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Diterpenoids from *Salvia glutinosa*, *S. austriaca*, *S. tomentosa* and *S. verticillata* roots

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Abstract

From the acetone extract of the dry roots of *Salvia glutinosa*, 15 diterpenoids were isolated and characterised. Of these, 12-deoxydanshenxinkun B and dihydroisotanshinone II are novel compounds and isotanshinone II is a new natural product. The acetone extracts of *S. austriaca*, *S. tomentosa* and *S. verticillata* all yielded 7α-acetoxyroyleanone and 7α-hydroxyroyleanone. In addition, royleanone and 6,7-dehydroxyroyleanone were isolated from *S. tomentosa*. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia austriaca*; *S. glutinosa*; *S. tomentosa*; *S. verticillata*; Labiatae; Roots; Diterpenoids; 12-Deoxydanshenxinkun B; Isotanshinone II; Dihydroisotanshinone II

1. Introduction

The genus *Salvia* (Labiatae) is a rich source of diterpenoids (Rodríguez-Hahn, Esquivel, Cárdenas & Ramamoorthy, 1992). *Salvia* species indigenous to Hungary are being investigated and diterpenoids isolated from *S. pratensis* L. (Nagy et al., 1998c), *S. nutans* L. (Nagy et al., 1999) and *S. glutinosa* L. (Nagy et al., 1998a, 1998b) have been reported. In the present communication we record the identification of such compounds from *S austriaca*, *S. tomentosa* and *S. verticillata*, none of which have been investigated previously for diterpenoids, and further compounds from *S. glutinosa*.

2. Results and discussion

The acetone extract of *Salvia glutinosa* was fractionated by flash column chromatography using mixtures of *n*-hexane and increasing amounts of ethyl acetate. The fractions were screened by TLC and those with similar composition were combined to give a total of nine fractions. These were subjected to gel filtration to eliminate high molecular weight contaminants. Further separation of the diterpenoids was achieved by preparative TLC, resulting in the isolation of 15 compounds. These were characterised mainly from spectroscopic data.

From fraction 1, five diterpenoids were isolated (**1**–**5**). Compound **1** was obtained as red needles. The IR spectrum of **1** exhibited an intense band at 1657 cm^{-1}, indicative of a quinone. The ^{1}H-NMR spectrum showed the characteristics of an abietane with a 12,14-*para*-quinone moiety. Six aromatic protons were recorded, five of which indicated that rings A and B were fully aromatic, with absorption for Me-18 at δ

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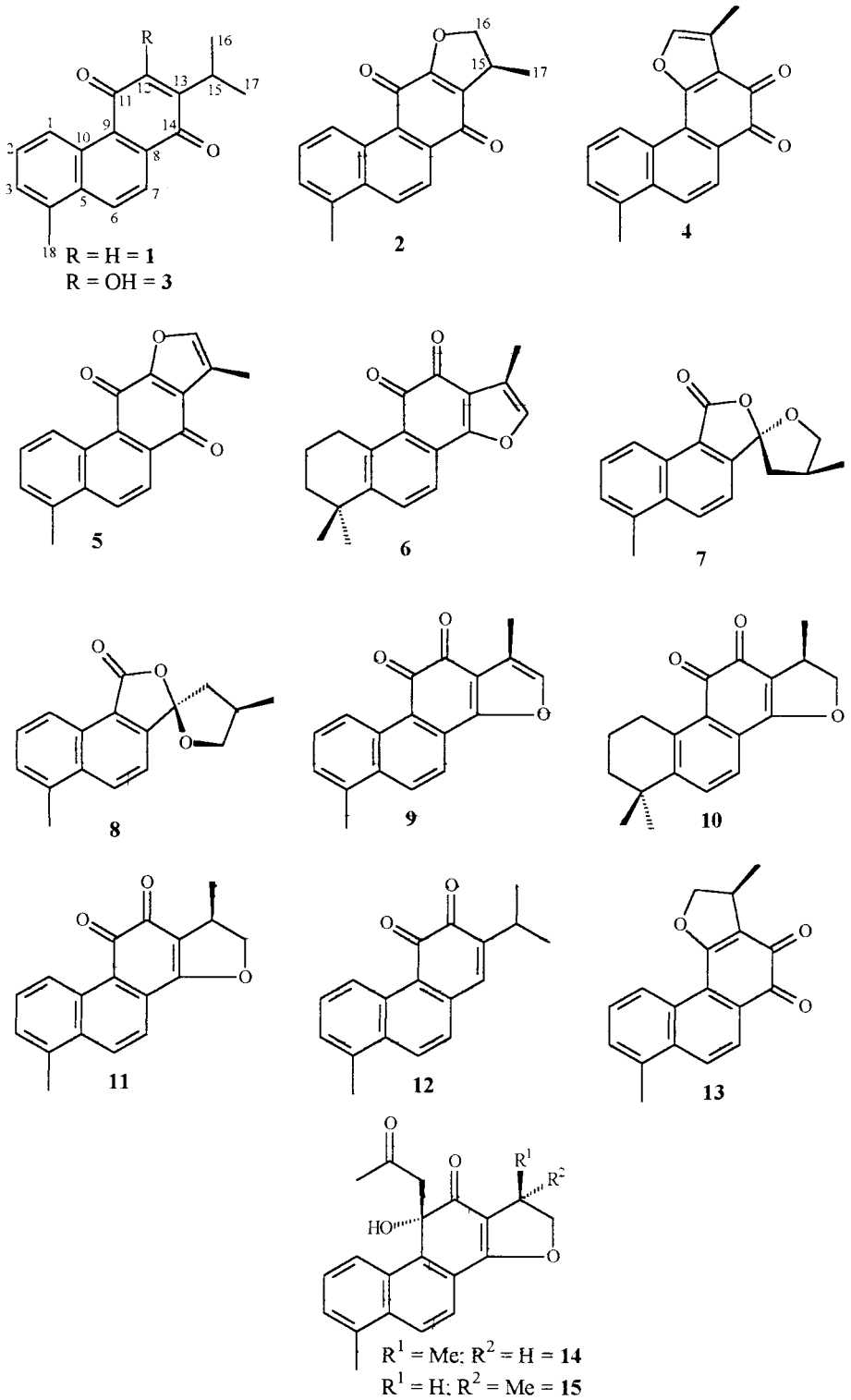

2.70. Signals for H-6 and H-7 were recorded at δ 7.38 (d, J = 8.5 Hz) and δ 8.29 (d, J = 8.5 Hz), respectively. The ^{1}H-NMR spectroscopic details of **1** were very similar to those of danshenxinkun B (**3**) (Fang, 1976), except that the H-12 (0 H) signal exhibited as a very broad singlet at δ 7.81 in **3** was replaced by a sharp aromatic proton singlet at δ 7.19. These data suggested that **1** was a 12-deoxy derivative of danshenxinkun B. Mass spectrometric measurement indicated a molecular formula $C_{18}H_{16}O_2$, which is in accordance

with the proposed structure. Thus, **1** is 12-deoxydanshenxinkun B (18,20-dinor-1,3,5(10),6,8,12-abietahexaene-11,14-dione), which we believe to be a new compound.

The ^{1}H-NMR spectroscopic and mass spectrometric characteristics of **2** and **3** were consistent with those reported for dihydroisotanshinone I (Kong, 1984) and danshenxinkun B (Fang, 1976), respectively, and these identities were attributed to them. Dihydroisotanshinone I has been recorded previously for *S. glutinosa* (Nagy et al., 1998b).

Compound **4** was isolated as orange crystals. Its ^{1}H-NMR spectrum was very similar to that of tanshinone I (**9**) (Kakisawa, Hayashi & Yamasaki, 1969), showing a total of six protons, five of which were indicative of unsaturated A and B rings, with absorption for Me-18 at δ 2.74. However, the signals for H-6 and H-7 in the spectrum of **4** appeared as an AB quartet (δ 8.36, 8.38, J = 8.8 Hz) instead of doublets at δ 8.34 (H-6) and δ 7.85 (H-7) in that of **9**. The mass spectrum of **4** revealed a molecular formula of $C_{18}H_{12}O_3$ and the fragmentation pattern obtained was very similar to that of tanshinone I (King & Read, 1961). The above data suggested that **4** was an isomer of tanshinone I. Kakisawa et al. (1969) reported the isolation of isotanshinone I, which had spectroscopic characteristics similar to those of tanshinone I. However, the spectral data (UV, IR, NMR and MS) of **4** were not consistent with those of isotanshinone I, indicating that **4** was another isomer of tanshinone I. Based on the available evidence, the only possible difference in the structure of **4** must be that ring D is fused to C-11 and C-12. Thus, **4** is 11,16-oxy-18,20-dinor-1,3,5(10),6,8,11,15-abietaheptaene-13,14-dione. This was produced by King and Read (1961) in an attempt to synthesise tanshinone I. However, this is the first record of **4** as a natural product and we have given it the trivial name isotanshinone II.

Insufficient (0.8 mg) **5** was isolated for a full spectroscopic characterisation of it to be made. However, UV spectroscopic, mass spectrometric and melting point data matched those of isotanshinone I (Kakisawa et al., 1969). The mass spectrum of **5** was reminiscent of tanshinone I-type diterpenoids, but the molecular ion was more abundant than that in the spectrum of tanshinone I. This is a reflection of the greater stability of the *para*-quinoid structure of **5** in comparison to *ortho*-isomers on electron impact. Moreover, the relative intensities of the ions at m/z 261 and 248 differed in the mass spectra of **5** and tanshinone I. Hayashi, Inouye, Ohashi and Kakisawa (1970) revealed that the predominant fission of a methyl group in the case of isotanshinone I can be attributed to ring A and not to the furan ring portion. On the basis of the available data, **5** was identified as isotanshinone I.

From fraction 2, four diterpenoids were isolated (**6**–**9**). From UV, IR and NMR spectroscopic and mass spectrometric data, these compounds were identified as tanshinone IIA (**6**), danshenspiroketallactone (**7**), *epi*-danshenspiroketallactone (**8**) and tanshinone I (**9**). Melting points of **6** and **9** helped to confirm their identities. Both **6** and **9** have been reported previously as constituents of *S. glutinosa* (Nagy et al., 1998b). Danshenspiroketallactone (**7**) and its epimer (**8**) have been isolated previously from *S. miltiorrhiza* (Kong, Liu, Teng & Rao, 1985; Luo, Chen, Lee & Snyder, 1988). However, Luo et al. (1988) concluded that **7** was formed either partly or entirely from **8** during silica gel chromatographic separations. Like them, we found that even after chromatographic separation, both **7** and **8** were contaminated with the other epimer.

No diterpenoid was isolated from fraction 3, but fraction 4 yielded three (**10**–**12**), two of which, cryptotanshinone (**10**) and 15,16-dihydrotanshinone I (**11**), have been reported previously for *S. glutinosa* (Nagy et al., 1998b). From melting point, mass spectrometric and UV, IR and NMR spectroscopic data, **12** was identified as R0-09-0680 {18,20-dinor-1,3,5(10),6,8,13-abietahexaene-11,12-dione}; this has been isolated earlier from *S. miltiorrhiza* (Onitsuka, Fujiu, Shiuma & Maruyama, 1983).

From combined fractions 5 and 6 three diterpenoids were obtained (**13**–**15**). Compound **13** was isolated as red crystals. Its IR spectrum exhibited carbonyl absorptions at 1630 and 1713 cm^{-1}. The ^{1}H-NMR spectrum showed great similarity to that of **2**, exhibiting five proton signals characteristic of fully unsaturated A and B rings, with an absorption for Me-18 at δ 2.75. The aliphatic region of the spectrum was consistent with those of both **2** and **11**. High resolution mass spectrometric measurement revealed a molecular formula for **13** of $C_{18}H_{14}O_3$ ($[M]^+$, m/z 278.0950; calculated for $C_{18}H_{14}O_3$ 278.0942); the fragmentation pattern was very similar to that of **2**. All these data strongly suggested that **13** was an isomer of both **2** and **11** and that ring D of **13** was fused to C-11 and C-12. Thus, we propose that **13** is 11,16-oxy-18,20-dinor-1,3,5(10),6,8,11-abietahexaene-13,14-dione, which appears to be a novel compound and which we have named dihydroisotanshinone II. Insufficient material was isolated to characterise the compound further.

Compounds **14** and **15** were danshenol-A and 15-*epi*-danshenol-A, which have been reported previously for *S. glutinosa* (Nagy et al., 1998a).

Acetone extracts of *S. austriaca*, *S. tomentosa* and *S. verticillata* were processed in a manner similar to that used for *S. glutinosa*. From all three species, 7α-acetoxyroyleanone and 7α-hydroxyroyleanone were isolated. Additionally, from *S. tomentosa*, royleanone and 6,7-dehydroroyleanone were obtained. Characterisation in all cases, as before, was based mainly on melting

point, spectroscopic (UV, IR and NMR) and mass spectrometric data. These compounds are found widely in the genus (Rodríguez-Hahn et al., 1992).

3. Experimental

Mps: uncorrected. ^{1}H and ^{13}C-NMR spectra, in $CDCl_3$, were obtained at either 400 or 270 MHz and either 100 or 67.8 MHz, respectively, with TMS as int. standard. Assignments were based on ^{1}H-^{1}H COSY, HMQC and HMBC techniques. Prep. TLC was conducted on 500 μm layers of silica gel 60F (Merck 5729 and 5715), activated at 110°C for 60 min. The developed chromatograms were viewed under UV light (λ 254 and 366 nm) to locate the bands and, if necessary, the edges of the chromatograms were sprayed with conc. H_2SO_4 and heated at 110°C for 5 min. The required bands were scraped from the plates and the compounds eluted with one of either $CHCl_3$, EtOAc, Me_2CO or MeOH.

3.1. Plant material

Salvia glutinosa L. (voucher sample S022) was collected from Galyatetõ, Hungary, and both *S. austriaca* Jacq. (voucher sample S023) and *S. verticillata* L. (voucher sample S021) from near Szeged, Hungary. *S. tomentosa* Mill. (voucher sample S024) was grown in Sofia, Bulgaria, by Dr. E. Genova. After harvesting, the roots were dried at room temp. and ground using an electric mill fitted with a 2 mm aperture screen. All the voucher samples are deposited in the Herbarium of the Department of Pharmacognosy, Albert Szent-Györgyi Medical University at Szeged.

3.2. Extraction and isolation of diterpenoids

Powdered *S. glutinosa* roots (930 g) were macerated with Me_2CO (12 l) for 5 days before the mixt was filtered. The plant material was pressed in an oil hydraulic press and the expressed liquid added to the filtrate. The combined solns were concd to dryness under reduced pressure at 40°C to yield a brown resinous residue (24.2 g). This was subjected to flash CC (60 × 8 cm) using silica gel 60 (650 g; 60–200 mesh; Merck). The column was eluted with mixts of *n*-hexane and increasing amounts of EtOAc and 50 ml frs were collected. After screening by TLC, frs with similar composition were mixed together to yield nine combined frs. Each of these was subjected to gel filtration on Sephadex LH-20, using Me_2CO as the eluting solvent, to eliminate high MW contaminants.

The diterpenoids were isolated from each fr by prep. TLC. The use of petrol (bp 40–60°C) EtOAc–Me_2CO (94:3:3) resulted in the separation of **1** (2.5 mg), **2** (3.5 mg) and a mixt of **3**, **4** and **5** from fr 1. Toluene–EtOAc (95:5) was used to isolate **3** (2.0 mg) from **4** (1.5 mg) and **5** (0.8 mg). Petrol (bp 40–60°C)-EtOAc–Me_2CO (94:3:3) divided fr 2 into **6** (2.0 mg), **9** (3.5 mg) and a mixt of **7** and **8**. Separation of these last two was achieved using toluene–EtOAc (93:7) to yield **7** (1.5 mg amorphous solid) and **8** (1.0 mg amorphous solid). No diterpenoid was isolated from fr 3. For fr 4, the use of toluene–EtOAc (93:7) led to the isolation of a mixt of **10**, **11** and **12**. CH_2Cl_2-EtOAc (99:1) separated **10** (11.0 mg) from a mixt of **11** and **12**. The last two, after recrystallisation from EtOAc, were isolated using CH_2Cl_2-EtOAc (90:1.5) to yield **11** (6.0 mg) and **12** (53.0 mg). Frs 5 and 6 were combined. A mixt of **13**, **14** and **15** resulted from the use of CH_2Cl_2-EtOAc (80:20). Further purification using $CHCl_3$-MeOH (95:5) led to the isolation of **13** (8.5 mg) and a mixt of **14** and **15**. After recrystallisation from EtOAc, these last two were separated (**14** 6.5 mg; **15** 7.0 mg) using toluene–EtOAc (5:4).

Powdered roots of *S. austriaca* (900 g), *S. tomentosa* (1200 g) and *S. verticillata* (960 g) were extracted in the same way as *S. glutinosa* to yield 15.0, 17.6 and 32.6 g residue, respectively. These were fractionated separately by flash column chromatography, as described earlier, but using mixts of petrol (bp 40–60°C) and increasing amounts of EtOAc for the *S. austriaca* extract, *n*-heptane and EtOAc mixts for *S. tomentosa*, and petrol (bp 40–60°C), benzene and EtOAc mixts for *S. verticillata*. The frs obtained were treated in the same way as described above. Prep. TLC of *S. austriaca* frs using benzene-EtOAc (93:7) and cyclohexane-EtOAc (95:5) yielded 7α-acetoxyroyleanone (**16**) and 7α-hydroxyroyleanone (**17**). For *S. tomentosa*, the use of toluene-EtOAc (99.8:0.2) and benzene-EtOAc (99:1) resulted in the isolation of **16**, **17**, royleanone and 6,7-dehydroroyleanone; and for *S. verticillata*, benzene–Me_2CO (85:15) and benzene-Me_2CO (87:13) yielded **16** and **17**.

The isolated compounds were recrystallised from either $CHCl_3$, $CHCl_3$-*n*-hexane mixts or MeOH.

3.3. 12-Deoxydanshenxinkun B [18,20-dinor-1,3,5(10),6,8,12-abietahexaene-11,14-dione] (*1*)

$C_{18}H_{16}O_2$. Red needles ($CHCl_3$-*n*-hexane), m.p. 187–197°C. UV $\lambda_{max}^{CHCl_3}$ nm: 289, 314 sh, 423; λ_{max}^{MeOH} nm: 236, 288, 310 sh, 421. IR ν_{max}^{KBr} cm^{-1}: 3440, 2921, 2851, 1657, 1461, 1361, 1217, 1086. CIMS *m*/*z*: 265 $(M+H)^+$. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 1.22 (6H, d, J = 7 Hz; Me-16 and -17), 2.70 (3H, s; Me-18), 3.08 (1H, sept; H-15), 7.19 (1H, s; H-12), 7.36 (1H, d, J = 7 Hz; H-3), 7.38 (1H, d, J = 8.5 Hz; H-6), 7.57 (1H, dd, J = 8.8, 7.0 Hz; H-2), 8.29 (1H, d, J = 8.5 Hz; 7-H), 9.25 (1H, d, J = 8.8 Hz; H-1).

3.4. Isotanshinone II [11,16-oxy-18,20-dinor-1,3,5(10),6,8,11,15-abietaheptaene-13,14-dione] (**4**)

$C_{18}H_{12}O_3$. Orange crystals ($CHCl_3$), m.p. 291–293°C. UV $\lambda_{max}^{CHCl_3}$ nm: 290, 323 sh, 362, 467; λ_{max}^{MeOH} nm: 230, 287, 326 sh, 358, 462. IR ν_{max}^{KBr} cm^{-1}: 2923, 1662, 1638, 1589, 1510, 1470, 1379, 1252, 1172. CIMS *m/z*: 276 $(M+H)^+$. EI MS (probe) 70 eV *m/z* (rel. int.): 275 (100), 260 (40), 246 (14), 232 (4), 218 (7), 204 (6), 189 (6), 173 (3). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 2.45 (3H, s, Me-17), 2.74 (3H, s; Me-18), 6.85 (1H, br s; H-16), 7.43 (1H, d; J = 6.8 Hz; H-3), 7.58 (1H, dd, J = 8.8, 6.8 Hz; H-2), 8.36 (1H, d, J = 8.8 Hz; H-6 or -7), 8.38 (1H, d, J = 8.8 Hz; H-6 or -7), 9.70 (1H, d, J = 8.8 Hz; H-1).

3.5. Dihydroisotanshinone II [11,16-oxy-18,20-dinor-1,3,5(10),6,8,11-abietahexaene-13,14-dione] (**13**)

$C_{18}H_{14}O_3$. Red crystals (MeOH), m.p. 247–249°C. UV $\lambda_{max}^{CHCl_3}$ nm: 293, 337, 394; λ_{max}^{MeOH} nm: 245 sh, 290, 337, 386. IR ν_{max}^{KBr} cm^{-1}: 2924, 2853, 1713, 1630, 1591, 1470, 1359, 1199, 1034. CIMS *m/z*: 279 $(M+H)^+$. EI MS (probe) 70 eV *m/z* (rel. int.): 278 (100), 263 (60), 250 (41), 235 (52), 222 (16), 207 (32), 179 (30). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 1.31 (3H, d, J = 7.7 Hz; Me-17), 2.75 (3H, s; Me-18), 3.50 (1H, m; H-15), 3.89 (1H, dd, J = 10.6, 7.0 Hz; H-16), 4.00 (1H, dd, J = 10.6, 7.7 Hz; H-16′), 7.47 (1H, d, J = 7.0 Hz; H-3), 7.63 (1H, dd, J = 8.8, 7.0 Hz; H-2), 8.27 (1H, d, J = 8.8 Hz; H-7), 8.42 (1H, d, J = 8.8 Hz; H-6), 9.43 (1H, d, J = 8.8 Hz; H-1).

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