Pergamon

PII: S0031-9422(96)00709-1

Phytochemistry, Vol. 44, No. 7, pp. 1297–1299, 1997

0031-9422/97 $17.00+0.00

DITERPENOIDS FROM THE ROOTS OF *SALVIA SCLAREA*

AYHAN ULUBELEN,* UFUK SÖNMEZ* and GÜLAÇTI TOPCU†

*Faculty of Pharmacy, University of Istanbul, 34452 Istanbul, Turkey; †TUBITAK Marmara Research Center, Research Institute for Basic Sciences, Department of Chemistry, P.O. 21, 41470, Gebze, Kocaeli, Turkey

(*Received* 13 *August* 1996)

Key Word Index—*Salvia sclarea*; Lamiaceae; triterpenoids; steroid; diterpenoids; sclareapinone; acetylsalvipisone.

Abstract—The roots of *Salvia sclarea* yielded two triterpenoids, 3-oxo-oleanolic acid, α-amyrin, a steroid sitosterol and 12 diterpenoids, two of which are new. The known diterpenoids are ferruginol, salvipisone, microstegiol, candidissiol, 2,3-dehydrosalvipisone, aethiopinone, 1-oxoaethiopinone, salvinolone, cryptojaponol, Δ^7-manool, and the new compounds are sclareapinone and acetylsalvipisone. The structures were established by spectral data and also by TLC comparison for the known compounds. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In a previous study with the whole plant of *Salvia sclarea* L. collected from central Turkey (Sivas), we isolated seven known and two new diterpenoids, together with sesquiterpenes and flavones [1]. In the present study with the roots of the same plant collected from a different location of central Turkey (Çorum), two triterpenoids, 3-oxo-oleanolic acid, α-amyrin and sitosterol as well as 12 diterpenoids were isolated. Five of the diterpenoids, ferruginol [2], salvipisone [3], microstegiol [4], candidissiol [5], 2,3-dehydrosalvipisone [1], were isolated from both collections, while the others, sclareol [6], manool [7], 7-oxoroyleanone [8] and 7-oxoferruginol-18-al [1], were only found in the first study. However, additional diterpenoids cryptojaponol [9], aethiopinone [10], 1-oxoaethiopinone [11], salvinolone [12], Δ^7-manool [13] as well as new diterpenoids sclareapinone (**1**) and acetylsalvipisone (**2**) were isolated in the present study. Sclareol is an important bioactive diterpene [14, 15], but it was not found in this collection; however, its dehydroxylated derivative Δ^7-manool was present. The structures of the known compounds were established by comparing their spectral data with literature values and by TLC comparison with authentic samples, except for Δ^7-manool. There is only one other study with *S. sclarea*, by a Russian group [16]; they have found tanshinone, isotanshinone, hydroxytanshinone, which were not present in the Turkish collections of the plant.

RESULTS AND DISCUSSION

The new diterpene sclareapinone (**1**) had a molecular formula $C_{20}H_{24}O_4$ as deduced from its HREI mass spectrum (*m/z* 328.1698, calc. 328.1674). The IR spectrum exhibited frequencies for a hydroxyl at 3400 cm^{-1}, an oxo group at 1725 cm^{-1} and an orthoquinoid group at 1680, 1640 and 1605 cm^{-1}, together with UV maxima at 432 (sh), 390, 332 nm, indicating an aethiopinone derivative for **1**. The ^1H NMR spectrum corroborated this suggestion with the signals at δ 7.06 (1H, *d*, $J = 8$ Hz, H-7), 6.95 (1H, *d*, $J = 8$ Hz, H-6), 6.80 (1H, *d*, $J = 1$ Hz, H-14), 2.90 (1H, *d* septet, $J = 1$ and 7 Hz, H-15), 2.25 (3H, *s*, Me-20), 1.45 and 1.38 (each 3H, *s*) (Me-18 and Me-19), 1.17 (3H, *d*, $J = 7$ Hz), 1.13 (3H, *d*, $J = 7$ Hz) (Me-16 and Me-17). Spin decoupling experiments showed the relations between C-1 and C-2 protons at δ 2.85 (1H, *m*, H-1β), 2.58 (1H, *m*, H-2a), 2.42 (1H, *ddd*, $J = 4$, 7 and 11 Hz, H-2b) and 2.0 (1H, *m*, H-1α). The chemical shifts of the two methyl groups (Me-18 and Me-19) as well as the lack of an isopropyl methine proton (H-4) suggested the presence of a hydroxyl group at C-4. The oxo group could be placed at C-1, C-2 or C-3; its being at C-2 was unlikely due to the absence of the signals for the isolated methylene groups in its ^1H NMR spectrum. As for C-1 and C-3 positions, the latter was evident from the mass degradation of the side chain, at *m/z* 268 $[M-C_3H_7O+H]^+$ (22%), 241 $[M-C_4H_7O_2]^+$ (40%), 227 $[M-C_5H_9O_2]^+$ (90%) and 213 $[M-C_6H_{11}O_2]^+$ (48%). The ^{13}C NMR spectrum supported the placement of the oxo group as well as the given structure (Table 1). Thus, **1** is 3-oxo-4-hydroxyaethiopinone and is named sclareapinone.

The second new compound was the acetyl derivative of salvipisone (**2**). Salvipisone was isolated in previous [1] and present studies of *S. sclarea* as well as from *S. candidissima* subsp. *occidentalis* [17]. A literature

Table 1. ^{13}C NMR data for compounds **1** and **2**

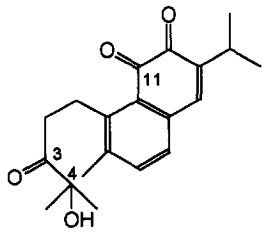
C	1	2
1	28.9 *t*	27.1 *t*
2	36.0 *t*	30.0 *t*
3	210.7 *s*	38.4 *t*
4	74.2 *s*	145.6 *s*
5	139.9 *s*	142.0 *s*
6	136.4 *d*	136.6 *d*
7	128.0 *d*	126.5 *d*
8	134.6 *s*	133.5 *s*
9	134.8 *s*	126.5 *s*
10	144.6 *s*	144.7 *s*
11	181.2 *s*	183.3 *s*
12	182.3 *s*	150.0 *s*
13	148.4 *s*	123.8 *s*
14	140.0 *d*	184.5 *s*
15	26.8 *d*	24.6 *s*
16	21.4 *q*	20.0 *q*
17	20.9 *q*	20.3 *q*
18	22.3 *q*	110.2 *t*
19	26.4 *q*	22.6 *q*
20	19.8 *q*	20.2 *q*
C=O	—	164.6 *s*
Me	—	21.1 *q*

survey revealed that there is no acetylsalvipisone occurring naturally. Compound **2** could be an artefact although we did not used any drastic acetylating agent; only ethyl acetate was used for the elution of the column. Nevertheless, the spectroscopic data for **2** is as follows. The HREI mass spectrum of **2** indicated a molecular formula $C_{22}H_{26}O_4$ (*m/z* 354.3822, calc. 354.3831). The ^1H NMR signals were quite similar to those of salvipisone at δ 7.90 (1H, *d*, $J = 8$ Hz, H-7), 7.49 (1H, *d*, $J = 8$ Hz, H-6), 4.80 (2H, *br s*, CH_2-18), 3.37 (1H, septet, $J = 7$ Hz, H-15), 2.43 (3H, *s*, Me-20), 2.05 (3H, *s*, OAc), 1.80 (3H, *s*, Me-19), 0.98 and 1.00 (each 3H, *d*, $J = 7$ Hz) (Me-16 and Me-17). The main difference between salvipisone and acetylsalvipisone was the observation of the presence of an acetyl group at δ 2.05 and the chemical shift of the two methyl doublets upfield compared to salvipisone. Hydrolysis of the acetyl group using 5% NaOH yielded salvipisone (TLC comparison), thus verifying the structure of **2**. The ^{13}C NMR spectrum of **2** correlated with the given structure (Table 1).

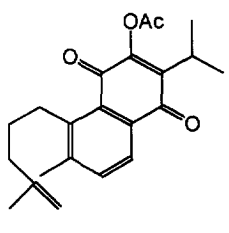
EXPERIMENTAL

General. UV: in MeOH; IR: in $CHCl_3$; ^1H and ^{13}C NMR in $CDCl_3$; HRMS: VG ZabSpec; Chromatotron Model 7924 T.

Plant material. Roots of *S. sclarea* were collected from central Turkey (Alacahöyük-Sungurlu, Çorum) and identified by Prof. Dr N. Özhatay. A voucher specimen (ISTE 68381) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.



1



2

Extraction and isolation. Dried roots of the plant (630 g) were extracted with distilled Me_2CO in a Soxhlet apparatus; the solvent was evap *in vacuo*, and 12 g residue was obtained and fractionated on a silica gel column (5 × 70 cm) using PE; a gradient of EtOAc was added up to 100%, followed by EtOH. The combined frs were further sepd on a Chromatotron using silica gel plates. When necessary prep. TLC plates were used to obtain pure compounds. The following compounds were isolated: **1** (15 mg), ferruginol (12 mg), Δ^7-manool (9 mg), microstegiol (15 mg), candidissiol (8 mg), **2** (12 mg), salvipisone (10 mg), 2,3-dehydrosalvipisone (15 mg), salvinolone (8 mg), aethiopinone (10 mg), 1-oxoaethiopinone (15 mg), cryptojaponol (20 mg), α-amyrin (18 mg), 3-oxo-oleanolic acid (10 mg) and sitosterol (25 mg).

Sclareapinone (**1**). Amorphous orange compound. UV λ_{max}^{MeOH} nm (log ε): 432 (sh), 390 (2.0), 332 (3.1), 230 (4.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2960, 2920, 2860, 1725, 1680, 1640, 1605, 1460, 1380, 1280, 1160, 1120, 1090, 1030. ^1H NMR ($CDCl_3$) given in text; ^{13}C NMR ($CDCl_3$): Table 1. HREIMS *m/z* (percent): 328.1698 $[M]^+$ (5), 310 $[M-H_2O]^+$ (15), 286 $[M-C_3H_6]^+$ (39), 268 $[M-C_3H_7O+H]^+$ (22), 241 $[M-C_4H_7O_2]^+$ (40), 227 $[M-C_5H_9O_2]^+$ (90), 213 $[M-C_6H_{11}O_2]^+$ (48), 173 (24), 128 (25), 78 (28), 69 (31).

Acetylsalvipisone (**2**). Amorphous orange compound. UV λ_{max}^{MeOH} nm (log ε): 425 (2.4), 360 (3.5), 280 (4.0), 245 (4.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3050, 3020, 2980, 2850, 1725, 1680, 1665, 1650, 1590, 1560, 1475, 1380, 1250, 1230, 1100, 980. ^1H NMR ($CDCl_3$) given in text; ^{13}C NMR ($CDCl_3$): Table 1. HREIMS *m/z* (percent): 354.3822 $[M]^+$ (18), 312 $[M-Ac+H]^+$ (100), 296 $[M-HOAc]^+$ (45), 271 $[M-C_6H_{11}]^+$ (20), 243 (15), 186 (8), 97 (12), 69 (75).

Hydrolysis of compound **2**. To a soln of **2** (5 mg) in MeOH, 2 ml 5% NaOH was added and refluxed for 2 hr. Deacetylated **2** was extracted with $CHCl_3$ and compared with authentic salvipisone on TLC plates; their ^1H NMR spectra were found to be identical.

Acknowledgement—This study was partly supported by the Research Fund of the University of Istanbul (Ö-150).

REFERENCES

1. Ulubelen, A., Topcu, G., Eriş, C., Sönmez, U.,

Kartal, M., Kurucu, S. and Bozok-Johansson, C., *Phytochemistry*, 1994, **36**, 971.
2. Cambie, R. C., Madden, R. J. and Parnell, J. C., *Australian Journal of Chemistry*, 1971, **24**, 217.
3. Rodriguez, B., Fernandez-Gadea, F. and Savona, G., *Phytochemistry*, 1984, **23**, 1805.
4. Ulubelen, A., Topcu, G., Tan, N., Lin, L.-J. and Cordell, G. A., *Phytochemistry*, 1992, **31**, 2419.
5. Ulubelen, A., Topcu, G. and Tan, N., *Tetrahedron Letters*, 1992, **33**, 7241.
6. Janot, M. M., *Annals of Chemistry*, 1932, **17**, 5.
7. Thomas, B. R., *Nature*, 1952, **170**, 1018.
8. Hensch, M., Ruedi, P. and Eugster, C. H., *Helvetica Chimica Acta*, 1975, **58**, 1921.
9. Kondo, T., Suda, M. and Tejima, M., *Yakugaku Zasshi*, 1962, **82**, 1252; *Chemical Abstracts*, 1962, **59**, 1685.
10. Boya, T. M. and Valverde, S., *Phytochemistry*, 1981, **20**, 1367.
11. Michavila, A., de la Torre, M. C. and Rodriguez, B., *Phytochemistry*, 1986, **25**, 1935.
12. Lin, L.-Z., Blasko, G. and Cordell, G. A., *Phytochemistry*, 1989, **28**, 177.
13. Bohlmann, F., Zdero, C., Hoffmann, E., Mahanta, P. K. and Dorner, W., *Phytochemistry*, 1978, **17**, 1917.
14. Bailey, J. A., Carter, G. A., Burden, R. S. and Wain, R., *Nature* (*London*), 1974, **255**, 328.
15. Severson, R. F., Culter, H. G., Cole, D. P., Jackson, D., Sission, V. A., Johnson, A. W., Herzog, G. A. and Stephenson, M. G., *Proceedings of the Plant Growth Regulation Society of America*, 1985, 175.
16. Romanova, A. S., Patudin, A. V., Pervykh, L. N. and Zobenko, L. P., *Khimiya Prirodnykh Soedinenii*, 1978, 515.
17. Ulubelen, A., Topcu, G. and Tan, N., *Phytochemistry*, 1992, **31**, 3637.