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ABIETANE DITERPENES FROM *SALVIA NAPIFOLIA*

AYHAN ULUBELEN,*† GÜLAÇTI TOPÇU,† UFUK SÖNMEZ,* M. IQBAL CHOUDHARY‡ and ATTA-UR-RAHMAN‡

*Faculty of Pharmacy, University of Istanbul, 34452 Istanbul, Turkey; †TUBITAK, Marmara Research Center, Department of Chemistry, PO Box 21, 41470, Gebze, Turkey; ‡H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, 75270, Pakistan

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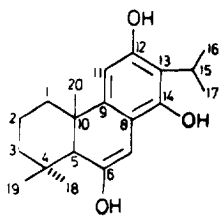
Key Word Index—*Salvia napifolia*; Labiatae; diterpenoids; 6,12,14-trihydroxyabieta-6,8,11,13-tetraen; 7,20-epoxyroyleanone; 1-oxoferruginol; 6-oxoferruginol; 11,12-dioxoabieta-8,13-dien.

Abstract—From the acetone extract of the roots of *Salvia napifolia*, eight known diterpenoids—horminone, 7-acetylhorminone, ferruginol, pachystazone, cryptanol, cryptojaponol, sugiol and microstegiol—and five new diterpenes—6,12,14-trihydroxyabieta-6,8,11,12-tetraen, 7,20-epoxyroyleanone, 1-oxoferruginol, 6-oxoferruginol and 11,12-dioxoabieta-8,13-dien— were obtained. The structures of the new and the known compounds were established by spectral methods.

INTRODUCTION

As part of our continuing studies of *Salvia* species grown in Turkey, we have now investigated *Salvia napifolia* jacq. Hort. Vindop. (syn. *S. verticillata* L. ssp. *napifolia* Jacq.). The plant is a perennial herb of widespread distribution in western Turkey. The roots of the plant yielded eight known diterpenes: horminone, acetylhorminone [1], ferruginol [2], pachystazone [3], cryptanol [4], cryptojaponol [5], sugiol [6] and microstegiol [7]; and five new diterpenes: 6,12,14-trihydroxyabieta-6,8,11,13-tetraen (**1**), 7,20-epoxyroyleanone (**2**), 1-oxoferruginol (**3**), 6-oxoferruginol (**4**) and 11,12-dioxo-abieta-8,13-dien (**5**). The identities of the known compounds were determined by comparing their spectral data (UV, IR, ^{1}H NMR) to those of literature values, and by TLC comparison with authentic samples.



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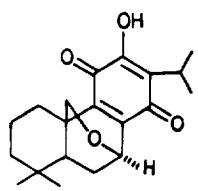

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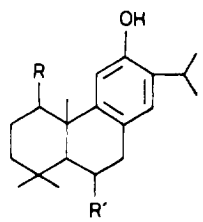
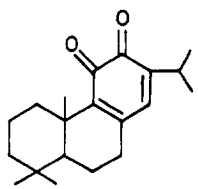


3 R=O; R'=H
4 R=H; R'=O



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RESULTS AND DISCUSSION

Compound **1** had a molecular formula of $C_{20}H_{28}O_3$, as indicated by its HR mass spectrum (*m/z* 316.2311; calc. 316.2308). The UV spectrum showed a conjugated aromatic system (332 nm), as observed for cryptanol [4] and the IR spectrum also indicated a conjugated aromatic system with absorptions at 1620, 1610, 1600 and 1560 cm^{-1}, while hydroxyl peaks were observed at 3450 and 3320 cm^{-1}. The ^{1}H NMR spectrum of **1** showed low field signals at δ6.87 (1H, *s*, H-7), 6.20 (1H, *s*, H-11) and 5.28 (1H, *br s*, OH) (disappeared on treatment with D_2O) and upper field signals at δ3.07 (1H, *septet*, J = 7Hz, H-15), 2.92 (1H, *dt*, J = 1.5, 2.0 and 10.0 Hz, H-1β), 2.60 (1H, *s*, H-5), 1.24 (6H, *s*) (Me-18 and Me-19), 1.16 (3H, *d*, J = 7.0 Hz) and 1.14 (3H, *d*, J = 7.0 Hz) (Me-16 and Me-17) and 1.09 (3H, *s*, Me-20). Although the chemical composition and the UV maximum were similar to those of cryptanol, the hydroxyl which is located at C-11 in cryptanol was placed at C-6 in compound **1** for the following reasons: the hydroxyl group at C-11 of cryptanol induced a downfield shift of H-1β to *ca* δ3.1–3.8 [8, 9], whereas in the case of **1** H-1β was observed at δ2.92 indicating the presence of a hydrogen at C-11. If the third hydroxyl was at C-7 instead of C-6, two doublets would

*†Author to whom correspondence should be addressed.

be expected to be observed for the C-5 and C-6 protons, but instead there were two sharp singlets at δ6.87 and 6.20 corresponding to H-7 and H-11, respectively, as well as a broad singlet at δ2.60 for H-5. The spectral data, therefore indicated a structure corresponding to 6,12,14-trihydroxyabieta-6,8,11,13-tetraen for compound **1**.

The HR mass spectrum of compound **2** indicated a molecular formula $C_{20}H_{26}O_4$ (*m/z* 330.1818; calc. 330.1831). The UV (400 nm) and the IR (1665,1657,1640 cm^{-1}) spectra showed the presence of a quinone system. The ^1H NMR spectrum contained signals at δ4.42 (1H, *dd*, J = 1.5 and 4.0 Hz, H-7α), 3.73 (1H, *d*, J = 7.0 Hz) and 3.65 (1H, *d*, J = 7.0 Hz) (oxymethylene protons), 3.16 (1H, *septet*, J = 7.0 Hz, H-15), 2.68 (1H, *dt*, J = 3.0, 4.0 and 12.0 Hz, H-1β), 1.22 (3H, *d*, J = 7.0 Hz), 1.18 (3H, *d*, J = 7.0 Hz) (Me-16 and Me-17), 0.92 (3H, *s*) and 0.88 (3H, *s*) (Me-18 and Me-19). Acetylation yielded a monoacetyl derivative showing a signal at δ2.32 which indicated the presence of an unsaturated acetyl group. Neither the methylene signals at δ3.73 and 3.65 nor the signal at δ4.42 (H-7) shifted on acetylation, thereby indicating the presence of an acetyl group attached to an olefinic carbon. The ^{13}C NMR spectrum (APT) (Table 1) showed the presence of four methyl, five methylene, three methine and eight quaternary carbons. Two of the latter signals were assigned to quinone carbonyls. Since there was only one olefinic hydroxyl group present in the molecule and the APT experiment showed a methylene carbon signal at δ65.4 as well as a methine carbon at δ69.2, these should both be attached to an ether function which would also account for the oxygen function. The ether function had to be between either Me-20 and C-7 or between Me-18 (or Me-19) and C-7. In the latter case, Me-19 (or Me-18) would be shifted downfield by greater than 1.0 ppm. When there is a functional group at C-10, the two methyl groups come close to each other and generally appear lower than 1.0 ppm, as observed in the present case. The stereochemistry of H-7 was deduced as α by studying a Dreiding model. All the ^{13}C NMR signals were in agreement with the given structure (Table 1). Based on the spectral data, the structure of compound **2** was established as 7,20-epoxyroyleanone.

Table 1. ^{13}C NMR of compounds **2**, **4** and **5**

C	2	4	5
1	35.6	35.4	37.0
2	18.5	18.7	18.2
3	40.9	41.9	40.8
4	32.9	32.2	33.4
5	45.4	58.0	46.4
6	22.9	207.2	23.7
7	69.2	42.5	33.4
8	134.2	135.8	148.3
9	147.8	149.3	154.0
10	39.1	41.4	39.3
11	184.2*	118.9	182.0
12	150.6	151.2	182.0
13	124.6	132.8	136.0
14	182.4*	126.5	118.3
15	24.2	26.7	25.0
16	19.6†	21.6*	20.4†
17	19.8†	21.1*	20.6†
18	33.1	32.3	33.1
19	22.9	22.8	22.0
20	65.4	22.5	20.1

*,†The assignments are interchangeable within columns.

The IR spectrum 1-oxoferruginol (**3**) showed the presence of an oxo group at 1730 cm^{-1}, and the UV spectrum indicated a simple aromatic compound as observed for ferruginol (267 nm). The HR mass spectrum of **3** indicated a molecular formula $C_{20}H_{28}O_2$ (*m/z* 300.2104; calc. 300.2089). The ^1H NMR spectrum showed two aromatic signals at δ7.40 (1H, *s*, H-11) and 7.18 (1H, *s*, H-14) along with other signals were at δ3.28 (1H, *septet*, J = 7.0 Hz, H-15), 1.28 (3H, *s*, Me-20), 1.17 and 0.87 (each 3H and *s*, Me-18 and Me-19) and 1.24 (6H, *d*, J = 7.0 Hz) (Me-16 and Me-17). The lack of a H-1β signal and the downfield appearance of H-11 (δ7.40) indicated an oxo group at C-1. Acetylation of compound **3** yielded a monoacetate (acetyl signal at δ2.32) which indicated that the hydroxyl at C-12 was acetylated (see Experimental). The spectral data suggested the given formula for compound **3**.

Compound **4**, 6-oxoferruginol, had a molecular formula $C_{20}H_{28}O_2$ (*m/z* 300.2110; calc. 300.2089) as derived from its HR mass spectrum. The ^1H NMR spectrum showed two aromatic proton signals at δ6.92 (1H, *s*, H-11) and 6.71 (1H, *s*, H-14) similar to that of ferruginol. Other signals were observed at δ3.19 (1H, *septet*, J = 7.0 Hz, H-15), 3.02 and 2.57 (each 1H, *d*, J = 14.0 Hz, H_2-7), 2.61 (1H, *s*, H-5), 2.47 (1H, *tt*, J = 3, 5 and 11 Hz, H-1β), 1.18 and 1.22 (each 3H, *d*, J = 7.0 Hz, Me-16 and Me-17), 0.91, 0.88 and 1.27 (each 3H, *s*) (Me-18, Me-19 and Me-20). The IR spectrum indicated a carbonyl at 1730 cm^{-1}. The carbonyl group had to be placed at one of the following positions C-2, C-3, C-6 or C-7. Position C-7 was unlikely since H-14 was observed at δ6.71. If at C-2, then the signals for two isolated methylene groups should have been observed, while if at C-3, the signal for C-2 should be shifted to *ca* 28–30 ppm in the ^{13}C NMR spectrum. The placement of the carbonyl group at C-6 was deduced from the observation of H-5 at δ2.61 as a singlet, the benzylic methylene group at δ3.02 and 2.57 as doublets, and the observation of the C-7 signal at δ42.5. The DEPT and HMBC (Table 2) experiments were in agreement with the given structure **4**.

The HR mass spectrum of compound **5** indicated a molecular formula $C_{20}H_{28}O_2$ (*m/z* 300.2070; calc. 300, 2089). Its UV spectrum showed a maximum at 350 nm and a shoulder at 395 nm. The IR spectrum indicated the presence of an *ortho* quinone, i.e. carbonyl groups at 1685 (sh) and 1645 cm^{-1}. The ^1H NMR spectrum showed a doublet at δ6.31 (1H, *d*, J = 1.5 Hz, H-14), while other signals were observed at δ2.98 (1H, *d septet*, J = 1.5 and

Table 2. HMBC experiments on compound **4** (500 MHz, in $CDCl_3$)

H	Correlated carbons
1β (2.47)	C-2, C-3, C-5
5 (2.61)	C-1, C-7, C-10
7α (2.57)	C-5, C-8, C-10, C-11, C-13, C-14
7β (3.02)	C-8, C-11
11 (6.92)	C-1, C-8, C-10
14 (6.71)	C-13, C-14
15 (3.19)	C-13, C-14, C-16
16 (1.18)	C-13, C-17
17 (1.22)	C-13, C-16
18 (0.91)	C-3, C-5
19 (0.88)	C-3, C-5
20 (1.27)	C-1, C-4

7 Hz, H-15), 2.70 (1H, *dt*, J = 3, 5, 11 Hz, H-1β), 1.28 (3H, *s*, Me-20), 1.10 (3H, *d*, J = 7 Hz), 1.01 (3H, *d*, J = 7 Hz) (Me-16 and Me-17), 0.91 (3H, *s*), 0.89 (3H, *s*) (Me-18 and Me-19). The ^{13}C NMR (APT) spectrum of **5** (Table 1) contained five methyl quartets, five methylene triplets, three methine doublets and six quaternary carbon singlets for seven C atoms. The signal at δ182.0 was attributed to two carbonyls, thus verifying the presence of an *ortho* quinone group in the structure. The spectral data established the structure of **5** as 11,12-dioxoabieta-8,13-dien.

EXPERIMENTAL

General. IR: $CHCl_3$; UV: MeOH; DEPT and HMBC experiments of **4** and **5** were recorded on a Bruker AMX 500 and other NMR experiments on a Bruker AC 200 in $CDCl_3$; HRMS: VG ZabSpec; prep TLC: Kieselgel $60F_{254}$ (E.Merck); CC: Silica gel and Sephadex LH-20 (Fluka).

Plant material. The roots of *S. napifolia* Jacq. were collected from Küçükçekmece (near Istanbul) in July 1993 and identified by Dr K. Alpinar (Istanbul). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 65138).

Extraction and isolation of the compounds. Powdered roots of the plant (1.5 kg) were extracted with Me_2CO in a Soxhlet. The extract was evaporated *in vacuo* to give 18 g of residue. The residue was fractionated by CC on silica gel, eluted with petrol, followed by a gradient of EtOAc, up to 100% and then with EtOH. The fractions were further separated on smaller silica gel columns and purified on Sephadex LH-20 columns using petrol –$CHCl_3$–MeOH (7:4:2) and when necessary on prep. TLC plates. The following compounds were obtained: cryptanol (9 mg), 7-acetylhorminone (70 mg), horminone (66 mg), ferruginol (26 mg), pachystazone (21 mg), sugiol (26 mg), **4** (17 mg), **1** (5 mg), **2** (17 mg), **3** (6 mg) and **5** (12 mg).

6,12,14-*Trihydroxyabieta*-6,8,11,13-*tetraen* (**1**). Amorphous, light yellow compound. UV λ_{max}^{MeOH} nm (log ε): 332 (4.0), 268 (3.8), 250 (sh), 208 (4.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3320, 2950, 2860, 1620, 1610, 1600, 1560, 1460, 1420, 1380, 1350, 1240, 1140, 1050; ^1H ($CDCl_3$) given in the text; HRMS 70 eV *m/z* (rel. int.): 316.2311 ($C_{20}H_{28}O_3$) $[M]^+$ (5), 284 $[M - 2xMe]^+$ (60), 236 (8), 152 (100), 126 (15), 96 (55), 69 (45), 57 (48).

7,20-*Epoxyroyleanone* (**2**). Amorphous compound. UV λ_{max}^{MeOH} nm (log ε): 400 (3.00), 270 (4.2), 216 (3.6); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2960, 2880, 1665, 1657, 1640, 1600, 1580, 1460, 1390, 1380, 1250, 1160, 1130, 1100, 1080; ^1H NMR ($CDCl_3$) given in the text; ^{13}C NMR ($CDCl_3$) given in Table 1; HRMS 70 eV *m/z* (rel. int.): 330.1818, ($C_{20}H_{26}O_4$) $[M]^+$ (100), 315 $[M - Me]^+$ (35), 300 $[M - 2xMe]^+$ (40), 289 (14), 245 (32), 187 (12), 118 (36), 87 (98), 69 (15).

1-*Oxoferruginol* (**3**). UV λ_{max}^{MeOH} nm (log ε): 267 (3.5), 220 (4.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2920, 2850, 1730, 1675, 1607, 1460, 1420, 1376, 1260; ^1H NMR ($CDCl_3$) given in the text; HRMS 70 eV *m/z* (rel. int.): 300.2104 ($C_{20}H_{28}O_2$) $[M]^+$ (18), 285 $[M - Me]^+$ (20), 269 (10), 236 (8), 215 (10), 185 (12), 123 (33), 109 (31), 95 (47), 83 (62), 69 (87), 57 (100).

1-*Oxoferruginylacetate* (**3a**). ^1H NMR ($CDCl_3$): δ0.92, 0.99 and 1.28 (9H, each *s*, H-18, H-19 and H-20), 1.21 and 1.23 (6H, each *d*, J = 6.9 Hz, H-16 and H-17), 2.32 (3H, *s*, OAc), 3.02, 1H, *septet*, H-15), 7.18 (1H, *s*, H-14), 7.60 (1H, *s*, H-11).

6-*Oxoferruginol* (**4**). UV λ_{max}^{MeOH} nm (log ε): 262 (3.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360, 2960, 2920, 2870, 1730, 1605, 1595, 1510, 1460, 1440, 1420, 1360, 1340, 1260, 1120, 1070; ^1H NMR ($CDCl_3$) given in the text; ^{13}C NMR: Table 1; HRMS 70 eV *m/z* (rel. int.): 300.2110 ($C_{20}H_{28}O_2$) $[M]^+$ (35), 301 $(M + 1)^+$ (100), 285 $[M - Me]^+$ (27), 248 (23), 233 (20), 1 76 (95), 83 (79), 71 (62).

11,12-*Dioxo-abieta*-8,13-*dien* (**5**). UV λ_{max}^{MeOH} nm (log): 395 (sh) (2.1), 350 (3.2), 257 (4.0), 211 (4.0); IR $\lambda_{max}^{CHCl_3}$ 2980, 2920, 2860, 1685, 1645, 1600, 1540, 1460, 1380, 1290, 1095; ^1H NMR ($CDCl_3$) given in the text; ^{13}C NMR ($CDCl_3$): Table 1; HRMS 70 eV *m/z* (rel. int.): 300.2070 ($C_{20}H_{28}O_2$) $[M]^+$ (70), 285 $[M - Me]^+$ (33), 255 (30), 243 (55), 204 (68), 83 (100), 69 (68).

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