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A NEW 3,4-SECOPENTACYCLIC TRITERPENOID FROM THE GENUS *SATUREIA**

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Key Word Index—*Satureia calamintha*; *S. graeca*; Labiatae; 3,4-secopentacyclic triterpenediol.

Abstract—The chemical analysis of the neutral extracts of *Satureia calamintha* and *S. graeca* aerial parts afforded, besides calaminthadiol, a new triterpene, isocalaminthadiol which belongs to the 3,4-seco-12-ursene class. The isolation of isocalaminthadiol and calaminthadiol may constitute a valid index for the chemotaxonomic characterization of the genus *Satureia*.

INTRODUCTION

In the course of our studies on the chemotaxonomic characterization of the genus *Satureia* (Labiatae), we examined the neutral petrol extracts of *Satureia calamintha* and *S. graeca*, herbaceous perennial plants, widely distributed in the Mediterranean area. In an earlier report on triterpenoid constituents of acid extracts, both species were found to contain, besides ursolic and oleanolic acids, the rarely occurring 3-epiursolic acid, which we have characterized on the basis of its spectrometric parameters [1, 2].

More recently we have isolated, from the neutral fractions, a new secopentacyclic triterpenoid, identified as 3,4-seco-4(23),12-ursadien-3,28-diol, calaminthadiol (**1**) [3].

RESULTS AND DISCUSSION

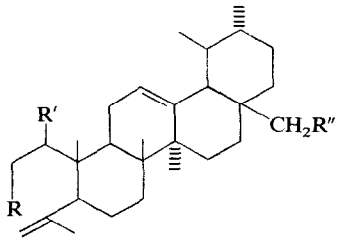
We now report the further investigation of the neutral extracts of *Satureia* species, which has revealed the presence of a second 3,4-secotriterpenoid, isolated by repeated column and thin layer chromatography and characterized by ^{1}H NMR and MS. The second substance, mp 195–197°, had the same molecular formula as calaminthadiol, $C_{30}H_{50}O_2$, gave a positive TNM and Liebermann–Burchard tests, and had an IR spectrum almost identical with that of calaminthadiol (**1**). It appeared to be a new triterpene of the 12-ursene class and was given the trivial name isocalaminthadiol (**2**). Evidence to support the structural assignment as 3,4-seco-4 (23), 12-ursadien-1, 28-diol is given below.

Isocalaminthadiol (**2**) formed a diacetate (**3**) and adsorbed 1 mol of hydrogen (Pd/C) to yield the dihydroisocalaminthadiol (**4**), which also could be converted into a diacetate (**5**). Oxidation of **4** with CrO_3–H_2SO_4 led to the keto acid (**6**), which reacted with CH_2N_2 to give the methyl ester (**7**). This sequence of reactions pointed out the presence in **2** of a hydrogenable double bond and of primary and secondary hydroxyl groups. The presence of a second double bond, trisubstituted and inert to the hydrogenation, was inferred by the ^{1}H NMR spectrum of **2**, which showed a one-proton triplet at δ 5.13, still present in the spectrum of dihydroisocalaminthadiol (**4**).

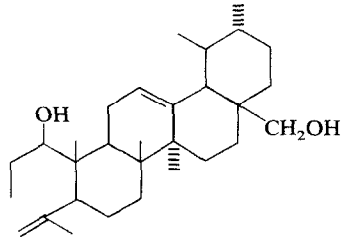
Evidence for the presence of the isopropenyl group in **2** was obtained from the ^{1}H NMR spectra of **2** and **3**, which displayed a three-proton singlet at δ 1.68–1.70 in **3** due to the C-24 methyl resonance of the side-chain isopropenyl group, and a doublet centred at δ 4.62, virtually unaltered in **3**, indicative of C-23 protons. Furthermore the spectrum of dihydroisocalaminthadiol (**4**) only exhibited the one proton signal at δ 5.13 in the olefinic region, and the C-24 methyl resonance was shifted upfield to 0.8–1.1 ppm.

The nature and positions of the two hydroxyl groups were deduced by examination of ^{1}H NMR and MS data of **2**, **3** and **5**. The ^{1}H NMR spectrum of **2** contained, in fact, an AB system centred at δ 3.35 with splitting of 11 Hz, typical of an isolated CH_2OH. This pattern was almost identical in the spectrum of the diacetate (**3**), but it was displaced downfield to δ 3.86. The presence of —CH(OH)CH_2— in **2** was inferred by the easy identification in the ^{1}H NMR spectrum of **5** of a one-proton quartet at δ 4.50 with splitting of 9.0 and 7.0 Hz (X component of the ABX system corresponding to —CH(OAc)CH_2— grouping).Conclusive information on the skeleton was obtained from the MS spectra. The fragmentation pattern of **2** exhibited the typical retro-Diels–Alder cleavage of the C ring, characteristic of the α- and β-amyrin skeletons. The data indicated that **2** was a dihydroxytriterpenoid of the α- or β-amyrin class with one hydroxyl in the D/E rings (*m/e* 234) and one hydroxyl in the A/B rings (*m/e* 207). A striking feature of the MS spectrum of **2** was the presence of a prominent fragment at *m/e* 411 ($M^+ - 31$): this suggested the presence of a

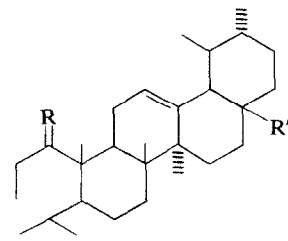
*Part 4 in the series "Constituents of the Genus *Satureia*". For Part 3 see ref. [3].



1 R = CH_2OH; R′ = H; R″ = OH
3 R = Me; R′ = R″ = OAc
8 R = Me; R′ = R″ = H



2



4 R =  ; R′ = CH_2OH

5 R =  ; R′ = CH_2OAc

6 R = O; R′ = COOH
7 R = O; R′ = COOMe

CH_2OH group at C-28 as confirmed by the base peak at *m/e* 203, which arose from *m/e* 234 by the loss of the C-28 function from a 12-ursene or a 12-oleanene derivative. The MS spectra of **2** and **4** were very similar to those of calaminthadiol (**1**) and its dihydro derivative, thus establishing the basic structure of the A and B moiety as being strictly correlated to that of a 3,4-secotriterpenoid, as compound **1**. The appearance of a medium-intensity peak at *m/e* 414 (M^+ – ethylene) was noteable; this type of fragmentation is reported for some secondary alcohols ($MeCH_2$—CH(OH)R) [4].

The isopropenyl double bond position in the 12-ursene-like skeleton was independently proved by the identity (mmp, GLC) of the diene (**8**), obtained from **2** via tosylation and $LiAlH_4$ reduction, with the 3,4-seco-4(23),12-ursadiene, synthesized from **1** by a parallel reaction sequence [5].

As regards the secondary hydroxyl group localization, its *gem*-proton resonated as a quartet in the ^1H NMR spectrum of **2**, so appearing spin-coupled only with two other protons. Thus the secondary OH must be at either C-1 or C-7. The choice of position C-1 and the subsequent attribution of the structure 3,4-seco-4(23),12-ursadien-1,28-diol to isocalaminthadiol (**2**) were supported by the high LIS observed for the C-25 methyl when the ^1H NMR spectrum of **2** was recorded in the presence of the LSR $Eu(fod)_3$ [2]. Also in accord with the C-1 position of the secondary OH was the loss of ethylene from the molecular ion observed in the MS spectrum of **2** (see above).

Because of the rareness of the 3,4-fission in the α- and β-amyrin skeletons, the isolation of the isomer diols **1** and **2** may be significant for the chemotaxonomic characterization of the genus *Satureia*. The lack of an oxygen function at fission of the A ring in **2** is unique among triterpenoids; the isolation of isocalaminthadiol from *S. calamintha* and *S. graeca* represents the first report of its occurrence in nature.

EXPERIMENTAL

Mps are uncorr. ^1H NMR spectra were recorded in $CDCl_3$ at 60 MHz, chemical shifts are in ppm (δ) from TMS as internal standard. MS were recorded at 70 eV. Optical rotations were recorded in $CHCl_3$ soln, IR spectra as Nujol mulls. Petrol had bp 40–60°. CC was performed with Al_2O_3 and TLC with Si gel.

Extraction of the leaves of S. calamintha *and* S. graeca. From 1 kg of air-dried leaves, worked up as in [3], *ca* 500 mg of pure isocalaminthadiol (**2**) were obtained by CC (elution with petrol–Et_2O, 1:1) and repeated PLC, violet colour on Liebermann–Burchard test, mp 195–197° (from EtOH); $[\alpha]_D^{20}$ +56.7° ($CHCl_3$, *c*, 1.5). Ir ν_{max} cm^{-1}: 3332, 1649 and 881. ^1H NMR: δ 0.8–1.1 (6×3H, *m*, Mes), 1.68 (3H, *s*, C-24), 3.1–3.9 (3H, *m*, C-1 and C-28), 4.57 and 4.68 (2H, *m*, C-23), 5.13 (1H, *t*, C-12). MS *m/e*: 442, 424, 414, 411, 255, 234, 221, 207, 203, 189, 175, 133. (Found: C, 81.5; H, 11.2. $C_{30}H_{50}O_2$ requires: C, 81.4; H, 11.1%).

1,28-*Diacetoxy*-3-*seco*-4(23),12-*ursadiene* (**3**). Under standard conditions (Py–Ac_2O), 50 mg of **2** gave quantitatively diacetylisocalaminthadiol (**3**), mp 169–171° (from EtOH). ^1H NMR: δ 0.8–1.1 (6×3H, *m*, Mes), 1.70 (3H, *s*, C-24), 2.03 and 2.05 (2×3H, *s*, Ac), 3.5–4.6 (3H, *m*, C-1 and C-28), 4.62 and 4.70 (2H, *m*, C-23), 5.17 (1H, *t*, C-12).

3,4-*Seco*-12-*ursen*-1,28-*diol* (**4**). Isocalaminthadiol (**2**) (50 mg) in EtOAc (15 ml) was hydrogenated at room temp. under atmos. pres. in the presence of 20 mg 10% Pd/C. Dihydroisocalaminthadiol (**4**) was obtained (45 mg), mp 178–180° (from EtOH). ^1H NMR: δ 0.8–1.1 (8×3H, *m*, Mes), 3.1–3.9 (3H, *m*, C-1 and C-28), 5.13 (1H, *t*, C-12).

1,28-*Diacetoxy*-3,4-*seco*-12-*ursene* (**5**). Under standard conditions (Py–Ac_2O), 50 mg of **4** gave quantitatively diacetyldihydroisocalaminthadiol (**5**), mp 155–158° (from MeOH). ^1H NMR: δ 0.8–1.1 (8×3H, *m*, Mes), 2.05 (2×3H, *s*, Ac), 3.5–4.6 (3H, *m*, C-1 and C-28), 5.16 (1H, *t*, C-12).

1-*Oxo*-3,4-*seco*-12-*ursen*-28-*oic acid* (**6**). Compound **4** (100 mg) dissolved in Me_2CO (20 ml) was oxidized by Jones' reagent at 5°. The keto acid (**6**) (80 mg) obtained was recrystallized from MeOH, mp 230–231°. IR ν_{max} cm^{-1}: 3390, 1709 and 1701.

Methyl 1-*oxo*-3,4-*seco*-12-*ursen*-28-*oate* (**7**). Compound **6** (50 mg), methylated by CH_2N_2, afforded the methyl ketoester (**7**), mp 150–151° (from MeOH). IR ν_{max} cm^{-1}: 1747 and 1715.

3,4-*Seco*-4(23),12-*ursadiene* (**8**). (i) Isocalaminthadiol (**2**) (50 mg) was treated with TsCl (350 mg) in dry Py (3 ml), and the mixture set aside at 0° for 16 hr. After addition of a few drops of H_2O at room temp., dil HCl(1:1) was added and the product extracted with Et_2O. The dried extracts gave the toluene sulphonate (50 mg), which was reduced by $LiAlH_4$ in

dry Et_2O under reflux. The crude product was purified by PLC, with petrol–Et_2O (8:2) as eluant. The hydrocarbon (**8**) crystallized as needles (20 mg), mp 118–120° (from $CHCl_3$–petrol). (Found: C, 87.4; H, 12.8. $C_{30}H_{52}$ requires C, 87.3; H, 12.7%). (ii) The same hydrocarbon (**8**) (25 mg) was obtained from calaminthadiol (**1**) (50 mg) as described above.

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