Pergamon

PII: S0031-9422(97)00042-3

Phytochemistry, Vol. 45, No. 6, pp. 1293–1294, 1997

0031-9422/97 $17.00+0.00

TERPENOIDS FROM *SALVIA GLUTINOSA*

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(*Received in revised form* 19 *November* 1996)

Key Word Index—*Salvia glutinosa*; Lamiaceae; triterpenoids; steroids; 1-oxo-7α-hydroxysitosterol; cytotoxic activity.

Abstract—A new sterodial compound 1-oxo-7α-hydroxysitosterol was isolated from the whole plant of *Salvia glutinosa* in addition to 11 known triterpenoids and three steroids. The structures were established by spectral data. Cytotoxic activity of the new compound and 7α-hydroxysitosterol were tested against P-388 and KB systems; only marginal activity was found.

INTRODUCTION

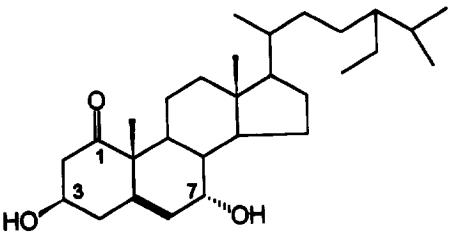
In continuation of our chemical and biological investigations of Turkish *Salvia* species, we have now studied an endemic plant *S. glutinosa* L. which yielded only triterpenic and steroidal compounds, one of them being new. The known triterpenoids were α-amyrin, α-amyrin acetate [1], 11-oxo-α-amyrin (3β-hydroxy-11-oxo-ursan-12-ene) [2], 11-oxo-β-amyrin (3β-hydroxy-11-oxo-olean-12-ene) [3], 3β-acetoxyolean-9,11-diene [4], ursolic and oleanolic acids, ursolic and oleanolic acid methyl esters [5], lupeol, erythrodiol 28-acetate [6] and the steroidal compounds stigmasterol, sitosterol, 7α-hydroxysitosterol [7] together with the new compound 1-oxo-7α-hydroxysitosterol (24-ethylcholest-5-ene-3β,7α-diol-1-one). According to the literature [8, 9] 3,7-dihydroxysteroidal compounds have bioactivity. The antitumour activity of 7-hydroxycampesterol and 7α-hydroxystigmasterol were established [8]. The similar compounds, ergosta-5,24(24′)-diene-3β,7α-diol and 24,24′-epoxy-ergost-5-ene-3β,7α-diol, showed activity against genetically engineered yeast (*Saccharomyces cerevisiae*) and P-388 murine lymphocytic leukemia [9]. Since the new compound **1** and one of the known compounds which have a 3,7-dihydroxysteroidal structure, were tested against P-388 murine lymphocytic leukemia and KB test systems, only marginal activity was established for both of them.

RESULTS AND DISCUSSION

The HREI mass spectrum of the new compound 24-ethylcholest-5-ene-3β,7α-diol-1-one (**1**) corresponded to a molecular formula $C_{29}H_{48}O_3$ (*m/z* 444.3562, calcd 444.3603). The IR spectrum showed signals for hydroxyl (3450 cm^{-1}), and a six-membered ring carbonyl (1712 cm^{-1}). The ^1H and ^{13}C NMR spectra indicated the structure of **1**. The broad singlet at δ 5.55 (1H, *br s*, H-6) and at δ 4.33 (1H, *br t*, $J = 2.5$ Hz, H-7) were typical signals for Δ^5-7α-hydroxyl group, studying a Dreiding model showed about a 100° angle between H-6 and H-7β, thus indicating the α-position of the hydroxyl group. A spin decoupling experiment showed the relation between H-6 and H-7. The location of the β-hydroxyl group at C-3 was evident with the signal at δ 4.19 (1H, *m*, H-3α). Both signals for H-3 and H-7 were somewhat more downfield than expected when compared to that of 7α-hydroxysitosterol (δ 3.85, *br s*, H-7 and δ 3.66, *m*, H-3); these chemical shift differences could be explained by the presence of an oxo group at C-1. In the ^1H NMR spectrum the methyl singlets were observed at δ 0.67 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19) and methyl doublets at δ 0.82 (3H, *d*, $J = 6.5$ Hz, Me-27), 0.85 (3H, *d*, $J = 6.5$ Hz, Me-26), 0.86 (3H, *t*, $J = 6.5$ Hz, Me-29), 0.91 (3H, *d*, $J = 7$ Hz, Me-21). The ^{13}C NMR (APT) indicated the presence of a six-membered ring carbonyl signal at δ 208.4 *s*. Unsaturated carbon atoms were at δ 142.4 *s* (C-5) and 132.5 *d* (C-6). The carbon atoms carrying the secondary hydroxyl groups were at δ 67.9 *d* (C-7) and 87.0 *d* (C-3), the downfield appearance of the signal of C-3 was also explained by the presence of a carbonyl group at C-1. The rest of the ^{13}C NMR signals was consistent with the suggested structure (see Experimental) and the side chain assignments were comparable with the literature data given for similar structures [10]. Acetylation of compound **1** yielded a diacetyl derivative (**1a**), the proton signals in the ^1H NMR spectrum for H-3 and H-7 were shifted

downfield to δ 4.70 (1H, *m*) and 5.05 (1H, *t*, $J = 4$ Hz), respectively, the acetyl signals were at δ 2.04 and 2.10, other signals were more or less similar.

In the literature [11], 7β-hydroxysteroidal compounds are reported to be highly active while their 7α-hydroxy counterparts have little or no cytotoxic activity. In order to confirm this finding the cytotoxic activity tests in P-388 and KB test systems were performed for two of our compounds having a 7α-hydroxyl group and both showed marginal activity, thus agreeing with the literature data.



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EXPERIMENTAL

General. The spectra were recorded with the following instruments. IR: Perkin–Elmer 980 in $CHCl_3$; NMR: Bruker AC-200 MHz (^1H) and 50.32 MHz (^{13}C) in $CDCl_3$; HRMS: VG ZabSpec; Optical rotation: Optical Act. Ltd; TLC: Kieselgel 60 F 25 (E. Merck) precoated plates; CC, silica gel 60; Sephadex LH-20 (Fluka).

Plant material. Salvia glutinosa L. was collected from the Turkish Black Sea area, Rize-Çamlihemşin at an altitude 1300–1800 m in July 1994. The plant was identified by Prof. S. Kurucu (Ankara), a voucher specimen was deposited in the Herbarium of the University of Ankara, Faculty of Pharmacy AEF 18853.

Extraction and isolation of the compounds. The powdered whole plant (930 g) was extracted with Me_2CO in a Soxhlet. The solvent was evaporated *in vacuo* to give 28 g of a crude residue which was then fractionated by silica gel CC (5 × 80 cm). The column was eluted with petrol, a gradient of EtOAc was added up to 100% followed by EtOH. The compounds were isolated in the following order: α-amyrin acetate (20 mg), **1** (22 mg), 11-oxo-α-amyrin (13 mg), 11-oxo-β-amyrin (8 mg), oleanolic acid methyl ester (12 mg), 3-acetoxyolean-9,12-dien (18 mg), erythrodiol 28-acetate (6 mg), ursolic acid methyl ester (7 mg), 7α-hydroxysitosterol (18 mg), lupeol (8 mg), α- amyrin (20 mg), ursolic acid (5 mg), oleanolic acid (8 mg), stigmasterol (12 mg), sitosterol (25 mg).

1-Oxo-7α-hydroxysitosterol. $[\alpha]_D + 5.3^\circ$ ($CHCl_3$, *c* 0.8). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2980, 2960, 1712, 1680, 1480, 1350, 1180, 1100. ^1H NMR ($CDCl_3$): given in the text. ^{13}C NMR ($CDCl_3$): C-1 208.4 *s*, C-2 39.6 *t*, C-3 87.0 *d*, C-4 42.4 *t*, C-5 142.4 *s*, C-6 132.5 *d*, C-7 67.9 *d*, C-8 36.1 *d*, C-9 45.8 *d*, C-10 37.0 *s*, C-11 23.0 *t*, C-12 39.6 *t*, C-13 42.4 *s*, C-14 50.8 *d* C-15 24.1 *t*, C-16 28.2 *t*, C-17 55.1 *d*, C-18 11.9 *q*, C-19 18.6 *q*, C-20 36.1 *d*, C-21 18.7 *q*, C-22 33.9 *t*, C-23 26.0 *t*, C-24 45.7 *d*, C-25 29.0 *d*, C-26 19.5 *q*, C-27 20.2 *q*, C-28 20.7 *t*, C-29 11.9 *q*. HREIMS *m/z* (rel.int.): 444.3562 $[M]^+$ ($C_{29}H_{48}O_3$)(5), 428 $[M-16]^+$ (100), 412 $[M-32]^+$ (45), 303 $[M-$side chain$]^+$ (5), 275 $[M-$side chain$-28]^+$ (12), 245 (15), 183 (12), 152 (40), 138 (12), 121 (23).

Acetylation of **1**. Compound **1** (10 mg) dissolved in 1 ml of pyridine, 1 ml of acetic anhydride was added and the mixture left at room temp overnight then evapd under a vacuum and purified by TLC to yield acetylated compound (7 mg) (**1a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2980, 2860, 1730, 1722 (sh), 1715, 1680, 1475, 1360, 1240, 1170. ^1H NMR ($CDCl_3$): δ 0.67 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19), 0.81 (3H, *d*, $J = 7$ Hz, Me-27), 0.85 (6H, *d*, $J = 7$ Hz, Me-26 and me-29), 0.91 (3H, *d*, $J = 7$ Hz, Me-21), 2.04 and 2.10 (each 3H, *s*, 2 × OAc), 4.70 (1H, *m*, H-3α), 5.05 (1H, *d*, $J = 4$ Hz, H-7β), 5.55 (1H, *d*, $J = 4$ Hz, H-6).

Cytotoxic activity. The new compound **1** and 7-hydroxysitosterol were tested against P-388 *in vitro* murine lymphocytic leukemia and KB test systems [9], only marginal activity was found.

Acknowledgements—This study was supported by the Research Fund of the University of Istanbul No 758. The authors thank Dr J.M. Pezzuto (Chicago, IL) for the cytotoxic activity tests.

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