PERGAMON

Phytochemistry 51 (1999) 809–812

PHYTOCHEMISTRY

12-Deoxy-6,7-dehydroroyleanone, 12-deoxy-6-hydroxy-6,7-dehydroroyleanone and 12-deoxy-7,7-dimethoxy-6-ketoroyleanone from *Salvia nutans* roots

Gábor Nagy[a], Gábor Günther[a], Imre Máthé[a,b], Gerald Blunden[c,*], Ming-he Yang[c], Trevor A. Crabb[c]

[a]*Faculty of Pharmacy, Albert Szent-Györgyi Medical University, H-6720, PO Box 121, Szeged, Hungary*
[b]*Institute of Ecology and Botany of the Hungarian Academy of Sciences, Alkotmány Street 15, H-2163, Vácrátót, Hungary*
[c]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth, Hampshire, PO1 2DT, UK*

Received 18 January 1999; accepted 17 February 1999

Abstract

From the roots of *Salvia nutans* seven diterpenoids have been isolated and characterized, primarily from spectroscopic data. Three of the compounds are new 12-deoxyroyleanones (12-deoxy-6,7-dehydroroyleanone, 12-deoxy-6-hydroxy-6,7-dehydroroyleanone and 12-deoxy-7,7-dimethoxy-6-ketoroyleanone). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia nutans*; Labiatae; Phytochemistry; Diterpenoids; 12-deoxyroyleanones; 12-deoxy-6; 7-dehydroroyleanone; 12-deoxy-6-hydroxy-6; 7-dehydroroyleanone; 12-deoxy-7; 7-dimethoxy-6-ketoroyleanone

1. Introduction

Numerous diterpenoids, based mainly on abietane and neo-clerodane skeletons, have been isolated from *Salvia* species (Labiatae) (Rodríguez-Hahn, Esquivel, Cárdenas & Ramamoorthy, 1992). A study is being made of the diterpenoid constituents of Salvia species indigenous to Hungary and in this communication we report the isolation of seven such compounds from *S. nutans* L., three of which appear to be novel.

2. Results and discussion

The acetone extract of *Salvia nutans* roots was fractionated by flash column chromatography using mixtures of *n*-heptane and increasing amounts of ethyl acetate. The fractions were screened by TLC and those with similar compositions were combined to give five fractions. These were further fractionated by preparative TLC to yield seven compounds (**1** to **7**), which were characterized, mainly from spectroscopic data. The ^{1}H and ^{13}C NMR spectra of all the isolated compounds showed features characteristic of royleanones, with resonances for the C-4 dimethyls, the angular Me-20, the isopropyl group and the ring C quininoid system.

Compound **1** was isolated as dark reddish-brown crystals. The IR spectrum exhibited carbonyl stretching vibrations at 1653 and 1673 cm^{-1}, reminiscent of an *ortho*-quinone moiety (Pouchert, 1981). The band at 3462 cm^{-1} suggested the presence of an hydroxyl group. In the COSY spectrum, cross peaks were shown between 7-C*H* (δ 4.40, 1 H, *m*) and the O*H* doublet at δ1.92 (1 H, $J=8.1$ Hz). The absorption for the single C-ring proton at C-14 was observed as a singlet at δ_H 7.06, with a corresponding δ_C at 134.9. These results (HMQC and HMBC) permitted the assignment of the two keto carbonyls to C-11 (δ_C 180.8) and C-12 (δ_C 181.5), respectively. Thus **1**

* Corresponding author. Tel.: +44-01705-843593; fax: +44-01705-843565.

PII: S0031-9422(99)00135-1

is 6-deoxysalviphlomone (7β-hydroxy-8,13-abietadiene,11,12-dione). This compound has been recorded once previously by Chang et al. for *Salvia miltiorrhiza* Bunge (Chang et al., 1990). Our data are in good agreement with theirs, but the ^{13}C NMR spectroscopic and mass spectrometric information, recorded here for the first time, permitted a more detailed characterization of the compound.

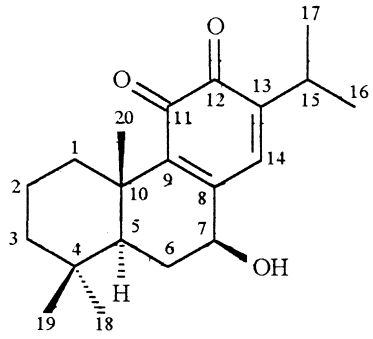
Compound **2** was obtained as yellow, plate-like crystals. On TLC examination, the compound displayed a yellow fluorescence under UV light (λ 366 nm), after spraying with concentrated sulphuric acid. The ^{1}H NMR spectrum of **2** was similar to that of 6,7-dehydroroyleanone (Matsumoto & Harada, 1979; Edwards, Feniak & Los, 1962). Two doublets were observed at δ 6.35 (1H, $J=6.8$ Hz) and δ 6.74 (1 H, $J=6.8$ Hz), which were assigned to H-6 and H-7. HMQC data showed the presence of two olefinic signals attributed to C-6 at δ 118.4 and C-7 at δ 133.3; these signals show cross peaks with the above protons. The ^{1}H NMR spectrum lacked the hydroxyl signal observed for C-12 in the spectrum of 6,7-dehydroroyleanone (Matsumoto & Harada, 1979; Edwards, Feniak & Los, 1962), but a sharp singlet was observed at δ 6.92, which indicated a single proton at C-12. COSY and HMBC data supported the identity of **2** as 12-deoxy-6,7-dehydroroyleanone (6,8,12-abietatriene-11,14-dione), which appears to be a new compound.

Compound **3** was isolated as brownish-yellow, plate-like crystals. When examined by TLC, the compound produced a bright yellow fluorescence under UV light (λ 366 nm), after spraying with concentrated sulphuric acid. All spectroscopic data suggested a 12-dehydroroyleanone-type compound. The IR spectrum displayed a strong signal at 3304 cm^{-1}, suggesting the presence of more than one hydroxyl function in the molecule. From the ^{13}C NMR spectra of **2** and **3**, comparison of the resonances of C-6 and C-7 showed a downfield shift in the value for C-6 of **3** from δ 118.4 in **2** to δ 144.9 in **3**, indicative of an hydroxyl group at C-6 of **3**. Moreover, in the ^{1}H NMR spectrum of **3**, no signal was recorded for H-6, and H-7 was observed as a singlet at δ 6.22 (1H). A singlet at δ 7.61 (1H), which showed no coupling to any of the carbon atoms in the HMQC spectrum, was assigned to the hydroxyl function at C-6; the rest of the ^{1}H NMR spectral data were consistent with those of **2**. ^{1}H–^{1}H COSY, HMQC and HMBC data were in good agreement with the proposed 12-deoxy-6-hydroxy-6,7-dehydro feature of **3**. Furthermore, the formula $C_{20}H_{26}O_3$ was deduced from high resolution mass spectrometric measurements, which is in accord with the proposed structure. On the basis of the available data, **3** was identified as 12-deoxy-6-hydroxy-6,7-dehydroroyleanone (6-hydroxy-6,8,12-abietriene-11,14-dione). This compound has not been reported previously.

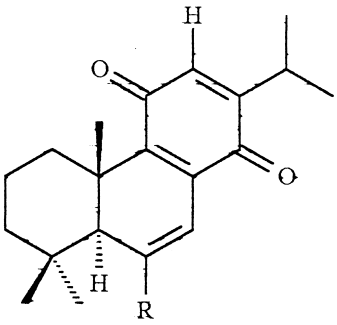
On the basis of melting points, spectroscopic and spectrometric characteristics, compounds **4**, **5** and **6** were identified as royleanone (Edwards, Feniak & Los, 1962), 7α-hydroxyroyleanone (Matsumoto & Harada, 1979) and 7α-acetoxyroyleanone (Matsumoto & Harada, 1979; Edwards, Feniak & Los, 1962), respectively.

Compound **7** was isolated as green needles, which on TLC examination produced a light green fluorescence under UV light (λ 366 nm), after spraying with concentrated sulphuric acid. All spectroscopic data suggested a royleanone-type compound with definite structural similarities to **2** and **3**. However, unlike these last two compounds, the IR spectrum exhibited absorption at 1739 cm^{-1}, indicative of an extra carbonyl function; this was substantiated by the resonance in the ^{13}C NMR spectrum at δ 203.2. The ^{1}H NMR spectrum of **7** showed the presence of two methoxyl singlets at δ 3.19 (3H) and δ 3.37 (3H). As rings A and C had the usual constitution of 12-deoxyroyleanones, such as **2** and **3**, the extra carbonyl and both the methoxyl groups have to be positioned at C-6 and C-7. Both ^{13}C and HMBC spectra revealed that C-6 and C-7 were quaternary carbons with signals at δ 203.2 and 96.9. Positioning the extra carbonyl group at C-6 is favoured by the IR absorption at 1739 cm^{-1}. Location of this group on the adjacent C-7 would make it part of the conjugated quinone system, which would result in a lower wave number. Furthermore, if an axial methoxyl group were located at C-6, severe steric hindrance would be expected between this and the C-20 and C-4 axial methyl groups. In the ^{13}C NMR spectrum of 7-oxoroyleanone, the 7-keto group has a marked upfield effect on the Me-20 resonance (δ 17.9) in comparison with the value for compounds such as **4** (δ 20.0). In **7** such a shift is not observed (δ 21.4). All these data prove that in **7** the extra keto function is located at C-6 and the two methoxyl groups at C-7. Thus **7** is 12-deoxy-7,7-dimethoxy-6-ketoroyleanone (7,7-dimethoxy-8,12-abietadiene-6,11,14-trione), which is a novel compound. ^{1}H–^{1}H COSY, HMQC and HMBC data were in good agreement with this assignment. High resolution mass spectrometric measurements showed a molecular formula of $C_{22}H_{30}O_5$, which is consistent with the proposed structure. Compound **7** could have been formed from the equivalent 7-keto compound by reaction with methanol.

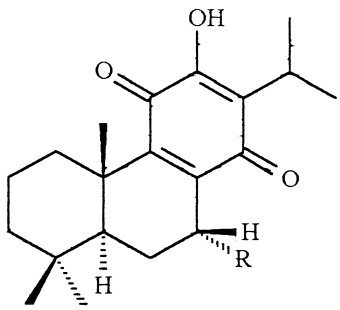
The presence of royleanones, 12-deoxyroyleanones and an *ortho*-quinone abietanoid diterpenoid in the same species provides a unique diterpenoid composition for *S. nutans*. From a phylogenetic viewpoint this species may be considered as a link between those species which contain royleanone and those with tanshinone (nor-abietane). Thus *S. nutans* deserves a special status in the section *Plethiosphace* Benth., and, through it, within the subgenus *Sclarea*.



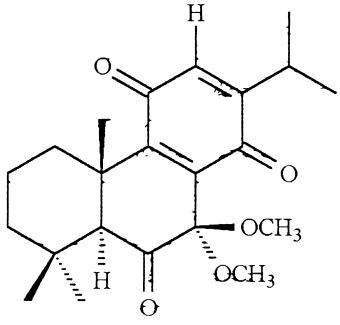
1



R = H = **2**
R = OH = **3**



R = H = **4**
R = OH = **5**
R = O.CO.CH_3 = **6**



7

3. Experimental

3.1. General

Mps: uncorrected. ^1H and ^{13}C NMR spectra, in $CDCl_3$, were obtained at either 400 or 270.06 and either 100 or 67.8 MHz, respectively, with TMS as internal standard. Assignments were based on ^1H–^1H COSY, heteronuclear multiquantum coupling spectroscopy (HMQC) and heteronuclear multiple bound coupling spectroscopy (HMBC) techniques.

3.2. Plant material

Salvia nutans L. was grown in the experimental fields of the Institute of Ecology and Botany of the Hungarian Academy of Sciences at Vácrátót. After harvesting, the roots were dried at room temperature and ground using an electric mill fitted with a 2 mm aperture screen. A voucher sample (SO25) of the plant is maintained in the Department of Pharmacognosy, Albert Szent-Györgyi Medical University.

3.3. Extraction and isolation of diterpenoids

Powdered *S. nutans* roots (500 g) were soaked in Me_2CO (5 l) for 5 days before the mixture was filtered. The plant material was pressed in an oil hydraulic press and the expressed liquid added to the filtrate. The combined solutions were evaporated to dryness under reduced pressure at 40° to yield a yellow-brown residue (17 g). This was subjected to flash CC (55 × 5 cm) using silica gel 60 (450 g; 60–200 mesh; Merck). Elution with mixtures of *n*-heptane and increasing amounts of EtOAc furnished 50 frs, each of 100 ml. After screening by TLC, frs with similar composition were combined to yield 5 frs (1–5). From combined fr 1, after prep. TLC on 500 μm layers of silica gel 60F (Merck No. 5729 and 5715), activated by drying at 110° for 60 min, and using cyclohexane–EtOAc (95:5) and C_6H_6–Me_2CO (94:6) as development solvents, compounds **1** and **2** were obtained. Prep. TLC using cyclohexane–EtOAc (97:3), C_6H_6–EtOAc (99.8:0.2) and C_6H_6–EtOAC (99.7:0.3) gave three further diterpenoids (**3**, **4** and **5**). From fr 2, prep. TLC using C_6H_6–EtOAc (99.7:0.3) and cyclohexane–EtOAc (95:5)

yielded **6** and **7**. The developed chromatograms were examined under UV light (λ 254 and 366 nm), followed by spraying the edges with concentrated H_2SO_4 to locate the diterpenoids. The separated bands were scraped from the plates and the compounds extracted with $CHCl_3$, EtOAc, Me_2CO or MeOH. The isolated compounds were recrystallized from $CHCl_3$.

*3.4. 6-Deoxysalviphlomone (7β-hydroxy-8,13-abietadiene,11,12-dione) (**1**)*

$C_{20}H_{28}O_3$. Reddish-brown crystals, mp 156–158°; UV $\lambda_{max}^{CHCl_3}$ nm: 242, 283, 421; λ_{max}^{MeOH} nm: 239 sh, 295, 424. ν_{max}^{KBr} cm^{-1}: 3462, 2958, 2922, 2863, 1673, 1653, 1559, 1457, 1393, 1325, 1256, 1077. EI MS (probe) 70 eV *m/z* (rel. int.): 316 $[M]^+$ (100), 301 (95), 283 (17), 273 (5), 259 (31), 241 (10), 231 (55), 219 (59), 205 (17), 191 (10). ^1H NMR (Chang et al., 1990). ^{13}C NMR ($CDCl_3$) δ ppm: 35.7 (C-1), 18.6 (C-2), 40.9 (C-3), 32.9 (C-4), 49.3 (C-5), 28.7 (C-6), 71.3 (C-7), 145.3 (C-8, -9, or -13), 146.8 (C-9, -8 or -13), 39.0 (C-10), 180.8 (C-11 or -12), 181.5 (C-12 or -11), 147.6 (C-13, C-8 or C-9), 134.9 (C-14), 27.2 (C-15), 21.4 (C-16), 21.4 (C-17), 21.7 (C-18), 33.2 (C-19), 20.1 (C-20).

*3.5. 12-Deoxy-6,7-dehydroroyleanone (6,8,12-abietatriene-11,14-dione) (**2**)*

$C_{20}H_{26}O_2$. Yellow, plate-like crystals, mp 150–152°; UV $\lambda_{max}^{CHCl_3}$ nm: 256, 295, 390; λ_{max}^{MeOH} nm: 265, 298, 395. ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.17 (3H, *s*, Me-16), 1.18 (6H, *s*, Me-17 and -20), 1.24 (1H, *m*, H-2), 1.26 (6H, *s*, Me-18 and -19), 1.44 (1H, *m*, H-3), 1.53 (1H, *m*, H-1), 1.64 (1H, *m*, H-2′), 1.65 (1H, *m*, H-3′), 2.03 (1H, *m*, H-5), 3.17 (1H, *sept*, H-15), 3.30 (1H, *m*, H-1′), 6.35 (1H, *d*, $J=6.8$ Hz, H-6), 6.47 (1H, *d*, $J=6.8$ Hz, H-7), 6.92 (1H, *s*, H-12).

*3.6. 12-Deoxy-6-hydroxy-6,7-dehydroroyleanone (6-hydroxy-6,8,12-abietatriene-11,14-dione) (**3**)*

$C_{20}H_{26}O_3$. Brownish-yellow, plate-like crystals, mp 125–127°; UV $\lambda_{max}^{CHCl_3}$ nm: 309 sh, 324, 337, 407; λ_{max}^{MeOH} nm: 304 sh, 321, 333, 398. ν_{max}^{KBr} cm^{-1}: 3304, 2963, 2926, 1665, 1615, 1454, 1359, 1275, 1178, 993, 910, 645. EI MS (probe) 70 eV *m/z* (rel. int.): 314.1921 $[M]^+$ (100), 299 (16), 286 (67), 271 (72), 253 (16), 245 (44), 231 (34), 217 (19), 203 (7), 187 (11). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.12 (3H, *s*, Me-20), 1.17 (3H, *s*, Me-16), 1.17 (1H, *m*, H-3), 1.18 (3H, *s*, Me-17), 1.27 (6H, *s*, Me-18 and -19), 1.41 (1H, *m*, H-3′), 1.60 (1H, *m*, H-2), 1.71 (1H, *m*, H-1), 1.73 (1H, *m*, H-2′), 2.60 (1H, *br s*, H-5), 2.93 (1H, *m*, H-1′), 3.08 (1H, *sept*, H-15), 6.22 (1H, *s*, H-7), 6.89 (1H, *s*, H-12), 7.61 (1H, *s*, O*H*-6). ^{13}C NMR ($CDCl_3$) δ ppm: 36.9 (C-1), 18.5 (C-2), 42.5 (C-3), 42.8 (C-4), 62.9 (C-5), 144.9 (C-6), 133.9 (C-7), 149.8 (C-8), 125.5 (C-9), 32.7 (C-10), 200.9 (C-11), 136.1 (C-12), 145.2 (C-13), 181.6 (C-14), 27.1 (C-15), 21.1 (C-16 or -17), 21.6 (C-17 or -16), 21.8 (C-18 or -20), 33.2 (C-19), 22.0 (C-20 or -18).

*3.7. 12-Deoxy-7,7-dimethoxy-6-ketoroyleanone (7,7-dimethoxy-8,12-abietadiene-6,11,14-trione) (**7**)*

$C_{22}H_{30}O_5$. Green needles, mp 145–147°; UV $\lambda_{max}^{CHCl_3}$ nm: 242 sh, 274 sh, 413, 592; λ_{max}^{MeOH} nm: 245 sh, 263 sh, 407, 620. ν_{max}^{KBr} cm^{-1}: 2964, 2871, 1739, 1663, 1464, 1387, 1143, 1080, 941, 904. EI MS (probe) 70 eV *m/z* (rel. int.): 374.2196 $[M]^+$ (10), 395 (12), 342 (100), 337 (85), 315 (16), 299 (36), 286 (49), 273 (37). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.99 (3H, *s*, Me-20), 1.12 (1H, *m*, H-3), 1.13 (3H, *s*, Me-16), 1.15 (3H, *s*, Me-17), 1.26 (1H, *m*, H-1), 1.28 (3H, *s*, Me-18), 1.36 (3H, *s*, Me-19), 1.36 (1H, *m*, H-3′), 1.55 (1H, *m*, H-2), 1.67 (1H, *m*, H-2′), 2.68 (1H, *br d*, $J=13.4$ Hz, H-1′), 2.86 (1H, *s*, H-5), 2.94 (1H, *sept*, $J=7$ Hz, H-15), 3.19 (3H, *s*, OMe-6), 3.37 (3H, *s*, OMe-7), 6.93 (1H, *s*, H-12). ^{13}C NMR ($CDCl_3$) δ ppm: 36.0 (C-1), 18.3 (C-2), 41.6 (C-3), 32.4 (C-4), 59.8 (C-5), 203.2 (C-6), 96.9 (C-7), 147.6 (C-8 or -9), 143.6 (C-9 or -8), 44.1 (C-10), 180.3 (C-11), 132.4 (C-12), 148.9 (C-13), 181.2 (C-14), 27.5 (C-15), 21.1 (C-16), 21.4 (C-17), 21.5 (C-18), 32.2 (C-19), 21.4 (C-20), 51.5 (C-21 or -22), 52.5 (C-22 or -21).

Acknowledgements

We thank Dr V.V. Miklóssy of the Institute of Ecology and Botany of the Hungarian Academy of Sciences for growing the plant material at Vácrátót. We are grateful to Ms M. Csepregi for her technical assistance.

References

Chang, H. M., Cheng, K. P., Choang, T. F., Chow, H. F., Chui, K. Y., Hon, P. M., Tan, F. V. L., Yang, Y., & Zhong, Z. P. (1990). Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* Bunee (Danshen). *Journal of Organic Chemistry*, *55*, 3537.

Edwards, O. E., Feniak, G., & Los, M. (1962). Diterpenoid quinones if *Inula royleana*. *Canadian Journal of Chemistry*, *40*, 1540.

Matsumoto, T., & Harada, S. (1979). The total synthesis of (+)-taxoquinone, (−)-7α-acetoxyroyleanone, (−)-dehydroroyleanone, (−)-horminone, (−)-7-oxoroyleanone, and (+)-inuroyleanol. *Bulletin of the Chemical Society of Japan*, *52*, 1459.

Pouchert, C. J. (1981). *The aldrich library of infrared spectra* (3rd ed.). Milwaukee: Aldrich Chemical Company Inc.

Rodríguez-Hahn, L., Esquivel, B., Cárdenas, J., & Ramamoorthy, T. P. (1992). The distribution of diterpenoids in *Salvia*. In R. M. Harley, & T. Reynolds, *Advances in Labiate science* (p. 335). Kew: Royal Botanic Gardens.