Phytochemistry, 1979, Vol. 18, pp. 1203–1205. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0701-1203 $02.00/0

CALAMINTHADIOL, A NEW 3,4-SECOTRITERPENOID FROM *SATUREIA CALAMINTHA* AND *SATUREIA GRAECA**

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(*Received* 8 *December* 1978)

Key Word Index—*Satureia calamintha*; *Satureia graeca*; Labiatae; 3,4-secopentacyclic triterpenoid; new ursane derivatives; sterols.

Abstract—A new triterpenoid, calaminthadiol, 3,4-seco-4(23),12-ursadien-3,28-diol, was isolated from the leaves of *Satureia calamintha* and *Satureia graeca*. Its structure was elucidated by spectroscopic methods and partial synthesis. The natural occurrence of this compound can be conclusive for the chemotaxonomic characterization of the genus because of the rarity of the 3,4-fission in the α- and β-amyrin skeletons. A biogenetic mechanism for its formation is proposed. Sitosterol, stigmasterol and campesterol were also isolated.

INTRODUCTION

Satureia calamintha and *Satureia graeca* (Labiatae) are quite common herbaceous perennial plants, widely distributed in the Mediterranean area. In Sicily, *S. calamintha* is largely used in folk medicine as a disinfectant and cauterizing agent. As part of our program of a chemosystematic investigation of the genus *Satureia*, we have undertaken the study of triterpenoids of the two species.

In previous work [1, 2], we have investigated the acid extracts of *S. calamintha* and we have reported the isolation of the rarely occurring 3-epiursolic acid, which was characterized, on the basis of its ^{1}H NMR parameters, together with several derivatives [3]. The investigation, now extended to *S. graeca*, has led to the detection from acid fractions of the same products (see Experimental).

We report here the structure elucidation of a new triterpenoid isolated by repeated chromatography of neutral extracts of both *S. calamintha* and *S. graeca* aerial parts. It was identified as 3,4-seco-4(23),12-ursadien-3,28-diol (calaminthadiol) on the basis of the spectrometric parameters of the title compound and its suitable derivatives. A partial synthesis of calaminthadiol was also achieved.

RESULTS

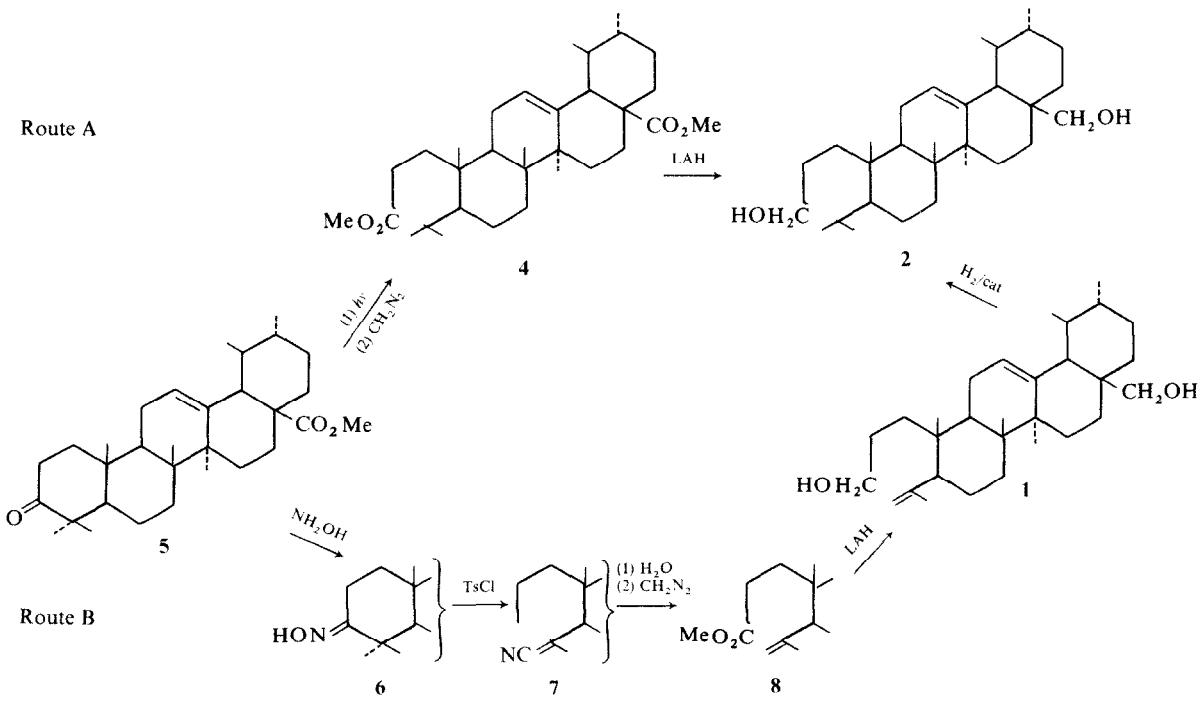
The isolated triterpenoid (**1**), mp 197–199° (from EtOH) $[\alpha]_D^{20}$ +87°, had the formula $C_{30}H_{50}O_2$ (M^+ 442). Its IR spectrum exhibited a broad absorption for OH at 3333 cm^{-1} and two bands for the $>C=CH_2$ group at 1648 and 880 cm^{-1}. Evidence for the presence of an isopropenyl group was also obtained by the ^{1}H NMR spectrum of **1**, which displayed two peaks at δ 4.66 and 4.83 (2H), and a three-proton singlet (1.75), attributable to the methyl hydrogens of the isopropenyl group. This resonance was lacking in the spectrum of dihydrocalaminthadiol (**2**), obtained by catalytic hydrogenation of **1**. In addition, the ^{1}H NMR spectrum of **1** showed another olefinic proton at δ 5.16, still present in the spectrum of **2**. The presence of an inert trisubstituted double bond in **1** was deduced.

Compound **1** gave a diacetyl derivative (**3**) whose ^{1}H NMR spectrum showed two acetoxymethyl signals at δ 2.02 and 2.03, and four carbinolic protons as a multiplet centred at 3.92. The primary nature of the two OH groups in the molecule was confirmed by the Jones' oxidation at 5° of **2**, which afforded, after methylation by CH_2N_2, the dihydrodiester (**4**).

Cogent information on the skeleton of calaminthadiol (**1**) was given by MS which showed strong peaks characteristic of 12-ursene and 12-oleanene skeletons [4]. In particular, the prominent fragment ion at *m/e* 234 ($C_{16}H_{26}O$), derived from the C-, D- and E-rings by a typical retro-Diels–Alder reaction, allowed one OH group to be located at the D- and E-rings. Furthermore, the fragment at *m/e* 234 yielded the peak at *m/e* 203, by the loss of CH_2OH, according to the known fragmentation in the angular C-17 position [5]. In addition, the *m/e* 207 peak ($C_{14}H_{23}O$) suggested the presence of the second OH in the A- and B-ring portion of the molecule. The shifts induced by Eu(fod)$_3$ on the proton resonances of **1** led us to observe the doublets of two secondary methyl groups, typical of the 12-ursene skeleton [3]. Moreover, the achieved simplification of the complex Me pattern displayed the presence of three tertiary methyl groups. The 12-ursene skeleton, suggested for **1** by LSR, restricted the presence of the isopropenyl group only to the A-ring, and required the fission of the A-ring.

The above data and results were consistent with the structure 3,4-seco-4(23),12-ursadien-3,28-diol (**1**). That calaminthadiol actually has structure **1** was proved by its partial synthesis from methyl ursonate (**5**) (Scheme 1). Compound **5** was converted into the corresponding oxime (**6**) then, by refluxing with tosyl chloride in pyridine [6], into the unsaturated seco-nitrile (**7**). Alkaline hydrolysis of **7** and subsequent methylation yielded the unsaturated diester (**8**), which was reduced by $LiAlH_4$ to the diol (**1**). Direct comparison with our natural product proved the identity (mmp, GLC, ^{1}H NMR and MS).

* Part 3 in the series "Constituents of the genus *Satureia*". For preceding papers see refs. [1–3].



Scheme 1. Chemical synthesis of calaminthadiol (**1**) and dihydrocalaminthadiol (**2**) from methyl ursonate (**5**).

The same identity was confirmed by the dihydrocalaminthadiol (**2**), which was identical by the above criteria to the synthetic 3,4-seco-12-ursen-3,28-diol obtained by photochemical cleavage [7] of **5** and subsequent reduction by $LiAlH_4$.

Sterol fractions isolated from the leaves were found by GLC to contain the ubiquitous sitosterol, stigmasterol and campesterol (see Experimental).

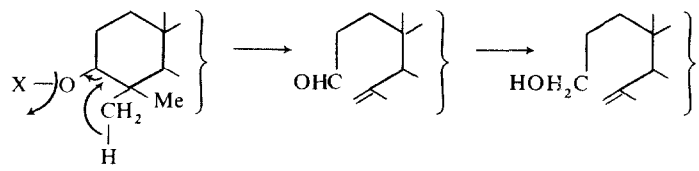
DISCUSSION

The isolation of calaminthadiol (**1**) may prove conclusive in the chemotaxonomic characterization of the genus *Satureia* because of the rareness of the 3,4-fission in the A-ring of α- and β-amyrin skeletons. Hitherto this structural feature has been reported only for the isomeric roburic and nyctanthic acids [6, 7]. Moreover, the presence of a primary alcoholic function at the 3,4-fission, as in calaminthadiol, is unique among triterpenoids.

That the tetracyclic calaminthadiol (**1**) occurs in *S. calamintha* and *S. graeca* together with the pentacyclic ursolic and 3-epiursolic acids is in good agreement with the biogenetic mechanism of C-3/C-4 bond cleavage, proposed for dammarenolic and nyctanthic acids [7]. In line with the aforesaid process, we suggest the biogenetic mechanism shown in Scheme 2 for the formation of **1**, where X is a suitable leaving group.

The process would start from uvaol or a similar 3-hydroxy-substituted product, affording a formyl group easily reducible to a primary carbinolic function.

Further work is in progress to verify the natural occurrence of **1** in other *Satureia* species to improve the chemotaxonomic correlations of the genus.



Scheme 2. Suggested mechanism for the biosynthesis of calaminthadiol (**1**).

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded at 60 MHz, chemical shifts are in ppm (δ) from TMS as internal standard. Optical rotations were recorded in $CHCl_3$ solns, IR spectra as Nujol mulls. Petrol had bp 40–60°. CC was performed with Al_2O_3 and TLC with Si gel. GLC was carried out using a OV17 capillary column (240°, H_2) with the TMSi ethers.

Extraction of the leaves of S. calamintha. Air-dried leaves (1 kg), collected in June near Messina, were powdered and extracted (Soxhlet) with petrol. Evapn of the solvent under red. pres. gave a dark green mass (40 g). It was dissolved in Et_2O and the acid constituents removed by adding a soln of 10% NaOH. The residual Et_2O soln yielded after solvent removal a brown semi-solid product (20 g) which was chromatographed over Al_2O_3 (400 g); petrol with increasing amounts of Et_2O was used as eluant. The fractions eluted by petrol–Et_2O (8:2) gave a mixture of sterols (1.1 g, green colour on Liebermann–Burchard test). GLC proved the occurrence of campesterol, stigmasterol and sitosterol (crossed injection with a mixture of the 3 sterols). The elution with petrol–Et_2O (1:1) yielded 1.5 g of a crude product which, by repeated PLC, gave pure calaminthadiol (**1**) (500 mg, violet colour on Liebermann–Burchard test), mp 197–199° (from EtOH); $[\alpha]_D^{20}$ +87° ($CHCl_3$; *c*, 1.5). IR ν_{max}^{Nujol} cm^{-1}: 3333, 1648 and 880. ^{1}H NMR: δ 0.8–1.2 (5 × 3H, *m*, Me's), 1.75 (3H, *s*, C-24), 3.0–3.7 (4H, *m*, C-3 and C-28), 4.66 and 4.83 (2H, *m*, C-23), 5.16 (1H, *m*, C-12). MS (70 eV) *m/e:* 442, 424, 414, 411, 396, 234, 221, 207, 203, 190, 189, 148, 133. (Found: C, 81.6; H, 11.1. $C_{30}H_{50}O_2$ requires: C, 81.4; H, 11.1%).

Extraction of Satureia graeca. Fresh leaves (1 kg, collected in June) were dried and treated in the same way as described for *S. calamintha*. Ursolic, 3-epiursolic and oleanolic acids were detected in the acid fractions. The same mixture of sterols was present in the neutral extracts with only a slight difference in percentage composition with respect to *S. calamintha*. Compound **1** was present in an estimated amount of 300 mg.

Hydrogenation of **1**. Calaminthadiol (50 mg) in EtOAc (15 ml) was hydrogenated at room temp. under atmos. pres. in the presence of 20 mg of 10% Pd/C. 3,4-Seco-12-ursen-3,28-diol (**2**) was obtained (50 mg), mp 184–186° (from EtOH). ^{1}H NMR: δ 0.7–1.5 (7 × 3H, *m*, Me's), 3.0–3.8 (4H, *m*, C-3 and C-28), 5.17 (1H, *m*, C-12).

Acetylation of **1**. Under standard conditions (Py–Ac_2O), 50 mg of **1** gave quantitatively 3,28-diacetoxy-3,4-seco-4(23),12-ursadiene (**3**), mp 162–164° (from MeOH). ^{1}H NMR: δ 0.8–1.2 (5 × 3H, *m*, Me's), 1.75 (3H, *s*, C-24), 2.02 and 2.03 (2 × 3H, *s*, Ac), 3.5–4.2 (4H, *m*, C-3 and C-28), 4.67 and 4.87 (2H, *m*, C-23), 5.22 (1H, *m*, C-12).

Oxidation of **2**. **2** (100 mg) was treated with Jones' reagent at 5°. The crude product, methylated by CH_2N_2, afforded methyl 3,4-seco-12-ursen-3,28-dionate (**4**) (85 mg), mp 171–173° (from MeOH). ^{1}H NMR: δ 0.7–1.4 (7 × 3H, *m*, Me's), 3.63 and 3.67 (2 × 3H, *s*, OMe), 5.28 (1H, *m*, C-12).

Partial synthesis. Route A. Methyl ursonate (**5**) (2 g) in HOAc–H_2O (9:1) at refluxing temp. was irradiated under N_2 for 12 hr in a reactor equipped with a medium pressure arc tube emitting in the range 254–366 nm. The crude product, obtained by solvent removal, was purified by CC (acid Al_2O_3, 100 g) and methylated by CH_2N_2 to give the dihydrodiester (**4**) (300 mg). **4** (100 mg) was subsequently reduced by $LiAlH_4$ in dry Et_2O at reflux and afforded dihydrocalaminthadiol (**2**) (90 mg).

Route B. Methyl ursonate oxime (**6**) (1 g) prepared from methyl ursonate and hydroxylamine chloride in EtOH was dissolved in dry Py (40 ml) and tosyl chloride (1.1 g) was added. After 4 hr at refluxing temp., dil HCl was added and the product was isolated with C_6H_6 and chromatographed on Al_2O_3 (50 g). Elution with C_6H_6 afforded methyl 3-cyano-3,4-seco-4(23), 2-ursadien-28-oate (**7**) (220 mg), mp 205–207° (from C_6H_6–ligroin). IR ν_{max}^{Nujol} cm^{-1}: 2257, 1640 and 880 (). Hydrolysis of **7** under reflux with 20% KOH in EtOH (25 ml) for 3 hr and subsequent acidification gave, by CH_2N_2 methylation, methyl 3,4-seco-4(23),12-ursadien-3,28-dionate (**8**) (200 mg). ^{1}H NMR: δ 0.7–1.2 (5 × 3H, *m*, Me's), 1.75 (3H, *s*, C-24), 3.62 and 3.63 (2 × 3H, *s*, OMe), 4.67 and 4.85 (2H, *m*, C-23), 5.28 (1H, *m*, C-12). Reduction of **8** with $LiAlH_4$ under the usual conditions gave calaminthadiol (**1**) (180 mg).

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