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DITERPENOIDS AND TRITERPENOIDS FROM *SALVIA MULTICAULIS*

AYHAN ULUBELEN*†‡, NUR TAN†, UFUK SÖNMEZ† and GÜLAÇTI TOPCU‡

†Faculty of Pharmacy, University of Istanbul, Istanbul 34452, Turkey; ‡TUBITAK, Marmara Research Center, Department of Chemistry, P.O. 21, 41470 Kocaeli, Gebze, Turkey

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Key Word Index—*Salvia multicaulis*; Lamiaceae; triterpenoids; diterpenoids; steroids; aromatic ester.

Abstract—Two new diterpenoids, 3-oxo-12-methoxy-14-hydroxyabieta-8,11,13-triene and 6-oxo-12-peroxyabieta-8,11,13-triene, a new steroid brassicasterone and a new aromatic ester 4,4′-*bis*benzoic acid heptyl ester were obtained from the roots of *Salvia multicaulis*. In addition six known triterpenoids, six diterpenoids and sitosterol were identified. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

In a previous study [1] of the roots of *Salvia multicaulis* Vahl. we isolated 16 terpenoidal compounds; seven of them were new diterpenoids and three showed high antitubercular activity against *Mycobacterium tuberculosis*. In the present study with the same plant collection we have obtained lupeone [2], 3α-acetylamyrin [3], 3α-acetyl-erythrodiol [4], hopanone [5], sitosterol, stigmast-4-en-3-one [6], ferruginol [7], ferruginol-18-al [8], 6-oxoferruginol [9], acetylhorminone [10], cryptanol [11], wiedelactone [12] as well as new compounds 3-oxo-12-methoxy-14-hydroxyabieta-8,11,13-triene (**1**), 6-oxo-12-peroxyabieta-8,11,13-triene (**2**), brassicasterone (**3**) and 4,4′-*bis*benzoic acid heptyl ester (**4**). The structures of the known compounds were established by comparing their spectral data to those of literature values and by TLC comparison with authentic samples.

RESULTS AND DISCUSSION

The HREI mass spectrum of the first new compound (**1**) indicated the molecular formula $C_{21}H_{30}O_3$ (*m/z* 330.2180, calcd 330.2196). The ^1H NMR spectrum of **1** showed the signals at δ 6.95 (1H, *s*, H-11), 3.82 (3H, *s*, OMe), 3.27 (1H, *septet*, $J = 7$ Hz, H-15), 2.70 (1H, *dt*, $J = 3,4,12$ Hz, H-1β), 1.20 (6H, *s*, Me-18 and Me-19), 1.17 (6H, *d*, $J = 7$ Hz, Me-16 and Me-17), 1.00 (3H, *s*, Me-20). The presence of a carbonyl group was evident from the IR (1715 cm^{-1}) and the ^{13}C NMR (δ 210.3) spectra. One of the oxygen functions was a methoxyl group placed at C-12, while another was a hydroxyl group which should be at C-14. If the hydroxyl group was placed at C-11 then the chemical shift of H1-β should be *ca.* δ 3.10–3.30 or even more [13]. The carbonyl group could be placed at one of the following positions C-1, C-2, C-3, C-6 and C-7. The lack of a resonance at around δ 13.40 eliminated a hydrogen bond between the C-14 hydroxyl and the C-7 carbonyl group and indicated that the carbonyl group could not be present at C-7 [14]. If the carbonyl group was situated at C-6, the benzylic protons of C-7 would have been observed *ca.* δ 2.5 and 3.0 as well divided doublets [9]. Because of the lack of isolated methylene groups for the C-1 and C-3 protons, the carbonyl group could not be placed at C-2. When a carbonyl is located at C-1, H-11 usually moves downfield at about δ 7.10–7.30 [9], but this was not observed in the ^1H NMR spectrum of **1**. Thus the only plausible location for the carbonyl group was C-3. The ^{13}C NMR spectrum of **1** indicated the presence of six methyl quartets; one methoxyl at δ 54.3, four methylene triplets, three methine doublets and eight quaternary carbons for the 21 C atoms of compound **1** (Table 1). The spectral data indicated the structure of compound **1** as 3-oxo-12-methoxy-14-hydroxyabieta-8,11,13-triene.

The HREI mass spectrum of compound **2** gave the molecular formula $C_{20}H_{28}O_3$ (*m/z* 316.2026, calcd 316.2038). The ^1H NMR revealed the structure of **2** quite clearly with the signals at δ 8.58 (1H, *s*, OOH), 6.77 (1H, *s*, H-11), 6.62 (1H, *s*, H-14), 3.35 (1H, *septet*, $J = 7$ Hz, H-15), 2.96 (1H, *d*, $J = 14$, H-7), 2.53 (1H, *d*, $J = 14$ Hz, H-7′). The last two signals showed the presence of a benzylic methylene group indicating the

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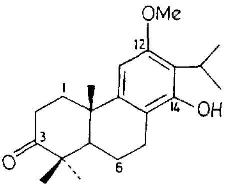
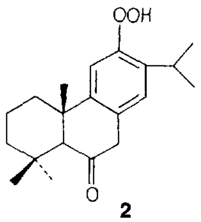
* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR of **1–3**

C	**1**	**2**	**3**
1	36.4 *t*	35.8 *t*	37.8 *t*
2	42.9 *t*	18.3 *t*	42.1 *t*
3	210.3 *s*	41.4 *t*	210.5 *s*
4	47.3 *s*	33.1 *s*	38.3 *t*
5	54.5 *d*	58.2 *d*	138.3 *s*
6	19.2 *t*	209.4 *s*	121.2 *d*
7	30.5 *t*	43.2 *t*	33.4 *t*
8	127.5 *s*	135.8 *s*	36.3 *d*
9	151.7 *s*	149.3 *s*	54.9 *d*
10	37.9 *s*	41.0 *s*	36.5 *s*
11	112.7 *d*	118.7 *d*	20.6 *t*
12	153.2 *s*	154.2 *s*	39.4 *t*
13	131.7 *s*	132.8 *s*	40.3 *s*
14	148.5 *s*	126.5 *s*	52.3 *d*
15	26.7 *d*	26.7 *d*	23.6 *t*
16	22.3 *q*	21.6 *q*	29.2 *t*
17	22.5 *q*	21.6 *q*	57.1 *d*
18	33.1 *q*	32.3 *q*	20.2 *q*
19	21.5 *q*	22.4 *q*	17.9 *q*
20	24.5 *q*	23.5 *q*	40.1 *d*
OMe	54.3 *q*	—	—
21	—	—	19.6 *q*
22	—	—	132.4 *d*
23	—	—	135.0 *d*
24	—	—	32.0 *d*
25	—	—	32.1 *d*
26	—	—	19.8 *q*
27	—	—	19.8 *q*
24^1	—	—	19.6 *q*



1



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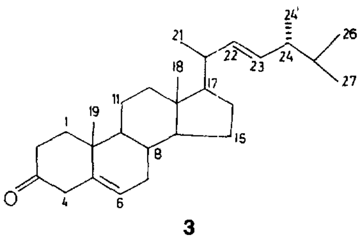

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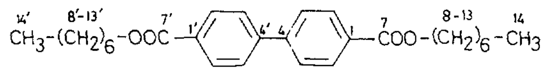

$CH_3-(CH_2)_6-OOC-C_6H_4-C_6H_4-COO-(CH_2)_6-CH_3$

4

presence of C-6 oxo group. The H-1β was evident with its typical signal at δ 2.40 (1H, *dt*, $J = 3,5,11$ Hz). The methyl groups were observed at δ 1.28 (3H, *s*, Me-20), 1.12 (3H, *d*, $J = 7$ Hz), 1.08 (3H, *d*, $J = 7$ Hz) (Me-16 and Me-17), 0.88 (6H, *s*, Me-18 and Me-19). The presence of the carbonyl group was observed in the IR (1718 cm^{-1}) and in the ^{13}C NMR (δ 209.4) spectra. In order to prove the presence of a peroxy group, KI solution was added to the acidified solution of **2**, I_2 was formed, giving a light yellow colour to the solution which turned to blue by the addition of starch solution. The ^{13}C NMR spectrum showed the presence of five methyl quartets, four methylene triplets, four methine doublets and seven quaternary carbon singlets for the 20 C atoms (Table 1). The spectra data of compound **2** was quite similar to those of 6-oxoferruginol [9] with a peroxy group at C-12 instead of a hydroxyl.

The new steroidal compound brassicasterone (**3**) had the molecular formula $C_{28}H_{44}O$ (*m/z* 396.3300, calcd 396.3391). The IR spectrum showed the signal at 1708 cm^{-1} for a carbonyl group, there was no signal to indicate the presence of a hydroxyl group. A literature survey revealed the presence of brassicasterol [15] but no mention of its 3-oxo derivative. The ^1H NMR spectrum of **3** showed the signals at δ 5.35 (1H, *br t*, $J = 4$ Hz, H-6), 5.17 (1H, *dd*, $J = 4$ and 10 Hz), 5.10 (1H, *dd*, $J = 5$ and 10 Hz) (H-22 and H-23). The methyl signals were at δ 0.67 (3H, *s*, Me-19), 0.89 (3H, *s*, Me-18), 0.76, 0.93 (each 3H, *d*, $J = 6.8$ Hz) (Me-21, Me-24′), 0.97 (6H, *d*, $J = 7$ Hz) (Me-26, Me-27). The ^{13}C NMR spectrum showed the presence of six methyl quartets, nine methylene triplets, nine methine doublets and four quaternary carbons, one being a carbonyl group at δ 210.5, counting 28 C atoms. The spectral data indicated the given structure for compound **3**.

The last new compound was an aromatic ester (**4**) with the molecular formula $C_{28}H_{38}O_4$ (*m/z* 438.2764, calcd 438.2770). The IR spectrum of **4** showed an ester carbonyl with the signal at 1735 cm^{-1}, and aromatic absorbances at 1610, 1600 and 1520 cm^{-1}. The ^1H NMR indicated the structure of **4** giving the signals at δ 7.08 (4H, *d*, $J = 9$ Hz, H-2, H-6, H-2′, H-6′), 6.77 (4H, *d*, $J = 9$ Hz, H-3, H-5, H-3′, H-5′), 4.22 (4H, *t*, $J = 7$ Hz, CH_2-8 and CH_2-8′), 2.86 (4H, *t*, $J = 7$ Hz, CH_2-9 and CH_2-9′), 2.28 (4H, *t*, $J = 7$ Hz, CH_2-10 and CH_2-10′), 1.70 (4H, *m*, CH_2-11 and CH_2-11′), 1.60 (4H, *m*, CH_2-12 and CH_2-12′), 1.26 (4H, *m*, CH_2-13 and CH_2-13′), 0.89 (6H, *t*, $J = 7$ Hz, Me-14 and Me-14′). Spin decoupling experiments showed the relation between methylene groups.

EXPERIMENTAL

General. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ in 200 MHz and 50.32 MHz instrument. The mass spectra of all the new compounds were recorded in an HRMS. For the sepn of the compounds silica gel columns, VLC and chromatotron and when necessary prep. plates were used.

Plant material. The roots of *Salvia multicaulis* were collected from southern Turkey (Adana-Armutluk) district at an altitude 1850 m in June 1995. The plant was identified by Prof. Neriman Özhatay (Istanbul), a voucher specimen (ISTE 68325) is deposited in the Herbarium of the Faculty of Pharmacy University of Istanbul.

Extraction and isolation. The dried and powdered roots of the plant (600 g) was extracted with Me_2CO in a Soxhlet, filtered and evapd to dryness *in vacuo*. The residue (12 g) was fractionated on a silica gel column (4 × 60 cm) eluting with petrol, a gradient of EtOAc was added up to 100% followed by EtOH. The oily frs were discarded and the similar frs were combined and sepd on VLC and/or on chromatotron using silica gel plates. Frs 5–7 (petrol EtOAc, 9:1, from a silica gel column) upon sepn on chromatotron and cleaning on prep. TLC yielded the following compounds; lupeone (10 mg), 3α-acetylamyrin (15 mg), 3α-acetylerythrodiol (8 mg), ferruginol (20 mg), **1** (12 mg), acetylhorminone (15 mg), ferruginol 18-al (15 mg), **2** (8 mg), cryptanol (6 mg). Frac. 8–10 (Pe:EtOAc 8:2) yielded wiedelactone (5 mg), sitosterol (25 mg), **3** (15 mg), α-amyrin (20 mg), lupeol (20 mg), **4** (15 mg), stigmast-4-en-3-one (12 mg).

Peroxide test. Compound **2** (5 mg) was dissolved in EtOH, a few drops of 0.1 N HCl and a few drops of 1% KI aq. sol. were added. A yellow colour was formed which turned to blue by the addition of starch soln.

3-*Oxo*-12-*methoxy*-14-*hydroxyabieta*-8,11,13-*triene* (**1**). UV λ_{max}^{MeOH} nm: 278 (3.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 3050, 2950, 2880, 1715, 1690, 1605, 1480, 1320, 1240, 1170 and 1080. ^1H NMR ($CDCl_3$) given in the text. ^{13}C NMR ($CDCl_3$) given in Table 1. HREIMS *m/z* (rel. int.): 330.2180 $[M]^+$ (5), 314 $[M-16]^+$ (38), 299 $[M-OMe]^+$ (12), 271 $[299-CO]^+$ (45), 255 (50), 227 (16), 191 (20), 148 (26), 104 (14), 95 (15), 83 (100), 69 (22), 57 (42).

6-*Oxo*-12-*peroxyabieta*-8,11,13-*triene* (**2**). UV λ_{max}^{MeOH} nm: 270 (3.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3040, 2950, 2860, 1710, 1695, 1620, 1480, 1380, 1200, 1150 and 1080. ^1H NMR ($CDCl_3$) given in the text. ^{13}C NMR ($CDCl_3$) given in Table 1. HREIMS *m/z* (rel. int.): 316.2026 $[M]^+$ (6), 286 $[M-CO]^+$ (15), 283 $[M-OOH]^+$ (12), 228 (15), 193 (18), 105 (50), 95 (53), 83 (70), 69 (100), 57 (42).

Brassicasterone (**3**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2850, 1708, 1620, 1480, 1450, 1357, 1210, 1100, 890. ^1H NMR ($CDCl_3$) given in the text. ^{13}C NMR ($CDCl_3$) given in Table 1. HREIMS *m/z* (rel. int.): 396.3300 $[M]^+$ (8), 313 $[M-C_6H_{11}]^+$ (4), 284 $[M-C_8H_{16}]^+$ (6), 218 (16), 149 (100), 111 (14), 97 (20), 83 (22), 71 (32).

4,4′-*Bisbenzoic acid heptyl ester* (**4**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3050, 2960, 2925, 1735, 1610, 1600, 1520, 1480, 1390, 1260, 1180, 1110, 830 and 720. ^1H NMR ($CDCl_3$) given in the text. HRMS *m/z* (rel. int.): 438.2764 $[M]^+$ (68), 423 $[M-Me]^+$ (26), 414 (42), 396 (24), 329 (16), 285 (16), 273 (100), 232 (90), 217 (20), 203 (14), 189 (20), 135 (84), 121 (94), 107 (78), 95 (56), 81 (44), 69 (64).

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