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TERPENOIDS FROM *SALVIA LANIGERA*

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Key Word Index—*Salvia lanigera*; Lamiaceae; abietane-type diterpenes; oleanane-type triterpenoids.

Abstract—Two new terpenoids, 12-methoxycarnosic acid and 3β-hydroxyoleanan-13$\beta \rightarrow$ 28 lactone, have been isolated from the leaves of *Salvia lanigera*, together with two known diterpenes and two triterpenes.

INTRODUCTION

Our previous studies on the terpenoid constituents of *Salvia lanigera* [1, 2] showed the presence of diterpenes **1**–**3** and the triterpenes oleanolic (**4**) and ursolic (**5**) acids. Terpenes **1**, **3**–**5** were found to be major components of the extracts in which they occurred; the diterpene **2**, however, was present only in small amounts [3]. Re-examination of the fractions which gave **1**–**5** has yielded a new abietane diterpene which we have named 12-methoxycarnosic acid (**6**) and the new triterpene **7**.

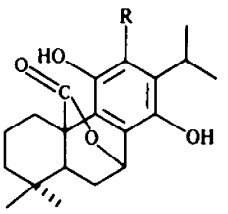
RESULTS AND DISCUSSION

The petrol extract of the leaves of *S. lanigera* on chromatographic separation yielded a new diterpene (**6**) as an oil, $[\alpha]_D^{20}$ +83.5° (chloroform; *c* 0.06). This compound was found to have the molecular formula $C_{21}H_{30}O_4$ (EIMS *m/z* 346.2149 $[M]^+$). Its IR spectrum revealed the presence of a carboxylic acid group (3300–2700 and 1690 cm^{-1}) and a free hydroxyl group (3490 cm^{-1}). The ^1H NMR spectrum of **6** showed signals for the following: CH– (δ3.1, *m*), H_2C–Ar (δ3.55, *m*, 2H-7), MeO(δ3.66, *s*), aromatic proton (δ6.5) and four methyl groups, two MeC (δ0.95 and 0.84) and two CH_3CH (δ1.17, *d*, *J* = 7 Hz). On decoupling the ^1H NMR signal centred at δ3.1, the doublet at δ1.17 collapsed into a singlet, whilst on irradiation at δ1.17, the multiplet at δ3.1 was changed to a singlet, confirming the presence of an isopropyl group. The ^1H NMR spectral data, together with the other spectroscopic features clearly indicated an abeitane-type structure with two oxygen groups on the aromatic ring. The mass spectrum of **6** revealed the presence of fragments at *m/z* 344, 302 and 300. The base peak (*m/z* 300) was attributed to the ion $[M-COOH-H]^+$ formed by the ready loss of the carboxyl group at C-10 as CO_2 [1, 4]. Evidence in favour of structure **6** for the new diterpene was provided by its ^{13}C NMR spectrum, which showed 21 signals (Table 1). The resonance of the unsubstituted carbon atom (δ118.0) was known to be characteristic of an aromatic carbon remote from an oxygen substituent [5]. Further evidence for the proposed structure came from the methylation of **6** to give **8**, which on esterification gave the known ester **9** [6]. Structure **6** seemed to be more likely than the alternative **10** by biogenetic analogy to similar compounds isolated from plants of the same genus, i.e. *S. bicolor* [7] and *S. canariensis* [8]. Further evidence in support of structure **6** was provided by Wenkert's studies [9], which demonstrated that a hydroxyl group at C-12 produces a solvent shift of approximately 0.4 ppm in the chemical shift of the methyls of an isopropyl group. The ^1H NMR spectrum of **6** measured in $CDCl_3$ and pyridine-d_5 revealed only a very small difference in the chemical shift of the methyls of the isopropyl group.

The terpenoid fraction, obtained after silica gel column chromatography of the methanolic extract, yielded **4** and **5** and other triterpenes. Attempts to separate this mixture further into its constituents on repeated column chromatography met with no success. Therefore, the mixture was acetylated and worked up in the usual manner to give a crystalline product, which on chromatography afforded the monoacetates of oleanolic and ursolic acids and the new compound **7**.

Table 1. ^{13}C NMR spectral data of compounds **6** and **8** ($CDCl_3$)

C	**6**	**8**
1	31.99	32.10
2	20.01	20.20
3	41.60	41.4
4	34.05	34.3
5	61.5	61.8
6	20.37	20.2
7	34.05	34.0
8	134.0	134.4
9	125.5	125.5
10	48.0	48.02
11	147.83	147.7
12	142.48	142.6
13	139.37	139.3
14	118.0	118.1
15	26.36	26.5
16	23.7	23.8
17	23.4	23.6
18	32.7	32.7
19	18.49	18.7
20	178.61	176.4
OMe	54.13	54.2, 51.6



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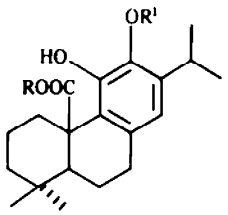
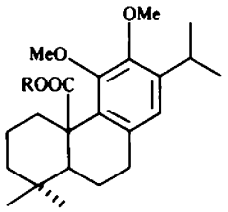
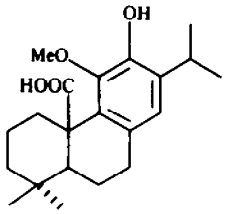
2 R = OH



3 R = Me, R¹ = H
6 R = H, R¹ = Me



8 R = H
9 R = Me



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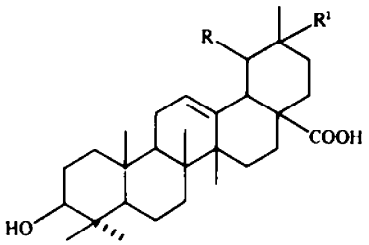
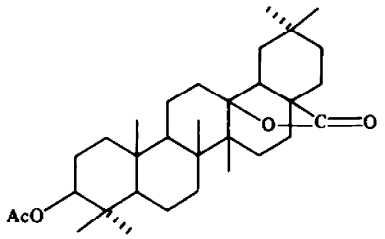


4 R = H, R¹ = Me
5 R = Me, R¹ = H



7

Compound 7, mp 282° (chloroform), analysed for $C_{32}H_{50}O_4$ (*m/z* 498 $[M]^+$). It gave a positive Liebermann–Burchard test for triterpenes and a yellow (turning to violet) colour with Noller's reagent [10]. Its IR spectrum exhibited absorption bands at 1765 and 1725 cm^{-1} (carbonyl) and 1390, 1375 cm^{-1} (geminal dimethyl). The band at 1765 cm^{-1} suggested 7 was a γ-lactone. There was no IR evidence for the presence of a double bond or a hydroxyl group. The ^1H NMR and ^{13}C NMR spectra of 7 were very characteristic of the oleanane skeleton (Table 2). The ^1H NMR spectrum displayed singlets for seven C-methyl groups (see Experimental). A singlet (3H) at δ2.05 was attributed to an acetoxyl methyl group, and a double of doublets centred at δ4.5 (1H) to a proton on the acetoxyl-bearing carbon. Assuming that the compound was of the oleanolic type then the acetoxyl group had to be at C-3, and the lactone function was located in one of the remaining rings (C, D and E). The placement of the acetoxyl group at C-3 in 7 was based on biogenetic grounds as well as on the ^1H NMR data. The coupling constants of the proton on the acetoxyl-bearing carbon (J = 8 and 2 Hz) in 7 indicated it to be 3α-axial, i.e. the hydroxyl was a 3β-equatorial group [3, 11]. Furthermore, the ^{13}C signals of the carbons in the A and B rings of 7 agreed well with those of the monoacetate of oleanolic acid (Table 2).

Table 2. ^{13}C NMR spectral data of compounds 4 (as the acetate) and 7

C	4 Acetate	7	C	4	7
1	38.01	37.80	16	23.50	21.80
2	28.50	27.88	17	46.48	50.34
3	80.80	80.70	18	40.83	44.03
4	37.67	37.43	19	45.36	38.60
5	55.20	55.04	20	30.70	31.30
6	18.12	18.20	21	33.70	34.17
7	32.40	33.28	22	32.40	31.50
8	39.22	38.63	23	28.02	27.80
9	47.56	50.58	24	16.60	16.41
10	36.90	36.75	25	17.15	17.10
11	22.80	23.80	26	16.60	16.10
12	122.45	19.61	27	25.87	18.30
13	143.50	91.70	28	184.2	180.20
14	41.47	42.20	29	33.60	33.44
15	27.60	26.50	30	23.50	23.60
$\underline{C}H_3CO$–	21.30	20.80	$CH_3\underline{C}O$–	170.90	170.91

The presence of a singlet at δ91.7 in the ^{13}C NMR spectrum of 7 showed that the oxygen-bearing carbon in

the lactone function was tertiary (Table 2) and was either C-13 or C-18. In the ^{1}H NMR spectrum of **7**, the most downfield signal in the methyl region at δ1.2 was assigned to C-26 [12] due to the strong anisotropic effect of the lactone group at C-28 while the C-27 methyl appeared at δ1.12. The presence of the carbonyl group of the lactone at C-28 ruled out C-18 for the oxygen-bearing carbon in the lactone function. Thus the structure of the natural product was elucidated as 3β-hydroxyolean-13$\beta \rightarrow$28 lactone.

EXPERIMENTAL

Mps: uncorr.; IR: KBr discs; EIMS: direct inlet, 70eV; ^{1}H NMR and ^{13}C NMR: 100 MHz, $CDCl_3$, TMS as internal standard. Known compounds were compared with authentic samples by mmp, TLC in at least two solvent systems, IR and NMR.

Plant material. This was the same as that used in the previous study [2].

Extraction and isolation. Air-dried leaves (1 kg) were extracted with petrol (3 l.) for 10 days at room temp. and filtered. The marc was then treated with MeOH (2 l.) for 1 week. Both extracts were processed separately. The petrol extract (20 g) was separated as reported earlier [1], and column chromatographic fractions (silica gel, 150 ml each) obtained with petrol–$CHCl_3$ (3:1 and 1:1) were collected and rechromatographed (CC, silica gel, 70 g). Elution was performed with petrol (8 × 100 ml), petrol–$CHCl_3$, 3:1 (8 × 100 ml) and petrol–$CHCl_3$, 1:1 (7 × 100 ml). Compound **3** (90 mg) crystallized out from the fractions eluted with petrol–$CHCl_3$ (3:1). The mother liqour of the eluates was purified on TLC to give compound **6** (48 mg). Salvigenin was obtained from the first petrol–$CHCl_3$ (1:1) eluates [3], while isocarnosol (200 mg) was separated from the remainder of these eluates.

Identification of **6**. Thick oil, $[\alpha]_D^{20}$ +85.5° ($CHCl_3$; *c* 0.06); IR ν_{max}^{KBr} cm^{-1}: 3490, 3300–2700, 1690, 1610, 1580, 1500, 1415, 1390, 1380, 1240; MS *m/z* (rel. int.): 346.2149 $[M]^+$ (17), 344 (14), 302 (10), 300 (100), 285 (21), 244 (29), 229 (21), 91 (17), 44 (17), 43 (17); ^{1}H NMR ($CDCl_3$): δ0.84 (3H, *s*), 0.95 (3H, *s*), 1.17 (3H, *d*, *J* = 7 Hz), 1.19 (3H, *d*, *J* = 7 Hz), 2.85 [*d* (*br*), 2H], 3.1 (*m*, 1H), 3.55 (*m*, 2H-7), 3.66 (3H, *s*), 6.48 (1H, *s*, H-14), 8.0 [*s* (*br*), 2H]; ^{13}C NMR: see Table 1.

Methylation of **6**. A mixture of **6** (25 mg) in Me_2CO, K_2CO_3 (0.2 g) and MeI (0.5 ml) was refluxed for 4 hr. K_2CO_3 was removed by filtration and the Me_2CO evaporated to yield **8** (oil). IR ν_{max} cm^{-1}: 3200–2700, 1700, 1600, 1500, 1390, 1380, 1210, 1170; ^{1}H NMR ($CDCl_3$): δ0.77 (3H, *s*), 0.97 (3H, *s*), 1.18 (3H, *d*, *J* = 6.9 Hz), 1.2 (3H, *d*, *J* = 6.9 Hz), 3.1 (1H, *m*), 3.48 (*m*, 2H-7), 3.62 and 3.72 (each *s*, 3H), 6.42 (1H, *s*, H-14); ^{13}C NMR: see Table 1.

Compound **9** *from compound* **8**. Ethereal CH_2N_2 treatment of **8** for 2 hr at 10° yielded a colourless oil; IR and ^{1}H NMR data identical with those [13] for compound **9**. This compound was also identical (TLC, UV, IR and ^{1}H NMR) in all respects with the substance obtained from methylation of compound **3**, employing the same procedure as that for methylation of **6**.

Isolation of triterpenes. The MeOH extract was concentrated to give a greenish residue (150 g). Part of the residue (20 g) was taken and acetylated [C_5H_5N–Ac_2O (1:1), 40 ml] for 3 days. The crude product was then chromatographed on a column of silica gel (400 g). Elution with petrol–$CHCl_3$(1:1) gave 10 fractions (150 ml each). The last 5 fractions were mixed together, reduced in vol. to approximately 30 ml and mixed with 20 ml hexane and $CHCl_3$ (3:1). The crystalline product which formed overnight was filtered and recrystallized ($CHCl_3$) to give white needles of **7** (27 mg). Elution of the column with MeOH–$CHCl_3$ (1:3) afforded the acetates of oleanolic and ursolic acids.

Identification of compound **7**. White needles ($CHCl_3$), $C_{32}H_{50}O_4$ ($[M]^+$ at *m/z* 498), mp 282°; IR ν_{max}^{KBr} cm^{-1}: 2970, 1765, 1725, 1465, 1450, 1390, 1375, 1240, 1150, 1030; ^{1}H NMR ($CDCl_3$): δ0.86 (9H, *s*), 0.89 (3H, *s*), 0.99 (3H, *s*), 1.12 (3H, *s*), 1.2 (3H, *s*), 2.05 (3H, *s*), 4.5 (1H, *dd*, *J* = 8 and 2 Hz); ^{13}C NMR ($CDCl_3$): see Table 2.

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