Phytochemistry, 1978, Vol 17, pp. 1961–1965 ©Pergamon Press Ltd. Printed in England 0031-9422/78/1101-1961 $02.00/0

SECOBEYERENE DITERPENES FROM *BEYERIA CALYCINA**

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(*Received* 21 *March* 1978)

Key Word Index—*Beyeria calycina* var. *minor*; Euphorbiaceae; secobeyerene diterpenes; photochemistry; *Calonectria decora*; microbiological hydroxylation; biosynthesis.

Abstract—Two new secobeyerene acids have been isolated from *Beyeria calycina* var. *minor* and their structures identified as *ent*-6α,17-dihydroxy-3,4-secobeyer-15-en-3-oic acid and (4*S*)-*ent*-18-hydroxy-3,4-secobeyer-15-ene-3,17-dioic acid. Distinct pathways are involved in the formation of the former compound and the major seco acid component.

INTRODUCTION

The presence of the acetoxy ketol (**1**), the acetoxy keto acid (**2**) along with the A-ring secobeyerene (**3**) in *Beyeria calycina* var. *minor*† was reported in 1968 [1]. Interest in the mechanism of the ring fission process *in vivo* prompted us to carry out feeding experiments which showed incorporation into intact plants of the substrates *ent*-beyer-15-ene [17,19-$^3H_2$] and *ent*-beyer-15-en-19-ol [17-$^3H_1$] [2]. In a recent extension to this work, we have applied more modern isolation procedures to the plant material which is now shown to contain small quantities of two new seco compounds **4** and **6** which were isolated as the esters **5** and **7** after methylation of the acidic fraction and chromatography.

This paper describes the assignment of structure and stereochemistry to these esters, together with a feeding experiment indicating quite distinct pathways to the seco acid **3** and the 6-hydroxydihydro analogue **4**.

RESULTS AND DISCUSSION

The more abundant ester **5** was hydrolysed to the acid **4**, $C_{20}H_{32}O_4$ which shows singlets in the NMR spectrum, each for two protons, at δ 5.83 and 3.68 which are characteristic of the CD ring substitution of the congener **3**. The spectrum also showed a multiplet at δ 4.12 which is also seen in spectra of 6β-hydroxybeyerenes and is assigned to the 6α-H. Other features of the NMR spectrum include a singlet for the tertiary methyl at δ 0.78 and signals attributable to two secondary methyls. Confirmation of the structure thus indicated was sought by correlation with authentic material. The simplest route appeared to be by ring opening of the naturally occurring acetoxy ketol **1** by an established method and this was achieved as follows. The acetoxy ketol (**1**) was acetylated and converted to the oxime (**9**) which gave the nitrile under conditions of the Beckmann rearrangement [3]. Hydrolysis of this nitrile gave the seco acid (**10**) characterized as the methyl ester (**11**) which showed the spectroscopic properties of a 3,4-secobeyer-4(18)-ene compound. Hydrogenation of the acid then gave the saturated acid (**12**) identical to that obtained by hydrogenation of the naturally occurring material (**4**).

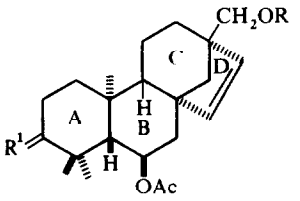
We have shown before that some 3,17-dioxygenated beyerenes are hydroxylated at C-6 by *Calonectria decora* [4] and another correlation was sought by hydroxylation of the known diol (**13**) [1] with this organism. After separation of the metabolites thus formed, the triol (**14**) was obtained in 20% yield. A reference sample of **14** was obtained by reduction of the acid (**10**) with $LiAlH_4$. This sequence confirmed the 6β-hydroxylation by *C. decora* on the one hand, and also secured the presence of the 15-ene in **4**.

The other minor seco acid (**6**) was isolated as the dimethyl ester (**7**), $C_{22}H_{34}O_5$. The NMR spectrum of **7** included a three proton singlet at δ 0.75 and a three proton doublet at δ 1.22, assigned to tertiary and secondary methyl groups, respectively, a six proton singlet at δ 3.63 for two methyl ester groups and a two proton singlet at δ 5.48 for two vinyl protons. A multiplet between δ 3.1 and 4.2 was shown by INDOR and decoupling experiments to be due to an AB spin system with J_{AB} = 10 Hz and with one extra coupling to the A and B proton (J = 3 and 10 Hz). These signals were attributed to a secondary hydroxymethylene group. On the basis of the spectral data and by analogy with the other resin constituents, the structure **7**, with undefined stereochemistry at C-4, was indicated and this was established by reduction of the derived tosylate (**15**) with $LiAlH_4$ to give the seco diol (**16**) which was hydrogenated to the known [1] saturated diol (**17**). The assignment of the Δ^{15} double bond in the diol (**16**) and hence in the seco diester (**7**) followed from the AB quartet (J_{AB} = 6 Hz) at δ 5.69 in the NMR spectrum of **16**, signals of this type being generally observed in the spectra ($CHCl_3$) of 17-hydroxybeyer-15-enes.

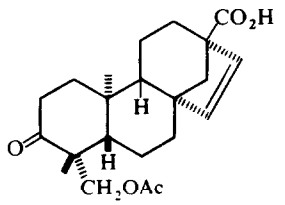
The structural feature remaining in doubt was the stereochemistry at C-4 and it was decided to prepare both epimers preparatory to settling this problem. To this end, the seco diester (**18**) was epoxidized with *m*-

*Part 25 in the series "The Chemistry of the Euphorbiaceae". Part 24 in (1976) *Aust. J. Chem.* **29**, 1809.

†Previously [1, 2] referred to as *B. leschenaultii* var. This has now been classified (Airy Shaw, H. K. (1971) *Kew Bull.* **26**, 67).



	R	R^1
1	H	O
9	Ac	NOH



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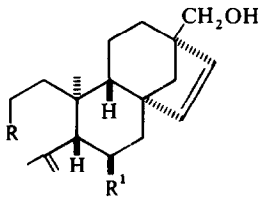
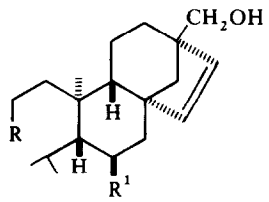
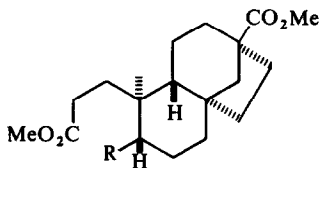
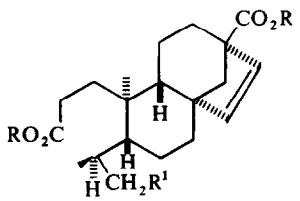


	R	R^1
3	CO_2H	H
10	CO_2H	OH
11	CO_2Me	OH
13	CH_2OH	H
14	CH_2OH	OH



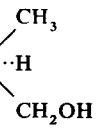
	R	R^1
4	CO_2H	OH
5	CO_2Me	OH
12	CO_2H	OH (15,16-H_2)
16	CH_2OH	H
17	CH_2OH	H (15,16-H_2)

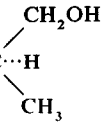


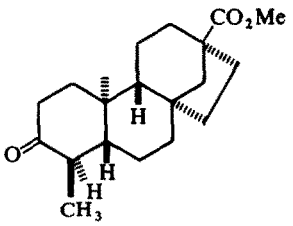


	R	R^1
6	H	OH
7	Me	OH
15	Me	OTs

18 R = 

19 R = 

20 R = 



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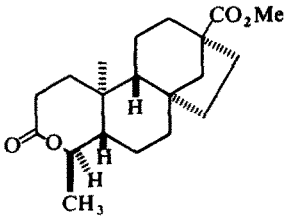

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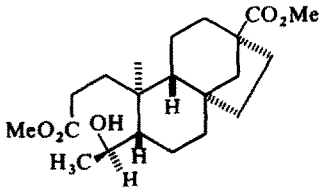

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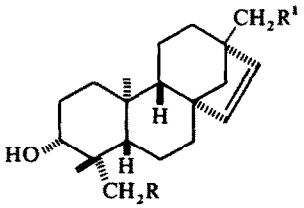

	R	R^1
24	OH	H
25	H	OH

chloroperbenzoic acid. The crude epoxide was then treated with BF_3-etherate to give a mixture whose NMR spectrum indicated similar quantities of epimeric aldehydes. Reduction with $NaBH_4$ and chromatography gave the separate isomers **19** and **20** readily distinguished by their NMR spectra. The more polar product (**20**) was identical to that prepared by hydrogenation of the diester (**7**) derived from the plant. The stereochemistry of **20** was then determined as follows. The norketoester (**21**), available from beyerol [5], was oxidized to the lactone (**22**) with *m*-chloroperbenzoic acid. Hydrolysis of the lactone and methylation gave the hydroxyester (**23**) which was identical to that prepared by oxidation of the seco diester (**19**) to the aldehyde with chromic acid–pyridine, followed by Baeyer–Villiger oxidation and hydrolysis. Since these oxidations are known [6] to proceed with retention of configuration the chirality at C-4 in the natural seco compound (**6**) is *S*.

There are many examples of natural A-ring seco acids with unsaturation at C-4. On the other hand analogous substances with C-4 saturated are very rare [7] although they are easily made by photolytic fragmentation of 3-oxo derivatives [8].

In considering the origin of the seco acids, the similarity in the level and position of oxygenation between the co-occurring acetoxy ketol (**1**) and the seco acid (**4**) on the one hand and the acetoxy keto acid (**2**) and the seco diacid (**6**) on the other is apparent and it seemed possible that ring opening might result from photolysis of these ketones during extended exposure of the resin on the leaf surface to the intense sunlight of the natural habitat. In line with this proposition, photolysis of the acetoxy ketol (**1**) and the methyl ester of **2** gave, after hydrolysis, and in the latter case methylation [9], the seco acid (**4**) and the seco diester (**7**), respectively. The retention of configuration of C-4 in the latter process will be discussed elsewhere [9].

There is no simple way to exclude a biochemical mechanism for the formation of **4** and the acid derived from **7**. However, we have shown [2] that the isopropenyl analogue (**3**) is labelled by *ent*-beyer-15-en-19-ol [17-^3H_1] during a few days under screening from UV radiation and we would predict negligible labelling for the dihydro analogue if it were derived by irradiation of the surface resin. To test this proposition a sample of *ent*-3β,19-dihydroxybeyer-15-ene [$3,19\text{-}^3H_2$] (**24**)* was prepared from the unlabelled diol by mild oxidation to give the keto aldehyde and then reduction with tritiated $LiBH_4$ [10]. When this substrate was fed to *B. calycina* var. *minor* and the resin harvested ten days later, significant labelling was detected in the seco acid (**3**), but after dilution and crystallization all activity was lost from the 6β-hydroxy-dihydro analogue (**4**). Similar results were obtained when *ent*-3β,17-dihydroxybeyer-15-ene [$3,17\text{-}^3H_2$] (**25**)* was used as a substrate.

*Evidence for the relative distribution of label (*ca.* 1:1) is given in the Experimental.

EXPERIMENTAL

General experimental details are as described previously [2, 11]. The NMR spectra were recorded for $CHCl_3$ solns at 60 MHz, unless otherwise stated.

Extraction of Beyeria calycina *var.* minor. Dried plant (700 g) was extracted with Et_2O and the acidic fraction (39 g) methylated (CH_2N_2) and chromatographed on Al_2O_3 (Act. 3, 800 g). Gradient elution with $CHCl_3$–petrol gave **2** (1.5 g) followed by the ester of **3** (14 g) and more polar material which was saponified, methylated and rechromatographed as above to give a further crop of this ester (6 g) and polar material which was crystallized from EtOAc. The ppt. (400 mg) was saponified and crystallized from EtOAc to give needles of ent-6α,17-*dihydroxy*-3,4-*secobeyer*-15-*en*-3-*oic acid* (**4**, 0.3 g), mp 167–169°, $[\alpha]_D$ +14 (*c*, 0.8, EtOH) (Found: C, 71.2; H, 9.5. $C_{20}H_{32}O_4$ requires: C, 71.4; H, 9.6%). IR ν_{max}^{Nujol} cm^{-1}: 3440, 3200, 1680, 745; NMR (C_5H_5N): δ 0.78 (*s*, 20-H_3), 3.68 (*s*, 17-H_2), 4.12 (*m*, $W_{\frac{1}{2}}$ = 25 Hz, 6-H), 5.83 (*s*, 15-, 16-H); MS *m/e*; 318 (M^+ -18). The mother liquors from crystallization of the ester of **4** were separated by PLC to give (4S)-*dimethyl*-ent-18-*hydroxy*-3,4-*secobeyer*-15-*ene*-3,17-*dioate* (**7**, 60 mg) which crystallized from Et_2O–pentane as needles, mp 56–58° (Found: M-18, 360.230. $C_{22}H_{32}O_4$ requires: 360.230). IR $\nu_{max}^{CS_2}$ cm^{-1}: 3610, 1740; NMR (C_5H_5N; 90 MHz): δ 0.75 (*s*, 20-H_3), 1.22 (*d*, *J* = 6.5 Hz, 19-H_3), 3.63 (*s*, OMe), 3.40 (*t*, $J_{18a,18b} = J_{18a,4}$ = 10 Hz, 18-H_a), 3.94 (*dd*, $J_{18b,4}$ = 3 Hz, $J_{18a,18b}$ = 10 Hz, 18-H_b), 5.84 (*s*, 15-, 16-H); MS *m/e*: 378 (M^+).

Ent-6α,17-*dihydroxy*-3,4-*secobeyeren*-3-*oic acid* (**12**). The acetoxy ketol (**1**, 6 g) [1] was acetylated with Ac_2O–C_5H_5N and the crude product treated with $NH_2OH.HCl$ and Py in refluxing EtOH. The *oxime* (**9**) crystallized from $CHCl_3$–petrol as plates, mp 164–165°; $[\alpha]_D$ −70° (*c*, 1.0, MeOH) (Found: C, 68.9; H, 8.4; N, 3.2. $C_{24}H_{35}O_5N$ requires: C, 69.0; H, 8.4; N, 3.3%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1740; NMR ($CDCl_3$): δ 0.76, 1.17, 1.36 (*s*, 18-, 19-, 20-H_3), 2.05 (*s*, CH_3CO—), 3.99 (*s*, 17-H_2), 5.76 (AB*q*, *J* = 6 Hz, 15-, 16-H); MS *m/e*: 417 (M^+). The oxime (**9**, 1 g) and TsCl (0.6 g) in C_5H_5N (15 ml) were refluxed for 16 hr. Chromatography of the product on Al_2O_3 (Act. grade 3, 20 g) gave the crude nitrile (0.45 g) which was hydrolysed with boiling 10% KOH in EtOH to give the acid (**10**) which was characterized as the *methyl ester* (**11**, 0.4 g) which crystallized from Et_2O as needles, mp 145–147°, $[\alpha]_D$ +23° (*c*, 0.5, $CHCl_3$) (Found: C, 72.1; H, 9.0. $C_{21}H_{32}O_4$ requires: C, 72.4; H, 9.3%). IR $\nu_{max}^{CS_2}$ cm^{-1}: 3600, 1740, 900, 750; NMR: δ 0.79 (*s*, 20-H_3), 1.85 (*s*, 19-H_3), 3.49 (*s*, 17-H_2), 3.66 (*s*, OMe), 3.96 (*m*, 6-H), 4.86, 5.19 (*br.ss*, 18-H_2), 5.72 (AB*q*, *J* = 6 Hz, 15-, 16-H); MS *m/e*: 348 (M^+). The seco ester (**11**) was hydrogenated using PtO_2 in AcOH and the product saponified with 5% NaOH. The recovered *dihydroxy acid* (**12**) crystallized from Me_2CO–pentane as needles, mp 180–181° $[\alpha]_D$ −26° (*c*, 0.4, EtOH) (Found: C, 71.0; H, 10.0. $C_{20}H_{34}O_4$ requires: C, 71.0; H, 10.1%). IR ν_{max}^{Nujol} cm^{-1}: 3450, 1705; NMR (C_5H_5N): δ 0.95 (*s*, 20-H_3), 3.59 (*s*, 17-H_2), 3.93 (*m*, 6-H); MS *m/e*: 320 (M^+-18). The acid (**12**) was identical with a sample prepared by hydrogenation of **4** in EtOH using Pd/C.

Metabolism of the seco diol (**13**) *by* Calonectria decora. The diol (200 mg) in EtOH (10 ml) was incubated with cultures of *C. decora* for 3 days using conditions described previously [12]. The organic product was recovered by filtering the mycelia, washing the medium and mycelia with EtOAc, and combining the 2 extracts. The EtOAc soln was washed with H_2O, dried (Na_2SO_4) and evapd. PLC of the residue gave a fraction which crystallized from EtOAc as plates of the *seco triol* (**14**, 40 mg), mp 150°, $[\alpha]_D$ +18° (*c*, 0.3, C_5H_5N) (Found: C, 73.8; H, 10.1. $C_{20}H_{32}O_3$ (0.25 $C_4H_8O_2$) C, 73.6; H, 10.0%). The presence of between 0.25–0.50 molar ratio of EtOAc as solvent of crystallization was confirmed by NMR. NMR (C_5H_5N): δ 0.85 (*s*, 20-H_3), 2.00 (*s*, 19-H_3), 3.75 (*s*, 17-H_2), 3.83 (*t*, *J* = 6.5 Hz, 3-H_2), 4.22 (*m*, $W_{\frac{1}{2}}$ = 25 Hz, 6-H), 4.97, 5.15 (*br*, *s*, 18-H_2), 5.92 (*s*, 15-, 16-H); MS *m/e* 320.2351 (M^+, $C_{20}H_{32}O_3$), 302.2246 (M^+-18, $C_{20}H_{30}O_2$), 236.1779 (M^+-C_5H_8O, fragmentation of 5–10 and 6–7 bonds, $C_{15}H_{24}O_2$). The compound was identical with a sample prepared by reduction of the dihydroxy acid (**10**) with $LiAlH_4$ in Et_2O.

Ent-3,4-*secobeyeran*-3,17-*diol* (**17**). The diester (**7**, 20 mg) was converted to the tosylate in C_5H_5N at room temp. and the crude product reduced with $LiAlH_4$ (50 mg) in THF (10 ml). PLC gave ent-3,4-*secobeyer*-15-*ene*-3,17-*diol* (**16**, 15 mg) as a gum (Found: M^+ 306.252. $C_{20}H_{34}O_2$ requires: 306.256). IR $\nu_{max}^{CS_2}$ cm^{-1}: 3610, 750; NMR: δ 0.72 (*s*, 20-H_3), 0.81, 0.94 (*d*, *J* = 7 Hz, 18-, 19-H_3), 3.50 (*s*, 17-H_2), 3.64 (*t*, *J* = 7 Hz, 3-H_2), 5.69 (AB*q*, *J* = 6 Hz, 15-, 16-H). The diol (**16**) in EtOH was hydrogenated (Pd/C) to give the diol (**17**) identical with authentic material [1].

(4R)- *and* (4S)-*Dimethyl*-ent-3,4-*seco*-18-*hydroxybeyerane*-3,17-*dioate* (**19** *and* **20**). *Ent*-3,4-secobeyer-4(18)-ene-3,17-dioic acid [1] was methylated and the ester (**18**, 1 g) treated with *m*-chloroperbenzoic acid (0.8 g) in $CHCl_3$ overnight. The epoxide mixture was recovered as an oil, dissolved in toluene and BF_3–Et_2O (10 ml) added at −5° under N_2. After 0.5 hr the aldehyde mixture was isolated NMR: δ 9.58 (*s*, C<u>H</u>O), 9.72 (*d*, *J* = 3 Hz, C<u>H</u>O). Reduction with $NaBH_4$ (1 g) in EtOH gave a product which was adsorbed on Al_2O_3 (Act. grade 3, 20 g). Gradient elution with $CHCl_3$–petrol gave (4R)-*dimethyl*-*ent*-18-*hydroxy*-3,4-*secobeyerane*-3,17-*dioate* (**19**, 150 mg) as a gum. (Found: M-18, 362.237. $C_{22}H_{34}O_4$ requires: 362.245). NMR: δ 0.78 (*d*, *J* = 7 Hz, 19-H_3), 0.92 (*s*, 20-H_3), 3.34 (*m*, $W_{\frac{1}{2}}$ = 10 Hz, 18-H_2), 3.62 (*s*, OMe). Saponification in KOH–EtOH gave the *diacid* which crystallized from EtOAc as plates, mp 215–218° (Found: C, 67.7; H, 9.1; M-18: 334.213. $C_{20}H_{32}O_5$ requires: C, 68.2; H, 9.2%; $C_{20}H_{30}O_4$: 334.214). NMR: δ 0.87 (*d*, *J* = 7 Hz, 19-H_3), 0.93 (*s*, 20-H_3), 3.63 (*d*, *J* = 7 Hz, 18-H_2). Further elution gave the 4S-*isomer* (**20**, 70 mg) as a gum (Found: M-18, 362.235. $C_{22}H_{34}O_4$ requires: 362.245) NMR: δ 0.94 (*s*, 20-H_3), 1.04 (*d*, *J* = 7 Hz, 19-H_3), 3.4 (*m*, 18-H_2), 3.64, 3.66 (*s*, OMe). Saponification gave the *diacid* which crystallized from EtOAc as plates mp 106–109° (Found: M-18, 334.215 $C_{20}H_{30}O_4$ requires: 334.214). NMR (C_5H_5N): δ 0.95 (*s*, 20-H_3), 3.7 (*m*, 18-H_2). The diester (**20**) and derived acid were identical with samples obtained by hydrogenation of the hydroxy diester (**7**) and the naturally occurring acid (**6**).

(4R)-*Dimethyl*-ent-4-*hydroxy*-18-*nor*-3,4-*secobeyerane*-3,17-*dioate* (**23**) 1. The norketo ester [5] (**21**, 0.3 g) was set aside in $CHCl_3$ (10 ml) with *m*-chloroperbenzoic acid (0.3 g) overnight. Isolation of the neutral product with Et_2O gave the lactone (**22**, 0.28 g) as plates, mp 168–170°, $[\alpha]_D$ +24° (*c*, 0.9, $CHCl_3$) (Found: C, 70.9; H, 8.9. $C_{20}H_{30}O_4$ requires: C, 71.2; H, 8.8%) IR $\nu_{max}^{CS_2}$ cm^{-1}: 1730; NMR: δ 1.03 (*s*, 20-H_3), 1.25 (*d*, *J* = 7 Hz, 19-H_3), 3.65 (*s*, OMe), 4.66 (*m*, $W_{\frac{1}{2}}$ = 17 Hz, 4-H); MS *m/e*: 334 (M^+). The lactone (**22**, 0.2 g) was saponified in the usual way and methylated (CH_2N_2) to give, after PLC, the hydroxy diester (**23**, 0.15 g) as an oil which was characterized as the *p*-bromobenzoate which crystallized from MeOH as needles, mp 92–94°, $[\alpha]_D$ −31° (*c*, 0.9, $CHCl_3$) (Found: C, 61.4; H, 6.8. $C_{27}H_{37}O_6Br$ requires: C, 61.2; H, 6.8%). IR $\nu_{max}^{CS_2}$ cm^{-1}: 1720, NMR: δ 0.98 (*s*, 20-H_3), 1.29 (*d*, *J* = 7 Hz, 19-H_3), 3.55, 3.66 (*s*, OMe). MS *m/e*: 550, 548 (M^+).

2. The hydroxy diester (**19**, 50 mg) was oxidized by the method of ref. [13] and the crude aldehyde treated with *m*-chloroperbenzoic acid (50 mg) in $CHCl_3$ overnight. The neutral product was saponified and methylated as above to give, after PLC, the hydroxy diester (**23**, 30 mg). The *p*-bromobenzoate was identical with the sample described above.

Photolysis of ent-6α-*acetoxy*-17-*hydroxybeyer*-15-*ene*-3-*one* (**1**). A soln of **1** (0.5 g) in AcOH (45 ml) and H_2O (5 ml) was refluxed under N_2 in pyrex for 2 days while irradiated by an external Hanovia 1L photochemical reactor. The acid (**4**, 240 mg) recovered after saponification of the product crystallized from EtOAc as needles, mp 167–169° undepressed by the sample obtained from the plant.

Ent-3β,19-*dihydroxybeyer*-15-*ene*[3,19-$^3H_2$] (**24**). Unlabelled diol (150 mg) [14] was oxidized with Jones' reagent at 0° and the crude product dissolved in THF (1 ml). Tritiated H_2O (5 Ci/ml; 9 μl) and a soln of $LiBH_4$ (0.33 M, 1.5 ml) were boiled for 20 min under N_2 and the keto aldehyde added. After 1 hr further reflux the soln was poured into Et_2O and the Et_2O

soln was washed with 2% aq. HCl and H_2O, dried and the solvent evapd. PLC of the residue gave the tritiated diol which was crystallized from $CHCl_3$–petrol to constant sp. act. (5.23×10^7 dpm/mg; 1.59×10^{10} dpm/mmol). A sample of the labelled diol (1.3 mg) was diluted to 204 mg and benzoylated with PhCOCl (140 mg) in C_5H_5N. The hydroxy benzoate separated by chromatography on Al_2O_3 was crystallized from $CHCl_3$–petrol to constant sp. act. (2.33×10^8 dpm/mmol) and was identical with a sample of unlabelled ent-3*β*-*hydroxy*-19-*benzoyloxybeyer*-15-*ene* (needles, mp 142–143°, $[\alpha]_D$ +40° (*c*, 0.4, $CHCl_3$) (Found: C, 79.6; H, 8.8. $C_{27}H_{36}O_3$ requires: C, 79.4; H, 8.9%). IR $\nu_{max}^{CS_2}$ cm^{-1}: 3600, 1715; NMR: δ 0.77, 1.00, 1.25 (*s*, 17-, 18-, 20-H_3) 3.33, (*m*, $W_{\frac{1}{2}}$ = 17 Hz, 3-H), 4.48 (AB*q*, J_{AB} = 12 Hz, 19-H_2), 5.57 (AB*q*, $^4J_{AB}$ = 6.5 Hz, 15-, 16-H), MS *m/e*: 408 (M^+). Oxidation of the labelled hydroxy benzoate with Jones' reagent gave the keto benzoate which was crystallized from C_6H_6–petrol to constant sp. act. (1.12×10^8 dpm/mmol). A sample of unlabelled ent-19-*benzoyloxybeyer*-15-*en*-3-*one* showed the following properties: mp 155–157° (plates), $[\alpha]_D$ +35° (*c*, 1.4, $CHCl_3$) (Found: C, 80.0; H, 8.9. $C_{27}H_{34}O_3$ requires: C, 79.8; H, 8.4%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1705; NMR: δ 1.02, 1.07, 1.27 (*s*, 17-, 18-, 20-H_3), 4.52 (AB*q*, J_{AB} = 11 Hz, 19-H_2), 5.62 (AB*q*, J_{AB} = 6.5 Hz, 15-, 16-H); MS *m/e*: 406 (M^+).

Feeding of ent-3*β*,19-*dihydroxybeyer*-15-*ene*[3,19-$^3H_2$] (**24**). Feeding of the diol (4.6 mg; 5.23×10^7 dpm/mg) was carried out as described previously [2]. The plant extract gave an acidic fraction which was methylated. The fraction corresponding to the dihydroxy ester (**5**) was saponified and diluted with the dihydroxy acid (**4**) which lost all activity after several crystallisations from EtOAc. The fraction corresponding to the hydroxy ester (**3**) was reduced with $LiAlH_4$ to give the diol (**13**, 12.7 mg) which was diluted with unlabelled material (31.7 mg), purified by TLC and crystallized to constant sp. act. (279 dpm/mg; 8.47×10^4 dpm/mmol; 0.01% incorporation). The diacid [1] obtained from the diol with Jones' reagent had constant sp. act. (276 dpm/mg; 9.06×10^4 dpm/mol) after crystallization from C_6H_6.

Ent-3*β*,17-*dihydroxybeyer*-15-*ene*[3,17-$^3H_2$] (**25**). The tritiated compound was prepared by reduction of methyl *ent*-3-oxobeyer-15-en-17-oate with tritiated $LiBH_4$ prepared by the method of ref. [10]. The tritiated diol was recrystallized from C_6H_6 to a constant sp. act. (1.0×10^8 dpm/mg; 3.04×10^{10} dpm/mmol). A portion (0.53 mg) of the tritiated diol was diluted with unlabelled material (152 mg) and converted to the corresponding 3-hydroxy-17-benzoate which, after chromatography on neutral Al_2O_3, was recrystallized from $CHCl_3$–petrol to constant sp. act. (2.86×10^5 dpm/mg; 1.17×10^8 dpm/mmol) and was identical with a sample of unlabelled ent-3*β*-*hydroxy*-17-*benzoyloxybeyer*-15-*ene* (prisms, mp 177–178°, $[\alpha]_D$ +67° (*c*, 0.8, $CHCl_3$) (Found: C, 79.3; H, 8.9. $C_{27}H_{36}O_3$ requires: C, 79.4; H, 8.9%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1710, NMR: δ 0.75, 0.78, 1.00 (*s*, 18-, 19-, 20-H_3), 3.22 (*m*, $W_{\frac{1}{2}}$ = 17 Hz, 3-H), 4.20 (*s*, 17-H_2), 5.74 (AB*q*, J_{AB} = 6.5 Hz, 15-, 16-H); MS *m/e*: 408 (M^+). Oxidation of the labelled hydroxy benzoate with Jones' reagent gave the keto benzoate which was crystallized from C_6H_6–petrol to constant sp. act. (1.37×10^5 dpm/mg; 5.56×10^7 dpm/mmol). A sample of unlabelled ent-17-*benzyloxybeyer*-15-*en*-3-*one* showed the following properties: mp 136–138° (plates), $[\alpha]_D$ +58 (*c*, 0.3, $CHCl_3$) (Found: C, 80.0; H, 8.6. $C_{27}H_{34}O_3$ requires: C, 79.8; H, 8.4%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1700; NMR: δ 0.93 (3H) and 1.08 (6H) (18-, 19-, 20-H_3), 4.24 (*s*, 17-H_2), 5.80 (AB*q*, J_{AB} = 6.5 Hz, 15-, 16-H); MS *m/e* 406 (M^+).

Feeding of ent-3*β*,17-*dihydroxybeyer*-15-*ene*[3,17-$^3H_2$] (**25**). The diol (3.58 mg, 1.0×10^8 dpm/mg) in Me_2CO (80 μl) was applied to the plant as described previously [2]. The resin components were then isolated as described above. The dihydroxy acid (**4**, diluted to 38.5 mg) lost all radioactivity after several recrystallizations from EtOAc. The seco diol (**13**, 2.5 mg) prepared by reduction with $LiAlH_4$ of the fraction corresponding to the hydroxy ester (**3**) was diluted with unlabelled material to 43 mg and recrystallized from C_6H_6 to constant sp. act. (207 dpm/mg; 6.3×10^4 dpm/mmol; 0.005% incorporation). The diol was then oxidized to the diacid which lost all radioactivity after crystallization from C_6H_6.

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