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DITERPENOIDS FROM *SALVIA OXYODON* AND *SALVIA LAVANDULIFOLIA*

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Key Word Index—*Salvia oxyodon*; *Salvia lavandulifolia*; Labiatae; diterpenoids; abietane derivatives.

Abstract—Three new diterpenoids have been detected in *Salvia oxyodon* and identified as 3β-hydroxy-dehydroabietic acid, 3β-acetoxy-abieta-8(14)-en-18-oic acid 9α,13α-endoperoxide and 3β-hydroxy-abieta-8(14)-en-18-oic acid 9α,13α-endoperoxide. *Salvia lavandulifolia* yielded two known compounds ursolic acid and galdosol.

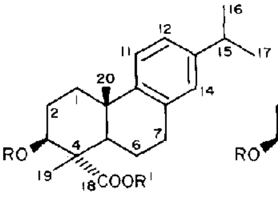
INTRODUCTION

Quite a few diterpenoids and triterpenoids have been isolated from *Salvia* species in recent years [1–7]. We report here the isolation (as their methyl ester derivatives) and structure determination of three new diterpene acids and two known triterpenes (ursolic and crataegolic acids) obtained from *Salvia oxyodon*, a plant endemic to the Andalusian region of Spain. A similar study carried out with *S. lavandulifolia*, collected in central Spain, yielded ursolic acid and galdosol [1], the latter isolated and identified as its dimethyl ether derivatives.

RESULTS AND DISCUSSION

Chromatography of the crude acetone extract obtained from *S. oxyodon* afforded fractions enriched in diterpenic and triterpenic acids. These fractions were methylated with diazomethane and further chromatographed yielding compounds **1b**, **2a** and **3a**, plus the methyl esters of crataegolic acid and ursolic acid. Compounds **1b** and **3a** are difficult to separate as free alcohols and they were transformed into their acetyl (**1a**, **2a**) derivatives prior to isolation. The ^{1}H NMR spectrum of **1a** (mp 160–161°, $[\alpha]_D^{22} = 53.7°$) displayed four singlets (3H each) at δ1.12, 1.15, 1.20 and 1.22 and a characteristic signal for three aromatic protons at 6.8–7.1. The mass spectrum of **1a** revealed ions at m/z 372 $[M]^+$ and 330 $[M-42]^+$ and 312 $[M-60]^+$. These results point to a dehydroabietic acid derivative of molecular formula $C_{23}H_{32}O_4$ [8, 9]. There was a secondary acetoxyl group (3H, *s*, at δ2.0 and 1H, *m*, at δ5.2), which must be located on C-2, C-3 or C-6. Positions C-1 and C-7 were excluded after considering the normal shift shown by the three aromatic protons [8]. The location of the acetyl group at C-3 (β-configuration) was sustained by the ^{13}C NMR data of compound **1a**. The assignments, shown in Table 1 for **1a**, have been derived taking into consideration the data published for the 3β-hydroxy-abieta-8,11,13(14),15-tetraene-18-oic acid methyl ester and dehydroabietic acid [10]. Compound **1a** has been prepared by incubation of methyl dehydroabietate with *C. sasakii* [11].

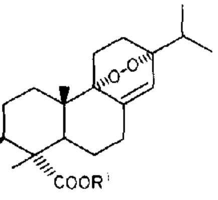
The mass spectrum of **2a** revealed a molecular ion at m/z 406 $[M]^+$ corresponding to $C_{23}H_{34}O_6$. The ^{1}H NMR spectrum of **2a** showed signals corresponding to a carbomethoxyl and acetyl groups (see Experimental). No hydroxyl absorption was observed in its IR spectrum. These data point to the presence of ether linkages in **2**. Other relevant data from the ^{1}H NMR spectrum were four singlets (3H each) observed at δ0.9, 1.0, 1.1 and 1.3 and the presence of an olefin proton singlet at 6.2. The proton geminal to the acetoxyl group appeared as a 1H quartet centered at 5.2 ($J_a = 10.5$ Hz, $J_b = 4.5$ Hz). These data were compatible with an abietane skeleton for **2a**, which was also suggested on biogenetic grounds. The ^{13}C NMR spectrum of **2a** showed two signals (Table 1) assignable to oxygen-linked quaternary carbon atoms



1 R=R'=H

1a R=Ac, R'=Me

1b R=H, R'=Me



2 R=Ac, R'=H

2a R=Ac, R'=Me

3 R=R'=H

3a R=H, R'=Me

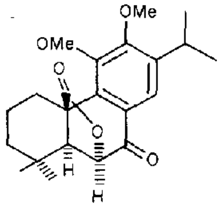


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Table 1. ^{13}C NMR spectral data of compounds **1a**, **2a**, **3a** and **4** [δ-values (ppm), 22.5 MHz, $CDCl_3$, TMS as int. standard]

Carbon No.	**1a**	**2a**	**3a**	**4**	Carbon No.	**1a**	**2a**	**3a**	**4**
1	36.1 t	24.9 t	25.0 t	27.6 t	11	123.7 d	17.0 t	17.0 t	151.4 s
2	23.8 t	23.0 t	26.0 t	19.0 t	12	123.7 d	24.2 t	24.2 t	157.2 s
3	76.7 d	77.1 d	74.8 d	37.9 t	13	145.7 s	79.3 s*	79.4 s*	136.0 s
4	52.0 s	51.3 s	53.0 s	32.5 s	14	126.3 d	127.3 d	127.1 d	122.7 d
5	45.4 d	39.3 d	39.2 d	60.9 d	15	33.3 d	32.1 d	32.1 d	27.1 d
6	21.0 t	22.0 t	22.0 t	80.6 d	16	23.8 q	19.2 q	19.6 q†	22.8 q*
7	29.8 t	28.9 t	29.2 t	189.4 s	17	23.8 q	19.3 q†	19.3 q†	23.0 q*
8	133.7 s	143.8 s	143.8 s	125.5 s	18	175.7 s	176.0 s	177.2 s	31.6 q
9	146.9 s	80.5 s*	80.6 s*	143.9 s	19	11.6 q	12.3 q	11.3 q	22.0 q
10	36.5 s	38.8 s	39.0 s	49.6 s	20	24.4 q	17.4 q	17.4 q	176.3 s
COOMe	52.0 q	52.3 q	52.2 q	—	OCOMe	169.4 s	169.9 s	–	
OMe	—	—	—	60.6 q 60.4 q	OCOMe	21.0 q	21.1 q	—	—

*,† Values with the same sign may be interchangeable.

(δ 80.5 *s*; 79.3 *s*) and excluded the presence of –CO– functions, other than the COOMe and OCOMe, already mentioned. Since there are no hydroxyl groups in the molecule which can be acetylated, and its molecular formula requires two more oxygen atoms and one more unsaturation, the presence of an endoperoxide grouping was postulated. Using suitably substituted abietane and pimarane models [12, 13], it is possible to assign the ^{13}C NMR spectrum of **2a** as indicated in Table 1. The relative stereochemistry of the endoperoxide grouping was assigned considering this ^{13}C NMR data. An oxygen atom on C-9 (α-configuration) should cause a γ-gauche effect on the chemical shifts of C-1, C-5 and C-7 and this was actually observed (see Table 1). There was also an upfield effect on the chemical shift of C-20. Although **2a** was prepared by methylation and acetylation of the corresponding acidic fractions, the natural acetyl derivative, **2**, was also present in the original mixture and **2a** was consequently isolated simply by methylation of certain fractions of the total crude acid mixture.

Inspection of the spectroscopic information obtained with **3a** (mp 158–161°) (see Experimental) suggested the structure indicated. This prediction was confirmed since acetylation of **3a** under normal conditions afforded **2a**. The ^{13}C NMR spectrum of **3a** confirmed the assigned structure and helped to reduce ambiguities in the assignment of the spectra of both compounds, as shown in Table 1. The absolute stereochemistry of these compounds was assigned on biogenetic grounds, although it can be pointed out that **1a** and dehydroabietic acid both have positive specific rotations (53.7° and 62.0°, respectively), while compounds **2a** and the 9,13-endoperoxide derivative of abietic acid both show negative specific rotations (−54.6° and −73.9°, respectively) [14].

There has been some uncertainty on the correct relationship between these two *Salvia* species and some authors have subordinated *S. oxyodon* to *S. lavandulifolia*. It is expected that the results reported here will contribute to a clarification of this question.

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. Optical rotations were measured on a Perkin-Elmer 142 spectropolarimeter. IR spectra were obtained in KBr pellets, unless otherwise stated, and MS were determined at 70 eV. ^{1}H and ^{13}C NMR spectra were measured at 90 and 22.5 MHz, respectively, in $CDCl_3$ soln with TMS as int. standard. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant materials were collected in June 1980, in Sierra de Cázulas (Granada, Spain) (*S. oxyodon*), and in the proximities of Madrid in June 1978 (*S. lavandulifolia*). Voucher specimens of both plants were deposited in the Herbarium of the Faculty of Pharmacy (Universidad Complutense de Madrid).

Extraction. A similar procedure was used for the extraction of *S. oxyodon* Webb et Heldreich and *S. lavandulifolia* Wahl. Dried and finely powdered plant material (1 kg) were extracted with Me_2CO (6 l.) at room temp. for 2 days. After filtration the solvent was evaporated yielding a gum which was subjected to chromatography.

Isolation of the components of S. oxyodon. The crude extract (40 g) was chromatographed on a short column [15] over Si gel 60G, eluting with $CHCl_3$–MeOH (97.5:2.5) (250 ml fractions). Ursolic acid was the main component in fractions 6–8. Careful TLC of fractions 9–16 showed a series of minor components accompanying it. A second short CC of these latter fractions allowed the separation of ursolic acid, crataegolic or maslinic acid and mixtures of crataegolic acid with **1**–**3**. Treatment of these fractions with CH_2N_2–Et_2O and further chromatography allowed the separation of crataegolic acid methyl ester from compounds **1b**, **2a** and **3a**. Part of compounds **2a** and **3a** separated spontaneously from the final fractions of this chromatography. To achieve the isolation of **1b**, it was necessary to acetylate the mixtures containing it. Compounds **1a** and **2a** were then chromatographically separated by short CC on Si gel 60G with hexane–EtOAc (9:1). Ursolic acid and crataegolic acid (2α-hydroxyoleanolic acid) were characterized as their methyl esters and acetyl derivatives through mp, $[\alpha]_D$, IR, ^{1}H NMR and ^{13}C NMR spectra.

Diterpenoid **1a**. This compound was obtained via the procedure described above. **1a** (250 mg after crystallization from hexane–EtOAc): mp 160–161°; $[\alpha]_D^{22} = 53.7°$ ($CHCl_3$; *c* 0.10); IR ν_{max}^{Nujol} cm^{-1}: 1740, 1610, 1500, 1240, 1125, 1050, 1038, 1025, 980, 880, 827. MS (75 eV, direct inlet) *m/z* (rel. int.): 372 $[M]^+$ (14), 357 (1.5), 330 (3.3), 312 (7.5), 297 (90), 253 (13), 237 (100), 195 (50). ^{1}H NMR ($CDCl_3$): δ 1.17 (6H, *d*, $J = 6$ Hz, H-16, H-17), 1.15 (3H, *s*, H-19), 1.20 (3H, *s*, H-20), 2.0 (3H, *s*, OAc), 2.6–2.9 (3H, complex signal), 3.6 (3H, *s*, OMe), 5.2 (1H, *m*, H-3), 6.8–7.1 (3H, *m*, H-11, H-12, H-14). ^{13}C NMR ($CDCl_3$): see Results and Discussion and Table 1.

Diterpenoid **2a**. 115 mg (after crystallization from hexane–EtOAc): mp 153–155°; $[\alpha]_D^{22} = -54.6°$ ($CHCl_3$; *c* 0.03); IR ν_{max}^{KBr} cm^{-1}: 1735, 1500, 1470, 1385, 1370, 1245, 1200, 1180, 1125, 1040, 995, 920, 890, 830. MS (75 eV, direct inlet) *m/z* (rel. int.): 406 $[M]^+$ (6.7), 388 (12.5), 374 (100), 320 (45), 314 (14), 303 (45), 299 (79), 271 (39), 243 (75), 226 (30), 215 (50), 201 (25), 197 (30), 187 (45), 180 (62). ^{1}H NMR ($CDCl_3$): δ0.95 (6H, *d*, J = 6 Hz, H-16, H-17), 1.1 (3H, *s*, H-19), 1.3 (3H, *s*, H-20), 2.0 (3H, *s*, OAc), 2.3–2.6 (3H, complex signal), 3.6 (3H, *s*, OMe), 5.2 (1H, *q*, J_a = 10.5 Hz, J_b = 4.5 Hz, H-3), 6.2 (1H, slightly broadened singlet, H-14). ^{13}C NMR ($CDCl_3$): see Table 1.

Diterpenoid **3a**. 118 mg (after crystallization from hexane–EtOAc): mp 158–161°. ^{1}H NMR ($CDCl_3$): δ0.95 (6H, *d*, J = 6 Hz, H-16, H-17), 1.1 (3H, *s*, H-19), 1.3 (3H, *s*, H-20), 3.6 (3H, *s*, OMe), 3.95 (1H, *m*, H-3), 6.1 (1H, slightly broadened singlet, H-14). ^{13}C NMR ($CDCl_3$): see Table 1.

Isolation of the components of S. lavandulifolia. The crude extract (60 g) was subjected to dry CC over Si gel (2 kg, Merck No. 7734, deactivated with 15% H_2O). Elution with hexane–EtOAc (3:1) afforded ursolic acid (characterized as the acetyl derivative of its methyl ester). Further chromatography of the final fractions containing ursolic acid, once subjected to treatment with CH_2N_2–Et_2O, afforded the dimethyl ether derivative of galdosol (**4**).

Dimethyl derivative of galdosol. 160 mg (after crystallization from hexane–EtOAc): mp 115–118°. UV λ_{max}^{EtOH} nm (ε): 243 (11 000), 275 (10 000). ^{1}H NMR ($CDCl_3$): δ0.96, 1.0 (6H, *s*, *s*, H-18, H-19), 1.2 (6H, *d*, J = 7 Hz, H-16, H-17), 2.4 (1H, *s*, H-5), 3.9, 3.88 (6H, *s*, *s*, two OMe), 4.7 (1H, *s*, H-6), 7.8 (1H, *s*, H-14). ^{13}C NMR ($CDCl_3$): see Table 1.

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