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SECONDARY METABOLITES FROM *SATUREJA* SPECIES. NEW TRITERPENOID FROM *SATUREJA ACINOS*

JAVIER ESCUDERO, J. CRISTÓBAL LÓPEZ, ROSA MARIA RABANAL, and SERAFÍN VALVERDE*

Instituto de Química Orgánica, C.S.I.C., Juan de la Cierva, 3, Madrid 6, Spain

The chemistry of the genus *Satureja* (syn. *Calamintha*) (Labiatae) has not been thoroughly studied. Previous reports (1-4) indicate the presence of ursolic, epiursolic, and oleanolic acids, and two 3,4-seco-triterpenoids, calainthadiol and isocalaminthadiol (3,4). We report here the content of secondary metabolites of three species, *Satureja acinos* (L.) Scheele, *Satureja montana* L., and *Satureja obovata* Lag., collected in central Spain.

Besides a new triterpene, of the ursene type, other known triterpenes, such as oleanolic, ursolic, or crataegolic acid, have been isolated from these species. β-Sitosterol-β-D-glucoside is common to the three species. Naringenin 7-*O*-rutinoside and eriodictyol 7-*O*-rutinoside has been isolated from *S. acinos* and *S. montana*, respectively. The presence of these flavanone glycosides was previously described in the peels of navel and valencia oranges (5) and lemons (6), respectively, and the structure of the naringenin glycoside (narirutin) confirmed by synthesis (7).

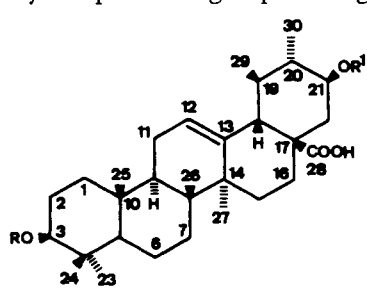
RESULTS AND DISCUSSION

Chromatography of the crude Me_2CO extract obtained from *S. acinos* afforded compound **1** plus ursolic acid and the glucoside of β-sitosterol. The two latter compounds were identified by comparison of their physical constants and spectral data with those of authentic specimens and further confirmed by the preparation of derivatives.

The ^{1}H-nmr spectrum of **1** displayed five singlets and two doublets (3H each) at δ 1.08 (s), 1.07 (d, J=5 Hz), 0.98 (s), 0.94 (s), 0.91 (d, J=6.5 Hz), 0.83 (s) and 0.78 (s) and one olefinic proton at 5.27 (t, J=3.7 Hz). The mass spectrum of **1** showed a molecular peak at *m/z* 472 compatible with a molecular formula of $C_{30}H_{48}O_4$.

Other important fragments at *m/z* 246 (60) and 201 (100) could be explained (8) by a typical fragmentation of urs-12-enes or olean-12-enes with a carboxyl group attached to C-17 and a hydroxyl group on rings D/E. The presence of hydroxyl and carboxyl groups was also confirmed by ^{1}H-nmr signals at δ 3.39 (1H) and 3.19 (1H) and ir bands at 3420, 3200, and 1685 cm^{-1}. A ^{13}C-nmr signal found in the spectrum of **1** (δ 180.1 ppm) also confirmed the carboxylic acid nature of this compound.

Compound **1** did not afford the corresponding methyl ester when treated with CH_2N_2 in Et_2O solution. It could be acetylated yielding the diacetate derivative **1a**. The ^{1}H-nmr spectrum of **1a** confirmed the presence of two acetoxyl groups and gave an indication about



1 R=R¹=H
1a R=R¹=Ac

their probable location. Besides a 3β-acetoxyl group (1H at δ 4.42, complex signal showing vicinal coupling) characteristic of these triterpenoids, a second proton geminal to another acetoxyl group (δ 4.63) showed a sextet with two large axial-axial coupling (J=11.1 Hz) and an axial-equatorial coupling (J=4.4 Hz). There are only two possible locations (C-6 and C-21) which could account for this type of coupling, in the structure we have assumed as a working hypothesis. The lack of important fragments at *m/z* 248 and 203 in the mass spectrum of **1** precludes its location on C-6 (9). The data obtained from the ^{13}C nmr of **1a** finally points to C-21 (β-configuration) as the location of the second acetoxyl group. Comparison of this spectrum with the ^{13}C-nmr spectra of ursolic acid (10) or its acetyl derivative (11) shows the identity of all signals assigned to the A/B/C rings carbon atoms, while some of the ring E signals have undergone shifts which would result from the attachment of an -OAc group on C-21 (β-configuration): C-19 (-1.0 ppm), C-20 (4.0), C-21 (43.0), and C-30 (-4.0) (12). This is also the case with the C-Me signals obtained in the ^{1}H-nmr spectrum of **1** as compared with those reported for methyl ursolate (13). In addition, a double resonance experiment with **1a** established the vicinal position of this acetoxyl group to the proton coupled to one of the C-methyl doublets. Consequently, structure **1** was assigned to this triterpenoid.

A material insoluble in $CHCl_3$ and also present in the crude extract of *S. acinos* was identified as naringenin 7-(6″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside), considering the ^{1}H- and ^{13}C-nmr data obtained with the natural compound and its peracetylated derivative and the glc results obtained with the TMSi derivatives of the sugar moiety. Although previously isolated (5) and synthetized (7), to our knowledge (14-16) no ^{13}C-nmr data of this glycoside has been previously published (see Experimental section). This is also the case for eriodictyol 7-(6″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) previously found in lemon peels (6) and isolated now from *S. montana*. Finally, *S. obovata* has only yielded two known compounds: oleanolic acid and the β-D-glucoside of β-sitosterol present in the three species studied.

These chemical findings could also have some bearing on the botanical classification of *S. acinos*, also named *Acinos arvensis* (Lam.) Daudy.

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 142 spectropolarimeter. Mps were determined on a Kofler apparatus and are uncorr. Ir spectra were obtained in KBr pellets and ms was 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 internal standard, unless otherwise stated. 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 August, 1980, in Sierra Parda (Albacete, Spain). Voucher specimens of the 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 the three plants: *S. acinos*, *S. montana*, and *S. obovata*. Dried and powdered plant materials (1.5 kg) were extracted with Me_2CO (6 liters) at rt for 2 days. After filtration, the solvent was evaporated, yielding gums which were subjected to separation procedures.

ISOLATION OF THE COMPONENTS OF *S. ACINOS*.—The crude extract (20.7 g) was chromatographed on a short column (17) over Si gel 60 G, eluting with $CHCl_3$-MeOH (95:5) (100 ml fractions). Ursolic acid was the main component (1.0 g) in fractions 4-6 (ir, α_D, mp identical to authentic sample). The β-D-glucopyranoside of β-sitosterol (50 mg) was isolated from fractions 25-30 (ir, ^{1}H nmr 360 MHz, [α_D] identical to those of authentic material). A third component, triterpenoid **1**, (30 mg) was found in fractions 17-19.

An insoluble residue obtained when attempting to dissolve the crude extract was further purified on a short column using $CHCl_3$-MeOH (90:10) yielding a flavanone glycoside identified as naringenin 7-O-rutinoside.

TRITERPENOID **1**.—**1** (30 mg, after crystallization from hexane: EtOAc): mp 292-295°;

$[\alpha]^{20}D$=37.3° (MeOH, c 0.10); ir ν max KBr 3420, 3200, 1685, 1465, 1390, 1380, 1260, 1045, 1030, 1005, 760, 745 cm^{-1}. Ms (direct inlet) *m/z* (rel. int.) 472 $[M]^+$, 455 (2), 454 (8), 439 (1), 436 (2), 427 (1), 421 (1), 410 (2), 399 (3), 393 (1), 372 (2), 354 (2), 264 (8), 255 (5), 247 (15), 246 (60), 231 (20), 207 (40), 201 (100), 190 (40). ^1H nmr (360 MHz, $CDCl_3$: CD_3OD) δ 5.27 (1H, t, J=3.7 Hz, H-12), 3.39 (1H, t, J=5.4 Hz, H-21), 3.19 (1H, t, J=8.6 Hz, H-3) and seven C-Me groups at 1.08 (s) (H-27), 1.07 (d, J=5.0 Hz) (H-29)[a], 0.98 (s) (H-23), 0.94 (H-25) (s). 0.91 (d, J=6.5 Hz) (H-30)[a], 0.83 (s) (H-26), 0.78 (s) (H-24). Compound **1** failed to provide the methyl ester derivative when treated with CH_2N_2 in Et_2O. It could be acetylated under ordinary conditions yielding a diacetate (**1a**). ^1H nmr (360 MHz) δ 5.27 (1H, t, J=3.5 Hz, H-12), 4.63 (1H, sextet, J_{20} 11.1 Hz, J_{22ax} 11.1 Hz, J_{22eq}, 4.4 Hz, H-21), 4.42 (1H, complex, H-3), two -OCO-CH_3 groups at 1.98 and 2.14.

^{13}C nmr (ppm) C-1 38.4 (t), C-2 23.5 (t). C-3 80.8 (d), C-4 37.8 (s), C-5 55.4 (d), C-6 18.2 (t), C-7 33.0 (t), C-8 39.6 (s), C-9 47.5 (d), C-10 37.0 (s), C-11 23.6 (t), C-12 126.8 (d), C-13 137.5 (s), C-14 42.0 (s), C-15 29.8 (t), C-16 25.1 (t), C-17 48.6 (s), C-18 52.1 (d), C-19 38.1 (d), C-20 43.6 (d), C-21 73.7 (d), C-22 41.0 (t), C-23 28.2 (q), C-24 15.6* (q), C-25 15.8* (q), C-26 16.8 (q), C-27 23.7 (q), C-28 180.1 (s), C-29 17.2[a] (q), C-30 17.3[a] (q).

Two -CO- signals at 171.1 and 170.7 and two acetyl methyl groups at 21.4 and 21.3.

NARINGENIN 7-(6″-*O*-α-L-RHAMNOPYRANOSYL-β-D-GLUCOPYRANOSIDE).—^{13}C nmr (DMSO-d_6) ppm C-2 79.0 (d), C-3 42.2 (t), C-4 197.5 (s), C-5 163.6 (s), C-6 97.1 (d), C-7 165.6 (s), C-8 96.0 (d), C-9 163.1 (s), C-10 103.2 (s), C-1′ 129.1 (s), C-2′ 128.9 (d), C-3′ 115.8 (d), C-4′ 158.1 (s), C-5′ 115.8 (d), C-6′ 129.9 (d); C-1″ 101.0 (d), C-2″ 73.4 (d), C-3″ 76.8 (d), C-4″ 70.2 (d), C-5″ 76.1 (d), C-6″ 66.5 (t); C-1‴ 100.0 (d), C-2‴ 70.8 (d), C-3‴ 71.3 (d), C-4‴ 72.7 (d), C-5‴ 68.8 (d), C-6‴ 18.2 (q). The glucoside was acetylated under ordinary conditions: ^{13}C nmr ppm 188.5 (s), 169.9 (s), 169.8 (s), 169.7 (s), 169.7 (s), 169.3 (s), 169.1 (s), 169.0 (s), 169.0 (s), 163.7 (s), 161.9 (s), 151.9 (s), 151.0 (s), 135.9 (s), 127.4 (d), 127.4 (d), 122.0 (d), 122.0 (d), 109.7 (s), 105.9 (d), 102.3 (d), 98.0 (d), 97.6 (d), 78.9 (d), 73.3 (d), 72.7 (d), 70.9 (d), 70.9 (d), 69.4 (d), 69.1 (d), 66.7 (d), 66.2 (t), 44.7 (t), 20.6 (q, eight signals), 17.3 (q).

ISOLATION OF THE COMPONENTS OF *S. MONTANA*.—The crude extract (10.7 g) was chromatographed on a short column eluting with $CHCl_3$-MeOH (95:5). The following known compounds were identified, oleanolic acid (750 mg), as the acetylated derivative of its methyl ester: (mp, $[\alpha]D$ and ir spectra and comparison with authentic sample (18); β-D-glucoside of β-sitosterol (50 mg) (previously isolated and identified from *S. acinos*), and crataegolic acid (mp, ir, and ^1H nmr). An insoluble residue was further purified on a very short column using $CHCl_3$-MeOH (90:10). It was identified as eriodictyol-7-*O*-rutinoside.

ERIODICTYOL 7-(6″-*O*-α-L-RHAMNOPYRANOSYL-β-D-GLUCOPYRANOSIDE).—^{13}C nmr (DMSO-d_6) ppm C-2 78.5 (d), C-3 44.7 (d), C-4 196.9 (s), C-5 162.9 (d), C-6 96.5 (d), C-7 165.0 (d), C-8 95.5 (d), C-9 162.5 (s), C-10 99.5 (s), C-1′ 129.2 (s), C-2′ 114.4 (d), C-3′ 145.1 (s), C-4′ 145.7 (s), C-5′ 115.4 (d), C-6′ 118.1 (d). The sugar moiety gave signals adscribable, within experimental error, to the rutinose disaccharide. The glucoside was acetylated under standard conditions. ^{13}C nmr ppm 188.4 (s), 170.0 (s), 169.9 (s), 169.7 (s), 169.7 (s), 169.3 (s), 169.3 (s), 169.1 (s), 168.0 (s), 168.0 (s), 163.8 (s), 161.9 (s), 151.9 (s), 142.4 (s), 142.4 (s), 137.0 (s), 124.3 (d), 123.8 (d), 121.5 (d), 109.6 (s), 106.2 (d), 102.2 (d), 98.0 (d), 97.4 (d), 78.3 (d), 73.2 (d), 72.4 (d), 70.9 (d), 70.9 (d), 69.4 (d), 69.0 (d), 66.6 (d), 66.2 (t), 44.7 (t), 20.5 (q), 17.2 (q).

ISOLATION OF THE COMPONENTS OF *S. OBOVATA*.—Applying similar procedures to those described previously, the following components were isolated: oleanolic acid (mp, ir and $[\alpha]D$ of the methyl ester of the acetylated derivative). β-D-Glucoside of β-sitosterol (ir, ^1H and ^{13}C nmr of its peracetylated derivative).

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