Pergamon

Phytochemistry, Vol. 42, No. 6, pp. 1641–1646, 1996

0031-9422/96 $15.00 + 0.00

S0031-9422(96)00231-2

SESQUITERPENOIDS AND DITERPENOIDS FROM THE LIVERWORT *JUNGERMANNIA TRUNCATA*

MALCOLM S. BUCHANAN, JOSEPH D. CONNOLLY,* AZIZOL A. KADIR† and DAVID S. RYCROFT

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.; †Malaysian Forest Research Institute, Kepong, Kuala Lumpur, Malaysia

(*Received* 23 *January* 1996)

Key Word Index—*Jungermannia truncata*; Jungermanniales; Jungermanniaceae; Hepaticae; liverwort; sesquiterpenoids; diterpenoids; *ent*-kaurane; pimarane; halimane; gymnomitrane; nor-gymnomitrane, ^{1}H and ^{13}C parameters.

Abstract—Twelve *ent*-kaurane diterpenoids, nine of which are new, have been isolated from the liverwort *Jungermannia truncata*, collected in Malaysia. The extract also contained new gymnomitrane and nor-gymnomitrane sequiterpenoids together with known pimarane and halimane diterpenoids. The structures were established mainly by ^{1}H and ^{13}C NMR spectroscopy. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Jungermannia species of liverwort are rich sources of diterpenoids [1]. *Jungermannia truncata* Nees has been investigated once previously (collected in Japan) and was found to contain two known *ent*-kaurane diterpenoids, *ent*-11α-hydroxy-16-kauren-15-one (**12**) and *ent*-(16*S*)-11α-hydroxykauran-15-one [2]. In this paper, we report the isolation and characterization of nine new *ent*-kaurane diterpenoids (**1–9**) and new gymnomitrane (**10**) and nor-gymnomitrane (**11**) sesquiterpenoids, together with previously known kaurane (**12–14**), pimarane (**15**) and halimane (**16**) diterpenoids, from the ether extract of a Malaysian collection of *J. truncata*.

RESULTS AND DISCUSSION

Compound **1**, $C_{20}H_{30}O_2$ (*m/z* 302.2239, $[M]^+$), is a crystalline compound and was present in very high yield (about 20% of the crude extract). The IR spectrum indicated the presence of a hydroxyl (ν_{max} 3625, 3486 cm^{-1}) and an α,β-unsaturated carbonyl in a five-membered ring (ν_{max} 1727, 1645 cm^{-1}). The UV spectrum showed a band at λ_{max} 232 nm, supporting the presence of a conjugated carbonyl. The ^{1}H NMR spectrum contained signals for three tertiary methyl groups (δ_H 0.80, 0.86, 1.06), a deshielded proton at δ_H 3.05 (*m*; H-13), a secondary carbinol proton at δ_H 4.03 (*dd*, J = 12.6, 4.5 Hz), two exomethylene protons (δ_H 5.91, *t*, J = 1.1 Hz; 5.23, *t*, J = 1.1 Hz) and a hydroxyl proton (δ_H 2.78, *br s*, exchangeable with D_2O). The ^{13}C NMR spectrum indicated the presence of 20 carbons (Table 1): three methyls, seven methylenes, three methines, three quaternary carbons, one carbonyl carbon (δ_C 210.0, *s*), one oxygenated methine (δ_C 70.5) and two olefinic carbons (δ_C 149.1, *s*, 114.5, *t*). The above spectral data indicate that compound **1** is tetracarbocyclic and are consistent with a hydroxylated 16-kauren-15-one structure.

The location and stereochemistry of the hydroxyl group was determined as follows. The multiplicity and coupling constants (δ_H 4.03, *dd*, J = 12.6, 4.5 Hz) of the carbinol methine proton indicate that the hydroxyl group is equatorial and must be attached to C-1, C-3 or C-7. Irradiation of the carbinol methine proton caused the neighbouring equatorial proton at δ_H 1.81 (*ddd*, J = 12.6, 4.5, 1.8 Hz) to collapse to a doublet of doublets (J = 12.6, 1.8 Hz) and the neighbouring axial signal at δ_H 1.38 (*q*, J = 12.6 Hz) to collapse to a triplet (J = 12.6 Hz). Thus the neighbouring methylene protons have only one further vicinal coupling. This information reveals the presence of the partial structure shown in Fig. 1. NOE difference experiments revealed NOEs between H-7 and both H-5β and H-9β. Thus, these results establish the hydroxyl group as 7α. The above data indicate that the structure of **1** is *ent*-7β-hydroxy-16-kauren-15-one, assuming that the compound belongs to the *ent*-series. This matter will be discussed below.

Compound **2** has the molecular formula $C_{20}H_{32}O$ (*m/z* 288.2456, $[M]^+$) and is clearly closely related to **1**. Its NMR spectra contained signals for an isolated exomethylene group (δ_H 4.82, *m*; 4.76, *m*; δ_C 1.55, *s*, 108.4, *t*) and a methylene group (δ_H 2.65, *dt*, J = 16.8, 2.7 Hz; 1.92 *br d*, J = 16.8 Hz; δ_C 43.2) instead of the proton and carbon signals belonging to the enone moiety in **1**. Moreover, the enone IR absorption bands

*Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR data for *ent*-kauranes **1–9**

C	1	2	3	4	5	6	7	8	9
1	39.1 t	40.2 *t*	39.1 *t*	32.5 *t**	40.1 *t*	40.1 *t*	34.1 *t*	34.4 *t*	34.5 *t*
2	18.0 *t*‡	18.5 *t*†	18.2 *t*†	25.0 *t*	18.5 *t*	18.5 *t*	18.5 *t*‡	18.6 *t*†	18.6 *t*†
3	41.4 *t*	41.8 *t*	41.6 *t*	75.8 *d*	41.7 *t*	41.7 *t*	41.5 *t*	41.6 *t*	41.6 *t*
4	32.9 *s*	33.2 *s*	33.1 *s*	37.5 *s*	33.1 *s*	33.0 *s*	32.9 *s*	33.0 *s*	33.2 *s*
5	52.4 *d*	53.1 *d*	52.8 *d*	48.1 *d*	52.6 *d*	52.5 *d*	53.0 *d*	53.0 *d*	52.9 *d*
6	27.5 *t**	29.4 *t**	28.3 *t*	18.1 *t*‡	27.9 *t**	27.4 *t**	29.1 *t*	29.9 *t*	29.7 *t*
7	70.5 *d*	75.2 *d*	71.6 *d*	32.4 *t**	73.8 *d*	72.1 *d*	71.9 *d*	71.2 *d*	71.4 *d*
8	58.3 *s*	49.8 *s*	58.5 *s*	52.4 *s*	52.7 *s*	51.1 *s*	58.3 *s*	58.9 *s*	58.3 *s*
9	51.7 *d*	55.9 *d*	51.8 *d*	52.2 *d*	53.0 *d*	45.6 *d*	52.0 *d*	51.6 *d*	52.1 *d*
10	39.5 *s*	39.3 *s*	39.3 *s*	39.8 *s*	39.5 *s*	38.6 *s*	42.5 *s*	42.6 *s*	42.8 *s*
11	17.7 *t*‡	17.9 *t*†	17.7 *t*†	18.2 *t*‡	17.9 *t*	17.9 *t*	18.2 *t*‡	18.3 *t*†	18.4 *t*†
12	32.6 *t*	33.6 *t*	25.2 *t*	33.4 *t*	33.2 *t*	33.2 *t*	24.0 *t*	28.1 *t*	32.0 *t*
13	37.4 *d*	43.3 *d*	34.3 *d*	38.1 *d*	41.4 *d*	40.0 *d*	34.6 *d*	37.0 *d*	37.8 *d*
14	27.6 *t**	30.5 *t**	28.3 *t*	36.6 *t*	28.5 *t**	29.2 *t**	28.3 *t*	27.0 *t*	28.6 *t*
15	210.0 *s*	43.2 *t*	224.2 *s*	209.3 *s*	84.4 *d*	75.5 *d*	224.7 *s*	226.0 *s*	209.6 *s*
16	149.1 *s*	155.1 *s*	48.3 *d*	149.5 *s*	160.8 *s*	157.8 *s*	48.5 *d*	46.8 *d*	149.7 *s*
17	114.5 *t*	108.4 *t*	9.9 *q*	114.4 *t*	107.7 *t*	105.0 *t*	10.0 *q*	15.3 *q*	114.6 *t*
18	33.3 *q*	33.5 *q*	33.4 *q*	28.4 *q*	33.6 *q*	33.5 *q*	34.0 *q*	34.0 *q*	34.0 *q*
19	21.3 *q*	21.5 *q*	21.4 *q*	22.0 *q*	21.6 *q*	21.6 *q*	22.3 *q*	22.4 *q*	22.4 *q*
20	17.5 *q*	17.7 *q*	17.8 *q*	17.3 *q*	17.7 *q*	17.7 *q*	61.2 *t*	61.3 *t*	61.3 *t*

*†‡Assignments may be interchangeable in each vertical column.

Identification of compounds and signal assignment were helped by comparing NMR data with published data [2, 4, 10–15].

of **1** were absent in the spectrum of **2**. These spectral data indicate that **2** is a 15-deoxy derivative of **1**. The structure of **2** is, therefore, represented as *ent*-7β-hydroxy-16-kaurene. Although **2** is a new natural product it should be noted that it has been synthesized previously [3].

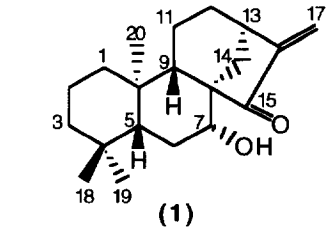
HR-mass spectrum of compound **3** gave the molecular formula $C_{20}H_{32}O_2$ (*m/z* 304.2421, $[M]^+$). The IR spectrum showed the presence of a hydroxyl (ν_{max} 3625, 3470 cm^{-1}) and a saturated five-membered ketone (ν_{max} 1740 cm^{-1}). The NMR data are similar to those of **1** except for the presence of a saturated ketone (δ_C 224.2), a secondary methyl (δ_H 1.09, *d*, J = 7.0 Hz; δ_C 9.9) and a methine (δ_H 2.21, *quintet*, J = 7.0 Hz; δ_C 48.3) in place of the proton and carbon signals from the enone moiety of **1**. The stereochemistry of the C-17 methyl was determined by consideration of J(H-13, H-16) and NOEs from {H-13}. Models show that for a 17α-methyl J(H-13, H-16β) is close to zero Hz (dihedral angle ~90°) and a significant NOE is expected for 3H-17 from {H-13} whereas for a 17β-methyl J(H-13, H-16α) is *ca* 7 Hz (dihedral angle ~30°) and it is H-16α which will experience a large NOE from {H-13}. Since in the case of **3**, J(H-13, H-16) is 7.0 Hz and irradiation of H-13 affords a large NOE at H-16 the 17-methyl group is β and the configuration at C-16 is *R*(*ent-S*). The compound **3** is *ent*-16(*S*)-7β-hydroxy-kauran-15-one.

The molecular formula of the next compound **4** was established as $C_{20}H_{30}O_2$ (*m/z* 302.2247, $[M]^+$) by HR-mass spectroscopy. Its NMR data are similar to those of *ent*-3β-hydroxy-16-kauren-15-one, a compound isolated from the liverwort *Jungermannia vulcanicola* [2]. Differences in the NMR data can be rationalised in terms of these compounds being C-3 epimers. The multiplicity and coupling constant values of H-3 (δ_H 3.40, *br t*, J = 2.5 Hz) are consistent with its equatorial nature. Also irradiation of H-3α gives NOEs at 3H-19 and 3H-18. The above spectroscopic data established the structure of **4** as *ent*-3α-hydroxy-16-kauren-15-one.

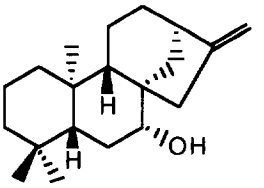
Compound **5** has the molecular formula $C_{20}H_{32}O_2$ (*m/z* 304.2386, $[M]^+$). Its NMR data are similar to those of **1** except for the presence of an oxygenated methine (δ_H 4.07, *br s*; δ_C 84.4) instead of a C-15 carbonyl group. Irradiation of H-15 (δ_H 4.07) resulted in NOEs at H-9β and H-17Z indicating a C-15α-hydroxyl group. Comparison of the ^{13}C NMR data of **5** and *ent*-15β,7α-dihydroxy-16-kaurene, which has been isolated from the liverwort *Plagiochila pulcherrima* [4], reveals strong similarities. Differences in chemical shift can be explained if they are C-7 epimers. On the basis of the above findings compound **5** is *ent*-15β,7β-dihydroxy-16-kaurene.

The HRMS of **6** gave the same molecular formula $C_{20}H_{32}O_2$ (*m/z* 304.2396, $[M]^+$) as that of **5**. On comparison of the ^1H and ^{13}C NMR data of **6** with those of **5** it is clear that these compounds are C-15 epimers. Irradiation of H-15 (δ_H 4.45, *br t*, J = 2.5 Hz; δ_C 75.5, *d*) resulted in a NOE at H-14*R*, thus also indicating a β-hydroxyl group at C-15. The above results reveal that **6** is *ent*-15α,7β-dihydroxy-16-kaurene.

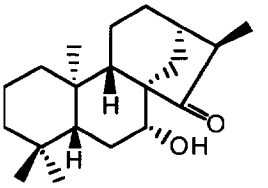
Compound **7** has the molecular formula, $C_{20}H_{32}O_3$ (*m/z* 320.2332, $[M]^+$). Spectroscopically it resembles **3** except for the presence of a hydroxymethyl group (δ_H 4.04, *s* 2H; δ_C 61.2, *t*) in place of a tertiary methyl group. NOEs were observed at H-14*S*, H-6α and 3H-19 on irradiation at δ_H 4.04. These are consistent with the replacement of the C-20 tertiary methyl group in **3** by a hydroxymethyl group in **7**. Thus **7** is *ent*-16(*S*)-7β,20-dihydroxykauran-15-one.



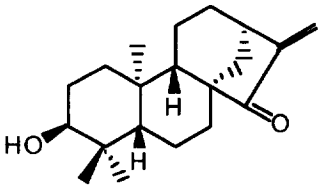
(1)



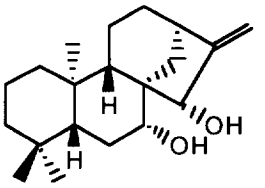
(2)



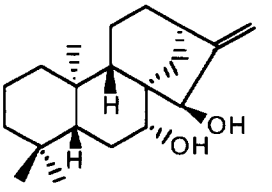
(3)



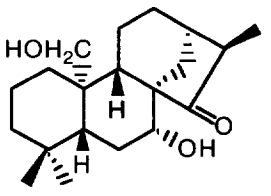
(4)



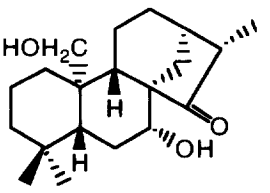
(5)



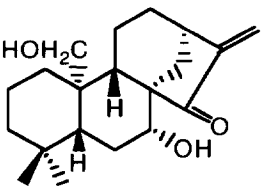
(6)



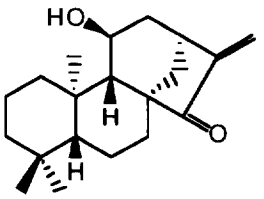
(7)



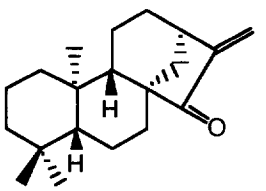
(8)



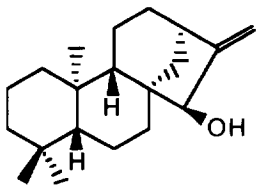
(9)



(12)



(13)



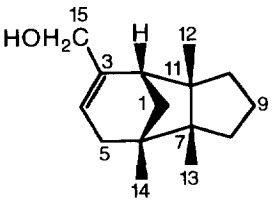
(14)

The next fraction contained a mixture of three compounds **6**, **8** and **9**. There was too little material to attempt further separation. The GC of this mixture established the presence of the three diterpenoids in the ratio 1(**6**): 3(**8**): 1(**9**). It was evident from the ^{1}H and ^{13}C NMR spectra of the mixture that one of the diterpenoids was **6**. Careful analysis of the NMR data enabled the structures of the remaining two diterpenoids to be established.

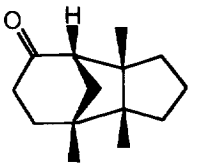
The GC-mass spectrometry of compound **8** gave a molecular ion at *m/z* 320. The ^{1}H and ^{13}C NMR data of **8** closely resemble those of **7** and any dissimilarity can be explained by their being C-16 epimers. The methine H-16 was identified within a multiplet of overlapping signals by NOE difference and homonuclear decoupling experiments. Decoupling of the secondary methyl group (δ_H 1.08, *d*, $J = 7.6$ Hz) caused a broad quartet at δ_H 2.15 ($J = 7.6$ Hz, H-16) to collapse into a broad singlet. In a NOE difference experiment irradiation of the secondary methyl group (3H-17) showed the position of the broad quartet of H-16. The configuration at C-16 is *S* (*ent*-16*R*) since *J*(H-13, H-16) is *ca* zero Hz. The secondary methyl at C-16 is therefore α. On the basis of these findings structure **8**, *ent*-(16*R*)-7β,20-dihydroxykauran-15-one, is assigned to this compound.

Compound **9** did not give a molecular ion on GC-mass spectrometry analysis. However, comparison of its ^{1}H and ^{13}C NMR spectra with those of **1**, **7** and **8** readily revealed its structure as *ent*-7β,20-dihydroxy-16-kauren-15-one **9**.

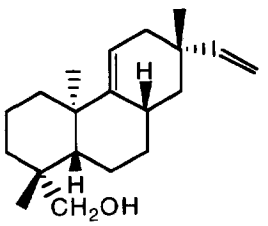
The previously known *ent*-11α-hydroxy-16-kauren-15-one (**12**) [1, 5], *ent*-16-kauren-15-one (**13**) [1, 6] and *ent*-15α-hydroxy-16-kaurene (**14**) [6] were also isolated from this extract and identified by comparison of their spectroscopic data with published data [5, 6]. Although the absolute stereochemistry of the new kauranes **1**–**9** and known kauranes **12** and **13** has not been established we assume that they belong to the *enantio*-series on the basis of the co-occurrence of the known *ent*-kaurene **14** [6]. The sign of the specific rotation of **14** ($[\alpha]_D$ −44, lit. $[\alpha]_D$ −70) [6] from the



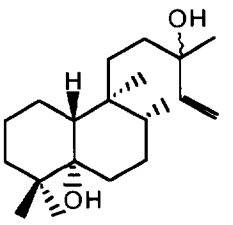
(10)



(11)



(15)



(16)

present extract is the same as for **1** ($[\alpha]_D$ −112). In general *ent*-kauranes have negative rotations.

The next two components of the extract proved to be sequiterpenoid derivatives with a gymnomitrane skeleton. The molecular formula of **10** was determined as $C_{15}H_{24}O$ ($[M]^+$ at *m/z* 220.1818) by HR-mass spectrometry. The IR spectrum showed the presence of a hydroxyl group (ν_{max} 3619, 3349 cm^{-1}). The ^1H NMR spectrum contained signals for three tertiary methyls (δ_H 1.00, 0.91, 0.85), two protons attached to an oxygenated carbon (δ_H 3.98, m, 2H) and an olefinic proton (δ_H 5.48, (*m*). The ^{13}C NMR spectrum (Table 2) showed the presence of a trisubstituted double bond (δ_C 143.5, *s*, 121.2, *d*) and a primary alcohol (δ_C 67.3). There were further signals for three methyl groups, five methylene groups, one methine and three quaternary carbons. These data indicate a tricyclic sesquiterpenoid containing three tertiary methyl groups, a trisubstituted double bond and a primary alcohol. These data are consistent with structure **10**, 3-gymnomitren-15-ol. Irradiation of H-2 (δ_H 1.81, *d*, $J = 4.4$ Hz) affords NOEs at 2H-15 and 3H-12 supporting the attachment of the hydroxymethyl group at C-3.

Table 2. ^{13}C NMR data for compounds **10** and **11**

C	10	11
1	42.8 *t*	41.8 *t*
2	47.0 *d*	63.3 *d*
3	143.5 *s*	215.2 *s*
4	121.2 *d*	33.6 *t*
5	40.3 *t*	37.1 *t*
6	43.9 *s*	43.7 *s*
7	55.5 *s*	54.4 *s*
8	37.2 *t*	36.0 *t*
9	27.2 *t*	26.5 *t*
10	38.3 *t*	37.8 *t*
11	58.4 *s*	55.9 *s*
12	27.5 *q*	27.3 *q*
13	24.7 *q*	24.8 *q*
14	23.6 *q*	23.5 *q*
15	67.3 *t*	

Identification of compounds and signal assignment were helped by comparing NMR data with those of other gymnomitranes [16, 17].

The HR-mass spectrometry of compound **11** gave the molecular formula $C_{14}H_{22}O$ ($[M]^+$ at *m/z* 206.1663). The IR spectrum showed the presence of a six-membered ketone (ν_{max} 1707 cm^{-1}). The ^1H NMR spectrum has signals for three tertiary methyls (δ_H 1.07, 0.99, 0.95) while the ^{13}C NMR spectrum shows fourteen carbons: three methyls, six methylenes, one methine, three quaternary carbons and a carbonyl carbon (δ_C 215.2, *s*). Compound **11** is therefore a tricyclic nor-sesquiterpene with three tertiary methyls and one ketone group. These data are consistent with a nor-gymnomitrane skeleton containing a carbonyl group at C-3 and thus **11** is 15-nor-3-gymnomitrone. This is the first example of a nor-gymnomitrane derivative as a natural product. However, compound **11** has previously been formed as a synthetic intermediate [7].

Two other types of diterpenoid, a pimarane and a halimane, were also obtained from this extract of *J. truncata*. Pimara-9(11),15-dien-19-ol (**15**) is a new constituent of liverworts although it has been found in the Korean medicinal plant, *Acanthopanax koreanum* [8]. Halimane diterpenoids are rare in nature, but pleuroziol (**16**) has been isolated previously from the liverwort *Pleurozia gigantea* [9]. These two compounds were identified by comparison of their spectroscopic data with published data [8, 9].

The present study of *J. truncata* enhances further the reputation of the Jungermanniales as rich sources of diterpenoids. This extract was particularly fruitful in *ent*-kauranes.

EXPERIMENTAL

General. TLC: over Merck precoated silica gel 60 F_{254} and visualised under UV light (254 nm) and by spraying with 25% H_2SO_4 and heating. Flash CC and PLC: Silica gel GF_{254}. Mps uncorr. GC: CP Sil 5 CB (chrompack) fused silica capillary column (25 m × 0.32 mm i.d. × 0.12 μm) and FID. The Grob-type injector was operated in the split mode (50:1) and the He carrier and make up gas flow rate was 2 ml min^{-1}. A linear temperature programme was used in which the column temperature was programmed from 80° (2 min) to 240° (5 min) at 5°C min^{-1}. The injection port and detector temperature were 255° and 260°, respectively. GC-MS: HP1 fused silica capillary column (12.5 m × 0.2 mm i.d. × 0.33 μm). Injection and temperature programing conditions are identical to those for GC above. Measured at 70 eV. NMR spectra (^{1}H, 200 MHz; ^{13}C, 50 MHz) were recorded for $CDCl_3$ solutions; chemical shifts are relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0. Assignment of ^{1}H NMR signals was aided by homonuclear decoupling and NOE difference experiments. Multiplicities were determined by DEPT experiments. IR spectra were measured for CCl_4, UV for EtOH and $[\alpha]_D$ for $CHCl_3$ solns. EIMS was measured at 70 eV.

Plant material. Jungermannia truncata was collected by JDC in the grounds of the Forest Research Institute of Malaysia, Kepong in January 1993. Identification was carried out by Dr R. Grolle, Jenna. Voucher specimens are held in Jena and in the Department of Chemistry, University of Glasgow.

Extraction and isolation. The ground material, which was contaminated with soil, was extracted with Et_2O to give a crude extract (2.2 g) which was subjected to chromatography on silica gel using a petrol–Et_2O gradient to give 7 fractions. These fractions were rechromatographed by PLC over silica gel (petrol–Et_2O, CH_2Cl_2–Et_2O, *n*-hexane–CH_2Cl_2) to give the following constituents in order of increasing polarity: *ent*-16-kauren-15-one (**13**) (12 mg), *ent*-15α-hydroxy-16-kaurene (**14**) (45 mg) $[\alpha]_D$ −44 ($CHCl_3$, *c* 1.00), 15-nor-3-gymnomitrone (**11**) (6 mg), *ent*-7β-hydroxy-16-kaurene (**2**) (4 mg), 3-gymnomitrene-15-ol (**10**) (7 mg), pimara-9(11),15-dien-19-ol (**15**) (6 mg), pleuroziol (**16**) (3 mg), *ent*-7β-hydroxy-16-kauren-15-one (**1**) (380 mg), *ent*-16(*S*)-7β-hydroxykauran-15-one (**3**) (1 mg), *ent*-3α-hydroxy-16-kauren-15-one (**4**) (7 mg), *ent*-15β-7β-dihydroxy-16-kaurene (**5**) (4 mg), *ent*-15α,7β-dihydroxy-16-kaurene (**6**) (30 mg); a mixture (3 mg) of **6**, *ent*-16(*R*)-7β,20-dihydroxykauran-15-one (**8**) and *ent*-7β,20-dihydroxy-16-kauren-15-one (**9**); *ent*-11α-hydroxy-16-kauren-15-one (**12**) (1 mg) and *ent*-16(*S*)-7β,20-dihydroxykauran-15-one (**7**) (1 mg).

ent-7β-*Hydroxy*-16-*kauren*-15-*one* (**1**). Crystals from petrol–Et_2O, mp 142–144°. $[\alpha]_D$ −112 ($CHCl_3$, *c* 1.16). HR-MS: *m/z* 302.2239 $[M]^+$ calculated for $C_{20}H_{30}O_2$: 302.2246. EIMS *m/z* (rel. int.): 302 $[M]^+$ (100), 274 (44), 246 (15), 225 (21), 180 (20), 165 (30), 152 (40), 135 (61), 109 (49), 91 (45), 79 (52). UV λ_{max} nm: 232. IR ν_{max} cm^{-1}: 3625, 3486 (OH), 1727 (C=O), 1645 (C=C). ^{1}H NMR: δ_H 5.91 (*t*, *J* = 1.1 Hz, H-17*Z*); 5.23 (*t*, *J* = 1.1 Hz, H-17*E*); 4.03 (*dd*, *J* = 12.6, 4.5 Hz, H-7β); 3.05 (*m*, H-13); 2.78 (*br s*, OH); 2.03 (*m*, 2H-14); 1.92 (*tdd*, *J* = 13.4, 7.0, 3.0 Hz, H-12α); 1.81 (*ddd*, *J* = 12.6, 4.5, 1.8 Hz, H-6β); 1.74 (*m*, H-1α); 1.66 (*br q*, *J* = 14.0 Hz, H-2α); 1.65 (*m*, H-12β); 1.38 (*q*, *J* = 12.6 Hz, H-6α); 1.13 (*br d*, *J* = 8.5 Hz, H-9β); 1.06 (*s*, 3H-20); 0.88 (*dd*, *J* = 12.6, 1.8 Hz, H-5β); 0.86 (*s*, 3H-18); 0.80 (s, 3H-19); 0.68 (*td*, *J* = 12.4, 3.4 Hz, H-1β). ^{13}C: NMR see Table 1.

ent-7β-*Hydroxy*-16-*kaurene* (**2**). Amorphous solid. HR-MS: *m/z* 288.2456 $[M]^+$ calculated for $C_{20}H_{32}O$: 288.2453. EIMS *m/z* (rel. int.): 288 $[M]^+$ (100), 270 (52), 255 (16), 190 (24), 164 (28), 149 (16), 123 (42), 109 (31), 91 (40), 79 (53). IR ν_{max} cm^{-1}: 3627, 3509 (OH); 1657 (C=C). ^{1}H NMR: δ_H 4.82 (*m*, H-17*Z*); 4.76 (*m*, H-17*E*); 3.46 (*dd*, *J* = 12.2, 4.2 Hz, H-7β); 2.68 (*m*, H-13); 2.65 (*dt*, *J* = 16.8, 2.7 Hz, H-15α); 1.92 (*br d*, *J* = 16.8 Hz, H-15β); 1.80 (*ddd*, *J* = 12.2, 4.2, 1.7 Hz, H-6β); 1.78 (*br d*, *J* = 12.4 Hz, H-9β); 1.67 (*m*, H-14*R*); 1.37 (*q*, *J* = 12.2 Hz, H-6α); 1.02 (*s*, 3H-20); 0.86 (*s*, 3H-18); 0.86 (*dd*, *J* = 12.2, 1.7 Hz, H-5β); 0.81 (*s*, 3H-19); 0.71 (*td*, *J* = 13.0, 4.0 Hz, H-1β). ^{13}C NMR: See Table 1.

ent-(16S)-7β-*Hydroxykauran*-15-*one* (**3**). Amorphous solid. HR-MS: *m/z* 304.2421 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402. EIMS *m/z* (rel. int.): 304 $[M]^+$ (100), 286 (25), 271 (15), 246 (63), 152 (39), 123 (56), 109 (54), 93 (32), 81 (56). IR ν_{max} cm^{-1}: 3625, 3470 (OH); 1740 (C=O). ^{1}H NMR: δ_H 3.94 (*dd*, *J* = 12.4, 4.6 Hz, H-7β); 2.46 (*m*, H-13); 2.21 (*q*, *J* = 7.0 Hz, H-16α); 2.09 (*dd*, *J* = 12.1, 1.3 Hz, H-14*S*); 1.95 (*br dd*, *J* = 12.1, 4.5 Hz, H-14*R*); 1.80 (*ddd*, *J* = 12.4, 4.6, 1.8 Hz, H-6β); 1.37 (*q*, *J* = 12.4 Hz, H-6α); 1.09 (*d*, *J* = 7.0 Hz, 3H-17); 1.07 (*s*, 3H-20); 1.02 (*br d*, *J* = 8.8 Hz, H-9β); 0.93 (*dd*, *J* = 12.4, 1.8 Hz, H-5β); 0.87 (*s*, 3H-18); 0.81 (*s*, 3H-19); 0.68 (*td*, *J* = 13.0, 4.0 Hz, H-1β). ^{13}C NMR: see Table 1.

ent-3α-*Hydroxy*-16-*kauren*-15-*one* (**4**). Amorphous solid. HR-MS: *m/z* 302.2247 $[M]^+$ calculated for $C_{20}H_{30}O_2$: 302.2246. EIMS *m/z* (rel. int.): 302 $[M]^+$ (100), 284 (43), 269 (64), 246 (20), 149 (85), 136 (29), 121 (39), 107 (42), 91 (57). UV λ_{max} nm: 229. IR ν_{max} cm^{-1}: 3630, 3500 (OH), 1725 (C=O), 1650 (C=C). ^{1}H NMR: δ_H 5.93 (*t*, *J* = 1.1 Hz, H-17Z); 5.24 (*t*, *J* = 1.1 Hz, H-17*E*); 3.40 (*br t*, *J* = 2.5 Hz, H-3α); 3.03 (*m*, H-13); 2.40 (*d*, *J* = 11.9 Hz, H-14*S*); 1.93 (*td*, *J* = 13.0, 2.5 Hz, H-2α); 1.88 (*tdd*, *J* = 13.2, 6.1, 2.6 Hz, H-12); 1.68 (*m*, H-12); 1.56 (*m*, H-2β); 1.32 (*m*, H-14*R*); 1.09 (*s*, 3H-20); 0.95 (*s*, 3H-18); 0.84 (*s*, 3H-19). ^{13}C NMR: see Table 1.

ent-15β,7β-*Dihydroxy*-16-*kaurene* (**5**). Amorphous solid. HR-MS *m/z* 304.2386 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402. EIMS *m/z* (rel. int.): 304 $[M]^+$ (100), 286 (96), 271 (42), 258 (30), 162 (37), 131 (24), 122 (64), 105 (49), 91 (91), 83 (24), 71 (25). IR ν_{max} cm^{-1}: 3600, 3405 (OH). ^{1}H NMR: δ_H 5.15 (*br s*, H-17*Z*); 5.07 (*br s*, H-17*E*); 4.07 (*br s*, H-15β); 3.85

(*dd*, $J = 12.4$, 4.7 Hz, H-7β); 2.81 (*m*, H-13); 2.04 (*br dd*, $J = 12.2$, 5.3 Hz, H-14*R*); 1.83 (*ddd*, $J = 12.4$, 4.7, 1.5 Hz, H-6β); 1.79 (*br d*, $J = 13.5$ Hz, H-1α); 1.65 (*br d*, $J = 12.2$ Hz, H-14*S*); 1.42 (*m*, H-2β); 1.41 (*q*, $J = 12.4$ Hz, H-6α); 1.03 (*s*, 3H-20); 0.89 (*s*, 3H-18); 0.88 (*dd*, $J = 12.4$, 1.5 Hz, H-5β); 0.82 (*s*, 3H-19). ^{13}C NMR: see Table 1.

ent-15α,7β-*Dihydroxy*-16-*kaurene* (**6**). Amorphous solid HR-MS: *m/z* 304.2396 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402. EIMS *m/z* (rel. int.): 304 $[M]^+$ (98), 286 (50), 271 (25), 246 (63), 152 (40), 137 (22), 123 (100), 109 (88), 91 (88), 81 (91). IR ν_{max} cm^{-1}: 3630, 3424 (OH). ^{1}H NMR: δ_H 5.09 (*m*, H-17*Z*); 4.96 (*br d*, $J = 2.5$ Hz, H-17*E*); 4.45 (*br t*, $J = 2.5$ Hz, H-15α); 3.56 (*dd*, $J = 11.6$, 4.5 Hz, H-7β); 2.69 (*m*, H-13); 1.80 (*ddd*, $J = 11.6$, 4.5, 1.7 Hz, H-6β); 1.56 (*m*, 2H-14); 1.37 (*q*, $J = 11.6$ Hz, H-6α); 1.30 (*br d*, $J = 8.5$ Hz, H-9β); 1.03 (*s*, 3H-20); 0.87 (*br d*, $J = 11.6$, *H*-5β); 0.86 (*s*, 3H-18); 0.81 (*s*, 3H-19). ^{13}C NMR: see Table 1.

ent-(16S)-7β,20-*Dihydroxykauran*-15-*one* (**7**). Amorphous solid. HR-MS: *m/z* 320.2332 $[M]^+$ calculated for $C_{20}H_{32}O_3$: 320.2351. EIMS *m/z* (rel. int.): 320 $[M]^+$ (7), 274 (48), 191 (12), 173 (12), 163 (14), 149 (29), 137 (23), 123 (28), 109 (44), 95 (100). IR ν_{max} cm^{-1}: 3630, 3584, 3458 (OH); 1734 (C=O). ^{1}H NMR: δ_H 4.04 (*s*, 2H-20); 4.00 (*dd*, $J = 11.7$, 4.9 Hz, H-7β); 2.46 (*m*, H-13); 2.23 (*q*, $J = 7.0$ Hz, H-16α); 2.20 (*br d*, $J = 12.0$ Hz, H-14*S*); 2.08 (*m*, H-14*R*); 1.77 (*ddd*, $J = 11.7$, 4.9, 1.8 Hz, H-6β); 1.52 (*br d*, $J = 12.5$ Hz, H-9β); 1.46 (*q*, $J = 11.7$ Hz, H-6α); 1.10 (*d*, $J = 7.0$ Hz, 3H-17); 1.06 (*dd*, $J = 11.7$, 1.8 Hz, H-5β); 0.90 (*s*, 3H-18); 0.84 (*s*, 3H-19); 0.60 (*td*, $J = 13.0$, 4.5 Hz, H-1β). ^{13}C NMR: see Table 1.

Mixture of **6**, **8**, *and* **9**. GC of a gummy mixture isolated from the extract identified the existence of three diterpenoids in the ratio 1 (**6**, RR_t (min) = 31.71): 3 (**8**, RR_t (min) = 34.50): 1 (**9**, RR_t (min) = 34.82). From the ^{1}H and ^{13}C NMR of the mixture one of the diterpenoids was obviously **6** and careful analysis of the NMR data enabled the structures of the remaining two diterpenoids **8** and **9** to be established.

ent-(16R)-7β,20-*Dihydroxykauran*-15-*one* (**8**). GC-MS *m/z* (rel. int.): 320 $[M]^+$ (14), 302 (4), 292 (17), 274 (100), 259 (16), 203 (19), 163 (30), 149 (55), 125 (42), 109 (53), 81 (58), 55 (81), 41 (92). ^{1}H NMR: δ_H 4.05 (*br s*, 2H-20); 3.95 (*dd*, $J = 11.8$, 4.8 Hz, H-7β); 2.15 (*br d*, $J = 13.0$ Hz, H-1α); 2.15 (*br q*, $J = 7.6$ Hz, H-16β); 1.08 (*d*, $J = 7.6$ Hz, 3H-17); 0.90 (*s*, 3H-18); 0.85 (*s*, 3H-19); 0.61 (*td*, $J = 13.0$, 5.0 Hz, H-1β). ^{13}C NMR: see Table 1.

ent-7β,20-*Dihydroxy*-16-*kauren*-15-*one* (**9**). ^{1}H NMR: δ_H 5.94 (br s, H-17*Z*); 5.26 (*br s*, H-17*E*); 4.12 (*dd*, $J = 11.7$, 5.1 Hz, H-7β); 4.05 (*br s*, 2H-20); 3.08 (*m*, H-13); 2.15 (*br d*, $J = 13.0$ Hz, H-1α); 0.92 (*s*, 3H-18); 0.87 (*s*, 3H-19); 0.61 (*td*, $J = 13.0$, 5.0 Hz, H-1β). ^{13}C NMR: see Table 1.

3-*Gymnomitren*-15-*ol* (**10**). Gum. $[\alpha]_D$ +27° ($CHCl_3$, *c* 0.17). HR-MS: *m/z* 220.1818 $[M]^+$ calculated for $C_{15}H_{24}O$: 220.1827. EIMS *m/z* (rel. int.): 220 $[M]^+$ (15), 189 (12), 136 (9), 124 (52), 111 (13), 106 (39), 95 (100), 79 (42), 67 (16). IR ν_{max} cm^{-1}: 3619, 3349 (OH). ^{1}H NMR: δ_H 5.48 (*m*, H-4); 3.98 (*m*, 2H-15); 2.22 (*m*, H-5α); 1.93 (*br dd*, $J = 18.8$, 3.4 Hz, H-5β); 1.93 (*ddd*, $J = 10.8$, 4.4, 1.0 Hz, H-1*S*); 1.81 (*d*, $J = 4.4$ Hz, H-2); 1.43 (*d*, $J = 10.8$ Hz, H-1*R*); 1.00 (*s*, 3H-12); 0.91 (*s*, 3H-13); 0.85 (*s*, 3H-14). ^{13}C NMR: see Table 2.

15-*Nor*-3-*gymnomitrone* (**11**). Gum. HR-MS: *m/z* 206.1663 $[M]^+$ calculated for $C_{14}H_{22}O$: 206.1671. EIMS *m/z* (rel. int.): 206 $[M]^+$ (15), 188 (23), 137 (25), 121 (15), 110 (46), 95 (100), 81 (48). IR ν_{max} cm^{-1} 1707 (C=O). ^{1}H NMR: δ_H 2.36 (*m*, 2H-4); 2.21 (*d*, $J = 4.5$ Hz, H-2); 2.08 (*ddd*, $J = 12.1$, 4.5, 2.9 Hz, H-1*S*); 1.70 (*d*, $J = 12.1$ Hz, H-1*R*); 1.07, 0.99, 0.95 (all *s*, 3H-12/3H-13/3H-14); ^{13}C NMR: see Table 2.

Acknowledgement—We are grateful to Dr R. Grolle, Jena, for identification of *J. truncata*.

REFERENCES

1. Asakawa, Y. (1982) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W., eds), Vol. 42, p. 1. Springer, Vienna.
2. Nagashima, F., Toyota, M. and Asakawa, Y. (1990) *Phytochemistry* **29**, 2169.
3. Node, M., Kajimoto, T., Ito, N., Tamada, J., Fujita, E. and Fuji, K. (1986) *J. Chem. Soc., Chem. Commun.* 1164.
4. Fukuyama, Y., Toyota, M. and Asakawa, Y. (1988) *Phytochemistry* **27**, 1425.
5. Connolly, J. D. and Thornton, I. M. S. (1973) *J. Chem. Soc., Perkin Trans. I* 736.
6. Matsuo, A., Kodama, J., Nakayama, M. and Hayashi, S. (1977) *Phytochemistry* **16**, 489.
7. Welch, S. C., Chayabunjonglerd, S. and Rao, A. S. C. P. (1980) *J. Org. Chem.* **45**, 4086.
8. Kim, Y. H. and Chung, B. S. (1988) *J. Nat. Prod.* **51**, 1080.
9. Asakawa, Y., Lin, X., Tori, M. and Kondo, K. (1990) *Phytochemistry* **29**, 2597.
10. Wehrli, F. W. and Nishida, T. (1979) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W., eds), Vol. 36, p. 1. Springer, Vienna.
11. Hutchison, M., Lewer, P. and Macmillan, J. (1984) *J. Chem. Soc., Perkin Trans. I* 2363.
12. Lopes, L. M. X., Bolzani, V. D. S., Trevisan, L. M. V. and Grigolli, T. M. (1990) *Phytochemistry* **29**, 660.
13. Herz, W. and Sharma, R. P. (1976) *J. Org. Chem.* **41**, 1021.
14. Takeda, Y., Ichihara, J., Takaishi, Y., Fujita, T., Shingle, T. and Kusano, G. (1987) *J. Chem. Soc., Perkin Trans. 1* 2403.
15. Hao, H., Yunlong, X. and Hundong, S. (1989) *Phytochemistry* **28**, 2753.
16. Connolly, J. D., unpublished results, University of Glasgow.
17. Connolly, J. D., Harding, A. E. and Thorton, I. M. S. (1974) *J. Chem. Soc., Perkin Trans. I* 2487.