oriented trimethylsiloxy grouping and the 7′-methylene was removed. Both of these processes have been described in our earlier work.[1] Unfortunately the resulting 12a-trimethylsilyl ether of rotenolone-6′-norketone, compound **13**, did not react with the carbanion generated from tris(trimethylsilyl)methane, anion **14**.[5,6] With triphenyl(trimethylsilylmethylene)phosphorane **15**,[7] although reaction occurred and the molecular ion of the required product **16** could be observed in the crude product, after chromatographic separation and purification the two products isolated were found to be the desilylated olefin **17** and the rotenolone **18**. Attention was then turned to the use of sulfur chemistry, which gave better results.



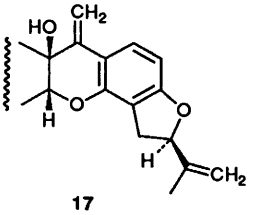
13



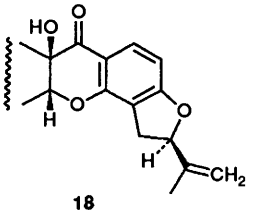
16

$(Me_3Si)_3C^-$ **14**

$Me_3SiHC = PPh_3$ **15**



17

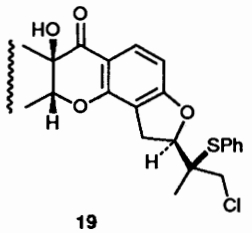


18

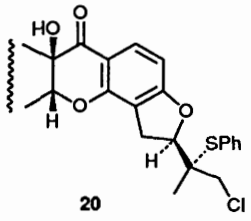
A solution of (6a*R*,12a*R*,5′*R*)-rotenolone **18**, as its trimethylsilyl ether in dichloromethane, was treated with benzenesulfenyl chloride to give, after desilylation, a crystalline product, the NMR spectrum of which showed C-8′ methyl singlets at δ 1.21 and 1.28. This proved to be the anti-Markownikov adduct[8] derived from both *re*- and *si*-addition, producing C-6′ diastereoisomers **19** and **20** having slightly different methyl chemical shifts. These are kinetically favoured, formed by the attack of chloride ion at the less hindered carbon of the bridged episulfenium ion.[9–11] Isomerisation to the thermodynamically stable diastereoisomers **21** and **22** having the desired Markownikov orientation of groups was accomplished by heating of the initial adducts in dimethylformamide (DMF) or tetrahydrofuran (THF) containing triethylamine (0.25 mol equiv.) at 60–65 °C for up to 60 h. The base does not cause dehydrohalogenation but prevents acid-catalysed dehydration across the B/C-ring junction. When acetonitrile was used as solvent one of the possible C-6′ diastereoisomers was found to predominate, and under these conditions the equilibrium mixture contained >90% of the Markownikov adduct.*

Dehydrohalogenation was carried out by treatment with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in anhydrous DMF at 55–60 °C for 16 h, and gave (6a*R*,12a*R*,5′*R*)-7′-(phenylthio)-rotenolone **23** as a mixture of (*E*)/(*Z*) isomers (85:15). Other hindered bases could be used (diethylaminopyridine, diazabicyclooctane, lithium cyclohexylisopropylamide, potassium *tert*-butoxide) but prolonged heating (>16 h) formed the diene **24** in varying amounts. (Presumably the sulfide **23** rearranges to the allyl sulfide with a tetrasubstituted double bond, and subsequent deprotonation at C-4′ and 1,4-elimination of thiophenol generates the diene.) The (*E*)/(*Z*) isomers constituting the sulfide **23** were separated by reversed-phase HPLC on a C-18 column with 85% methanol in water as eluent. The geometry of the two isomers was readily identified from their NMR spectra. In one, the 5′-proton (double doublet) resonated (δ 5.31) at much the same position as in rotenone itself (δ 5.24), but in the other there was a drastic downfield shift to δ 5.97. Examination of models shows that this large

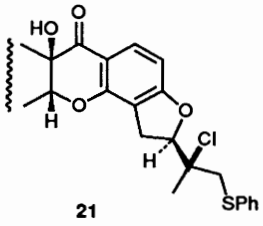
* When either (6a*R*,12a*R*,5′*R*)-7′-chloro-6-phenylthio-6′,7′-dihydrorotenolone (**19**/**20**) or (6a*R*,12a*R*,5′*R*)-6′-chloro-7′-phenylthio-6′,7′-dihydrorotenolone (**21**/**22**) is treated with W2 Raney nickel the product is (6a*R*,12a*R*,5′*R*)-rotenolone, formed in good yield. This suggests a possible procedure for masking/demasking of a double bond, though the stereochemistry and scope of the reaction has not been investigated.



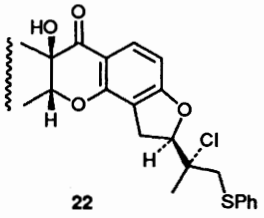
19



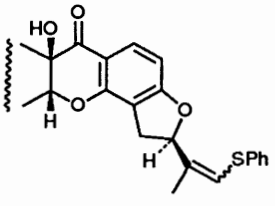
20



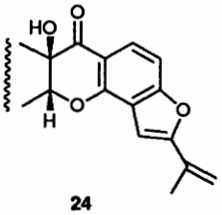
21



22



23

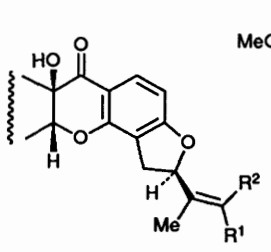


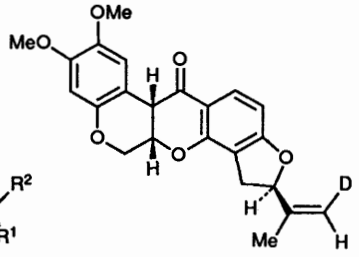
24

deshielding by the phenylthio substituent is possible only in the (*Z*)-olefin **25**.

(*E*)-7′-(Phenylthio)rotenolone **26** was then desulfurised stereospecially[12] with deactivated [$^2H_2$]-Raney nickel which had been made by shaking W2-Raney nickel[13] with deuterium gas in 1,4-dioxane for 40 h,[14] the gas being replaced with fresh deuterium at repeated intervals. The product was purified by HPLC to give (6a*R*,12a*R*)-(*E*)-[7′-^{2}H]rotenolone **27** in 40% yield, but mass spectrometric monitoring of the molecular ions at 410 and 411 indicated a deuterium incorporation of only ~60%. The Raney nickel apparently contained tenaciously held hydrogen and it is possible that the dioxane solvent provided a further ^{1}H source. Incorporation was not improved upon in a further experiment using a fresh batch of deuteriated Raney nickel. Although the above product **27** was useful for some experimental purposes we wished to have a much higher specifically placed isotopic concentration. Since the only problem appeared to be the attainment of a high deuterium content in the Raney nickel, the experimental order was changed.

Our earlier methodology was used to prepare [7′-$^2H_2$]rotenolone **30**,[1] as its trimethylsilyl ether, using iodo[$^2H_3$]methane having a deuterium content of >99%. The methylenation gave, after desilylation, the rotenolone **30** carrying >95% deuterium at each of the 7′-methylene positions. [7′-$^2H_2$]Rotenolone was now converted into its (*E*)-[7′-^{2}H] 7′-phenyl sulfide **28** through isomerisation of the initially formed β-chloro phenyl sulfide: dedeuteriochlorination proceeded normally when 2 mol equiv. of DBN were used. Raney nickel (hydrogen-containing) desulfurisation[12] of the product now gave (*Z*)-[7′-^{2}H]rotenolone **29** having a 95% incorporation of deuterium with >90% stereospecificity for the label position. The NMR spectrum showed a signal at δ 4.9 due to the 7′*E* proton, whilst the signal at δ 5.1 due to the 7′*Z* proton was effectively undetectable.



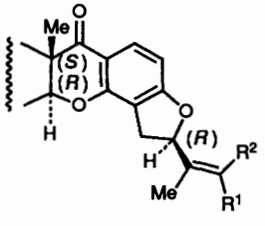


25 R^1 = H, R^2 = SPh
26 R^1 = SPh, R^2 = H
27 R^1 = D, R^2 = H
28 R^1 = SPh, R^2 = D
29 R^1 = H, R^2 = D
30 R^1 = R^2 = D

6

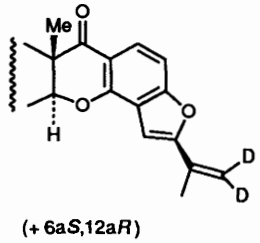
The final step in the synthesis was now carried out by reduction of the 12,12a-α-ketol **29** with removal of the blocking hydroxy group, using zinc and acetic acid,[4] the reaction being monitored by reversed-phase TLC with 85% methanol in water as developer. The product was purified by PLC to give crystalline (*Z*)-[7′-^{2}H]rotenone **6**, m.p. 163–164 °C, in 50% yield, δ 4.93 with the resonance at δ 5.07 barely detectable. The ^{2}H content was >95% of that expected, with a positional integrity of >90%.

For certain chemical experiments a rotenoid having a permanently unenolisable C-12,12a system would be useful, and to this end some work was carried out on the C-12a-methyl derivative formed when C-methylation of rotenone is carried out with iodomethane and sodium hydride.[15,16] The major product is a blocked representative of the unnatural *trans*-B/C fusion and, because of β-elimination from the enolate anion during the methylation, it is a mixture of two diastereoisomers, (6a*S*,12a*R*,5*R*)- and (6a*R*,12a*S*,5′*R*)-**31**, with closely similar properties. The mixture was converted as before, by removal of the 7′-methylene and Wittig reaction of the resulting ketone with [$^2H_2$]methylenetriphenylphosphorane, into the dideuterio compound **32**. Treatment with benzenesulfenyl chloride again gave the anti-Markownikov addition product, which isomerised on heating to give the Markownikov product.



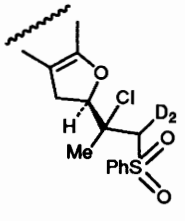
(+ 6a*S*,12a*R*,5′*R*)

31 R^1 = R^2 = H
32 R^1 = R^2 = D

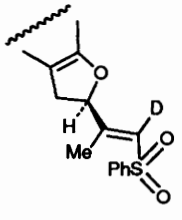


(+ 6a*S*,12a*R*)

33



34



35

Unexpectedly, however, the usual elimination step gave only low yields of the desired vinyl compound having phenylthio

substitution when 1 mol of DBN was used, and effectively none when 2 mol of DBN were employed. The main product was the diene **33**. It appears that deprotonation occurs preferentially in the *trans*–series in ring-E at C-5′ rather than at C-7′, and this is followed by 1,4-elimination of the benzenethiol residue. In both the *trans*- and *cis*-B/C series the acidity of the C-7′ proton would be expected to be similar and the cause of the difference would seem to be the greater shielding of the α-face in the natural *cis*-series. The B-ring residue in the latter has its 6-methylene axially oriented to ring-C, contrasting with the more planar *trans*-fusion. Increasing the acidity of the C-7′ proton in the *trans*-series should, however, help achieve the desired elimination, and the sulfide was therefore converted into the sulfone **34** with *m*-chloroperbenzoic acid (MCPBA).[17] Treatment with 1.1 mol of DBN at 40 °C for 1 h was then carried out and the products were separated by HPLC on μ-Porasil with ethyl acetate–hexane (1:4) as eluent. First eluted was a mixture of allyl and vinyl sulfones and this was then followed by crystalline (*E*) 12a-methyl-[7-^{2}H]rotenone 7′-phenyl sulfone **35** as a single pure diastereoisomer, though it is not known if it is the (6a*S*,12a*R*,5′*R*)- or the (6a*R*,12a*S*,5′*R*)-form. Unfortunately, despite a number of attempts (W2 Raney nickel at various temperatures, Al/Hg, Na/Hg, or $LiAlH_4$), stereospecific desulfurisation has proved unsatisfactory. It was concluded that, for many chemical purposes, a model based on compound **29**, with its natural *cis*-B/C fusion and C-12a blocked by an ether function, provided a satisfactory alternative for a stereochemical study of ring-E and its appendage. The main disadvantage of such a model is acid sensitivity, leading to formation of 6a,12a-didehydro-compounds.

Experimental

HPLC was carried out using a Waters system, mainly with RAD PAK (10 cm × 8 mm) columns. NMR spectra, normally run on solutions in $CDCl_3$, were recorded using Bruker AM400, WB250 and WP80SY spectrometers. *J*-Values are given in Hz. IR data refer to KBr discs.

(6aR,12aR, 5′R)-7′-*Chloro*-6′-*phenylthio*-6′,7′-*dihydrorotenolone* **19/20**.—Thiophenol (1.14 g 0.01 mol) was added in part to a rapidly stirred suspension of *N*-chlorosuccinimide (1.37 g, 0.01 mol) in dry (P_2O_5 distilled) dichloromethane (120 cm^3), heated gently to initiate reaction.[10] The mixture was cooled to 0 °C and the remaining thiophenol was added during 20 min. The solution was then cooled to −10 °C and a solution of rotenolone 12a-trimethylsilyl ether[1] (4.5 g, 9.3 mmol, see below) in dichloromethane (10 cm^3) added in one portion. After warming to room temperature, the solvent was removed under reduced pressure and the residue was protodesilylated, chromatographed on silica, and eluted with ethyl acetate–hexane (2:3). The *title compound* **19/20** (4.15 g, 80%) was crystallised from a concentrated acetone solution at 20 °C when diethyl ether was gradually added, as prisms, m.p. 111–112 °C (Found: C, 63.15; H, 6.05%; M$^+$, 554.113. $C_{29}H_{27}ClO_7S$-$C_4H_{10}O$ requires C, 63.0; H, 5.95%; $C_{29}H_{27}ClO_{27}S$ requires *M*, 554.117); ν_{max}(KBr)/cm^{-1} 1680 and 1610; δ_H 1.21 and 1.28 (3 H, s, 8′-H_3), 3.3 (2 H, m, 4′-H_2), 3.71 and 3.81 (each 3 H, s, OMe), 4.62 (3 H, m, 6-H_2 and 6a-H), 5.69 (1 H, t, *J* 9, 5′-H), 6.42 (1 H, d, *J* 10, 10-H), 6.52 (1 H, s, 4-H), 6.62 (1 H, s, 1-H), 7.44 (5 H, m, SPh) and 7.86 (1 H, d, *J* 10, 11-H). This product was also prepared directly from benzenesulfenyl chloride made from diphenyl disulfide and sulfuryl dichloride.[8] When crystallised from diethyl ether and dried *in vacuo* the material had a 'clean' ^{1}H NMR spectrum and was suitable for use without further purification.

(6aR,12aR,5′R)-6′-*Chloro*-7′-*phenylthio*-6′,7′-*dihydrorotenolone* **21/22**.—A rapidly stirring suspension of the 7′-chloro-6′-phenylthio compound (above) (4.15 g, 7.49 mmol) in acetonitrile (50 cm^3) containing triethylamine (0.25 cm^3) was heated at 60–65 °C for 70 h. The solvent was removed to give an equilibrium mixture containing ~90% of the title compound, largely as one C-6′ diastereoisomer, ν_{max}/cm^{-1} 1680 and 1610; δ_H 1.63 (3 H, s, 8′-H_3), 3.68 (3 H, s, OMe), 3.79 (3 H, s, OMe), 4.50 (1 H, s, 12a-OH), 4.55 (3 H, m, 6a-H and 6-H_2), 5.12 (1 H, dd, *J* 9, 5′-H), 6.48 (1 H, d, *J* 10, 10-H), 6.47 (1 H, s, 1- or 4-H), 6.54 (1 H, s, 4- or 1-H), 7.34 (5 H, m, SPh) and 7.80 (1 H, d, *J* 10, 11-H).

(6aR,12aR,5′R)-(E)- *and* -(Z)-*Rotenolone* 7′-*Phenyl Sulfide* **26** *and* **25** (≡**23**).—The 7′-chloro-6′-phenylthiorotenolone **19/20** (above) (1 g, 1.8 mmol) was heated in dry DMF (20 cm^3) in the presence of triethylamine (60 mm^3) under nitrogen for 70 h at 55–60 °C. The product was cooled, DBN (0.5 g) was added, and the mixture was heated at 55–60 °C for 16 h (under N_2). The product was poured into water and worked up by extraction (Et_2O) and chromatography on silica [eluent ethyl acetate–hexane (1:3)] to give mixed (*E*)/(*Z*) (85:15) rotenolone vinyl sulfides **23** (0.7 g, 75%). These were separated by reversed-phase HPLC on C-18 μ-Bondapak with 85% methanol in water as eluent. The (*E*)-isomer **26** formed a powdery solid from chloroform–propan-2-ol (Found: C, 67.4; H, 5.4. $C_{29}H_{26}O_7S$ requires C, 67.15; H, 5.05%); ν_{max}(KBr)/cm^{-1} 1680 and 1610; δ_H 1.83 (3 H, s, 8′-H_3), 3.20 (2 H, m, 4′-H_2), 3.72 (3 H, s, OMe), 3.82 (3 H, s, OMe), 4.38 (1 H, s, 12a-OH), 4.60 (3 H, m, 6-H_2 and 6a-H), 5.31 (1 H, t, *J* 8.5, 5′-H), 6.37 (1 H, s, 7′-H), 6.48 (1 H, s, 4-H), 6.53 (1 H, d, *J* 9, 10-H), 6.54 (1 H, s, 1-H), 7.30 (5 H, m, SPh) and 7.83 (1 H, d, *J* 9, 11-H).

The (*Z*)-isomer **25** had δ_H 1.88 (3 H, s, 8′-H_3), 2.20 (2 H, abx, 4′-H_2), 3.73 (3 H, s, OMe), 3.83 (3 H, s, OMe), 4.47 (1 H, s, 12a-OH), 4.60 (3 H, m, 6-H_2 and 6a-H), 5.97 (1 H, t, *J* 8.5, 5′-H), 6.14 (1 H, s, 7′-H), 6.49 (1 H, s, 4-H), 6.54 (1 H, s, 1-H), 6.54 (1 H, d, *J* 9, 10-H), 7.3 (5 H, m, SPh) and 7.83 (1 H, d, *J* 9, 11-H).

(E)-/(Z)-*Rotenolone* 7′-*Phenyl Sulfide* 12a-*Trimethylsilyl Ether*.—A solution of (*E*)/(*Z*)-rotenolone 7′-phenyl sulfide (0.2 g, 0.34 mmol) in pyridine (5 cm^3) was stirred overnight with hexamethyldisilazane (HMDS) (1 cm^3) and chlorotrimethylsilane (TMSCl) (0.25 cm^3). The product was poured into water, then extracted with chloroform, and the extracts were washed successively with aq. copper sulfate (3 ×), water, and brine. Drying, evaporation, and crystallisation from methanol–diethyl ether gave the (E)-/(Z)-12a-*trimethylsilylated ether* (0.2 g, 91%) as needles, m.p. 162–164 °C (Found: C, 65.0; H, 5.85%; M$^+$, 590.178. $C_{32}H_{34}O_7SSi$ requires C, 65.05; H, 5.8%; *M*, 590.179); ν_{max}(KBr)/cm^{-1} 1690 and 1605; δ_H 0.07 (9 H, s, $SiMe_3$), 1.82 and 1.87 [3 H, s, (*E*/*Z*)-8′-H_3], 3.11 (2 H, abx, 4′-H_2), 3.72 (3 H, s, OMe), 3.82 (3 H, s, OMe), 4.52 (3 H, s, 6-H_2 and 6a-H), 5.27 [t, (*E*), 5′-H], 5.95 [t, (*Z*), 5′-H], 6.12 [s, (*Z*), 7′-H], 6.35 [s, (*E*), 7′-H], 6.46 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.6, 10-H), 6.62 (1 H, s, 1-H), 7.30 (5 H, m, SPh) and 7.82 (1 H, d, *J* 8.6, 11-H).

(6aR,12aR,5′R)-(E)-[7′-^{2}H]*Rotenolone* **27**.—Deuteriated W2 Raney nickel was prepared[14] by shaking the nickel in purified 1,4-dioxane under deuterium for 40 h. The gas was replaced at 3 h intervals for the first 21 h. (*E*)-Rotenolone 7′-phenyl sulfide **26** was then refluxed [as below for the (*Z*)-isomer] with the deuteriated Raney nickel and the deuteriated rotenolone so produced was analysed by monitoring of the molecular ions at M$^+$ 411 ($C_{23}H_{21}{}^2HO_7$) and at M$^+$ 410 ($C_{23}H_{22}O_7$) relative to the base peak at *m*/*z* 208. This showed a 60% incorporation of dueterium. In view of the low incorporation the experiment was repeated after a fresh batch of deuteriated Raney nickel had been prepared, but the incorpora-

tion was not improved. The use of deuteriated 1,4-dioxane as solvent might improve the deuterium incorporation.

(6aR,12aR,5'R)-[7'-$^2H_2$]-*Rotenolone Trimethylsilyl Ether.*—(6a*R*,12a*R*,5'*R*)-Rotenolone **18** was made by oxidation (dichromate) as described earlier[18] in 54% yield, m.p. 87–88 °C as the mono-methanol solvate (lit.,[18] m.p. 88 °C). The methanol of solvation can be removed by dissolving the solvate in benzene and evaporating under reduced pressure. The unsolvated rotenolone was silylated with HMDS and TMS-Cl in pyridine, to give needles from methanol (76% yield), m.p. 153–154 °C (lit.,[1] 153.5–154 °C).

Under conditions similar to those given below, the 7'-methylene group was removed by osmium tetraoxide–periodate oxidation to give the silylated norketone **13** (34%), as needles from THF, m.p. 114–115 °C (lit.,[1] 113–114 °C).

A Wittig reagent was prepared under nitrogen from [$^2H_3$]-methyltriphenylphosphonium iodide (7.9 g, 0.019 mol) and butyllithium in hexane (1.5 mol dm^{-3}; 12.6 cm^3). Reaction with the silylated norketone **13** (4.0 g, 0.0097 mol), and purification of the product by crystallisation from methanol, gave the title compound (2.5 g, 63%), m.p. 152–153 °C (lit.,[1] 153.5–154 °C) (Found: M^+, 484.186. Calc. for $C_{26}H_{28}{}^2H_2O_7Si$: *M*, 484.189). By using the same methodology as above, this compound was converted into (*E*)-[7'-^{2}H]rotenolone 7'-phenyl sulfide **28** (M^+, 519.145. $C_{29}H_{25}DO_7S$ requires *M*, 519.146).

(6aS,12aS,5'R)-(Z)-[7'-^{2}H]*Rotenone* **6**.—Raney nickel (W2, 6 g) in acetone (60 cm^3) was refluxed under nitrogen (30 min) and cooled to room temperature. A solution of (*E*)-[7'-^{2}H]rotenolone 7'-phenyl sulfide **28** (150 mg, 0.29 mol) in acetone (5 cm^3) was added to the stirred mixture. The progress of the reaction was monitored by HPLC (C_{18} reversed-phase, elution with 85% methanol in water). Reaction times varied between 2 and 4 h and, if necessary, more deactivated Raney nickel was added. After filtration through Kieselguhr, the filtrate was evaporated and the product was purified by HPLC as above to give (6aR,12aR,5'*R*)-(Z)-[7'-^{2}H]*rotenolone* **29** (0.48 g, 40%), m.p. 88–89 °C (from MeOH) (mono-methanol solvate) (Found: M^+, 411.142. $C_{23}H_{21}{}^2HO_7$ requires *M*, 411.143). The deuterium content was >95% with >90% in the correct label position (NMR). The (*Z*)-H stereoisomer's 7'-H signal was barely detectable at δ 5.1.The strong signal at δ 4.9 (2 H, m) is due to the 7'-(*E*)-proton plus the 6a-H.

A solution of the rotenolone **29** (48 mg, 0.117 mmol) in glacial acetic acid (8 cm^3) was added to a suspension of zinc dust (5 g) in glacial acetic acid under nitrogen and the mixture was stirred rapidly under reflux. The reaction progress was monitored by HPLC as above. Further zinc dust (2 g) was added at intervals of 2 h and reaction was complete in 8 h. The mixture was filtered, water (20 cm^3) was added, followed by solid sodium hydrogen carbonate to neutralise the acetic acid, and the product was isolated by extraction with chloroform. Evaporation, and purification by preparative TLC [silica gel; $CHCl_3$–MeOH (99:1)] gave (6a*S*,12a*S*,5'*R*)-(*Z*)-(7'-^{2}H)rotenone **6** (26 mg, 56%), m.p. 163–164 °C (from EtOH) (lit.,[19] for undeuteriated material, 163 °C); δ_H 1.77 (3 H, s, 8'-H_3), 2.96 (1 H, dd, *J* 15.8 and 8.1, 4'-H^a), 3.32 (1 H, dd, *J* 15.8 and 9.8, 4'-H^b), 3.81 and 3.77 (2 × 3 H, s, OMe), 3.84 (1 H, d, *J* 4.0, 12a-H), 4.18 (1 H, d, *J* 12.1, 6-H^a), 4.61 (1 H, dd, *J* 12.1 and 3.1, 6-H^b), 4.93 [2 H, m, 7'-H (*E*) proton and 6a-H], 5.24 (1 H, dd, *J* 15.9, 8.1 and 9.8, 5'-H), 6.45 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.6, 10-H), 6.77 (1 H, s, 1-H) and 7.84 (1 H, d, *J* 8.6, 11-H).

(6aS,12aR,5'R)-/(6aR,12aS,5'R)-12a-*Methylrotenone* **31**.—Sodium hydride (2.6 g, 0.11 mol) was added in portions to a stirred solution of rotenone (25 g, 63.4 mmol) and iodomethane (25 cm^3) in dry DMF (400 cm^3) at 20 °C under nitrogen. After 6 h, the mixture was poured into 2 mol dm^{-3} hydrochloric acid (400 cm^3) and extracted with diethyl ether. The extracts were washed successively with water and brine, dried ($MgSO_4$) and evaporated to leave a red oil, which was chromatographed on a short silica column (eluent:ethyl acetate–hexane, 1:4). The product was then purified by preparative HPLC on Porasil and elution with ethyl acetate–hexane (1:4) gave, after crystallisation from acetone–ethanol, the title mixture of two diastereoisomers (17 g, 66%) as large prisms, m.p. 127–128 °C.

(6aS,12aR,5'R)-/(6aR,12aS,5'R)-12a-*Methylrotenone 6'-Norketone.*—12a-Methylrotenone diastereoisomers (18 g, 44 mmol), THF (250 cm^3), and water (40 cm^3) were stirred with osmium tetraoxide (100 mg) for 30 min and then sodium metaperiodate (21 g, 98 mmol) was added during 1.5 h. The mixture was stirred (24 h; 20 °C), then filtered through Celite, the filtrate being treated with aq. sodium dithionite (4 g in 50 cm^3) and stirred for 15 min. The solution was extracted with chloroform and the combined extracts were filtered through Celite to remove insoluble osmium residues. The filtrate was now washed successively with aq. sodium thiosulfate, saturated aq. sodium hydrogen carbonate, water, and brine, then dried ($MgSO_4$), and evaporated to form a foam, which was crystallised from acetone–ethanol to give the *title mixture of diastereoisomers* (14.5 g, 80%). Recrystallisation from THF gave large prisms, m.p. 151.5–152.5 °C (Found: C, 67.55; H, 5.45%; M^+, 410.138. $C_{23}H_{22}O_7$ requires C, 67.3; H, 5.4%; *M*, 410.137); ν_{max}/cm^{-1} 1720, 1685 and 1610; δ_H 1.48 and 1.49 (3 H, s, 12a-Me), 2.33 (3 H, s, 8'-H_3), 3.39 (2 H, abx, 4'-H_2), 3.82 and 3.93 (each 3 H, s, OMe), 4.33 (2 H, m, 6-H^a and 6a-H), 4.73 (1 H, m, 6-H^b), 5.22 (1 H, m, 5'-H), 6.39 (1 H, s, 4-H), 6.67 (1 H, d, *J* 8.6, 10-H), 7.67 (1 H, s, 1-H) and 7.89 (1 H, d, *J* 8.6, 11-H).

Deuteriomethylenation of (6aS,12aR,5'R)-/(6aR,12aS,5'R)-12a-*Methylrotenone 6'-Norketone.*—[$^2H_3$]Methyltriphenylphosphonium iodide (9.2 g, 22.6 mmol) in anhydrous THF (200 cm^3) was treated with butyllithium (15.0 cm^3; 15 mol dm^{-3} solution) and stirred for 10 min under nitrogen. The 12a-methylrotenone diastereoisomers (above) (9.1 g, 22.3 mmol) were added and the mixture was stirred at 20 °C for 2 h. The product was poured into water, then extracted with diethyl ether, and the product was worked up in the usual way and purified by chromatography on silica, with ethyl acetate–hexane (1:4) as eluent. Crystallisation from acetone–ethanol gave (6aS,12aR,5'R)-/(6aR,12aS,5'R)-12a-*methyl*-[7'-$^2H_2$]*rotenone* **32** (7.0 g, 76%) as prisms, m.p. 126.5–127.5 °C (Found: M^+, 410.168. $C_{24}H_{22}{}^2H_2O_6$ requires *M*, 410.166); δ_H 1.50 (3 H, s, 12a-Me), 1.80 (3 H, s, 8'-H_3), 3.19 (2 H, m, 4'-H_2), 3.83 and 3.94 (each 3 H, s, OMe), 4.36 (2 H, m, 6-H^a and 6aH), 4.75 (1 H, m, 6-H^b), 4.96 and 5.01 (each 0.06 H, s, together 7'-H_2), 5.36 (1 H, m, 5'-H), 6.39 (1 H, s, 4 H), 6.61 (1 H, d, *J* 8.5, 10-H), 7.70 and 7.71 (each 0.5 H, s, 1-H) and 7.87 (1 H, d, *J* 8.5, 11-H).

Treatment of (6aS,12aR,5'R)-/(6aR,12aS,5'R)-12a-*Methyl*-[7'-$^2H_2$]*rotenone with Benzenesulfenyl Chloride.*—12a-Methyl-[7'-$^2H_2$]rotenone (2.1 g, 5.12 mmol) was treated with benzenesulfenyl chloride (0.74 g, 5.14 mmol) under the usual conditions and the adduct was heated at 140 °C for 20 min under reduced pressure. The residue was taken up in DMF (25 cm^3) and the solution was treated with DBN (1.3 g, 10.5 mmol) and was then heated for 17 h at 65 °C under nitrogen. The product was poured into water and extracted with diethyl ether. The extracts were washed, dried ($MgSO_4$), and evaporated. Column chromatography on silica and elution with 7.5% ethyl acetate in hexane gave (6aS,12aR)-/(6aR,12aS)-12a-*methyl*-[7'-$^2H_2$]-4',5'-*didehydrorotenone* **33**, small prisms from ethyl acetate–hexane, m.p. 179–180 °C (Found: C, 70.75; H, 5.7%; M^+,

408.158. $C_{24}H_{20}{}^2H_2O_6$ requires C, 70.6; H, 5.9%; *M*, 408.154); ν_{max}/cm^{-1} 1685, 1610 and 1595; λ_{max}(EtOH)/nm 265 (log ε 4.64), 284infl (4.54) and 296infl (4.44); δ_H 1.53 (3 H, s, 12a-Me), 2.13 (3 H, s, 8′-H_3), 3.84 and 3.95 (each 3 H, s, OMe), 4.44 (2 H, m, 6a-H and 6-H^a), 4.86 (1 H, dd, *J* 10.8, 4.7, 6-H^b), 5.22 and 5.80 (each 0.1 H, s, together 7′-H_2), 6.41 (1 H, s, 4-H), 6.78 (1 H, s, 4′-H), 7.17 (1 H, d, *J* 8.7, 10-H), 7.72 (1 H, s, 1-H) and 7.91 (1 H, d, *J* 8.7, 11-H).

(6aS,12aR,5′R)-/(6aR,12aS,5′R)-12a-*Methyl*-[7′-^{2}H]*rotenone 7′-Phenyl Sulfone* **35**.—12a-Methyl-[7′-$^2H_2$]rotenone **32** (0.4 g, 0.97 mmol) was treated with benzenesulfenyl chloride (0.14 g, 0.97 mmol) under the usual conditions and the product was heated at 140 °C for 20 min under reduced pressure. MCPBA (0.39 g, 2.15 mmol) was added during 5 min to a solution of the product in dichloromethane (10 cm^3) at 0 °C and the mixture was stirred at room temperature for 1 h to form chloro sulfone **34**. Aq. sodium sulfite (10%; 2 cm^3) was added and the mixture was poured into diethyl ether (30 cm^3). The organic layer was washed successively with 10% aq. sodium carbonate (10 cm^3) and saturated brine, and dried ($MgSO_4$). The solution containing chloro sulfone **34** was evaporated and the foam was dissolved in dichloromethane (10 cm^3) and stirred with DBN (0.13 g, 1.05 mmol) at 40 °C for 1.5 h. The mixture was poured into 2% hydrochloric acid (25 cm^3), and diethyl ether (50 cm^3) was added to extract the product. The organic layer was washed with brine, dried ($MgSO_4$), evaporated, and chromatographed on silica; elution with ethyl acetate–hexane (1:4) allowed isolation of the mixture of vinyl and allyl sulfones. The latter mixture was further separated by semi-preparative HPLC on silica with ethyl acetate–hexane (1:4) as eluent. First eluted was a mixture of the allyl sulfone and one of the diastereoisomers of (6a*S*,12a*R*,5′*R*)- or (6a*R*,12a*S*,5′*R*)-(*E*)-12a-methyl-[7′-^{2}H]rotenone phenyl sulfone. This was followed by pure (6aS,12aR,5′R)- or (6aR,12aS,5′R)-(E)-12a-*methyl*-[7′-^{2}H]*rotenone 7′-phenyl sulfone* **35** as prisms from ethyl acetate–hexane, m.p. 117–119 °C (Found: C, 65.55; H, 5.3%; M^+, 549.158. $C_{30}H_{27}{}^2HO_8S$ requires C, 65.55; H, 5.35%; *M*, 549.159); ν_{max}(KBr)/cm^{-1} 1690, 1615 and 1510; λ_{max}(EtOH)/nm 235 (log ε 4.08) and 250infl (3.63); δ_H 1.50 (3 H, s, 12a-Me), 2.23 (3 H, s, 8′-H_3), 3.26 (2 H, abx, 4′-H_2), 3.83 and 3.94 (each 3 H, s, OMe), 4.35 (2 H, m, 6a-H and 6-H^a), 4.74 (1 H, dd, *J* 10.6 and 5.2, 6-H^b), 5.27 (1 H, dd, *J* 7.8, 5′-H), 6.39 (1 H, s, 4-H), 6.59 (1 H, dd, *J* 8.6, 10-H), 7.64 (3 H, m, SPh), 7.67 (1 H, s, 1-H), 7.87 (1 H, d, *J* 8.6, 11-H) and 7.94 (2 H, m, SPh).

Acknowledgements

One of us (S. J. P.) thanks the SERC for a research studentship.

References

1 D. Carson, M. W. Cass, L. Crombie, I. Holden and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1982, 773.
2 D. H. G. Crout and J. A. Corkill, *Tetrahedron Lett.*, 1977, 4355.
3 D. J. Aberhart and C. Tann, *J. Chem. Soc., Perkin Trans. 1*, 1979, 939.
4 K. Utimoto, M. Kitai and H. Nozaki, *Tetrahedron Lett.*, 1975, 2825.
5 H. Sakurai, K. Nishiwaki and M. Kira, *Tetrahedron Lett.*, 1973, 4193.
6 R. L. Merker and M. J. Scott, *J. Am. Chem. Soc.*, 1963, **85**, 2243.
7 H. Gilman and R. A. Tomasi, *J. Org. Chem.*, 1962, **27**, 3647.
8 W. H. Mueller and P. E. Butler, *J. Am. Chem. Soc.*, 1968, **90**, 2075.
9 W. A. Thaler, W. H. Mueller and P. E. Butler, *J. Am. Chem. Soc.*, 1968, **90**, 2069.
10 P. B. Hopkins and P. L. Fuchs, *J. Org. Chem.*, 1978, **43**, 1208.
11 K. D. Gunderman, *Angew. Chem., Int. Ed. Engl.*, 1963, **2**, 674.
12 P. J. Babidge and R. A. Massy-Westropp, *Aust. J. Chem.*, 1981, **34**, 1745.
13 R. Mozingo, *Org. Synth.*, 1941, **21**, 15.
14 N. A. Khan, *Science*, 1953, **117**, 130.
15 G. R. Brown and D. Wright, *Experientia*, 1976, **32/33**, 277.
16 M. J. Begley, L. Crombie, A. Hamid bin A. Hadi and J. L. Josephs, *J. Chem. Soc., Perkin Trans. 1*, 1989, 204.
17 R. A. Benkeser, M. L. Burrows, L. E. Nelson and J. V. Swisher, *J. Am. Chem. Soc.*, 1961, **83**, 4385.
18 L. Crombie and P. J. Godin, *J. Chem. Soc.*, 1961, 2861.
19 J. J. Boam, R. S. Cahn and R. F. Phipers, *J. Chem. Soc.*, 1938, 513.

Paper 2/01161A
Received 3rd *March* 1992
Accepted 6th *March* 1992