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ACIDIC *ENT*-KAURANOIDS FROM THE METABOLISM OF *ENT*-KAURA-2,16-DIEN-19-OL IN *GIBBERELLA FUJIKUROI*

I. F. COOK, P. R. JEFFERIES and J. R. KNOX*
Department of Organic Chemistry, University of Western Australia Nedlands, Western Australia, 6009

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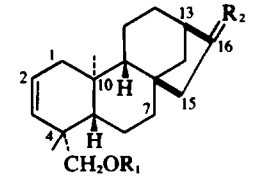
Abstract—Three acidic *ent*-kauranoid metabolites have been obtained as the methyl esters (**7**, **8**, and **9**) from incubation of the [17-^{14}C]-labelled dienol (**1**) with *Gibberella fujikuroi*. Spectroscopic studies of the triol ester (**7**) and chemical degradation to B-ring cleaved products establish the assigned structure (**7**). The structures of the other two metabolite esters are indicated to be **8** and **9** from the spectroscopic data.

In some previous papers[1,2] we have reported briefly structures for some of the gibberellin metabolites resulting from incubation of the dienol **1** and its hemisuccinate **2** with the fungus *Gibberella fujikuroi* and we now wish to discuss a group of *ent*-kauranoid metabolites which have also been obtained from the incubation of **1**. This substrate and its [17-^{14}C]-labelled derivative were obtained from the diol **3** through the intermediates **4**, **5** and **6**.



1: $R_1 = H$, $R_2 = CH_2$

2: $R_1 = -C(=O)-CH_2CH_2-CO_2H$, $R_2 = CH_2$

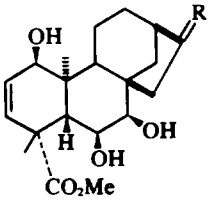
5: $R_1 = -C(=O)-Ph$, $R_2 = CH_2$

6: $R_1 = -C(=O)-Ph$, $R_2 = O$

3: R = H

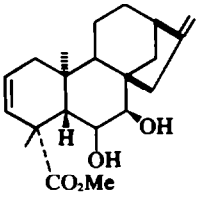
4: R = C(=O)–Ph

From preliminary studies, using the labelled material, it was decided to incubate **1** with washed fungal mycelium for a period of 7 days, which represented a balance between a convenient time and a high percentage conversion to acidic metabolities. In fact, the percentage conversion was found to be sensitive to substrate loading: 66% conversion for a loading of 13·3 mg/400 ml of culture medium compared with 12% for a loading of 200 mg/400 ml. Preparative work was conducted with a weakly radioactive substrate to facilitate the isolation of metabolites which were most readily purified as their methyl ester derivatives. These were obtained by treatment of the crude acidic metabolites with diazomethane. Repeated column and thick layer chromatography of the crude ester fraction then gave the three metabolite esters **7**, **8** and **9**.



7: R = CH_2

8: R = C(CH₃)(OH)



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These esters were recognized as C-19 oxidized derivatives of the dienol **1** as their NMR spectra showed signals attributed to the 4- and 10-Me groups, a methoxycarbonyl group and the vinylic protons at C-2, C-3 and C-17. Table 1 presents a list of the chemical shifts of these protons.

A molecular formula, $C_{21}H_{30}O_5$, was obtained for the most abundant metabolite (the triol-ester, **7**) from the precise mass of the M-18 ion. The triol-ester showed absorptions in the IR spectrum due to hydroxyl, ester and olefin groupings. The NMR spectrum measured in deuteropyridine showed carbinol methine resonances at δ 3·83 broad singlet), δ 3·95 (d, J = 5 Hz) and at δ 4·66 (a

Table 1. Chemical shifts of protons (δ)

Protons	Solvent	Triol-ester 7	Tetrol-ester 8	Diol-ester 9
4-Methyl	C_5D_5N	1·71	1·71	1·73
	$CDCl_3$	1·51	1·51	1·51
10-Methyl	C_5D_5N	1·18	1·22	1·11
	$CDCl_3$	·90	·90	·95
17-Protons	C_5D_5N	4·90	1·38	4·88
	$CDCl_3$	4·86	1·25	4·86
2-,3-Protons	C_5D_5N	H_2: 5·98 H_3: 5·90	H_2: 6·00 H_3: 5·92	5·70
	$CDCl_3$	5·80	5·75	5·61
Methoxy-carbonyl	C_5D_5N	3·73	3·60	3·73
	$CDCl_3$	3·73	3·60	3·73

partly obscured doublet of doublets). The latter signal was clearly observed, centered at δ 4·15 (J = 2, 10 Hz) in the spectrum measured in deuterochloroform. Double resonance analysis showed that this carbinol methine proton is coupled to a proton, resonating as a doublet centered at δ 3·02 (J = 10 Hz), while the magnitude[3] of the coupling demonstrates their diaxial relationship. The remaining small coupling shown by the carbinol methine proton suggests that the other C atom flanking the secondary OH group carries a single proton in an equatorial position.[3] This proton was shown by double resonance studies to be the carbinol methine proton responsible for the broadened singlet at δ 3·83 in the deuteropyridine NMR spectrum. The structural feature shown in Fig 1 is thus indicated for the triol-ester 7.

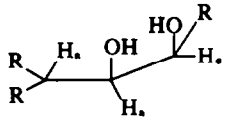


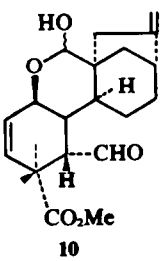
Fig 1.

Chemical support for this *vic*-glycol grouping resulted from the sodium metaperiodate cleavage of 7 to give an unstable product formulated as the aldehyde (10). This product was then immediately converted to the γ-lactone (11) by treatment with sodium borohydride. The high resolution mass spectrum of 11 is consistent with a molecular formula of $C_{20}H_{26}O_4$.

The mass spectrum of 11 also showed a prominent peak at *m/e* 181 which is attributed to an ion, incorporating the A-ring, resulting from the cleavage of the C-9, C-10 bond with transfer of a hydrogen. Related ions in the mass spectra of B-ring seco diterpenes have been observed for fujenal[4] and a hemiacetal-aldehyde derived from *ent* - 6α,7α,19 - trihydroxykaur - 16 - ene.[5] The IR spectrum of 11 showed absorptions due to an OH and a γ-lactone grouping. The NMR spectrum lacked resonances characteristic of a methoxycarbonyl group, demonstrating that C-19 is involved in the formation of the γ-lactone grouping. The *cis*-glycol grouping of 7 can thus be confidently located at the C-6 and C-7 positions. A one proton singlet at δ 4·62 in the NMR spectrum of 11 is attributed to a hemiacetal methine proton thus apparently accounting for the failure of the 7-aldehyde group to undergo reduction during sodium borohydride treatment of 11.

The location and configurational assignment of the remaining OH in 7 follows from an analysis[6] of the pattern observed for H-1, H-2 and H-3 in the NMR spectrum measured in deuteropyridine. H-2 and H-3 appeared as six lines forming the AB part of an ABX pattern. The X part is attributed to the signal from the proton geminal to the remaining OH group which appeared as a slightly broadened doublet at δ 3·95 (peak separation = 5 Hz). The coupling of H-1 to H-2 and H-3 was demonstrated by irradiation of the doublet at δ 3·95 which caused the H-2 and H-3 signals to collapse to a simple AB quartet (J_{AB} = 10 Hz). It follows that the triol-ester (7) possesses a 1-hydroxy-Δ^2-system.

The doublet observed for the allylic carbinol methine proton of 7 compares with the doublet (J = 6 Hz) observed[7,8] for the 12β - quasi - equatorial proton of 12α - hydroxy - $\Delta^{9(11)}$ - steroids and contrasts with the broadened singlet seen for the 12α - quasi - axial proton of the 12β - epimer. Moreover there is a close similarity between the values of the allylic-vinylic coupling constant ($J_{1\alpha,2}$) derived from Karplus equations using dihedral angles (ϕ) measured from a Dreiding model and $J_{1,2}$ derived from a mathematical analysis of the ABX pattern (Table 2). The former type of analysis has been found in the conduritols[9] and the amaryllidaceae alkaloids[10] to yield values of $J_{1,2}$ which are in good agreement with observed values. Consequently the OH group of 7 is assigned a quasi axial 1β-configuration. (Although the systematic naming of compounds in this paper follows the Rowe proposed system[11] in which an *ent* operator inverts the stereochemical designation of substituents we otherwise continue the recent practice[12,13] of specifying α- and β- stereochemistry according to the structural representations.)



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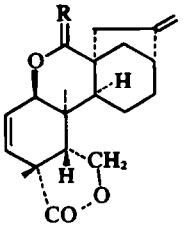


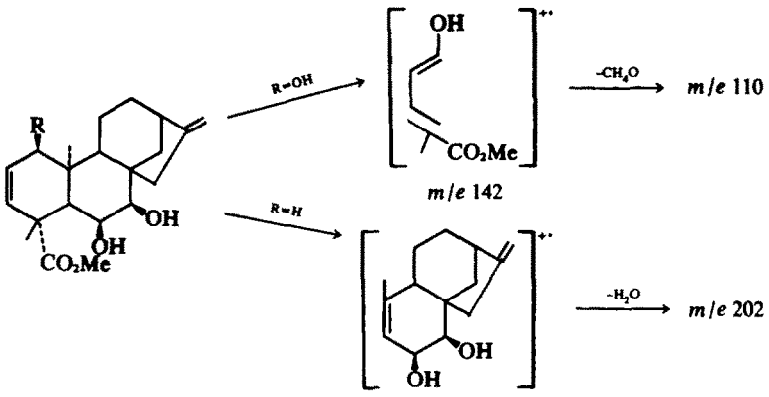
11: R = 

12: R = O

Table 2. Theoretical and observed coupling constants for 7

Derivation	$J_{1\alpha,2}$(Hz)	$J_{1\beta,2}$(Hz)
[a] $J = 11\cos^2\phi$	6·4	3·3
[b] $J = 10\cos^2\phi$	5·8	2·9
Analysis of ABX of 7	$J_{1,2}$ = 5·4	

[a] Ref 9. [b] Ref 10.



SCHEME 1

Ions at *m/e* 142 and *m/e* 110 in the mass spectrum of **7** are also in agreement with the assignment of a 1-OH group as they can be derived from the retro-Diels Alder fragmentation of the A-ring (Scheme 1).

The same ion in which charge is retained on the A-ring fragment is also obtained from the metabolite ester **8**. Corresponding ions are not abundant in Δ^2 - *ent* - kaurenes lacking 1-hydroxylation (e.g. **9**) as the charge is retained on the complementary fragment.

Additional support for a 1-OH group in **7** was obtained from oxidation of **11**. The MS of the product showed the loss of 2 H atoms consistent with the formation of the δ-lactone grouping in the dilactone (**12**). The δ-lactone functionality is supported by an additional absorption in the IR spectrum at 1725 cm^{-1}. This verified the hemiacetal group of **11** which can most reasonably be viewed to involve the 7-aldehyde group and the 1-OH group. Products of this type have good analogy with the chemistry of δ-caesalpin.[14,15]

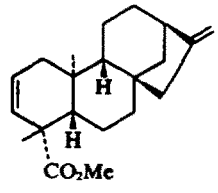
The triol-ester was thus formulated as methyl *ent* - 1α,6α,7α - trihydroxykaura - 2,16 - dien - 19 - oate (**7**).

The mass spectrum of the second *ent* - kauranoid metabolite, the tetrol–ester (**8**), showed a molecular ion at *m/e* 380. The NMR spectrum showed most of the resonances characteristic of **7** and an additional three proton singlet (δ 1·25 in $CDCl_3$ and δ 1·38 in C_5D_5N) (Table 1). This coupled with the absence of resonance due to the C-17 vinylic protons indicated that **8** is the hydrated exocyclic methylene derivative of **7**. The base peak (*m/e* 43) of **8** supports this assignment as this ion is the base peak of the hydrated gibberellins GA_2 and GA_{10} and has been attributed[16] to a fragment ion ($MeC{\equiv}O^+$) derived from C-16 and C-17. The configuration of the C-16 OH group of **8** is assumed to be exo (α), the same as other C-16, C-17 hydrated derivatives previously isolated[17-20] from the fungus. A notable feature of the NMR spectrum of **8** measured in deuteropyridine is the unusually low field position (δ 2·98) of a signal attributed to the 13-proton. This may be accounted for by the close promimity of the 16α-OH group to the 13-proton.

The tetrol ester was thus formulated as methyl *ent* - 1α,6α,7α,16β - tetrahydroxykaur - 2 - en - 19 - oate (**8**).

The remaining metabolite ester (**9**) is established as a dihydroxy ester by the molecular formula ($C_{21}H_{30}O_4$) and by the spectroscopic data. The carbinol methine proton signals in the $CDCl_3$ and C_5D_5N NMR spectra closely resemble those of H-6 and H-7 for **7** and **8**. The 6β,7β - dihydroxylation pattern thus indicated is supported by decoupling and INDOR measurements for the C_5D_5N solution. These verified the mutual coupling of the carbinol methine protons and demonstrated that a doublet at δ 2·45 accounts for the expected interaction with H-5. A further point in accord with the assigned structure (**9**) is the position of the 4β-Me signal which is the same as in **7** and **8** but at lower field than for the diene ester (**13**) (δ 1·51 versus δ 1·28). This deshielding can reasonably be attributed to a peri-like interaction between the 4β-methyl and the 6β-hydroxyl group.[21-23]

A feature of the deuteropyridine NMR spectra of the metabolites **7**, **8** and **9** is the low-field position of the 5-proton resonance whereas most *ent*-kaurenes have the resonance observed in the "methylene hump". Table 3 presents a listing of the chemical shifts for the metabolites and for the known 6β,7β-dihydroxylated *ent*-kaurenes **14** and **15**.[24,25] An important source of the deshielding is the interaction with the 7β-OH since it is known that such 1,3-diaxial interactions in chloroform solution have a paramagnetic effect[3] which is enhanced in pyridine solution.[26] Comparison of the chemical shifts of **9** and the



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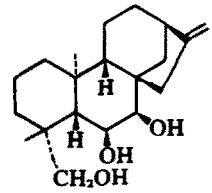

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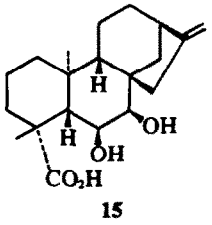
Table 3. Chemical shifts of 5-protons (δ, pyridine)

Triol-ester **7**	3·02
Tetrol-ester **8**	3·02
Diol-ester **9**	2·45
ent-6α,7α,19-Trihydroxykaur-16-ene (**14**)	2·22
ent-6α,7α-Dihydroxykaur-16-en-19-oic acid (**15**)	(2·38)

A-ring saturated compounds **14** and **15** demonstrates that the Δ^2-double bond has little effect on the position of resonance. On the other hand, a 1β-OH group introduced allylically to the Δ^2-double bond (**7** and **8** versus **9**) has a marked deshielding influence (0·57 ppm). This is not surprising because the stereochemical disposition of the 1β-OH group relative to the 5-proton is likely to resemble a 1,3-diaxial relationship.



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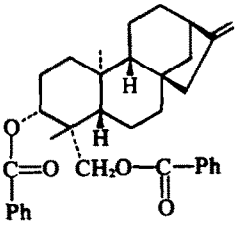

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EXPERIMENTAL

For general experimental details see Ref 27.

Synthesis of ent - *Kaura* - 2,16 - *dien* - 19 - *ol* (**1**) *and* [17-^{14}C] - *labelled* (**1**)

ent - 19 - *Benzoyloxy* - 3β - *hydroxykaur* - 16 - *ene* (**4**). *ent* - 3β,19 - *Dihydroxykaur* - 16 - *ene* (3, 24·0 g) was dissolved in dry pyridine (200 ml), PhCOCl (14 g) added and the mixture left at r.t. for 24 h. Work up of the mixture in the usual way gave a residue (36 g) which was dissolved in benzene–light petroleum (1:1) and absorbed on Al_2O_3 (500 g). Elution with benzene–light petroleum (1:4) gave *ent*-3β,19 - dibenzoyloxykaur - 16 - ene (**16**, 8·1 g) which crystallized from light petroleum as needles, m.p. 162–163° $[\alpha]_D$ −59° (*c*, 1·2); (Found: C, 79·7; H, 8·0. $C_{34}H_{40}O_4$ requires: C, 79·7; H, 7·9%); ν_{max}: 3050, 1720, 1660, 870, 710 cm^{-1}; NMR (δ): 4·75 (5H, m, CH_2–OR,  , ); 7·46, 8·00 (10H, PhH). Elution with benzene–light petroleum (1:1) gave *ent* - 19 - *benzoyloxy* - 3β - *hydroxykaur* - 16 - *ene* (**4**, 18·0 g) which crystallized from MeOH aq as cubes, m.p. 135–136°, $[\alpha]_D$ −48° (*c* 1·15) (Found: C, 79·0; H, 9·0. $C_{27}H_{36}O_3$ requires: C, 79·4; H, 8·9%); ν_{max}; 3600, 3050, 1720, 870, 710 cm^{-1}; NMR (δ): 3·22 (1H, ); 4·27, 4·51 (2H, AB, J = 11 Hz $-CH_2OR$); 7·46, 8·00 (5 PhH).

ent - 19 - *Benzoyloxykaura* - 2,16 - *diene* (**5**). The monobenzoate (**4**, 14 g) in dry pyridine (100 ml) was treated with freshly distilled $POCl_3$ (25 ml) and left at r.t. for 12 h. The mixture was heated for 1 h at 90°, cooled, and worked up in the usual way to give a residue (13·6 g) which was chromatographed on Al_2O_3 (200 g). Elution with benzene–light petroleum (1:4) gave the *diene–benzoate* (**5**, 10·2 g) which crystallized from MeOH as prisms m.p. 133–134°, $[\alpha]_D$ −128° (*c*, 1·5). (Found: C, 83·0; H, 8·8. $C_{27}H_{34}O_2$ requires: C, 83·0; H, 8·8%); ν_{max}: 3050, 3005, 1720, 870, 710 cm^{-1}; NMR (δ): 4·11, 4·36 (2H, AB, J = 11 Hz $-CH_2O$–R), 5·59 (2H, ), 7·46, 8·00 (5H, PhH).

ent - 19 - *Benzoyloxy* - 17 - *nor* - *kaur* - 2 - *en* - 16 - *one* (**6**). The diene–benzoate (**5**, 1·6 g) in a soln of dioxan (100 ml) and H_2O (30 ml) was treated with OsO_4 (100 mg) and $NaIO_4$ (2·6 g). After 12 h the reaction mixture was worked up in the usual way to give a residue (1·5 g) which was filtered through Al_2O_3 (25 g) in benzene. The *nor-ketone* (**6**, 1·2 g) thus obtained was crystallized from benzene–light petroleum as plates, m.p. 179–180°, $[\alpha]_D$ −120° (*c*, 0·44). (Found: C, 79·6; H, 8·3. $C_{26}H_{32}O_3$ requires: C, 79·6; H, 8·2%); ν_{max}: 3005, 1740, 1720, 710 cm^{-1}; NMR (δ): 4·22, 4·48 (2H, AB, J = 11 Hz, $-CH_2$–OR); 5·70 (2H, ); 1·99 (2H, s, H-15); 7·46, 8·00 (5H, PhH).

ent - 19 - *Benzoyloxy* - [17-^{14}C] - *kaura* - 2,16 - *diene* (17-^{14}C - labelled **5**). A suspension of $Ph_3\overset{+}{P}{}^{14}CH_3\bar{I}$ (6·8 g, 3·7 μc) in THF (100 ml) under an atmosphere of N_2, was treated with a 1·4 M soln of nC_4H_9Li (13·2 ml) in hexane. The mixture was stirred for 1 h and the nor-ketone (**6**, 1·89 g) added. After 15 h at r.t. the mixture was refluxed for 5 h, concentrated and worked up to give a residue which was chromatographed on Al_2O_3. Elution with benzene–light petroleum (1:4) gave the ^{14}C-diene–benzoate (**5**, 1·1 g, 1·0 μc/g) as prisms, m.p. and m.m.p. 133–134°.

ent - *Kaura* - 2,16 - *dien* - 19 - *ol* (**1**) *and* [17 - ^{14}C] *labelled* **1**. The ^{14}C-diene–benzoate (1·1 g) was dissolved in methanolic KOH (5%, 150 ml) and the mixture heated under reflux for 5 h. Work up in the usual way gave the ^{14}C-dienol (**1**, 0·75 g, 1·35 μc/g) which crystallized from EtOH aq as needles, m.p. and m.m.p. 73–74° (lit[28] m.p. 73–5°).

The unlabelled dienol was prepared from the unlabelled diene–benzoate in a similar manner.

Incubations. The mycelium from 400 ml of a 4 day culture of *G. fujikuroi* grown under standard conditions,[29] was resuspended in 5 × 100 ml conical flasks each containing a pH 4·4 phosphate buffer soln (60 ml). The ^{14}C-dienol (**1**, 2 mg, 2 × 10^6 cpm) was then added to each flask. At the incubation times indicated in Table 4, the contents of one of these flasks was worked up in the normal way to give a neutral and an acidic extract. The acidic extract was methylated with diazomethane. The mycelium from each of these experiments was homogenized with EtOH (3 × 100 ml) in a Waring blender for 10 min, filtered and the ethanolic filtrate concentrated to give a mycelial extract. The radioactivity of each fraction was determined in the normal way.

Incubation of the dienol (**1**, 16·4 g, 470 cpm/mg) with the fungus, at the rate of 200 mg/400 ml culture for 7 days, gave after the same workup a methyl ester fraction (6·73 g). Preliminary investigation used a third of this material, while the remainder was chromatographed on Al_2O_3 (135 g) to give **7**, **8** and **9** as listed below.

Methyl ent - 1α,6α,7α - *trihydroxykaura* - 2,16 - *dien* - 19 - *oate* (**7**). Elution of the metabolite ester chromatography with $CHCl_3$–benzene mixtures (1:4–3:7) gave *methyl* ent - 1α,6α,7α - *trihydroxykaura* - 2,16 - *dien* - 19 - *oate* (**7**) which crystallized from $CHCl_3$–light petroleum as needles (26 mg, 468 cpm/mg), m.p. 207–208°, $[\alpha]_D$ +56° (*c*, 0·5) (EtOH); ν_{max}: Nujol 3455, 3420, 3370, 1695, 1670, 890 cm^{-1}; NMR (δ): 0·90, 1·51 (CH_3–C); 3·73 ($-CO_2CH_3$); 4·15 (1H, d of d, $J_{5,6}$ = 10 Hz, $J_{6,7}$ = 2 Hz, H-6); 4·86 (); 5·80 (); NMR (δ, C_5D_5N): 1·18, 1·71

Table 4. Incubation studies with **1**. Distribution of recovered radioactivity

Fraction	12 h %	24 h %	2 days %	4 days %	8 days %
Methyl esters	5	14	48	63	66
Neutrals	21	33	26	18	16
Mycelium	74	53	26	19	18

(CH_3–C<), 3·73 (CO_2CH_3); 4·66 (1H, d of d, $J_{5,6}$ = 10 Hz, $J_{6,7}$ = 2 Hz, H-6); 3·02 (1H, d, $J_{5,6}$ = 10 Hz, H-5); 2·98 (1H, m, H-13); 3·83 (1H, $W_{h/2}$ = 4 Hz, H-7); 4·90 (2H, m, >C=CH_2), 5·98, 5·90, 3·95 (ABX pattern, $J_{2,3}$ = 10 Hz, $J_{1,2}$ = 5·4 Hz, $J_{1,3}$ = 4 Hz, H-2, H-3, H-1). (Found: 344·200; $C_{21}H_{28}O_4$ requires: 344·199. Found: 142·062; $C_7H_{10}O_3$ requires: 142·063. Found: 110·035; $C_6H_6O_2$ requires: 110·037). MS: *m/e* 362 (5, M^+), 344 (92), 326 (10), 285 (42), 267 (98), 203 (89), 185 (100), 142 (68), 110 (72); *m/e* 142→110 metastable ion. Found: *m/e* 85·2, calculated: 85·21.

Transformations of the triol-ester (**7**). The triol-ester [7, 16·7 mg] was dissolved in dioxan (12 ml) and an aqueous soln of $NaIO_4$ (10% w/v, 4·2 ml) added. After 16 h the mixture was diluted with water and extracted with EtOAc. The residue thus obtained was treated with $NaBH_4$ (50 mg) in MeOH (2 ml) for 1·5 h. Preparative TLC of the products from this treatment in a benzene–acetone (4:1) solvent gave two components (R_f 0·55 and 0·45).

The less polar product was the gummy unreduced (7→1) cyclohemiacetal of methyl *ent* - 1α - hydroxy - 6,7 - dioxo - 6,7 - seco - kaura - 2,16 - dien - 19 - oate (**10**, 2·1 mg); ν_{max}: 3580, 1740, 1725 cm^{-1}; MS: *m/e* 360 (15, M^+), 342, (13), 332 (7), 327 (37), 314 (25), 301 (42), 283 (15), 165 (67), 149 (42), 119 (100).

The more polar material was the gummy (7→1) *cyclohemiacetal* of ent - 1α,6 - *dihydroxy* - 7 - *oxo* - 6,7 - *seco* - *kaura* - 2,16 - *dien* - 19 - *oic acid* 19→6 - *lactone* (**11**, 5·2 mg); ν_{max}: 3570, 1770 cm^{-1}; MS: *m/e* 330 (15, M^+), 312 (12), 286 (2), 284 (7), 181 (65), 149 (100), 121 (31). (Found: 330·182; $C_{20}H_{26}O_4$ requires: 330·183); NMR (δ): 3·37 (1H, d of d, $J_{5,6}$ = 4·5 Hz, $J_{6\alpha,6\beta}$ = 11 Hz, H-6β), 4·63 (1H, d, $J_{6\alpha,6\beta}$ = 11 Hz, H-6α), 4·62 (1H, s, H-7).

The γ-lactone (**11**, 14·2 mg) was dissolved in acetone and treated for 10 min with Jones reagent[30] (0·80 ml). Work up in the normal way and preparative TLC in an acetone–benzene (1:9) solvent gave a gum considered to be the dilactone (**12**, 4·2 mg). ν_{max}: 1770, 1725 cm^{-1}; *m/e* 328 (12, M^+), 310 (7), 163 (100), 145 (47), 120 (67), 119 (54).

Methyl ent - 1α,6α,7α,16β - *tetrahydroxykaur* - 2 - *en* - 19 - *oate* (**8**). The fraction eluted with MeOH–$CHCl_3$ (1:9) from the metabolite ester chromatography was subjected to preparative TLC (acetone) and recrystallized from $CHCl_3$–light petroleum to give the *tetrol-ester* [8, 9·4 mg, 460 cpm/mg] as needles, m.p. 148–151°, $[\alpha]_D$ −103° (*c*, 0·7) (THF); NMR (δ): 0·90, 1·25, 1·51 (CH_3–C<); 3·60 (CO_2CH_3); 4·21 (1H, d of d, $J_{5,6}$ = 10 Hz, $J_{6,7}$ = 2 Hz, H-6); 5·75 (2H, >C=C<); NMR (δ, C_5D_5N): 1·22, 1·38, 1·71 (CH_3–C<); 2·98 (1H, m, H-13); 3·02 (1H, d, $J_{5,6}$ = 10 Hz, H-5); 3·60 (CO_2CH_3); 3·95 (1H, $W_{h/2}$ = 3 Hz, H-7); 6·0, 5·92, 3·95 (ABX pattern, $J_{2,3}$ = 10 Hz, $J_{1,2}$ = 5·4 Hz, $J_{1,3}$ = −0·4 Hz, H-2, H-3, H-1 resp). MS: *m/e* 380 (21, M^+), 362 (70), 344 (26), 326 (18), 303 (56), 285 (63), 267 (54), 239 (15), 221 (49), 203 (97), 185 (83), 142 (98), 110 (72), 43 (100); *m/e* 142→110 metastable ion. Found: *m/e* 85·20, calculated: 85·21.

Methyl ent - 6α,7α - *dihydroxykaura* - 2,16 - *dien* - 19 - *oate* (**10**). In a second large scale metabolism of the ^{14}C-dienol [1, 12 g, 530 cpm/mg], an additional acidic fraction was obtained by extraction with 5% NaOH solution. Chromatography of the methyl esters (315 mg) derived from this fraction on Al_2O_3 (25 g) gave upon elution with benzene–light petroleum (1:1), a residue (105 mg) which crystallized from benzene–light petroleum to give the *diol-ester* (**9**, 35 mg, 520 cpm/mg) as needles, m.p. 157–159, $[\alpha]_D$ −6° (*c*, 0·82); ν_{max}: 3560, 3480, 3050, 1705, 1650, 890 cm^{-1}; NMR (δ): 0·95, 1·51 (CH_3–C<); 3·64 (1H, s, $W_{h/2}$ = 4 Hz, H-7); 3·73 (CO_2CH_3); 4·11 (1H, d of d, $J_{5,6}$ = 10 Hz, $J_{6,7}$ = 2 Hz, H-6); 4·86 (>C=CH_2); 5·61 (>C=C<). NMR (δ, C_5D_5N): 1·11, 1·71 (CH_3–C<); 3·73 (CO_2CH_3); 2·45 (1H, d, $J_{5,6}$ = 10 Hz, H-5); 3·86 (1H, s, $W_{h/2}$ = 3 Hz, H-7); 4·61 (1H, d of d, $J_{5,6}$ = 10 Hz, $J_{6,7}$ = 2 Hz, H-6); 4·88 (>C=CH_2); 5·70 (>C=C<). MS: *m/e* 346 (8, $\dot{M}$), 328 (100), 313 (3), 296 (12), 269 (61), 268 (37), 251 (10), 270 (15), 202 (92). (Found: 328·202; $C_{21}H_{28}O_3$ requires: 328·204).

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