DITERPENOIDS FROM *Scutellaria galericulata*

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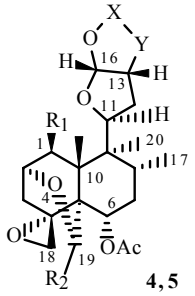
Neo-clerodane diterpenoids have attracted interest for their varied biological activities. In continuation of our systematic studies on *Scutellaria* plants [1–3], we have now investigated *S. galericulata* L.

The acetone extract of the aerial parts of *S. galericulata* was subjected to column chromatography to yield five compounds: **1**–**5**. The IR spectrum of **3** showed signals for the hydroxyl group (3444 cm^{-1}), oxirane (3050 cm^{-1}), and ester groups (1733, 1712, 1651, 1276, 1256 cm^{-1}), one of which is for a tigloyloxy moiety (1651 cm^{-1}). The ^{1}H NMR spectrum showed signals for an acetate δ 1.79 (3H, s) and tigloyloxy group δ_H 7.10 (qq, H-3′; 1.81 m, Me-4′; 1.89 m, Me-5′), as well as characteristic signals for the neo-clerodane structure (Me-17 at δ 0.84 d, $J_{17,8\beta}$ = 6.1 Hz, and Me-20 at δ 0.93 s) with a 4α,18-oxirane ring ($\delta_{HA\text{-}18}$ 2.44 d and $\delta_{HB\text{-}18}$ 3.00 d, J_{gem} = 4.4 Hz) and a 19,2α-hemiacetal function ($\delta_{H\text{-}19\alpha}$ 6.78 s, $\delta_{H\text{-}2\beta}$ 4.23 m). The ^{1}H NMR spectrum also showed signals for a 16,15-γ-lactone ($\delta_{HA\text{-}15}$ 3.94 ddd, $\delta_{HB\text{-}15}$ 4.09 td, J_{gem} = 8.4 Hz, $J_{15A,14A}$ = 7.8 Hz, $J_{15A,14BA}$ = 4.7 Hz, $J_{15B,14A}$ = 7.8 Hz, $J_{15B,14B}$ =2.3 Hz). In addition to these groups, **3** possesses two esterified equatorial alcohols at C-6α ($\delta_{H\text{-}6\beta}$ 4.64 d, $J_{6\beta,7\alpha}$ = 10.9 Hz) and C-7β ($\delta_{H\text{-}7\alpha}$ 5.19 dd, $J_{7\alpha,6\beta}$ = 10.9 Hz). On the basis of all the physical and spectroscopic data, compared with those described in the literature, compound **3** was established to be scutegalin D (16*R* and 16*S* epimers), because the ^{1}H NMR spectrum also showed a series of double signals for the H-19α (δ 6.78 s and 6.79 s) and CH_3-17 group (δ 0.84 d and 0.85 d).

Moreover, the signals at δ 5.32 (0.5H, dd, J_1 = 6.4, J_2 = 2.7 Hz) and 5.15 (0.5H, t, J = 2.7 Hz) were assigned to the two C-16 epimeric forms of the 16,15-hemiacetal group [4].

Compounds **1**, **2**, **4**, and **5** were neo-clerodanes with very similar structures. Their spectroscopic data showed the presence of a decalin ring system bearing one spiroepoxide substituent at C-4 (of C-1–C-10 fragment) and a furofuran system in the C-11–C-16 fragment (δ 4.08 dd, H-11α; 2.83 m, H-13β; 5.63 d, H-16β). The furofuran substructure was hexahydro in **1**, **2**, and **5** and tetradydro in **4** (3090, 1615 cm^{-1}; and at δ 4.79 t, H-14; 6.43 t, H-15, $J_{14,15}$ = 2.5 Hz, vinyl ether).



4, 5

4: R_1 = H, R_2 = OH, X-Y = CH=CH
5: R_1 = OH, R_2 = OTg, X-Y = CH_2-CH_2

The structures of compounds **1**, **2**, **4,** and **5** were established by comparing their physical (mp, R_f, $[\alpha]_D$) and spectral (IR, ^{1}H NMR, ^{13}C NMR) data with authentic samples such as 14,15-dihydrojodrellin T (**1**), scutegalin A (**2**), scutalbin A (**4**), and ajugapyrin A (**5**).

Compounds **1–3** have been isolated from *S. galericulata* growing in Spain [4, 5]. Scutalbin A, isolated previously from *S. albida* [6], was found for the first time in this species. Ajugapyrin A, isolated previously from Ajuga pyramidalis [7], was established for the first time in this plant and in the genus *Scutellaria*. The presence of **5** in the plants *Ajuga pyramidalis* and *Scutellaria galericulata* shows the close relation between the two genus.

The ^{1}H NMR spectrum of **5** run at 250 MHz was identical with that previously reported by Boneva et al. [7]. The additionally measured 600 MHz spectrum allowed corrections for some of the signal assignments and some of the J constants. Boneva et al. assigned δ_H 1.81 m to Me-5′ and 1.87 m to Me-4′. These signals in the 600 MHz spectrum are δ_H 1.81 (dq, $J_{3',4'}$ = 7.1 Hz, $J_{4',5'}$ = 1.1 Hz, 3H) and δ_H 1.87 qui (3H): this quintet is a consequence of the heavily overlapped doublet of quartets. Moreover, the signal of H-3′ is δ 7.06 qq (1H, $J_{3',4'}$ = 7.1 Hz, $J_{3',5'}$ = 1.4 Hz). Thus, the assignment of these signals has to be interchanged: δ 1.81 to Me-4′ and 1.87 to Me-5′. An analogous assignment for the tiglic acid moiety is given by [8, 9].

The signal in the 600 MHz spectrum at δ_H 1.39 (1H, ddd, $J_{7eq,7ax}$ = 13.0, 4.4, 3.1 Hz) can be assigned to 7β(eq) which is in agreement with [10].

In the literature there are no ^{13}C NMR data for ajugapyrin A (**5**). The measured ^{1}H broadband-decoupled ^{13}C NMR spectrum of **5** showed 27 signals and the DEPT spectrum displayed 21 resonances (six of them for CH_2). Some of the signals of tiglic acid and perhydrofurofuran moieties can be assigned from both spectra, and our assignments are in close agreement with data in [8].

Plant Material. The stems of *Scutellaria galericulata* L. were collected in June 2012 near Lovech, Bulgaria, and voucher specimens (No. 11927) were deposited in the Herbarium of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

Extraction and Isolation. Dried and finely powdered aerial parts of *Scutellaria galericulata* L. (300 g) were extracted with Me_2CO (3 × 2 L) at room temperature for a week. After filtration, the solvent was evaporated to dryness under redused pressure and low temperature (40°C), yielding a gum (3.2 g), which was dissolved in aq. Me_2CO (40% H_2O, v/v, 100 mL). The solution was cooled to 4°C for 24 h and filtered. The filtrate was extracted with $CHCl_3$ (3 × 30 mL), and the

organic layer was dried (Na_2SO_4) and evaporated in vacuum, giving a residue (0.9 g, bitter fraction). This residue was subjected to CC (silica gel Merck №7734, deactivated with 10% H_2O, w/w, 15 g). Elution with hexane–EtOAC (3:2) yielded 14,15-dihydrojodrellin T (**1**, 18 mg) and scutegalin A (**2**, 10 mg), and elution with hexane–EtOAc (1:1) gave scutegalin D (**3**, 15 mg). Finally, elution with hexane–EtOAc (1:1) yielded scutalbin A (**4**, 16 mg) and ajugapyrin A (**5**, 15 mg). All compounds were recrystallized from Me_2CO.

Structural Data. ^{1}H NMR spectra of **5** were recorded on a Bruker DRX-250 spectrometer operating at 250.13 MHz and a Bruker Avance II+ spectrometer operating at 600.130 MHz. ^{13}C NMR spectra of **5** were recorded on a Bruker Avance II+ 600 MHz NMR spectrometer operating at 150.903 MHz (^{13}C). TMS was used as internal standard and $CDCl_3$ as solvent. Chemical shifts (δ) are expressed in ppm. The IR spectra of **1–5** were registered in KBr pellet on a PerkinElmer 1750 FT-IR spectrometer from 4000 cm^{-1} to 450 cm^{-1} at resolution 4 cm^{-1} with nine scans.

14,15-Dihydrojodrellin T (**1**). Amorphous solid, mp 100–116°C. IR (KBr, ν_{max}, cm^{-1}): 2928, 1735, 1708, 1647, 1443, 1375, 1304, 1252, 1236, 1134, 1091, 1016, 975, 908.

Scutegalin A (**2**). Amorphous solid, mp 106–109°C. IR (KBr, ν_{max}, cm^{-1}): 2960, 1745, 1735, 1720, 1650, 1455, 1370, 1271, 1230, 1140, 1097, 1070, 1025, 970, 875, 790, 730, 645.

Scutegalin D (**3**). Amorphous solid, mp 109–112°C. IR (KBr, ν_{max}, cm^{-1}): 3444, 1733, 1712, 1651, 1451, 1376, 1276, 1256, 1245, 1143, 1075, 1053, 1024, 967, 939, 919, 878, 867.

Scutalbin A (**4**). Colorless needles from acetone, mp 159–161°C. IR (KBr, ν_{max}, cm^{-1}): 3460, 3092, 3052, 2962, 2938, 1731, 1664, 1614, 1468, 1442, 1433, 1381, 1366, 1273, 1099, 1069, 1024, 1008, 947, 899, 861, 798, 736, 593.

Ajugapyrin A (**5**). Colorless needles from acetone, mp 208–210°C ($CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3403, 2967, 2939, 2901, 2873, 1723, 1698, 1654, 1464, 1386, 1336, 1295, 1261, 1250, 1158, 1092, 1082, 1066, 1052, 1018, 986, 966, 942, 918, 899, 875, 835, 777, 732, 679, 628, 603, 568, 527, 485, 469. ^{13}C NMR (150.9 MHz, $CDC1_3$, δ, ppm): 170.02 ($O\underline{C}OCH_3$), 166.10 (C=O, C-1′), 138.71 (CH, C-3′), 128.74 (C-2′), 108.21 (CH, C-16), 91.05 (CH), 86.41 (CH), 71.03 (CH), 70.27 (CH), 68.33 (CH_2), 68.25 (CH), 65.92 (C), 44.15 (CH_2), 42.55 (C), 41.84 (CH), 41.26 (C), 40.75 (CH), 35.44 (CH), 33.36 (CH_2), 33.34 (CH_2), 32.64 (CH_2), 22.62 (CH_2), 21.01 (CH_3), 16.47 (CH_3), 14.58 (CH_3), 14.35 (CH_3), 11.94 (CH_3).

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