Mechanism and Stereochemistry of the Enzyme-catalysed Formation of a 2,2-Dimethylchromene Ring from a Prenylated Phenol: Conversion of Rot-2'-enonic Acid into Deguelin by Deguelin Cyclase

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The stereochemistry of the enzymic conversion of rot-2'-enonic acid into deguelin, mediated by deguelin cyclase, has been studied. Using both an enzyme preparation and seedlings of *Tephrosia vogellii*, it is shown that (6a*S*,12a*S*,5'*R*/*S*)-5'-hydroxy-4',5'-dihydro[6',6'-C^3H_3]deguelin is not an acceptable intermediate: no evidence for other oxygenated intermediates was found.

The (*pro-R*)- and (*pro-S*)-6'-methyl groups of deguelin were identified by synthesis from [(*E*)-4'-^{13}C]rot-2'-enonic acid. Addition of benzeneselenenyl chloride gives two diastereoisomeric 5'-(phenyl selenides) of 4',5'-dihydrodeguelin which are separated and their stereochemistry established by X-ray crystallography. Elimination of selenoxide from the (5'*S*)-stereoisomer then gives (6'*R*)-deguelin (δ_C 28.20): (6'*S*)-deguelin has δ_C 28.52.

Although a chemical conversion of [4'-^{13}C]rot-2'-enonic acid into labelled deguelin produces a 1:1 distribution of label between the (*pro-R*)- and (*pro-S*)-6'-methyls, the enzyme-mediated conversion results unexpectedly in a 76% incorporation into the (*pro-R*)- and 24% into the (*pro-S*)-form. The stereochemistry of the removal of the key 1'-hydrogens in rot-2'-enonic acid was therefore examined.

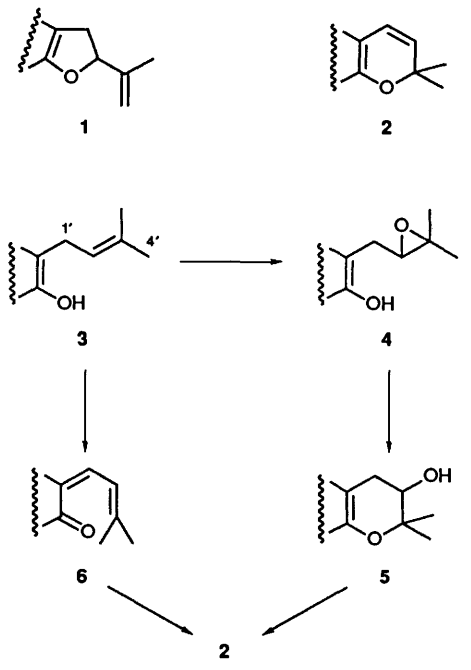
Addition of benzenesulfenyl chloride to deguelin gave a highly reactive chloro sulfide by *syn*-addition through attack from the less hindered β-face of the molecule. Treatment with sodium cyanoborohydride displaced the reactive chlorine with complete inversion to give (6a*S*,12a*S*,5'*S*)-5'-phenylthio-4',5'-dihydrodeguelin. Ring-E scission of the latter proceeded satisfactorily using sodium naphthalenide only after reduction of the 1,2-carbonyl to the alcohol: periodinane oxidation then produced rot-2'-enonic acid. Replacement of the unlabelled cyanoborohydride by cyanoborotritide gave the desired (6a*S*,12a*S*,1'*S*)-[1'-^{3}H]rot-2'-enonic acid. The (6a*S*,12a*S*,1'*R*)-[1'-^{3}H]-counterpart was made by first preparing [4'-^{3}H]deguelin by *syn*-elimination from the sulfoxide formed from (6a*S*,12a*S*,4'*R*,5'*S*)-5'-phenylthio-4',5'-dihydro-[4'-^{3}H]deguelin. Addition of benzenesulfenyl chloride to the [4'-^{3}H]deguelin, followed by a sequence parallelling that above, using unlabelled sodium cyanoborohydride, gave the required (6a*S*,12a*S*,1'*R*)-[1'-^{3}H]rot-2'-enonic acid.

Enzymic conversion of each [^{3}H]-labelled rot-2'-enonic acid into deguelin along with a [^{14}C]-labelled monitor, shows that a 73% loss of (*pro*-4'*S*-H) in rot-2'-enonic acid correlates with a 76% attainment of a (*pro*-6'*R*-Me) in deguelin, whilst a 27% loss of (*pro*-4'*R*-H) in the former correlates with a 24% attainment of a (*pro*-6'*S*-Me) in the latter. The possible enzymic mechanism of the reaction is discussed and related to a similar mechanism we have suggested for the enzymic formation of rotenone from rot-2'-enonic acid.

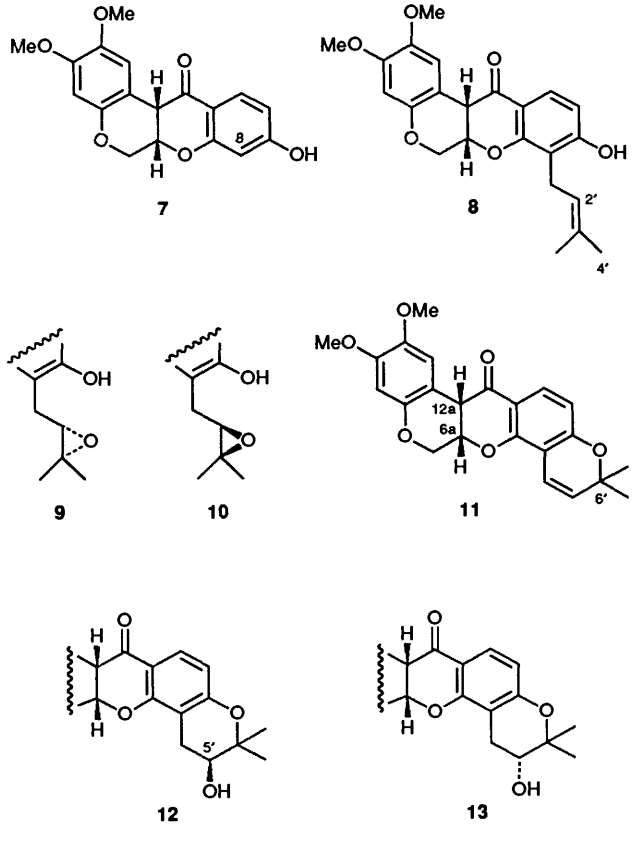
Two of the most ubiquitous structural features of natural products chemistry—particularly of flavonoid chemistry—are the 2-isopropenyl dihydrofuran system **1** and the 2,2-dimethylchromene system **2**. We have recently discussed the biosynthetic origins of the former which, like the latter, is represented in the rotenoid group of natural products.[1] It originates from the *ortho*-prenylated phenol **3**, and though little is known about the biosynthesis it is thought that the 2,2-dimethylchromene system has similar origins. Overall, the reaction is a formal dehydrogenation, and two mechanisms have been suggested, one proceeding through the epoxidised prenyl phenol **4** followed by cyclisation (**5**) and dehydration, the other involving a dienone (*o*-quinone methide) **6** which undergoes electrocyclisation to give the 2,2-dimethylchromene **2**.[2,3] There are several oxidative possibilities by which the dienone structure might be reached.

Biosynthesis in the rotenoid group involves, first, the construction of a rotenoid core, *e.g.* 9-demethylmunduserone **7**, followed by prenylation at C-8 to give rot-2'-enonic acid **8**.[4] The natural 2,2-dimethylchromene we wished to study was (6a*S*,12a*S*)-deguelin **11**, but it is not found in appreciable quantities in *Amorpha fruticosa* or *Derris elliptica*, the plants we used in our earlier biosynthetic work. We have therefore developed a satisfactory seedling system from *Tephrosia vogellii* which produces deguelin along with other rotenoids. Administration of [4'-^{3}H]- and [4'-^{14}C]-labelled (6a*S*,12a*S*)-rot-2'-enonic acid showed that they were well incorporated into deguelin (0.8–1.1%) after a 48 h interval, leaving no doubt as to the efficiency of compound **8** as a precursor. However, the (6a*S*,12a*S*,5'*R*/*S*)-alcohol **12**/**13**, made by epoxidation (**9**/**10**) and acid-catalysed cyclisation of [4'-^{3}H]-**8**, was not significantly incorporated into deguelin. There is, therefore, no evidence from whole-plant experimentation that the pathway proceeds *via* an hydroxylated intermediate of this type.[5]

The conversion of compound **8** into deguelin *via* a quinone methide intermediate (*cf.* **6**) might involve an isolable hydroxylated intermediate, followed by a dehydration step, or a direct dehydrogenation process, and it seemed highly desirable that these, and stereochemical features, should be studied as isolated enzymic processes rather than in a whole-plant system. A crude enzyme preparation from homogenised and centrifuged 3–5-day-old *T. vogellii* seedlings (grown 48 h in light) was found to convert the prenylated rot-2'-enonic acid **8** into the dimethylchromene deguelin **11** in almost quantitative yield, and this finding, along with the discovery that the soluble enzyme



(which we have named deguelin cyclase) was present in ungerminated seeds, has led us to isolate and characterise it. The isolation and properties of deguelin cyclase are described elsewhere.[6] It requires O_2 but no cofactors for activity, and is inhibited by chloride ion or by 1,10-phenanthroline and other chelating agents. Iron and copper were detected. It appears not to belong to the P450 group and seems to resemble the iron protein isopenicillin-N synthase.[7] The enzyme does not convert rotenonic acid into rotenone.

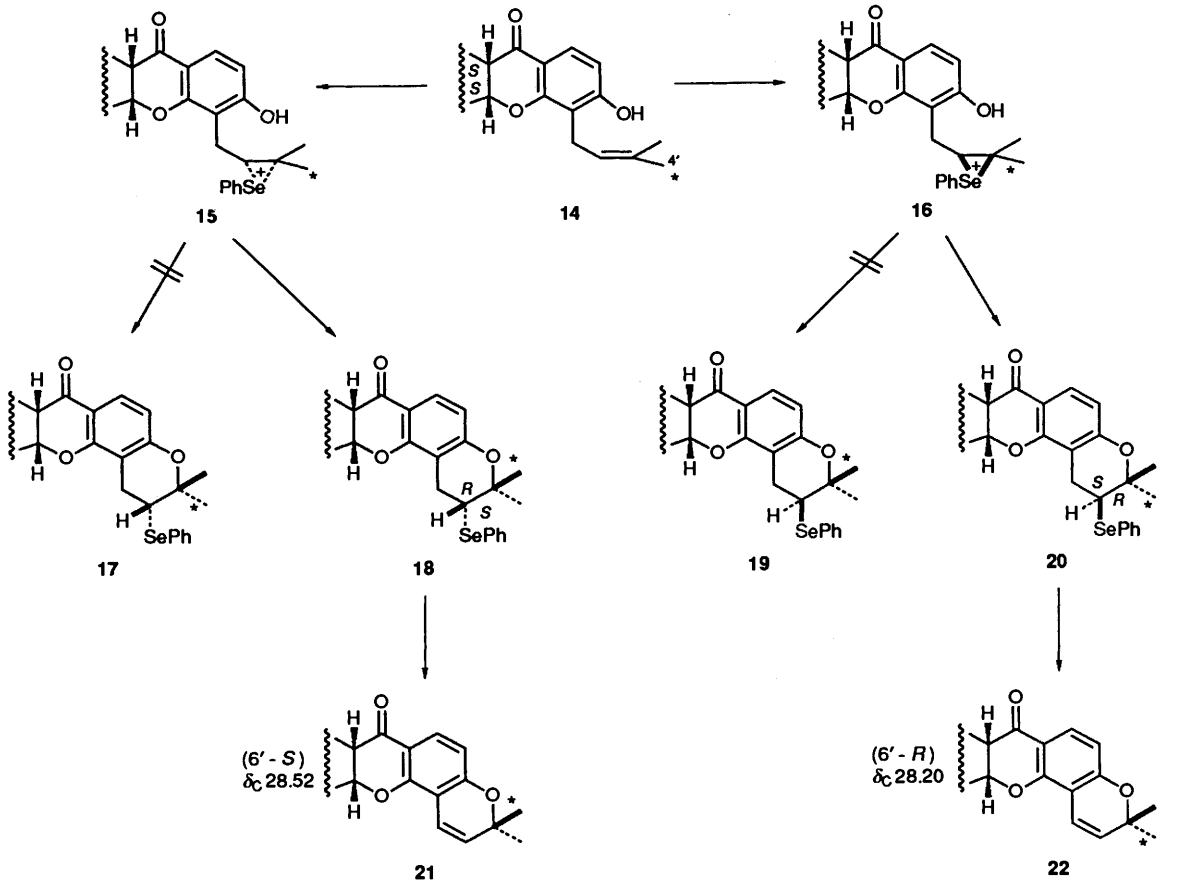


On incubating (6a*S*,12a*S*)-[4′-^{14}C]rot-2′-enonic acid **8** with the enzyme preparation, deguelin was formed but no hydroxylated intermediates could be detected using radioactively monitored HPLC. The [^{3}H-Me_2]-chromanol mentioned above, as mixed diastereoisomers (**12**/**13**), was again not converted into deguelin and we were led to the presumption that as in the conversion of compound **8** into rotenone, the cyclisation to form deguelin probably involved no oxygenated intermediates. With these preliminaries completed we turned our attention to the stereochemical problems involved in the conversion of rotenonic acid into deguelin.

Without further qualification, the methyl groups in a 2,2-dimethylchromene such as compound **2** are enantiotopic, but when placed in a chiral natural product such as deguelin **11** the two methyl groups become non-identical (diastereotopic) by virtue of the remote chirality at C-6a and C-12a. Each methyl has its own distinctive ^{1}H (δ 1.33 and 1.43) or ^{13}C (δ_C 28.20 and 28.52) NMR shift. It therefore appeared of interest to relate back each of these two methyl groups, formed after enzyme reaction, to each of the two *C*-methyl groups of the precursor rot-2′-enonic acid **8**. We have already solved the problem of identification and specific isotopic labelling of the (4′-*E*) or (5′-*Z*)-methyls of (6a*S*,12a*S*)-rot-2′-enonic acid,[8,9] and we must now identify the *C*-methyl NMR signals of (6a*S*,12a*S*)-deguelin with the appropriate (*pro-R*)- and (*pro-S*)-methyl groups. The strategy adopted was as shown in Scheme 1. The rot-2′-enonic acid used in the experiment was made by hydrogenolysis of [7′-^{13}C]rotenone and had a distribution of ^{13}C-label of 89% in C-4′ and 11% in C-5′. The latter labelling, whilst useful in providing a C-5′ marker, had to be corrected for.

(6a*S*,12a*S*)-[4′-^{13}C]Rot-2′-enonic acid (**14** = **8**), when treated with benzeneselenenyl chloride,[10,11] gave only two crystalline (phenylseleno)chromans, which were separated by HPLC. On examination of the ^{13}C NMR spectra (with correction for the initial label distribution) each (phenylseleno)chroman was found to be labelled in only one of its methyl groups. The (phenylseleno)chroman with m.p. 164–165 °C showed its labelled group at δ_C 27.73 with the second methyl at δ_C 23.25. The other, m.p. 185–186 °C, had its labelled methyl group at δ_C 28.49 with the second methyl at δ_C 21.91. Clearly the cyclisation was highly selective, proceeding *via* phenylselenonium ions **15** and **16** as expected. Attack with inversion therefore gives the *R,S*/*S,R* products **18** and **20** and not the *R,R*/*S,S* ones **17** and **19**. The problem of assigning the correct stereochemistry to the former pair was solved by determination of the X-ray single-crystal structure of the compound with m.p. 164–165 °C (carried out by Dr. M. J. Begley of our laboratory), which proved to be structure **20**. Oxidation by hydrogen peroxide, followed by elimination from the selenoxides, gave product **21** from **18** and product **22** from **20**. The chromene **21** therefore has the (6′-*pro-S*)-methyl at δ_C($CDCl_3$) 28.52 labelled, and its isomer **22** has the (6′-*pro-R*)-methyl at δ_C 28.20 labelled. This information allowed a study of the biosynthetic stereochemical problem to be undertaken.

To guard against any possible 'racemisation' at C-6′ caused by photochemical formation of *o*-quinone methides, work was conducted in the dark or in subdued light in the presence of a sample of deguelin specifically labelled at C-6′ as an external monitor. The monitor was examined at the end of the experiment to ensure that no positional scrambling of the ^{13}C-label had occurred. Subsequent studies have shown that the process is not initiated to any significant extent by normal sunlight over 5 days in ethanol under nitrogen. In $CDCl_3$ in air there was rapid deterioration but this was due mainly to oxidative processes. For quantitative work, the test sample (2 mg) of (6a*S*,12a*S*)-rot-2′-enonic acid, labelled (100%) at the 4′-(*E*)-methyl by ^{13}C-enrichment (90%), and made by the boron tribromide–cyanoborohydride route from [7′-^{13}C]rotenone,[9] was incubated (6 h) with a cell-free enzyme preparation from *T. volgellii* seeds at pH 7.6 and 25 °C. It was completely converted



Scheme 1 Identification and isotopic labelling of the (*R*)- and (*S*)-methyls of deguelin

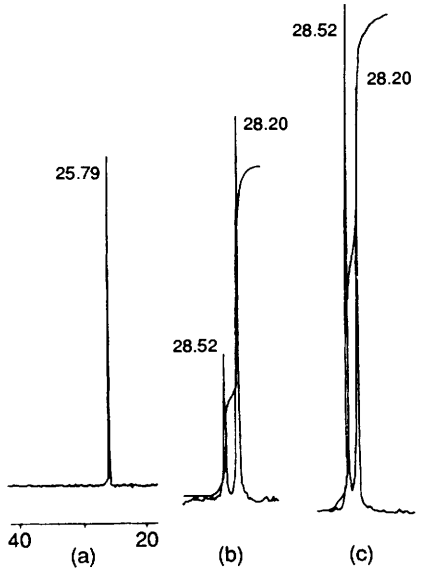


Fig. 1 ^{13}C Resonances from the NMR spectra of (a) [4'-^{13}C]rot-2'-enonic acid, (b) deguelin from the enzymic cyclisation, and (c) deguelin from the chemical oxidative cyclisation. Chemical-shift scales are not identical

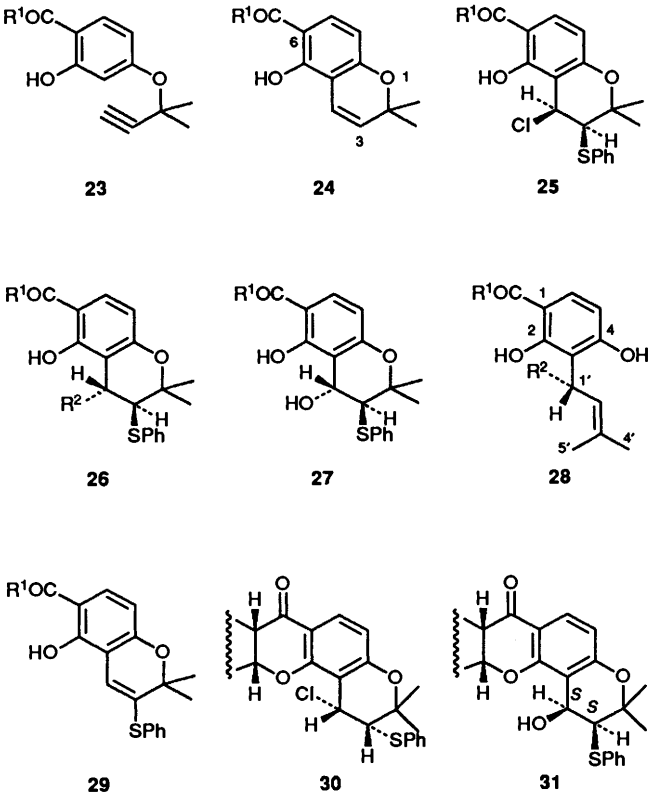
into (6a*S*,12a*S*)-deguelin, and ^{13}C NMR analysis [Cr(acetylacetonate)$_3$ and inverse gated decoupling] (Fig. 1) showed the label to be distributed 76% in the (C-6'-*pro*-*R*) and 24% in the (C-6'-*pro*-*S*) positions. (This differs a little from the value 73:27 given in our preliminary communication,[5b] as a correction has been applied for endogenous deguelin of normal ^{13}C abundance carried by the enzyme preparation.) A second experiment, carried out at the pH optimum of the enzyme (6.8) and temperature optimum (35 °C), gave an identical result.

A third experiment was carried out, using ^{13}C-labelled rot-2'-enonic acid made by the catalytic hydrogenolysis (Pd/pyridine) route from [7'-^{13}C]rotenone.[8] This method gave a sample of rot-2'-enonic acid with relative [^{13}C]-labelling 11% at C-5' and 89% at C-4'. Consequently a correction must be applied for this, thereby making the experiment less accurate than the first two. Correcting for the distribution (and for deguelin of natural abundance in the sample) gave enzymically formed (6a*S*,12a*S*)-deguelin having 72% of the [^{13}C]-label in the *pro*-*R* and 28% in the *pro*-*S*. There is thus, within experimental error, satisfactory agreement with the results from the first two experiments.

For comparison, [4'-^{13}C]rot-2'-enonic acid was cyclised to (6a*S*,12*S*)-deguelin by a chemical method using oxygen with palladium acetate and copper(II) chloride as catalyst.[12] The deguelin so produced showed, within experimental error, no discernible labelling bias as between the (*pro*-*S*)-and (*pro*-*R*)-methyl groups, suggesting that the chirality at C-6a and C-12a had little influence on the cyclisation process (Fig. 1). The result of the enzyme-mediated cyclisation therefore seemed at first sight rather puzzling as we had expected [^{13}C]-label distributions of either 50:50 or 100:0 in favour of either the (*pro*-*R*) or (*pro*-*S*)-methyls. As mentioned earlier the action of deguelin cyclase involves the overall removal of two hydrogen atoms from rot-2'-enonic acid, one being the hydrogen of the phenolic hydroxy group, the other being a hydrogen from the prochiral 1'-methylene of the prenyl side-chain. We therefore decided to study the stereochemistry of the removal of hydrogen from this centre, but first the (*pro*-*R*)/(*pro*-*S*) nature of the two hydrogens had to be established, along with a suitable labelling method.

The strategy employed, which should be generally applicable,

was the construction of the desired chiral centre on a ring system where stereochemical control is more effective, followed by ring scission at a late stage. Model reactions were first carried out using as substrates the readily available chromenes **24** (R^1 = OMe), made from the alkyne derivative **23** by electrocyclisation,[13] and **24** (R^1 = Me), made by chromenylation using 4,4-dimethoxy-2-methylbutan-2-ol.[14] Initial experiments on ring cleavage (see below) using selenium chemistry were less promising than those using sulfur chemistry so the chromene **24** (R = OMe) was allowed to react with benzenesulfenyl chloride in dichloromethane at −30 °C to give the highly reactive (±)-*cis*-chloro sulfide **25** (R^1 = OMe). The *cis*-geometry was shown by a pyran 3-H/4-H NMR coupling constant of ~2.7 Hz (*cf.* ~9 Hz for *trans*-geometry): such *syn*-electrophilic addition to aryl-conjugated double bonds *via* an ion-pair mechanism rather than a bridged ion is well precedented[15] and formation of the ion-paired species will be assisted by the electron release of the *ortho*-oxygen atoms. Aqueous work-up or chromatography on silica gave the inversion product at C-4, the 3,4-*trans*-hydroxy sulfide **27** (R^1 = OMe). Borohydride reduction of **25** (R^1 = OMe) in diglyme afforded the 3-sulfide **26** (R^1 = OMe, R^2 = H), as did reduction with sodium cyanoborohydride along with some elimination product **29**. On using deuterioborohydride, clean deuteriation with C-4-inversion was observed, giving the sulfide **26** (R^1 = OMe, R^2 = ^{2}H), $J_{3',4'}$ 10 Hz: its stereochemical purity was confirmed by the single ^{2}H NMR signal at δ_D 3.15. Finally, reductive ring scission using potassium naphthalenide[16] unmasked both the phenol and the double bond from substrate **26** (R^1 = OMe, R^2 = H) to give, as required, the corresponding dimethylallylphenol **28** (R^1 = OMe, R^2 = H).



23 **24** **25**

26 **27** **28**

29 **30** **31**

The sequence devised above was then applied to the natural enzyme substrate rot-2′-enonic acid.[17] A further supply of (6a*S*,12a*S*)-deguelin, $[\alpha]_D^{25}$ −30.4 (MeOH),* which it is difficult to isolate from *T. vogellii*, was made by reconstructive synthesis from natural rotenone *via* (6a*S*,12a*S*)-rot-2′-enonic acid **8** by using Anzeveno's cyclisation procedure.[11] Reaction of deguelin **11** with benzenesulfenyl chloride (Scheme 2) gave the *cis*-addition product **32**, which was reduced with sodium cyanoborohydride in dry hexamethylphosphoric triamide (HMPA) to form the chroman **33** without affecting the C-12 carbonyl. A pair of diastereoisomers (**30**/**32**) epimeric at C-4′,5′ had originally been hoped for from the addition reaction with benzenesulfenyl chloride, but the reaction in either dichloromethane or tetrahydrofuran (THF) over the temperature range −60 to +25 °C was found to be highly stereoselective for addition to the less hindered β-face. The stereochemistry at C-5′ as shown was firmly established by NMR comparison with the (5′*R*)- and (5′*S*)-seleno-analogues of established configuration (the NMR spectra are very similar), and these deductions were unequivocally confirmed by an X-ray analysis of compound **33** carried out in our laboratory by Dr. M. J. Begley.

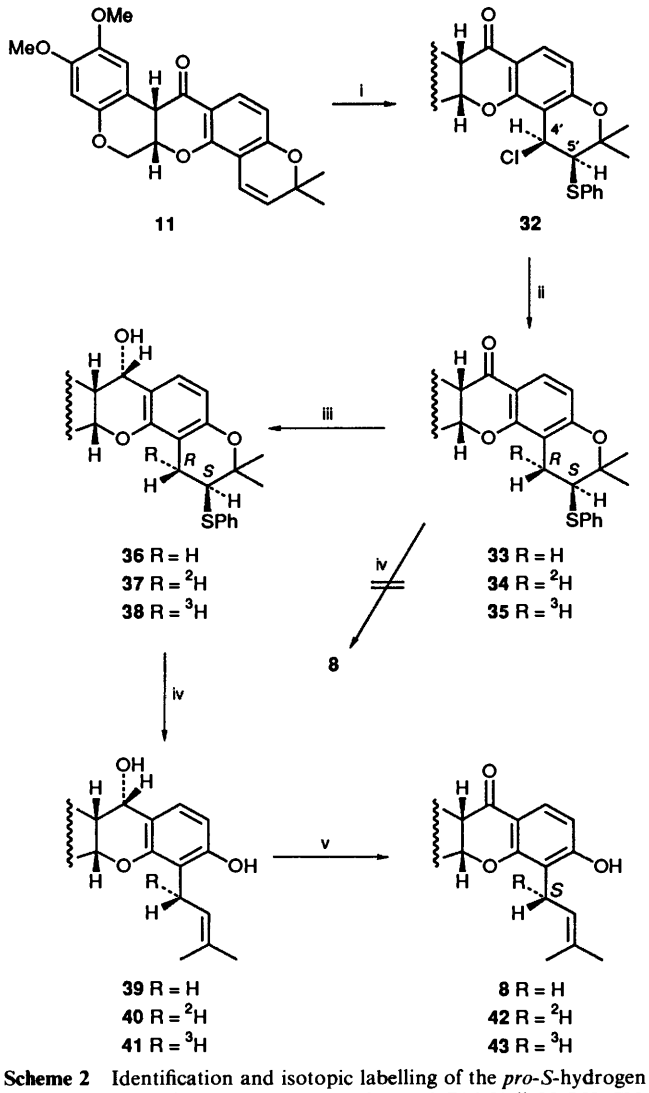
Unfortunately, reductive cleavage of the chroman **33** could not be effected cleanly with either potassium naphthalenide or with lithium naphthalenide over the temperature range −78 to +20 °C. Attempts using electrolytic cleavage at 1.5 and 1.3 V, employing a divided cell with a stirred pool of mercury as cathode and a saturated solution of tetraethylammonium bromide in dry dimethylformamide (DMF) as electrolyte, were also not successful. Carbonyl groups have remained unaffected in certain model compounds,[16] but it was felt that a possible seat of the difficulty in reductive ring scission was the rotenoid B/C system. The 12-carbonyl was therefore reduced with sodium borohydride to give the 12-hydroxy compound **36**. This latter was then smoothly reduced by potassium naphthalenide at −35 °C in 5 min to give the acid-labile phenol **39** which could be obtained in good yield by extraction from slightly alkaline solution. Finally, the 12-carbonyl group was reinstated by oxidation using the Dess–Martin reagent,[18] thereby giving (6a*S*,12a*S*)-rotenonic acid **8** in good yield.

The sequence from (6a*S*,12a*S*)-deguelin was now repeated, replacing the reduction of chloro compound **32** with sodium cyanoborohydride by sodium cyanoborodeuteride in dry HMPA, to give the 4′-deuterio compound **34**, precursor for **42**. Its stereochemistry was shown to be (4′*R*,5′*S*) by the diaxial coupling of the 4′,5′-hydrogen atoms, *J* 9.5 Hz ($J_{eq\text{-}ax}$ ~5 Hz), indicating an S_N2 reaction with clean inversion.† Using sodium cyanoborotritide, (6a*S*,12a*S*)-[1′*S*-^{3}H]rot-2′-enonic acid **43** was also prepared in the same way *via* intermediates **35**, **38** and **41**.

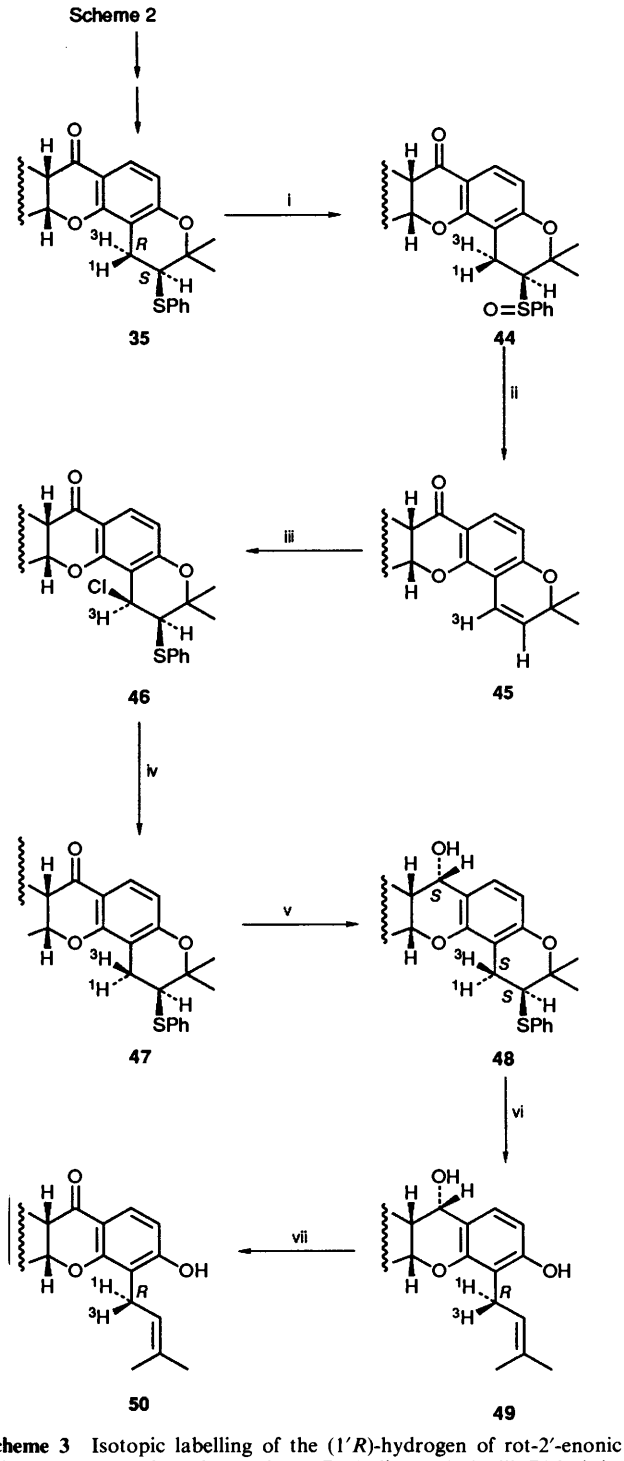
As mentioned above, it had been hoped that the stereochemistry of chlorosulfenylation of (6a*S*,12a*S*)-deguelin would be unspecific for the β-face, thereby giving us access by separation to *cis*-chloro sulfide **30**, the 4′,5′-epimer of the sulfide **32**, and hence access to tritium-labelled (1′*R*)-rot-2′-enonic acids. Since the reaction proved highly stereoselective, some other solution had to be found. Therefore (Scheme 3) the tritium-labelled sulfide **35** was oxidised with *m*-chloroperbenzoic acid (MCPBA) to give the sulfoxide **44**, a reaction that was followed by the pyrolytic elimination of benzenesulfenic acid in refluxing toluene, to give [4′-^{3}H]deguelin **45**. No tritium was lost in this *syn*-elimination and a reaction performed with a non-tritiated specimen gave a product having $[\alpha]_D^{25}$ −30.4, showing that no racemisation of the B/C system had occurred. The sulfone, as well as the sulfoxide, could be similarly pyrolysed to give optically pure deguelin. [4′-^{3}H]Deguelin was then converted into (1′*R*)-[1′-^{3}H]rot-2′-enonic acid **50** by the route outlined in Scheme 3.

* Following recent IUPAC recommendations, $[\alpha]_D$-values are now given in units of 10^{-1} deg cm^2 g^{-1}.

† In the reduction of chloro compound **32**, a side-product was formed by reaction with water, possibly on work-up. Curiously, unlike *trans*-**27**, it is the *cis*-β-form **31** having $J_{4',5'}$ 3.2 Hz, which appears to be formed from the chloro compound solvolytically with attack on the unbridged cation from the less hindered β-face of the bent rotenone structure.



Scheme 2 Identification and isotopic labelling of the *pro-S*-hydrogen of rot-2′-enonic acid. *Reagents and conditions:* i, PhSCl; ii, $NaBH_3CN$, $NaBD_3CN$, or $NaBT_3CN$; iii, $NaBH_4$; iv, $C_{10}H_7K$, −35 °C; Dess–Martin periodinane.



Scheme 3 Isotopic labelling of the (1′*R*)-hydrogen of rot-2′-enonic acid. *Reagents and conditions:* i, MCPBA; ii, pyrolysis; iii, PhSCl; iv, $NaBH_3CN$; v, $NaBH_4$; vi, $C_{10}H_7K$, −35 °C; vii, Dess–Martin periodinane

Biosynthetic oxidations of prenyl groups are a common feature of many natural products and we believe that the methods we have developed can be adapted to prenyl groups attached to other such structures to provide enabling tools for the study of stereochemistry and mechanism. With (1′*S*)- and (1′*R*)-(6a*S*,12a*S*)-[1′-^{3}H]rot-2′-enonic acids available, we were able to continue our study of the stereochemistry of the formation of deguelin.

Each tritiated rot-2′-enonic acid, *R* or *S*, was mixed with (6a*S*,12a*S*)-[4′-^{14}C]rot-2′-enonic acid to give a predetermined $^3H/^{14}C$ quotient, and incubated with *T. vogellii* enzyme preparation. The deguelin thus produced (Table 1) was isolated by HPLC and its $^3H/^{14}C$ quotient was measured. For the (1′*R*)-[1′-^{3}H]rotenonic acid supplied at a $^3H/^{14}C$ quotient of 3.56, the deguelin isolated in two experiments had $^3H/^{14}C$ quotients of 3.25 and 3.17 (mean 3.21) and the completeness of the two reactions, as judged from the [^{14}C]-count of unused rot-2′-enonic acid, was 96.0 and 97.3%. This indicates a relative quotient of (*S*-^{1}H)/(*R*-^{3}H) bond breaking of 90.2/9.8 but the selectivity is, of course, exaggerated because of isotope effects on the (*R*-^{3}H) cleavage.

Thus when (1′*S*)-[1′-^{3}H]rotenonic acid was supplied to the enzyme at a $^3H/^{14}C$ quotient of 4.44, the deguelin isolated had $^3H/^{14}C$ quotients of 2.52 and 2.35 (mean 2.44), the completeness of the reactions being 99.1 and 99.4% (Table 2). This indicates the relative rates of (*S*-^{3}H)/(*R*-^{1}H) to be 45/55. In this case the rate of (*S*-^{3}H) bond breaking is reduced by the ^{3}H isotope effect so that it is just slower than the (*R*-^{1}H) removal. Assuming that the kinetic isotope effect is the same for both 1′-hydrogen positions, it can be calculated that the relative rates of (*pro-S*-1′-H) removal/(*pro-R*-1′-H) removal is 73/27 and that a ^{3}H-isotope effect of 3.3 operates.

The labelling experiments carried out indicate that, within experimental error, there is a correlation between the enzymic loss of a (*pro-S*-1′)-hydrogen (73%) and the attainment of a (*pro-R*-6′)-methyl group (76%), and similarly the enzymic loss of a (*pro-R*-1′)-hydrogen correlates with the attainment of a

Table 1 Tritium retention in the formation of deguelin by administration of (1′*R*)-[1′-^{3}H]- and [4′-^{14}C]-rot-2′-enonic acids, **50** and **14**, to deguelin cyclase enzyme preparation

Expt.	Rot-2′-enonic acid administered (dpm) 10^{-3}[1′*R*-^{3}H]	10^{-3}[4′-^{14}C]	[^{3}H]/[^{14}C] Quotient	Incubation time (*t*/min)	Enzyme prepn. used[a] (*v*/cm^3)	Completeness of reaction[b] (%)	Deguelin produced [^{3}H]/[^{14}C] quotient
1	20.87	5.87	3.56	180	1.0	96.0	3.25
2	20.87	5.87	3.56	180	1.0	97.3	3.17
Mean			3.56				3.21

[a] In 0.1 mol dm^{-3} phosphate buffer. [b] Calculated from [^{14}C]-count of unused rot-2′-enonic acid.

Table 2 Tritium retention in the formation of deguelin by administration of (1′*S*)-[1′-^{3}H]- and [4′-^{14}C]-rot-2′-enonic acids, **43** and **14** to deguelin cyclise enzyme preparation

Expt.	Rot-2′-enonic acid administered (dpm) 10^{-3}[1′*S*-^{3}H]	10^{-3}[4′-^{14}C]	[^{3}H]/[^{14}C] Quotient	Incubation time (*t*/min)	Enzyme prepn. used[a] (*v*/cm^3)	Completeness of reaction[b] (%)	Deguelin produced [^{3}H]/[^{14}C] quotient
1	96.90	21.82	4.44	180	0.75	99.1	2.52
2	96.90	21.82	4.44	180	0.75	99.4	2.35
Mean			4.44				2.44

[a,b] As in Table 1.

(*pro-S*-6′)-methyl group. These data, together with the information we have gathered on deguelin cyclase, permit discussion of the mechanism involved.

Deguelin cyclase requires oxygen but no added cofactors and

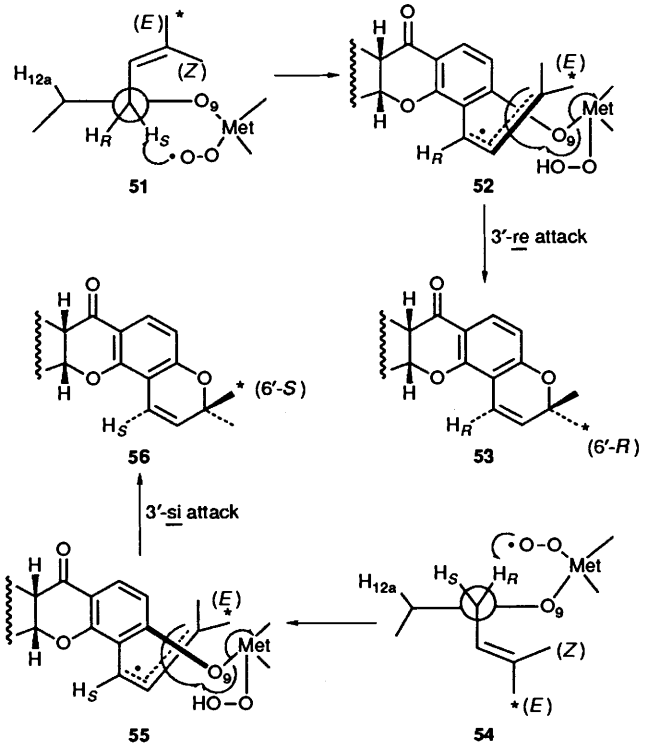
$$Lig_NMet^N + ArOH = Lig_{N-1}Met^NOAr + Lig^- + H^+$$

$$Lig_{N-1}Met^NOAr + O_2 = Lig_{N-1}Met^{N+1}(^*O_2)OAr \ (\mathbf{51})$$

$$\mathbf{51} \longrightarrow \mathbf{52} \longrightarrow \mathbf{53} + HOOMet^NLig_{N-1}$$

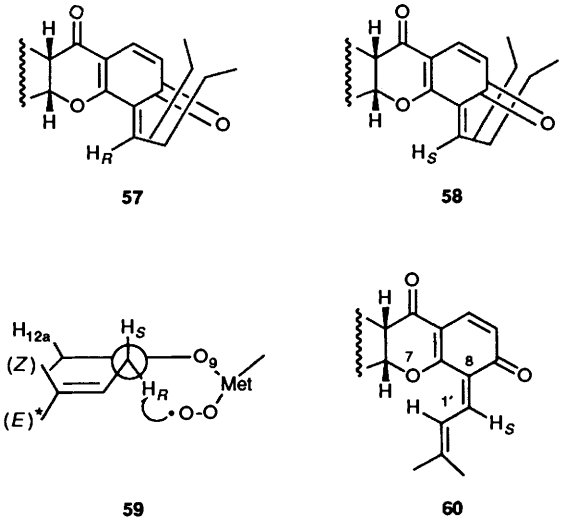
$$HOOMet^NLig_{N-1} + Lig^- + H^+ \longrightarrow H_2O_2 + Lig_NMet^N$$

Scheme 4 Suggested scheme for the dehydrogenation of rot-2′-enonic acid by deguelin cyclase

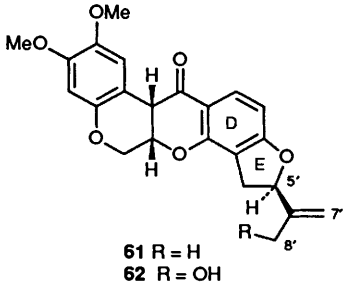


Scheme 5 Models for the enzymic formation of chromenes by deguelin cyclase

there is considerable evidence that a metal ion, probably iron, is involved at the active site.[6] A possible overall scheme for the enzyme's action is shown in Scheme 4 and a mechanistic model for conversion of the dimethylallylphenol rot-2′-enonic acid into the chromene deguelin is shown in Scheme 5. Removal of atom H_S in the enzyme–substrate complex **51** involving rotenonic acid [the substrate has (*E*)-labelling] leads to the radical **52**. 3′-*re*-Attack with rotation of the methyls then leads to the chromene **53** carrying H_R and with labelling in the (6′*R*)-methyl. The 73% loss of H_S correlates with the 76% attainment of (6′*R*)-methyl labelling. Similarly, the 27% loss of H_R (**54** and **55**) correlates *via* intermediate **55** with the 24% attainment of (6′-*S*)-methyl labelling **56**. It is possible that the radicals **52** and **55** could decompose to a state approaching the hypothetical dienones **57** and **58**, which now represent an extreme view of the situation, undergoing electrocyclisation to the chromene. Formula **59** shows the removal of H_R in a different and unfavourable conformation, and a similar diagram can be drawn for the removal of H_S. The transition state leading to product **60** would be compressed between O-7 and the 2′-hydrogen, and the product, shown in the extreme dienone form, is not capable of electrocyclisation without stereomutation about the 8,1′-double bond.

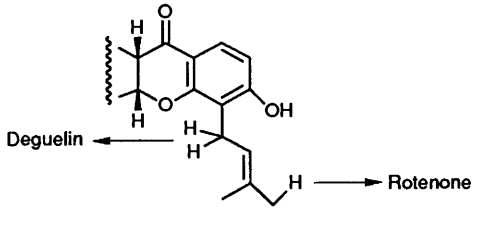


The results throw an interesting light on the precise overall stereochemistry of the *o*-prenylphenol-to-dimethylchromene cyclisation whilst revealing an 'untidy' situation insofar as the enzyme is concerned. Our expectation at the outset of this work was that the enzyme would be specific, or at least even-handed, as regards the attack on 1′-H_S and 1′-H_R and that a labelled methyl originating from the specifically labelled dimethylallyl substituent would emerge on the final chromene with either the label purely in the 6′*R* or the 6′*S* form, or evenly scrambled between the two. Assuming that a single cyclase is involved, it would appear that the enzyme is not evolutionarily perfected in this stereochemical sense and indeed there may be little evolutionary pressure for it to be so. Our stereochemical awareness of the enzyme–substrate complex comes only from the use of isotopes: from the point of view of the plant, the partition between removal of unlabelled 1′-H_R and 1′-H_S is of no consequence, starting material and cyclised product being the same in either case. However, evolutionary perfection could be envisaged if the enzyme structure were modulated so that a more precise sharpening of attack occurred with removal of, say, 1′-H_R to give a more efficient reaction than removal of either 1′-H_S or the existing 1′-H_R/1′-H_S system. This seems not to have been attained.



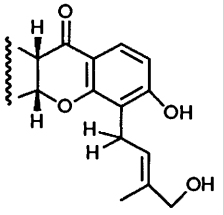
61 R = H
62 R = OH

In many classes of natural products, particularly the flavonoids,[19] dimethylchromenes of the deguelin type occur together with isopropenyldihydrofurans such as rotenone **61** and its hydroxylated relative amorphigenin **62**. There is circumstantial evidence that the biosynthesis of both types from a common prenylated precursor might involve similar enzymes and similar mechanisms removing either the 1′-H (chromene) or the 4′-H (isopropenyldihydrofuran) (see structure **63** = **8**). Indeed a microsomal preparation from elicitor-challenged soybean cell-suspension culture is reported to contain insoluble enzyme(s) of the P450 type which can convert the prenylated precursor pterocarpan into the dimethylchromene glyceolin II and into the isopropenyldihydrofuran glyceolin III.[20] The hypothetical metal-containing radical transition states (**52** and

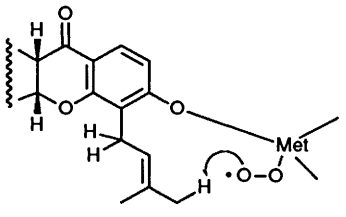


63 ≡ **8**

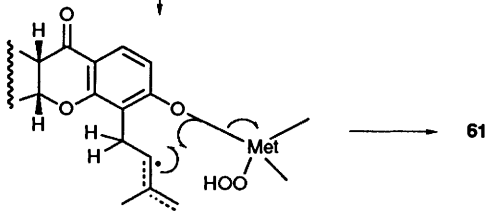
55) for formation of deguelin contain large and flexible pseudo-rings (*cf.* structures **51**/**52** and **54**/**55**) and models suggest that a similar transition metal-peroxyl radical is geometrically capable of abstracting a hydrogen atom from the (4′-*E*)-methyl group in complex **65** leading to the delocalised radical **66**, which is capable of forming the dihydrofuran ring as shown. It is also geometrically capable of removing a hydrogen atom from the (5′-*Z*)-methyl as is required in the conversion of 4′-hydroxy-rot-2′-enonic acid **64** into amorphigenin.[1] One may conjecture that the two enzymes have identical or very similar mechanisms of action, but that small differences in their amino acid sequence lead to clefts of different topology. The different positions of hydrogen abstraction in the substrate, forming dimethylchromenes or isopropenyldihydrofurans, would seem understandable on this basis.



64



65



66

Experimental

^{14}C and ^{3}H Radioactivity was measured using an Intertechnique SL 3000 or an LKB liquid scintillation counter. HPLC was carried out using a Waters system, mainly with RAD PAK (10 cm × 8 mm) columns. NMR spectra, normally run in $CDCl_3$, were recorded using Bruker AM400, WM250 and WP80SY spectrometers. For ^{2}H spectra a fluorine lock or an unlocked field was used, as necessary, with spectra run in $CHCl_3$. *J*-Values are given in Hz. IR data refer to KBr discs.

(6a*S*,12a*S*)-*Deguelin* **11**.—Benzeneselenenyl chloride (440 mg, 2.3 mmol) was added to a solution of rot-2′-enonic acid (830 mg, 2.095 mmol) in dry dichloromethane (10 cm^3) cooled to −35 °C under nitrogen.[11] The orange solution was stirred for 1 h at −35 °C and then for 1 h at room temperature. Evaporation gave a glass, which was dissolved in THF (30 cm^3) and treated with aq. hydrogen peroxide (30%; 0.45 cm^3) at 0 °C, and the mixture was stirred for 1 h and then at room temperature for 18 h. The product was diluted with diethyl ether (20 cm^3), washed successively with aq. sodium hydrogen carbonate (5%), and brine, dried (anhydrous $MgSO_4$), and evaporated to give a yellow solid. The latter was chromatographed on silica gel, and eluted with chloroform, to give (6a*S*,12a*S*)-deguelin as a pale yellow amorphous powder (680 mg, 82%); δ_H 1.39 (3 H, s, 6′-Me), 1.46 (3 H, s, 6′-Me), 3.78 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.84 (1 H, d, *J* 4.0, 12a-H), 4.19 (1 H, d, $J_{6,6}$ 12, 6-H), 4.64 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3, 6-H), 4.92 (1 H, m, 6a-H), 5.56 (1 H, d, $J_{5',4'}$ 10,

5′-H), 6.44 (1 H, s, 4-H), 6.46 (1 H, d, *J* 8.5, 10-H), 6.65 (1 H, d, $J_{5',4'}$ 10, 4′-H), 6.79 (1 H, s, 1-H) and 7.75 (1 H, d, *J* 8.5, 11-H).

5′-Hydroxy-4′,5′-dihydrodeguelin **12/13**.—A solution of rot-2′-enonic acid (250 mg, 0.631 mmol) in chloroform (2 cm^3) was added to an ice-cooled solution of MCPBA (150 mg, 0.75 mmol) in chloroform (2 cm^3). Toluene-*p*-sulfonic acid (PTSA) (50 mg) was added and the mixture was set aside for 3 h in an ice-bath. The mixture was poured into water and extracted with chloroform. The extracts were washed successively and thoroughly with saturated aq. sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated, and the product was chromatographed on silica with ethyl acetate–hexane (3:2) as eluent to give a mixture of 5′*R*/5′*S* diastereoisomers (66%). These were separated by HPLC on a Waters Z-module (silica) with ethyl acetate–hexane (3:2) as eluent, to give diastereoisomers A and B in elution order. *Diastereoisomer A* (59%) (Found: M^+, 412.153. $C_{23}H_{24}O_7$ requires M, 412.152) had δ_H 1.33 (3 H, s, CMe), 1.34 (3 H, s, CMe), 2.0 (1 H, s, OH), 2.69 (1 H, dd, *J* 6.0, 17.4, 4′-H^a), 2.95 (1 H, dd, *J* 5.1, 17.4, 4′-H^b), 3.77 and 3.81 (both 3 H, s, OMe), 3.8–3.9 obsc. (2 H, 5′- and 12a-H), 4.19 (1 H, br d, *J* 11.2, 6-H^a), 4.63 (1 H, dd, *J* 1.5, 11.2, 6-H^b), 4.92 (1 H, m, 6a-H), 6.44 (1 H, s, 4-H), 6.49 (1 H, d, *J* 8.8, 10-H), 6.78 (1 H, s, 1-H) and 7.75 (1 H, d, *J* 8.8, 11-H). *Diastereoisomer B* (41%) (Found: M^+, 412.151. $C_{23}H_{24}O_7$ requires *M*, 412.152) had δ_H 1.27 (3 H, s, CMe), 1.40 (3 H, s, CMe), 2.0 (1 H, s, OH), 2.77 (1 H, dd, *J* 4.8, 17.7, 4′-H^a), 2.90 (1 H, dd, *J* 5.0, 17.7, 4′-H^b), 3.77 and 3.81 (both 3 H, s, OMe), 3.8–3.9 obsc. (2 H, 5′- and 12a-H), 4.19 (1 H, dd, *J* 0.8, 12.1, 6-H^a), 4.64 (1 H, dd, *J* 3.1, 12.1, 6-H^b), 4.92 (1 H, m, 6a-H), 6.45 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.8, 10-H), 6.79 (1 H, s, 1-H) and 7.76 (1 H, d, *J* 8.8, 11-H).

5-Hydroxy-4′,5′-dihydro[6′,6′-C^3H_3]*deguelin* cf. **12/13**.—[4′-^{3}H]Rot-2′-enonic acid (50 mg, 0.13 mmol; 2.5 × 10^7 dpm/mg) was added to chloroform (800 mm^3) containing MCPBA (30 mg, 0.15 mmol) and the mixture containing the epoxides **9** and **10** was cooled to 0 °C. PTSA (10 mg) was added and the reaction mixture was allowed to warm to room temperature during 3 h. Work-up as above, followed by HPLC on silica, with hexane–ethyl acetate (65:35) as eluent, gave the title compound in 62% yield as two separable diastereoisomers in the ratio A:B 61:39 at an activity of 2.917 mCi/mmol. It was administered to the enzyme and the seedlings as the mixture.

Administration of Radioactive Precursors to Tephrosia vogellii *seedlings.—T. vogellii* seeds (National Seed Storage Laboratory, Colorado University, Ft. Collins, Colorado, USA) were sown on sterile, damp tissue paper in pre-sterilised seed trays. The trays were covered with aluminium foil, placed in an incubator, and watered daily. After three days the outer seed coat was removed from those seeds which had germinated and they were resown in separate trays and incubated for a further two days in the dark. The healthy seedlings were then removed from the trays, washed with deionised water, and placed in sterile plastic petri dishes (50 seedlings in each) to await administration of labelled compound.

The labelled precursor was made up in phosphate buffer (pH 8–9) and administered to the seedlings (usually 250). The seedlings were grown on under a strong halogen lamp for 48 h. The seedlings were then ground in ethanol–water [10 cm^3 (1:1)] and the wet pulp was added to ethanol (100 cm^3) and the mixture was boiled (10 min), and filtered. The extraction was repeated three times, when the extracts were combined and evaporated under reduced pressure. After addition of water, the product was extracted into chloroform and the extracts were evaporated. The residue was chromatographed on silica, with hexane–ethyl acetate (7:3) as eluent, and the rotenoid fraction was then separated and examined in detail by HPLC on silica gel, with hexane–ethyl acetate (9:1) as eluent. If necessary, samples were diluted with unlabelled material.

Preparation of a Cell-Free Homogenate containing Deguelin Cyclase Enzyme from T. vogellii *Seedlings.—T. vogellii* seeds were germinated on moist tissue paper at 25–30 °C (3–5 days). Healthy seedlings were subjected to strong light for 48 h. Seedlings (wet weight 50 g) were washed with water and ground to a pulp in sodium phosphate buffer [200 cm^3; pH 7.6; 0.1 mol dm^{-3}, with 1 mmol of dithiothreitol (1 mmol)]. The pulp was mixed with Polyclar AT (1 g per 2 g wet weight of seedlings), stirred for 1 h, and filtered through several layers of silk cloth. The filtrate was centrifuged at 18 000 *g* for 15 min and the supernatant was carefully decanted and similarly recentrifuged. The resulting homogenate was stored at 4 °C: all procedures were carried out at 4 °C.

Treatment of Rot-2′-enonic Acid **8** *with Benzeneselenenyl Chloride.*—Unlabelled rot-2′-enonic acid (157 mg, 0.396 mmol) was suspended in dry dichloromethane (5 cm^3) under nitrogen and cooled to −30 °C, when benzeneselenenyl chloride (82 mg, 0.424 mmol) was added. The orange solution was allowed to warm up to room temperature (1 h) and was then stirred for 2 h. Evaporation left an ~1:1 mixture of phenyl selenides contaminated with a small amount of benzeneselenenyl chloride. The diastereoisomers were separated by HPLC on μ-Porasil with ethyl acetate–hexane (1:9) as eluent. The least polar component (6aS,12aS,5′S)-5′-(*phenylseleno*)*dihydrodeguelin* (**20**, unlabelled) (89 mg, 42%), formed prisms from dichloromethane–methanol, m.p. 164–165 °C (Found: C, 62.7; H, 5.25. $C_{29}H_{28}O_6Se$ requires C, 63.15; H, 5.1%); $[\alpha]_D^{22}$ +70 (*c* 0.66, CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 1665; δ_H 1.43 (3 H, s, Me), 1.47 (3 H, s, Me), 2.85 (1 H, dd, $J_{4',4'}$ 17.6, $J_{4',5'}$ 9.2, 4′-H), 3.23 (1 H, dd, $J_{4',4'}$ 17.6, $J_{4',5'}$ 5.6, 4′-H), 3.41 (1 H, dd, $J_{4',5'}$ 5.6, $J_{4',5'}$ 9.2, 5′-H), 3.77 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.82 (1 H, d, obsc., 12a-H), 4.17 (1 H, d, *J* 12, 6-H), 4.58 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3.1, 6-H), 4.89 (1 H, m, 6a-H), 6.44 (1 H, s, 4-H), 6.44 (1 H, d, *J* obsc., 10-H), 6.78 (1 H, s, 1-H), 7.22–7.31 (3 H, m, SePh), 7.53–7.59 (2 H, m, SePh) and 7.64 (1 H, d, *J* 8.8, 11-H); δ_C 23.25 (CH_3, 6′-Me), 25.84 (CH_2, C-4′), 27.73 (CH_3, 6′-Me), 44.32 (CH, C-12a), 46.46 (CH, C-5′), 55.84 (CH_3, OMe), 56.40 (CH_3, OMe), 66.25 (CH_2, C-6), 72.43 (CH, C-6a), 79.33 (C, SeC_{ar}), 101.04 (CH, C-4), 104.98 (C, C-12b), 108.63 (C, C-8), 110.75 (CH, C-1), 112.16 (CH, C-10), 126.86, 127.83, 129.17 and 134.54 (6 × CH, C-11 and SePh), 143.94 (C, C-2), 147.53 (C, C-4a), 149.56 (C, C-3), 159.65 (C, C-7a), 160.27 (C, C-9) and 189.26 (C, C-12).

Crystal data: monoclinic, $C_{29}H_{28}O_6Se$·MeOH, *M* = 583.52.

The more polar (6aS,12aS,5′R)-5′-(*phenylseleno*)*dihydrodeguelin* (**18**, unlabelled) (96 mg, 44%), also crystallised from dichloromethane–methanol, m.p. 185–186 °C (Found: C, 63.25; H, 5.45. $C_{29}H_{28}O_6Se$ requires C, 63.15; H, 5.1%); $[\alpha]_D^{22}$ −315 (*c* 0.66, CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 1666; δ_H 1.32 (3 H, s, Me), 1.54 (3 H, s, Me), 2.32 (1 H, dd, $J_{4',4'}$ 17.5, $J_{4',5'}$ 11.5, 4′-H), 3.22 (1 H, dd, $J_{4',4'}$ 17.5, $J_{4',5'}$ 5.7, 4′-H), 3.37 (1 H, dd, $J_{4',5'}$ 11.5, $J_{4',5'}$ 5.7, 5′-H), 3.78 (3 H, s, OMe), 3.82 (3 H, s, OMe), 3.80–3.83 (1 H, obsc., 12a-H), 4.17 (1 H, d, *J* 12, 6-H), 4.61 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3.1, 6-H), 4.88 (1 H, m, 6a-H), 6.44 (1 H, d, *J* 8.8, 10-H), 6.47 (1 H, s, 4-H), 6.80 (1 H, s, 1-H), 7.25–7.32 (3 H, m, SePh), 7.57–7.62 (2 H, m, SePh), 7.73 (1 H, d, *J* 8.8, 11-H); δ_C 21.91 (CH_3, 6′-Me), 26.12 (CH_2, C-4′), 28.49 (CH_3, 6′-Me), 44.37 (CH, C-12a), 46.72 (CH, C-5′), 55.89 (CH_3, OMe), 56.37 (CH_3, OMe), 66.30 (CH_2, C-6), 72.57 (CH, C-6a), 79.68 (C, SeC_{ar}), 101.07 (CH, C-4), 104.98 (C, C-12b), 109.13 (C, C-8), 110.72 (CH, C-1), 112.08 (CH, C-10), 126.89, 127.92, 129.22 and 134.62 (6 × CH, C-11 and SePh), 129.50 (C, C-11a), 143.96 (C, C-2), 147.56 (C, C-4a), 149.59 (C, C-3), 159.44 (C, C-7a), 160.29 (C, C-9) and 189.26 (C, C-12).

(6aS,12aS)-*Deguelin from* (5′S)- *and* (5′R)-5′-(*Phenylseleno*)-

dihydrodeguelin.—A solution of unlabelled (5′*S*)-5′-(phenylseleno)dihydrodeguelin **20** (26.5 mg, 0.048 mmol) in THF (540 mm^3) at 0 °C was treated with aq. hydrogen peroxide (30%; 10 mm^3, ~0.90 mmol). The solution was stirred (1 h) at 0–5 °C and then at room temperature for 18 h. Diethyl ether (50 cm^3) was added and the ethereal extract was washed successively with aq. sodium hydrogen carbonate and brine, and dried ($MgSO_4$). Evaporation gave an oil, which was purified by HPLC on μ-Porasil with ethyl acetate–hexane (1:9) as eluent to give (6a*S*,12a*S*)-deguelin **11** (16 mg, 84%) as an oil, identical with authentic material; δ_H 1.39 (3 H, s, Me), 1.45 (3 H, s, Me), 3.77 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.84 (1 H, d, *J* obsc., 12a-H), 4.18 (1 H, d, *J* 12, 6-H), 4.64 (1 H, dd, $J_{6,6}$ 12, $J_{6,6a}$ 3.1, 6-H), 4.92 (1-H, m, 6a-H), 5.55 (1 H, d, *J* 10.1, 5′-H), 6.45 (1 H, s, 4-H), 6.45 (1 H, d, *J* 8.7, 10-H), 6.65 (1 H, d, *J* 10.1, 4′-H), 6.80 (1 H, s, 1-H) and 7.75 (1 H, d, *J* 8.7, 11-H). The (5′*R*)-isomer similarly gave (6a*S*,12a*S*)-deguelin, spectrally identical with authentic material.

Treatment of [4′-^{13}C]*Rot-2′-enonic Acid with Benzeneselenenyl Chloride*.—[4′-^{13}C]Rot-2′-enonic acid [17.8 mg, 0.045 mmol; 90% ^{13}C enrichment; ~85%(*E*) (δ_C 25.8)/15%(*Z*) (δ_C 17.8)], prepared by palladium-catalysed hydrogenation of [7′-^{13}C] rotenone, was mixed with unlabelled rot-2′-enonic acid (32.6 mg, 0.082 mmol) in dichloromethane (1.6 cm^3). The solution was treated with benzeneselenenyl chloride (26.4 mg, 0.137 mmol) at −30 °C. Treated as above, (5′*S*)-5′-(phenylseleno)dihydro-[6′*R*-^{13}C]deguelin **20** (30 mg, 43%) was obtained. It had δ_C 27.73 (6′*R*) with minor enrichment at δ_C 23.25 (6′*S*). Also obtained was (5′*R*)-5′-(phenylseleno)[6′*S*-^{13}C]deguelin **18** (29.2 mg, 42%); δ_C 28.49 (6′*S*) together with minor enrichment at δ_C 21.91 (6′*R*). The production of the two labelled compounds was verified by comparison of NMR spectra (above).

A similar experiment was carried out using rot-2′-enonic acid **8** labelled 100% at C-4′ with ^{13}C (90%). This was made by the boron tribomide reaction with [7′-^{13}C]rotenone which gives 4′-bromorot-2′-enonic acid: the latter was then treated with sodium cyanoborohydride as described in our earlier work. The ^{13}C signal (δ_C 25.79) is shown in Fig. 1.

(6a*S*,12a*S*)-[^{13}C]*Deguelin from* (5′*S*)- *and* (5′*R*)-5′-(*Phenylseleno)dihydro*[^{13}C]*deguelins*.—(5′*S*)-5′-(Phenylseleno)dihydro[6′*R*-^{13}C]deguelin **20** (from hydrogenolysis route: label distribution ~85:15 *E*:*Z*) (30 mg, 0.054 mmol) was treated as above with aq. 30% hydrogen peroxide (13 mm^3, ~0.12 mmol) in the dark. The product was isolated under subdued light to afford (6a*S*,12a*S*)-[6′*R*-^{13}C]deguelin **22** (18.1 mg, 84%), δ_C 28.20 (6′*R*-Me) (containing 86% of the label) and 28.52 (6′*S*-Me) (containing 14% of the label). The experiment was repeated using (5′*R*)-5′-(phenylseleno)dihydro-[6′*S*-^{13}C]deguelin **18** (29.2 mg, 0.053 mmol) to give (6a*S*,12a*S*)-[6′*S*-^{13}C]deguelin **21** (18.5 mg, 89%), δ_C 28.52 (6′*R*-Me) (containing 83% of the label) and 28.20 (6′*S*-Me) (containing 17% of the label).

[^{13}C]-*Labelled Deguelin by the Action of Deguelin Cyclase Enzyme Preparation on* [4′-^{13}C]*Rot-2′-enonic Acid*.—A cell-free homogenate was made as described above from *T. vogellii* seedings (51 g wet weight). The residual deguelin in the homogenate was 1.7 mg. The volume of the homogenate was made up to 75 cm^3 and samples (10 cm^3) were placed in each of six conical flasks. To each flask was added [4′-^{13}C]rot-2′-enonic acid made by the hydrogenolysis route (0.167 mg, with 89% of the label in C-4′ and 11% in C-5′). The flasks were incubated at 25 °C and shaken for 6 h. The homogenates were combined and extracted with diethyl ether. Unlabelled deguelin (7 mg) was added and the extract was dried ($MgSO_4$) and evaporated. The deguelin was purified by HPLC on silica with hexane–ethyl acetate (9:1) as eluent and the ^{13}C NMR spectrum of the purified material was recorded with the results described in the text.

In a similar way an experiment, employing [4′-^{13}C]rot-2′-enonic acid (2 mg) made by the 4′-bromorot-2′-enonic acid route and containing 100% of its ^{13}C at C-4′ (90% enriched), was carried out using homogenate from seedlings (81 g wet weight, carrying 10.1 mg of endogenous deguelin). The incubation time was 6 h, at 25 °C. The labelled material was completely converted into deguelin. The results (pH 7.6) are described in the text and in Fig. 1. No carrier deguelin was added. The experiment was repeated at pH 6.8 and 36 °C without any change in the result. ^{13}C-Enrichments were measured using $Cr(acac)_3$ (Hacac = pentane-2,4-dione) and inverse gated decoupling and appropriate corrections were applied for the content of deguelin of natural ^{13}C-abundance (or added material) in the sample (see text).

[^{13}C]-*Labelled Deguelin from Palladium Acetate/Copper*(II)/O_2-*Mediated Cyclisation of* (6aS,12aS)-[4′-^{13}C]*Rot-2′-enonic Acid*.—A mixture of (*E*)-[^{14}C]rot-2′-enonic acid (5 mg) and unlabelled material (25 mg), together with palladium(II) acetate (1 mg) and copper(II) chloride (6 mg) in methanol (2 cm^3)–water (0.2 cm^3), were stirred in a slow stream of oxygen at 65–70 °C for 16 h. The mixture was poured into brine and extracted with chloroform. Evaporation, followed by preparative TLC (PLC) on silica gel, with chloroform–propan-2-ol (99:1) as developer, gave [^{13}C]-labelled deguelin. A test sample co-chromatographed with authentic deguelin and gave the same blue colour with anisaldehyde spray reagent. It was examined by ^{13}C NMR spectroscopy (see text).

*Methyl 5-Hydroxy-2,2-dimethyl-*2H*-chromene-6-carboxylate* **24**, (R^1 = OMe).—Methyl 2,4-dihydroxybenzoate (20.96 g, 0.125 mol) was refluxed in dry acetone (250 cm^3) with 3-chloro-3-methylbutyne (19.21 g, 0.187 mol) and potassium carbonate (25.85 g, 0.187 mol) for 3 days. The product was poured into water, then extracted with chloroform, and the extracts were washed, dried (anhydrous $MgSO_4$), and evaporated to give an oil, which after chromatography on silica gel, with hexane–ethyl acetate (95:5) as eluent, gave *methyl* 4-(1,1-*dimethylprop*-2-*ynyloxy*)-2-*hydroxybenzoate* **23** (R^1 = OMe) (15.4 g, 53%) as needles from methanol, m.p. 90 °C (Found: C, 66.75; H, 6.2; M^+. 234.088. $C_{13}H_{14}O_4$ requires C, 66.65; H, 6.0%; *M*, 234.089).

The salicylate **23** (15.0 g, 0.064 mol) was refluxed in 1,2-dichlorobenzene (300 cm^3) under nitrogen for 24 h. After evaporation, the residue was chromatographed as above and the product was then distilled under reduced pressure (0.5 mmHg) to give the *title compound* **24** (R^1 = OMe) (13.26 g, 88%) as platelets from methanol, m.p. 80 °C (Found: C, 66.55; H, 6.1%; M^+, 234.090. $C_{13}H_{14}O_4$ requires C, 66.65; H, 6.00%; M^+, 234.089); δ_H 1.44 (6 H, s, 2-Me_2), 3.89 (3 H, s, CO_2Me), 5.56 (1 H, d, *J* 10, 3-H), 6.32 (1 H, d, *J* 8.8, 8-H), 6.71 (1 H, d, *J* 10, 4-H), 7.60 (1 H, d, *J* 8.8, 7-H) and 11.15 (1 H, s, chelated OH).

The corresponding *acid* **24** (R^1 = OH), prepared by hydrolysis, had m.p. 162–164 °C (Found: C, 65.25; H, 5.5. $C_{12}H_{12}O_4$ requires C, 65.45; H, 5.45%).

Methyl 5-Hydroxy-2,2-dimethyl-3-(phenylthio)chroman-6-carboxylate **26** (R^1 = OMe, R^2 = H).—Benzenesulfenyl chloride (0.80 g, 5.5 mmol) was added to a stirred solution of the chromene **24** (R^1 = OMe) (1.17 g, 5 mmol) in dry dichloromethane (25 cm^3) under nitrogen at −10 °C. After 1 h the solvent was evaporated off to give the chloro compound **25** (R^1 = OMe) as an oil.

The oil was dissolved in diglyme (25 cm^3) and the solution was stirred at 0 °C with sodium borohydride (400 mg, 10 mmol) for 30 min. The product was poured into water and worked up

with diethyl ether in the usual way. Chromatography on silica gel with light petroleum (boiling range 60–80 °C)–ethyl acetate (95:5) as eluent gave the *title compound* **26** (R^1 = OMe, R^2 = H) (1.28 g, 74%) as an oil (Found: C, 65.9; H, 6.15%; M^+, 344.1072. $C_{19}H_{20}O_4S$ requires C, 66.3; H, 5.8% *M*, 344.1082).

Hydrolysis with methanolic potassium hydroxide in the usual way gave the corresponding *acid* **26** (R^1 = OH, R^2 = H) (84%) as needles, m.p. 164.5 °C (from benzene–hexane) (Found: M^+, 330.090. $C_{18}H_{18}O_4S$ requires *M*, 330.093); δ_H 1.40 (3 H, s, 2-Me), 1.55 (3 H, s, 2-Me), 2.75 (1 H, dd, J_{ax} 10.0, J_{vic} 17.5, 4-H^{ax}), 3.14 (1 H, dd, J_{eq} 5.6, J_{vic} 17.5, 4-H^{eq}), 3.40 (1 H, dd, *J* 5.6, 10.0, 3-H^{ax}), 6.39 (1 H, d, *J* 8.9, 8-H), 7.2–7.5 (5 H, m, Ph), 7.2–7.5 (1 H, d, *J* 8.9, 7-H) and 10.86 (1 H, s, chelated 5-OH).

Methyl 5-Hydroxy-2,2-dimethyl-3-(phenylthio)[4-^{2}H]*chroman-6-carboxylate* **26** (R^1 = OMe, R^2 = ^{2}H).—In a similar experiment commencing with the chroman **25** (R^1 = OMe) (1.17 g, 5 mmol) and using sodium borodeuteride (400 mg, 9.5 mmol) for the reduction, the *4-deuterio compound* **26** (R^1 = OMe, R^2 = ^{2}H) was obtained (1.39 g, 80%) (Found: M^+, 345.113. $C_{19}H_{19}DO_4S$ requires *M*, 345.115); δ_H 2.72 (1 H, d, *J* 10.1, 4-H^{ax}) and 3.37 (1 H, d, *J* 10.1, 3-H^{ax}); δ_D 3.17.

Hydrolysis gave the *4-deuterio acid* **26** (R^1 = OH, R^2 = ^{2}H), m.p. 164.5 °C (Found: M^+, 331.099. $C_{18}H_{17}DO_4S$ requires *M*, 331.099); δ_H 1.40 (3 H, s, 2-Me), 1.54 (3 H, s, 2-Me), 2.73 (1 H, d, J_{ax} 10.1, 4-H^{ax}), 3.37 (1 H, d, *J* 10.0, 3-H^{ax}), 6.39 (1 H, d, *J* 8.9, 8-H), 7.2–7.5 (5 H, m, Ph), 7.69 (1 H, d, *J* 8.9, 7-H) and 10.82 (1 H, s, chelated 5-OH); δ_D 3.15.

The use of sodium cyanoborohydride in HMPA in place of sodium borohydride in diglyme led to some formation of the *elimination product* **29** (R^1 = OMe), m.p. 110 °C (from MeOH) (Found: C, 66.85; H, 5.3%; M^+, 342.092. $C_{19}H_{18}O_4S$ requires C, 66.65; H, 5.25%; M, 342.093), as well as the expected product **26** (R^1 = OMe, R^2 = H). The elimination product had δ_H 1.53 (6 H, s, 2-Me_2), 3.88 (3 H, s, CO_2Me), 6.35 (1 H, d, *J* 8.8, 8-H), 7.26 (1 H, s, 4-H), 7.60 (1 H, d, *J* 8.8, 7-H), 7.2–7.7 (5 H, m, SPh) and 11.07 (1 H, s, chelated 5-OH).

Potassium Naphthalenide.—Chips of potassium metal (1.1 g, 28.1 mmol) were added under a dry, inert atmosphere to a solution of naphthalene (4.2 g, 32.8 mmol) in dry THF (39 cm^3). A dark green colour formed and the mixture was stirred (2 h) to give a 0.94 mol dm^{-3} solution of potassium naphthalenide. For reaction purposes transfer was by syringe. Lithium naphthalenide was prepared similarly except that reaction was slower and the mixture was stirred for 3 h.

Treatment of the Chroman **26** (R^1 = OMe, R^2 = H) *with Potassium Naphthalenide.*—A solution of the chroman **26** (R^1 = OMe, R^2 = H) (235 mg, 0.68 mmol) in dry THF (5 cm^3) at −40 °C under nitrogen was treated with potassium naphthalenide (0.47 mol dm^{-3}). The solution was diluted with aq. potassium hydroxide (3 mol dm^{-3}; 20 cm^3) and quickly extracted with diethyl ether. The aqueous solution was acidified with conc. hydrochloric acid and was again extracted with diethyl ether. These extracts were washed, dried, and evaporated to give, after chromatography on silica gel [eluent hexane–ethyl acetate (95:5)], *methyl-3-(3′,3′-dimethylallyl)-2,4-dihydroxybenzoate* **28** (R^1 = OMe, R^2 = H) (89 mg, 55%) (Found: C, 66.1; H, 6.9%; M^+, 236.103. $C_{13}H_{16}O_4$ requires C, 66.1; H, 6.8%; *M*, 236.105); δ_H 1.74 [3 H, d, *J* 1.0, (*E*)-4′-H_3], 1.81 [3 H, s, (*Z*)-5′-H_3], 3.43 (2 H, br d, *J* 7, 1′-H_2), 3.89 (3 H, s, CO_2Me), 5.26 (1 H, t, with fine splitting, *J* 7, 1,2′-H), 6.35 (1 H, d, *J* 8.8, 5-H), 7.60 (1 H, d, *J* 8.8, 6-H) and 11.26 (1 H, s, chelated OH).

Methyl 4,5-Dihydroxy-2,2-dimethyl-3-(phenylthio)chroman-6-carboxylate **27** (R^1 = OMe).—Benzenesulfenyl chloride (320 mg, 2.2 mmol) in dichloromethane (20 cm^3) was added to a solution of the chromene **24** (R^1 = OMe) (468 mg, 2 mmol) in dichloromethane (20 cm^3) at −10 °C as above. The chloro compound was warmed with water to give the *title compound* (630 mg, 91%), which was obtained as an oil after chromatographic purification (silica gel; CH_2Cl_2). On prolonged storage the oil eventually crystallised (Found: C, 63.5; H, 5.6%; M^+, 360.102. $C_{19}H_{20}O_5S$ requires C, 63.35; H, 5.55%; *M*, 360.103); δ_H 1.44 (3 H, s, C-Me), 1.54 (3 H, s, C-Me), 3.42 (1 H, d, *J* 7.2, 3-H), 3.91 (4 H, s, CO_2Me and OH), 5.00 (1 H, dd, *J* 7.2, 2.2, 4-H), 6.39 (1 H, d, *J* 8.8, 8-H), 7.2–7.7 (6 H, m, 7-H and Ph) and 11.66 (1 H, s, chelated OH).

6-Acetyl-4-chloro-2,2-dimethyl-3-(phenylthio)chroman-5-ol **25** (R^1 = Me) *and its Hydrolysis Product* **27** (R^1 = Me).—A solution of 6-acetyl-2,2-dimethylchromen-5-ol **24** (R^1 = Me) (436 mg, 2 mmol) in dry dichloromethane (10 cm^3) at −10 °C was stirred with benzenesulfenyl chloride (316 mg, 2.2 mmol) in dry dichloromethane (20 cm^3) for 1 h. The solution was evaporated to give the addition product **25** (R^1 = Me) as an oil, δ_H 1.69 (6 H, s, 2-Me_2), 2.52 (3 H, s, COMe), 3.82 (1 H, d, *J* 2.7, 3-H), 5.38 (1 H, d, *J* 2.7, 4-H), 6.41 (1 H, d, *J* 8.9, 8-H), 7.2–7.7 (5 H, m, SPh), 7.62 (1 H, d, *J* 8.9, 7-H) and 13.5 (1 H, s, chelated 5-OH).

When this compound was warmed with water, the *hydrolysis product* **27** (R^1 = Me) was formed (592 mg, 86%), m.p. 113 °C (Found: M^+, 344.105. $C_{19}H_{20}O_4S$ requires *M*, 344.108); δ_H 1.44 (3 H, s, 2-Me), 1.56 (3 H, s, 2-Me), 2.49 (3 H, s, COMe), 3.42 (1 H, d, *J* 7.1, 3-H), 4.00 (1 H, br s, 4-OH), 5.01 (1 H, d, *J* 7.1, 4-H), 6.38 (1 H, d, *J* 8.9, 8-H), 7.2–7.7 (6 H, m, 7-H and SPh) and 13.6 (1 H, s, chelated 5-OH).

Benzenesulfenyl Chloride.—A small portion of thiophenol (100 mm^3) was added to a rapidly stirred suspension of *N*-chlorosuccinimide (1.37 g, 0.01 mmol) in dry dichloromethane (120 cm^3). The solution was warmed to initiate reaction and then thiophenol (1.06 cm^3, 0.01 mol) was added in portions with gentle heating of the mixture to sustain reflux. When all the thiophenol had been added the stirred solution was allowed to cool and gave an orange solution of benzenesulfenyl chloride (0.08 mol dm^{-3}).

Treatment of (6aS,12aS)*-Deguelin with Benzenesulfenyl Chloride, followed by Immediate Reduction with Sodium Cyanoborohydride.*—A solution of benzenesulfenyl chloride (80 mg, 0.55 mmol) in dry dichloromethane (4 cm^3) was added to a solution of (6a*S*,12a*S*)-deguelin (200 mg, 0.5 mmol) in dry dichloromethane (10 cm^3) at −35 °C and the mixture was stirred at that temperature for 1 h. The mixture was then allowed to warm to room temperature during 1 h and the solvent was evaporated off. The glass which remained was dissolved in dry HMPA (2.5 cm^3) and sodium cyanoborohydride (136 mg, 2.19 mmol) was added. After being stirred at room temperature for 2 h, the product was poured into water and thoroughly extracted with diethyl ether–hexane (4:1). The extracts were washed, dried ($MgSO_4$), and evaporated, and the pale yellow residue was purified and separated by PLC on silica gel with hexane–ethyl acetate (3:2, three developments) to give two products.

The less polar, (5′S)-*5′-phenylthio-4′,5′-dihydrodeguelin* **33** (110 mg, 43%) formed needles, m.p. 181–183 °C (from MeOH), which crystallised as the monomethanol solvate (Found: C, 66.8; H, 6.15. $C_{29}H_{28}O_6S$·MeOH requires C, 67.15; H, 6.0%. Found: M^+, 504.161. $C_{29}H_{28}O_6S$ requires *M*, 504.160); ν_{max}(KBr)/cm^{-1} 1675; δ_H 1.40 (3 H, s, 6′-Me), 1.45 (3 H, s, 6′-Me), 2.75 (1 H, dd, $J_{4',4'}$ 17.5, $J_{4',5'}$ 9.4, 4′-H), 3.15 (1 H, dd, $J_{4',4'}$ 17.5, $J_{4',5'}$ 5.6, 4′-H), 3.34 (1 H, dd, $J_{5',4'}$ 9.4, $J_{5',4'}$ 5.6, 5′-H), 3.76 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.83 (1 H, d, *J* 4.2, 12a-H), 4.16 (1 H, d, *J* 12.0, 6-H), 4.59 (1 H, dd, $J_{6,6}$ 12.0, $J_{6,6a}$ 3.2, 6-H), 4.91 (1 H,

m, 6a-H), 6.43 (1 H, s, 4-H), 6.47 (1 H, d, *J* 8.8, 10-H), 6.78 (1 H, s, 1-H), 7.2–7.35 (3 H, m, Ph 3″-, 4″- and 5″-H), 7.42 (2 H, d, *J* 8.0, Ph 2″- and 6″-H) and 7.74 (1 H, d, *J* 8.8, 11-H).

The more polar compound, (4′S,5′S)-4′-*hydroxy*-5′-*phenylthio*-4′,5′-*dihydrodeguelin* **31** (40 mg, 15%), formed needles, m.p. 183–184 °C (from MeOH) (Found: C, 67.0; H, 5.8. $C_{29}H_{28}O_7S$ requires C, 66.9; H, 5.4%) [Found ($M^+ - H_2O$) 502. $C_{29}H_{28}O_7S$ requires *M*, 520]; ν_{max}(KBr)/cm^{-1} 3440, 2922, 1670 and 1605; δ_H 1.48 (3 H, s, 6′-Me), 1.62 (3 H, s, 6′-Me), 3.49 (1 H, d, *J* 3.1, 5′-H), 3.77 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.85 (1 H, d, *J* 4.3, 12a-H), 4.17 (1 H, d, $J_{6,6}$ 12.0, 6-H), 4.42 (1 H, d, *J* 3.1, 4′-H), 4.54 (1 H, dd, $J_{6,6}$ 12.0, $J_{6,6a}$ 3.4, 6-H), 4.92 (1 H, m, 6a-H), 6.40 (1 H, s, 4-H), 6.50 (1 H, d, *J* 8.85, 10-H), 6.79 (1 H, s, 1-H), 7.35–7.4 (3 H, m, Ph 3″-, 4″- and 5″-H), 7.46 (2 H, d, *J* 7.0, Ph 2″- and 6″-H) and 7.83 (1 H, d, *J* 8.85, 11-H).

Oxidation of (5′S)-5′-*Phenylthio*-4′,5′-*dihydrodeguelin with* m-*Chloroperbenzoic Acid*.—A solution of MCPBA (60 mg, 0.35 mmol) in dry dichloromethane (2 cm^3) was stirred with (5′*S*)-5′-phenylthio-4′,5′-dihydrodeguelin (160 mg, 0.32 mmol) in dichloromethane (7.5 cm^3) at room temperature under nitrogen in the dark for 20 h. The mixture was diluted with chloroform (10 cm^3) and was washed successively with 5% aq. sodium hydrogen carbonate and water, and dried (anhydrous $MgSO_4$). Evaporation, and purification by PLC [silica gel; hexane–ethyl acetate (3:2, four developments)], gave, as the less polar product, (5′S)-5′-*phenylsulfinyl*-4′,5′-*dihydrodeguelin* (cf. **44**) (100 mg, 60%) as needles, m.p. 157–159 °C (from MeOH) [Found: C, 66.75; H, 5.5%; (M^+ – PhSOH), 394.145. $C_{29}H_{28}O_7S$ requires C, 66.9; 5.4%; (*M* – PhSOH) ($C_{23}H_{22}O_6$, 394.142]; δ_H 1.38 (3 H, s, 6′-Me), 1.84 (3 H, s, 6′-Me), 2.31 (1 H, dd, $J_{4',4'}$ 17.6, $J_{4',5'}$ 5.7, 4′-H), 2.68 (1 H, dd, $J_{5',4'}$ 12.3, $J_{5',4'}$ 5.7, 5′-H), 3.05 (1 H, dd, $J_{4',4'}$ 17.6, $J_{4',5'}$ 12.3, 4′-H), 3.76 (3 H, s, OMe), 3.79 (1 H, d, *J* 4.1, 12a-H), 3.84 (3 H, s, OMe), 4.14 (1 H, d, *J* 12.0, 6-H), 4.51 (1 H, dd, *J* 12.0, 3.3, 6-H), 4.78 (1 H, m, 6a-H), 6.45 (1 H, d, *J* 8.8, 10-H), 6.47 (1 H, s, 4-H), 6.72 (1 H, s, 1-H), 7.45–7.65 (5 H, m, Ph) and 7.70 (1 H, d, *J* 8.8, 11-H).

The more polar product was the corresponding *sulfone* (20 mg, 12%) [M^+ – $PhSO_4H$, 394. *M* – $PhSO_2H$ ($C_{23}H_{22}O_6$) requires *m*/*z*, 394]; δ_H 1.49 (3 H, s, 6′-Me), 1.77 (3 H, s, 6′-Me), 2.55 (1 H, dd, $J_{4',4'}$ 18.5, $J_{4',5'}$ 6.6, 4′-H), 2.84 (1 H, dd, $J_{4',4'}$ 18.5, $J_{4',5'}$ 5.5, 4′-H), 3.12 (1 H, dd, $J_{5',4'}$ 6.6, $J_{5',4'}$ 5.5, 5-H), 3.74 (3 H, s, OMe), 3.79 (4 H, s, OMe and 12a-H), 4.15 (1 H, d, $J_{6,6}$ 12.0, 6-H), 4.52 (1 H, dd, $J_{6,6}$ 12.0, $J_{6,6a}$ 3.2, 6-H), 4.75 (1 H, m, 6a-H), 6.29 (1 H, d, *J* 8.8, 10-H), 6.39 (1 H, s, 4-H), 6.72 (1 H, s, 1-H), 7.35–7.45 (3 H, m, Ph 3″-, 4″- and 5″-H) and 7.60 (3 H, m, Ph 2″- and 6″-H and 11-H).

(6aS,12aS)-*Deguelin* **11** *from Pyrolysis of the Sulfoxide*.—(6a*S*,12a*S*,5′*S*)-5′-Phenylsulfinyl-4′,5′-dihydrodeguelin (*cf.* **44**) (40 mg, 0.075 mmol) was refluxed under nitrogen in toluene (20 cm^3). Evaporation and PLC purification [silica; hexane–ethyl acetate (7:3, three developments)] gave optically pure (6a*S*,12a*S*)-deguelin as a pale yellow powder (25 mg, 85%), $[\alpha]_D^{25}$ –30.4 (*c* 0.56, MeOH); δ_H 1.39 (3 H, s, 6-Me), 1.46 (3 H, s, 6′-Me), 3.78 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.84 (1 H, d, *J* 4.0, 12a-H), 4.19 (1 H, d, *J* 12.0, 6-H), 4.64 (1 H, dd, *J* 12.0, 3.0, 6-H), 4.92 (1 H, m, 6a-H), 5.56 (1 H, d, *J* 10, 5′-H), 6.44 (1 H, s, 4-H), 6.46 (1 H, d, *J* 8.5, 10-H), 6.65 (1 H, d, *J* 10, 4′-H), 6.79 (1 H, s, 1-H) and 7.75 (1 H, d, *J* 8.5, 11-H).

Reduction of (6aS,12aS,5′S)-5′-*Phenylthio*-4′,5′-*dihydrodeguelin* **33** *with Sodium Borohydride*.—A solution of (6a*S*,12a*S*,5′*S*)-5′-phenylthio-4′,5′-dihydrodeguelin (50 mg, 0.1 mmol) in methanol (10 cm^3) was stirred with sodium borohydride (40 mg, 1.08 mmol) for 1 h at room temperature and was then poured into water and extracted with diethyl ether–hexane (5:1). Work-up gave (6a*S*,12*S*,12a*S*,5′*S*)-12-deoxo-12-hydroxy-5′-phenylthio-4′,5′-dihydrodeguelin **36** as an amorphous powder (45 mg, 90%); δ_H 1.38 (3 H, s, 6′-Me), 1.49 (3 H, s, 6′-Me), 2.73 (1 H, dd, $J_{4',4'}$ 17.6 ($J_{4',5'}$ 9.9, 4′-H), 3.14 (1 H, dd, $J_{4',4'}$ 17.6, $J_{4',5'}$ 5.7, 4′-H), 3.41 (2 H, m, 5′-H and 12a-H), 3.85 (3 H, s, OMe), 3.87 (3 H, s, OMe), 4.19 (1 H, m, 6-H), 4.56 (1 H, dd, $J_{6,6}$ 11.3, $J_{6,6a}$ 9.8, 6-H), 4.83 (1 H, m, 6a-H), 4.90 (1 H, d, *J* 3.0, 12-H), 6.46 (1 H, d, *J* 8.6, 10-H), 6.47 (1 H, s, 4-H), 6.69 (1 H, s, 1-H), 7.04 (1 H, d, *J* 8.6, 11-H), 7.25–7.38 (3 H, m, Ph 3″-, 4″- and 5″-H) and 7.46 (2 H, d, *J* 7.2, Ph 2″- and 6″-H).

(6aS,12aS)-*Rot*-2′-*enonic Acid* **8** from (6aS,12S,12aS,5′S)-12-*Deoxo*-12-*hydroxy*-5-*phenylthio*-4′,5′-*dihydrodeguelin* **36**.—A solution of potassium naphthalenide (0.47 mol dm^{-3}; ~2 cm^3, excess) was added to a stirred solution of 12-deoxo-12-hydroxy-5′-phenylthio-4′,5′-dihydrodeguelin (30 mg, 0.063 mmol) in dry THF (5 cm^3) at –40 °C until a green colour persisted. After the mixture had been stirred for 5–7 min at –40 °C, water was added and the product was extracted with diethyl ether. Purification by PLC on silica gel with hexane–ethyl acetate (3:2, three developments) gave the (12S)-*hydroxy reduction product of* (6aS,12aS)-*rot*-2′-*enonic acid compound* **39**, as needles (18 mg, 75%), m.p. 122 °C (from MeOH) [Found: 380.162. M – H_2O ($C_{23}H_{24}O_5$) requires *m*/*z*, 380.162]; ν_{max}(KBr)/cm^{-1} 3440, 2920, 1616 and 1510; δ_H 1.63 and 1.75 (6 H, s, 4′- and 5′-H_3), 3.27 (2 H, m, 1′-H), 3.35 (1 H, m, 12a-H), 3.74 (3 H, s, OMe), 3.75 (3 H, s, OMe), 4.16 (1 H, ddd, $J_{6,6}$ 10.1, $J_{6,6a}$ 5.2, $J_{6,12a}$ 1.1, 6-H), 4.51 (1 H, dd, $J_{6,6}$ 10.1, $J_{6,6a}$ 9.0, 6-H), 4.75 (1 H, m, 6a-H), 5.00 (1 H, d, *J* 4.1, 12-H), 5.16 (1 H, m, 2′-H), 6.36 (1 H, d, *J* 8.25, 10-H), 6.41 (1 H, s, 4-H), 6.94 (1 H, d, *J* 8.25, 11-H) and 7.05 (1 H, s, 1-H).

A solution of the 12-hydroxy compound **39** (65 mg, 0.163 mmol) in dichloromethane (4 cm^3) was stirred under nitrogen for 2 h with a solution of Dess–Martin periodinane (60 mg, 0.141 mmol) in dichloromethane (5 cm^3). The product was diluted with diethyl ether and washed successively with sodium thiosulfate in aq. sodium carbonate (0.1 mol dm^{-3}; 10 cm^3) followed by saturated aq. sodium hydrogencarbonate (10 cm^3) and water. After drying (anhydrous $MgSO_4$) and evaporation, the residue was purified by PLC on silica gel with hexane–ethyl acetate (3:2, two developments). (6a*S*,12a*S*)-Rot-2′-enonic acid **8** was obtained and was crystallised from chloroform–methanol as needles, m.p. 204 °C (24 mg, 40%). The NMR spectrum was identical with that of authentic material.

(4′R,5′S)-5′-*Phenylthio*-4′,5′-*dihydro*([4′-^{3}H]*deguelin* **35**.—A solution of (6a*S*,12a*S*)-deguelin (200 mg, 0.50 mmol) in dichloromethane (10 cm^3) was treated with benzenesulfenyl chloride (80 mg, 0.55 mmol) as before. Reduction of the product with sodium cyanoborotritide (~6 mCi) in HMPA (0.25 cm^3) followed by 'cold' sodium borohydride (136 mg, 2.19 mmol) gave, after PLC purification, (4′*R*,5′*S*)-5′-phenylthio-4′,5′-dihydro-[4′-^{3}H]deguelin **35** (105 mg, 41%), m.p. 181–183 °C (from MeOH); specific activity 4.46 × 10^9 dpm/mmol, and its (4′*S*)-4′-hydroxy derivative **31** (42 mg, 16%), m.p. 183–184 °C.

(6aS,12aS,1′S)-[1′-^{3}H]*Rot*-2′-*enonic Acid* **43**.—A solution of (4′*R*,5′*S*)-5′-phenylthio-4′,5′-dihydro-[4′-^{3}H]deguelin **35** (28 mg, 0.055 mmol) in methanol (4 cm^3) was treated with sodium borohydride (20 mg, 0.54 mmol) as above. Work-up gave (12*S*,4′*R*)-12-deoxo-12-hydroxy-4′,5′-dihydro-[4′-^{3}H]deguelin **38** as a powder (22 mg, 78%). It was diluted with 'cold' material (8 mg). Specific activity 4.4 × 10^9 dpm/mmol.

The 12-hydroxy compound **38** (25 mg, 0.049 mmol) as a solution in dry THF (2 cm^3) was treated as before with 0.47 mol dm^{-3} potassium naphthalenide in THF (~1.5 cm^3) to give the (1′*S*)-12-hydroxy-[1′-^{3}H]rot-2′-enonic acid derivative **41** (12 mg, 62%), m.p. 122 °C (from MeOH), which was diluted with 'cold' material (8 mg).

A solution of periodinane reagent (20 mg, 0.047 mmol) in dichloromethane (2 cm^3) was added to a solution of the hydroxy compound **41** (18 mg, 0.045 mmol). Work-up and PLC purification gave (6a*S*,12a*S*,1′*S*)-[1′-^{3}H]rot-2′-enonic acid **43** (12 mg, 67%) as needles, m.p. 204 °C (from MeOH), which was crystallised to constant count. Specific activity 1.27 × 10^9 dpm/mmol.

(6aS,12aS,4′R,5′S)-5′-*Phenylthio*-4′,5′-*dihydro*-[4′-^{2}H]-*deguelin* **34**.—Deguelin (225 mg, 0.57 mmol) was treated with benzenesulfenyl chloride (90 mg, 0.62 mmol) as before, and the product, as a solution in HMPA (2.5 cm^3), was treated with sodium cyanoborodeuteride (180 mg, 2.9 mmol) to give, after purification by PLC (6a,*S*,12a*S*,4′*R*,5′*S*)-5′-*phenylthio*-4′,5′-*dihydro*-[4′-^{2}H]*deguelin* **34** (140 mg, 48%) as the less polar product, m.p. 181–183 °C (M^+, 505. $C_{29}H_{27}DO_6S$ requires *M*, 505). In the ^{1}H NMR spectrum the signal for 4′-H lacked the usual geminal coupling and resonated at δ 2.74 as a 1 H-doublet, *J* 9.5; the 5′-H proton was a clear doublet at δ 3.33, *J* 9.5. The more polar product was (6a*S*,12a*S*,4′*S*,5′*S*)-4′-hydroxy-5′-phenylthio-4′,5′-dihydrodeguelin **31**, m.p. 183–185 °C.

(6aS,12aS)-[4′-^{3}H]*Deguelin* **45**.—A solution of deguelin (400 mg, 1.015 mmol) in dry dichloromethane (10 cm^3) at −35 °C was treated with benzenesulfenyl chloride (160 mg, 1.1 mmol) in dry dichloromethane (4 cm^3). The mixture was stirred (1 h) at −35 °C and then at room temperature (45 min). Evaporation gave a glass, which was dissolved in HMPA (2.5 cm^3) containing sodium cyanoborotritide (~6 mCi). 'Cold' cyanoborohydride (270 mg, 4.1 mmol) was then added to complete the reaction; the product was stirred at room temperature (2 h) and then poured into water. Work-up was followed by purification by PLC [silica; hexane–ethyl acetate (7:3, four developments)] to give (4′*R*,5′*S*)-5′-phenylthio-4′,5′-dihydro-[4′-^{3}H]deguelin **35** (160 mg, 32%) as needles, m.p. 181–183 °C (from MeOH) (specific activity 1.23 × 10^8 dpm/mmol). Also recovered in the chromatographic purification was (6a*S*,12a*S*,4′*S*,5′*S*)-4′-hydroxy-5′-phenylthio-4′,5′-dihydrodeguelin **31** (80 mg, 15%).

A solution of the tritiated product **35** (130 mg, 0.258 mmol) in dry dichloromethane (10 cm^3) was added to a solution of MCPBA (44 mg, 0.26 mmol) in dry dichloromethane (2 cm^3) and the mixture was stirred for 3 h at 20 °C, then diluted with chloroform. The solution was washed successively with aq. sodium hydrogencarbonate (5%) and water, and dried ($MgSO_4$). PLC purification [silica; hexane–ethyl acetate (3:2, two developments)] gave the (4′*R*,5′*S*)-[4′-^{3}H]sulfoxide **44** containing some [4′*R*-^{3}H]sulfone (total 120 mg, 89%), which was diluted with 'cold' sulfoxide (60 mg).

A solution of the mixture of sulfoxide **44** and its sulfone (180 mg) in toluene (20 cm^3) was refluxed (4 h) and the solvent was evaporated off to give a yellow residue. PLC purification [silica; hexane–ethyl acetate (3:2, three developments)] afforded optically pure (6a*S*,12a*S*)-[4′-^{3}H]deguelin **45** (120 mg, 88%) having specific activity 7.2 × 10^7 dpm/mmol. It was further diluted with 'cold' deguelin (80 mg).

(4′S,5′S)-5′-*Phenylthio*-4′,5′-*dihydro*-[4′-^{3}H]*deguelin* **47**.—A solution of (6a*S*,12a*S*)-[4′-^{3}H]deguelin (200 mg, 0.50 mmol) in dry dichloromethane (0.7 cm^3) was cooled to −35 °C and a solution of benzenesulfenyl chloride (80 mg, 0.55 mmol) in dry dichloromethane was added, the mixture being stirred for 1 h at −35 °C and then 1 h at room temperature. Evaporation gave a pale yellow residue **46**, which was dissolved in HMPA (2.5 cm^3), treated with sodium cyanoborohydride (170 mg, 2.74 mmol), and stirred at 20 °C for 90 min. Work-up, and purification by PLC as described earlier, gave the title compound **47** (84 mg, 33%). The corresponding (4′*R*)-hydroxy derivative (*cf.* **31**) (40 mg, 16%) was also isolated.

Reduction of (4′S,5′S)-5′-*Phenylthio*-4′,5′-*dihydro*-[4′-^{3}H]-*deguelin* **47** *with Sodium Borohydride*.—A solution of (4′*S*,5′*S*)-5′-phenylthio-4′,5′-dihydro-[4′-^{3}H]deguelin (74 mg, 0.147 mmol) in methanol (10 cm^3) was treated with sodium borohydride as described earlier and gave (6aS,12S,12aS,4′S,5′S)-12-deoxo-12-hydroxy-5′-phenylthio-4′,5′-dihydro-[4′-^{3}H]-deguelin **48** (68 mg, 93%) as a powder.

(6aS,12aS,1′R)-[1′-^{3}H]*Rot-2′-enonic Acid* **50**.—Potassium naphthalenide solution (0.47 mol dm^{-3}) was added in excess (~2.5 cm^3) to a solution of (6a*S*,12*S*,4′*S*,5′*S*)-12-deoxo-12-hydroxy-4′,5′-dihydrodeguelin **48** (65 mg, 0.128 mmol) in dry THF (5 cm^3) at −40 °C until a green colour persisted, when the mixture was stirred for 5 min at −40 °C. Customary work-up followed by PLC purification gave (12*S*,1′*R*)-12-hydroxy-[1′-^{3}H]rot-2′-enonic acid **49** (40 mg, 79%) as needles, m.p. 122 °C (from MeOH).

A solution of the latter (32 mg, 0.08 mmol) in dry dichloromethane (2 cm^3) was added to Dess–Martin periodinane reagent (37 mg, 0.088 mmol) in dry dichloromethane (2.5 cm^3) and the mixture was stirred for 3 h at room temperature. Work-up and customary purification by PLC afforded (6a*S*,12a*S*,1′*R*)-[1′-^{3}H]rot-2′-enonic acid **50** (20 mg, 63%) as needles, m.p. 204 °C (from MeOH). The sample was crystallised to constant activity (specific activity 2.3 × 10^7 dpm/mmol).

Preparation of a Cell-free Homogenate containing Deguelin Cyclase Enzyme from the Seeds of Tephrosia vogellii.—Powdered seeds of *T. vogellii* (100 g) were extracted with hexane (600 cm^3) at room temperature and then filtered. The residue was dried under reduced pressure and extracted with 0.1 mol dm^{-3} phosphate buffer (pH 6.95; 400 cm^3) containing Cleland's reagent (0.1 mmol) at 4 °C for 3 h. After centrifugation (4 °C; 18 000–20 000 rpm; 20 min) the solid residue was discarded. The supernatant (174 cm^3) was cooled to 0 °C and ammonium sulfate (56.72 g, 326 mg/cm^3) was added during 15 min (*i.e.*, 0–55% saturation). The solution was stirred at 0 °C (25 min) and then centrifuged. The residue was discarded and further ammonium sulfate (29.62 g, 161 mg/cm^3) was added during 15 min (55–88% saturation). The solution was stirred at 0 °C for 30 min and was then centrifuged at 4 °C (18 000–20 000 rpm; 20 min), the protein pellet which was formed then being resuspended in 0.1 mol dm^{-3} phosphate buffer (6 cm^3) containing 0.02% sodium azide. Application of the solution to a Sephadex G 25 M column [2.5 cm^3/column; elution with 0.1 mol dm^{-3} phosphate buffer (3.5 cm^3) containing 0.02% sodium azide] gave a fraction rich in deguelin cyclase enzyme, used in the biosynthetic work reported in Tables 1 and 2. For further details of the purification of deguelin cyclase see ref. 6.

Conversion of Labelled Rot-2′-enonic Acids into Labelled Deguelins by Deguelin Cyclase Enzyme.—A mixture of [1′-^{3}H]- and [4′-^{14}C]-rot-2′-enonic acids dissolved in ethanol (0.3–0.4 cm^3) was added to the cell-free homogenate (0.4–1.0 cm^3) in phosphate buffer (pH 6.95; 2 cm^3) containing deguelin cyclase and incubated at 32 °C for 2.3 h. Diethyl ether (2 cm^3) was added followed by 'cold' rot-2′-enonic acid and deguelin as carriers (2–4 mg). The products were extracted with diethyl ether–hexane (10:1) and the combined extracts were washed with water and evaporated. The reaction products were analysed by normal-phase HPLC [μ-Porasil; elution with hexane–ethyl acetate (4:1)]. The rot-2′-enonic acid and deguelin fractions were counted. Results are given in Tables 1 and 2 in which the enzyme reactions were allowed to go as

nearly as possible to completion. From the assumption that the hydrogen-isotope effect is the same for both the (1′*S*)- and the (1′*R*)-position, Tables 1 and 2 provide the data for two simultaneous equations from which the isotope effect and the relative extents of removal of ^1H from the two positions can be obtained.

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