DI TERPENOIDS WITH neo-CLERODANE SKELETON FROM SCUTELLARIA ALTISSIMA

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Abstract. Four neo-clerodane diterpenoids, scutecyprin, scupolin H, clerodin and scutecyprol A, have been isolated from the acetone extract of the aerial parts of S. altissima. The structures of these compounds were established by physical constants, spectroscopic means and by comparison with literature data.

Key Word Index. S. altissima; Labiatae; neo-clerodane diterpenes; scutecyprin, scupolin H, clerodin and scutecyprol A

INTRODUCTION

The plants from genus Scutellaria (Labiatae) are a rich source of neo-clerodane diterpenoids with potent insect antifeedant and antifungal activity [1–3].

Previous reports on isolation of neo-clerodanes from S. altissima provided 1:1 mixtures of the C-15 epimers of 15-hydroxy-hexahydrofurofuran derivatives, namely scutaltisin (1) [4] and scutalsin (2) [5] as the sole detectable diterpene constituents. However, our preliminary results showed the presence of a complex cocktail of these compounds in the acetone extract of S. altissima of a different region in Bulgaria.

Now we report on the isolation and structure elucidation of four neo-clerodane diterpenes from the stems of S. altissima gathered around Troian. Although the compounds have been reported previously, they were isolated from this species for the first time in this work.
RESULTS AND DISCUSSION

Work up the acetone extract of the aerial parts of the Bulgarian wild plant yielded four compounds scutecyprin (3), scupolin H (4), clerodin (5) and scutecyprol A (6). Their spectra revealed the presence of oxirane (3054–3050 cm⁻¹) and acetyl groups (1735 br. – 1712 and 1269, 1250 cm –1), but the absence of bands for a lactone or furan moiety. The least polar substance 3 displayed in addition bands for (E)-2-methyl-2-butenoyl ester (1710 and 1650 cm–1), compounds 4 and 5 for vinyl ether (3015 and 1618 cm–1) and 6 for hydroxyl group (3422 cm–1). The ¹H and ¹³C NMR spectra of compounds 3 and 6 showed the presence of a hexahydrofurofuran system (2H-15 at δ 3.88 m) while structures 3 and 4 possess tetrahydrofurofuran system in which the signals for double bond
C-14 and C-15 appeared as triplets at δ 4.80 and 6.45. The spectrum of 4 also displayed the presence of signal for methoxy instead of acetoxy group at δ 3.50 s, 3H, OMe-19β. All compounds 3–6 displayed other similar signals from common structural features for decalin ring of neo-clerodane structure (δ 0.90 d, 3H, J\textsubscript{17,8β} = 7.0 Hz, Me-17 and 1.16 s, 3H, Me-20) bearing 4α,18-oxirane ring (δ\textsubscript{HA-18} 2.43 d and δ\textsubscript{HB-18} 3.01 d, J\textsubscript{gem} = 4.4 Hz) and acetate group (δ\textsubscript{H} 1.80 s, 3H; δ\textsubscript{C} 170.00 s and 21.00 q). In addition a tigloyloxy group for compound 3 (δ\textsubscript{H} 7.08 qq, J\textsubscript{3′,4′} = 7.1 Hz, J\textsubscript{3′,5′} = 1.4 Hz, H-3′ 1.81 br d, 3H, J\textsubscript{4′,5′} = 7.1 Hz, Me-4′; δ\textsubscript{C-1′} 166.30, δ\textsubscript{C-2′} 128.40, δ\textsubscript{C-3′} 138.43, δ\textsubscript{C-4′} 14.51, δ\textsubscript{C-5′} 11.92) and a 19,2α-hemiacetal function for 3 and 4 (δ\textsubscript{H-19} 6.80 s, δ\textsubscript{H-2} 4.09 m; δ\textsubscript{C-19} 91.40 d, δ\textsubscript{C-2} 67.23 d). Compound 6 was TLC-homogeneous fraction. However, its \(^1\)H NMR spectrum displayed some duplicate distinct signals and the \(^{13}\)C NMR 42 lines (six of double intensity, see experimental), [δ 2.19 d and 2.23 brd (15-OH); 6.12 and 5.98/107.61 and 109.50 (d, H-16); 6.04 m and 5.81 dd/99.32 and 98.69 (H-15); 5.81 dd and 4.94 overlapped/83.81 and 83.41 (H-11α); 0.75 d and 0.82 d (Me-17)] pointing out the presence of a 1:1 (15R,15S)-epimeric mixture of 14-hydro-15-hydroxyclerodin. Based on physical constants (m.p., R\textsubscript{T}), IR, \(^1\)H NMR and \(^{13}\)C NMR spectral data, and comparison with literature data, compounds 3–6 were assigned to scutecyprin, scupolin H, clerodin and scutecyprol A [6–11].

Scutecyprin has not been received in crystal state up to now and the real temperature of melting is missing from the literature. We established it at 145–148°C.

**EXPERIMENTAL**

Plant material was collected during the flower’s blossoming period in June 2012, around village of Balkanets, near the town of Troian, Bulgaria. The plant was described by Prof. R. D. Mladenov and voucher specimens (n. 17494) were deposited in the Herbarium of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

The IR spectra of the compounds were registered in KBr pellet on a Perkin-Elmer 1750 FT-IR Spectrometer from 4000 cm\(^{-1}\) to 450 cm\(^{-1}\) at resolution 4 cm\(^{-1}\) with 9 scans. \(^1\)H NMR spectra were recorded with Bruker DRX-250 spectrometer, operating at 250.13 MHz. \(^{13}\)C NMR spectra were recorded on a Bruker DRX-250 spectrometer operating at 62.91 MHz. TMS was used as internal standard and CDCl\(_3\) as solvent. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz.

Dried and finely powdered stems of *Scutellaria altissima* L. (540 g) were extracted with Me\(_2\)CO (2×3 L) at room temperature for 1 week. After filtration, the solvent was evaporated to dryness under reduced pressure, yielding a gum (8 g), which was dissolved in 40% aq. Me\(_2\)CO (v/v, 100 mL). The soln. was cooled to 4°C for 24 hr and filtered. The filtrate was extracted with CHCl\(_3\) (4×50 mL). The organic extract was dried with Na\(_2\)SO\(_4\) and evaporated under vacuum (giving 1.5 g of a bitter residue). This residue was chromatographed (CC silica gel, deactivated with 10% H\(_2\)O, w/w, 22 g; CH\(_2\)Cl\(_2\)/MeOH solvent gradient from 10:0 to 9.9:0.1 as eluent). The collected eluates were pooled into two fractions based on TLC results: A (85 mg) and B (94 mg). CC of fraction A (silica
gel, 10 g, deactivated as above; 25 mL/fraction; CH₂Cl₂/hexane solvent gradient from 5:5 to 10:0 as eluent) yielded 3 (57 mg). Fraction B was subjected to preparative TLC (98:2 ratio of CH₂Cl₂/MeOH solvent mixture as eluent) to afford three homogenous on TLC spots. After recrystallization from acetone pure compounds were obtained: 4 (25 mg), 5 (8 mg) and 6 (17.3 mg).

**Scutecyprin (3).** Colorless prisms from acetone, mp 145–148°C. IR ν<sub>max</sub> (KBr) cm<sup>-1</sup>: 3050, 2961, 2930, 1731, 1710, 1650, 1454, 1373, 1263, 1246, 1083, 1022, 969, 939, 920, 880, 870, 733. <sup>1</sup>H NMR (170 MHz, CDCl<sub>3</sub>): δ 4.09 (m, 1H, H-2<sup>β</sup>), 4.62 (dd, 1H, J<sub>6β,7α</sub> = 11.3 Hz, J<sub>6β,7β</sub> = 4.5 Hz, H-6 β), 4.08 (dd, 1H, J<sub>11α,12α</sub> = 10.9 Hz, J<sub>11α,12β</sub> = 5.8 Hz, H-11α), 5.64 (d, 1H, J<sub>16β,13β</sub> = 5.2 Hz, H-16β), 0.90 (d, 3H, J<sub>17,8β</sub> = 7.0 Hz, Me-17), 2.43 (d, 1H, J<sub>18α,18B</sub> = 4.4 Hz, H-18A), 3.01 (d, 1H, J<sub>18β,18A</sub> = 4.4 Hz, H-18B), 6.80 (s, 1H, H-19α), 1.16 (s, 3H, Me-20), 1.80 (s, 3H, OAc), 7.08 (qq, J<sub>3',4'</sub> = 7.1 Hz, J<sub>5',5</sub> = 1.4 Hz, H-3'), 1.81 (br d, 3H, J<sub>4',3</sub> = 7.1 Hz, Me-4'), 1.89 (br s, 3H, Me-5'). <sup>13</sup>C NMR (62.9 MHz, CDCl<sub>3</sub>): δ: 28.41 (C-1), 67.23 (C-2), 36.92 (C-3), 60.64 (C-4), 41.43 (C-5), 68.27 (C-6), 32.64 (C-7), 35.08 (C-8), 55.31 (oMe), 22.06 (C-1), 24.89 (C-2), 28.41 (C-3), 55.31 (OMe).

**Scupolin H (4).** Colorless prisms from acetone, mp 205–207°C. IR ν<sub>max</sub> (KBr) cm<sup>-1</sup>: 3115, 3050, 2960, 1732, 1618, 1451, 1372, 1254, 1100, 1088, 1080, 1020, 990, 948, 904, 875, 778, 750, 739, 635, 601. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 4.10 (m, 1H, 2.9 Hz, H-2<sup>β</sup>), 4.62 (dd, 1H, J<sub>6β,7α</sub> = 11.3 Hz, J<sub>6β,7β</sub> = 4.5 Hz, H-6 β), 3.98 (dd, 1H, J<sub>11α,12α</sub> = 11.5 Hz, J<sub>11α,12β</sub> = 4.8 Hz, H-11α), 4.80 (t, 1H, J<sub>14,13</sub> = 2.5 Hz, J<sub>14,15</sub> = 2.4 Hz, H-14), 6.45 (t, 1H, J<sub>15,15</sub> = 2.4 Hz, H-15), 6.02 (d, 1H, J<sub>16β,13β</sub> = 5.1 Hz, H-16β), 0.89 (d, 3H, J<sub>17,8β</sub> = 6.0 Hz, Me-17), 2.38 (d, 1H, J<sub>18α,18B</sub> = 4.4 Hz, H-18A), 2.94 (d, 1H, J<sub>18α,18A</sub> = 4.4 Hz, H-18B), 5.12 (s, 1H, H-19α), 1.12 (s, 3H, Me-20), 2.02 (s, 3H, OAc), 3.50 (s, 3H, OMe-19B). <sup>13</sup>C NMR (62.9 MHz, CDCl<sub>3</sub>): δ: 29.01 (C-1), 66.73 (C-2), 37.02 (C-3), 60.84 (C-4), 42.73 (C-5), 68.24 (C-6), 33.64 (C-7), 35.68 (C-8), 41.04 (C-9), 41.25 (C-10), 85.61 (C-11), 32.33 (C-12), 45.72 (C-13), 102.00 (C-14), 146.72 (C-15), 108.21 (C-16), 16.47 (C-17), 50.19 (C-18), 91.40 (C-19), 14.00 (C-20), 170.00 (OOCOCH<sub>3</sub>), 21.00 (OOCOCH<sub>3</sub>), 166.30 (C-1'), 128.40 (C-2'), 138.43 (C-3'), 14.51 (C-4'), 11.92 (C-5').

**Clerodin (5).** Colorless needles from acetone, mp 167-169°C. IR ν<sub>max</sub> (KBr) cm<sup>-1</sup>: 3080, 1735, 1725, 1616, 1362, 1250, 1240, 1080, 1017, 1006, 855, 736, 635, 601. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 4.64 (dd, 1H, J<sub>6β,7α</sub> = 11.5 Hz, J<sub>6β,7β</sub> = 4.3 Hz, H-6 β), 3.98 (dd, 1H, J<sub>11α,12α</sub> = 11.5 Hz, J<sub>11α,12β</sub> = 4.7 Hz, H-11α), 3.52 (m, 1H, H-13), 4.77 (t, 1H, J<sub>14,13</sub> = 2.4 Hz, J<sub>14,15</sub> = 2.4 Hz, H-14), 6.42(t, 1H, J<sub>15,15</sub> = 2.4 Hz, J<sub>15,14</sub> = 2.4 Hz, H-15), 5.95 (d, 1H, J<sub>16β,13β</sub> = 6.1 Hz, H-16β), 0.79 (d, 3H, J<sub>17,8β</sub> = 6.2 Hz, Me-17), 2.18 (d, 1H, J<sub>18α,18B</sub> = 4.1 Hz, H-18A), 2.94 (d, 1H, J<sub>18α,18A</sub> = 4.1 Hz, H-18B), 4.32 (d, 2H, J<sub>18β,19α</sub> = 2.4 Hz, H-19), 0.92 (s, 3H, Me-20), 1.90 (s, 3H, OAc), 2.05 (s, 3H, OAc). <sup>13</sup>C NMR (62.9 MHz, CDCl<sub>3</sub>): δ: 22.06 (C-1), 24.89 (C-2), 39.52 (C-3), 65.04 (C-4), 45.53 (C-5), 71.89 (C-6), 33.34 (C-7), 36.08 (C-8), 40.04 (C-9), 48.25 (C-10), 84.51 (C-11), 32.63 (C-12), 45.92 (C-13), 107.60 (C-14), 146.76 (C-15), 55.31 (OAc).
101.81 (C-16), 14.03 (C-17), 48.59 (C-18), 61.60 (C-19), 16.28 (C-20), 170.02 (OCoCH₃), 170.82 (OCoCH₃), 21.12 (OCoCH₃) 21.11 (OCoCH₃).

Scutecyprol A (6). Colorless prisms from acetone, mp 188–191°C. IR νmax (KBr) cm⁻¹ (a + b): 3422, 3054, 2877, 1734, 1712, 1374, 1269, 1238, 1087, 1028, 1013, 899, 635, 603.

Scutecyprol A (6).

1H NMR (250 MHz, CDCl₃): δ 2.19 (d, 1H, J= 4.0 Hz, 15-oH), 6.12 (d, 1H, J=5.4 Hz, H-16), 6.04 (m, 1H, H-15), 5.18, 4.46 (d, each 1H, J= 12.3, H₂-19), 4.94 (dd, 1H, J₆₇,₇α = 11.8 Hz, J₆₇,₇β = 4.5 Hz, H-6), 4.18 (dd, 1H, J₆₇,₇α = 11.9 Hz, J₃₄,₄₃B = 4.8 Hz, H-3), 2.23 (s, each 3H, oAc x 2), 0.97 (s, 3H, Me-20), 0.75 (d, 3H, J₁₇,₈b = 6.0 Hz, Me-17). 13C NMR (62.9 MHz, CDCl₃): 22.45 (C-1), 25.20 (C-2), 33.22 (C-3), 65.34 (C-4), 46.13 (C-5), 72.17 (C-6), 34.04 (C-7), 36.01 (C-8), 40.54 (C-9), 48.72 (C-10), 83.81 (C-11), 32.58 (C-12), 41.02 (C-13), 39.85 (C-14), 99.32 (C-15), 107.61 (C-16), 16.57 (C-17), 48.30 (C-18), 61.90 (C-19), 14.21 (C-20), 170.58 (OCoCH₃), 169.71 (OCoCH₃), 21.30 (OCoCH₃). Compound 6b: 1H NMR (250 MHz, CDCl₃): δ 2.23 (brd, 1H, J= 1.7 Hz, 15-oH), 5.98 (d, 1H, J=5.4 Hz, H-16), 5.81 (dd, 1H, J= 4.8, 2.5 Hz, H-15), 5.18, 4.48 (d, each 1H, J= 12.2, H₂-19), 4.96 (dd, 1H, J₆₇,₇α = 11.7 Hz, J₆₇,₇β = 4.4 Hz, H-6), 4.94 (overlaped, 1H, H-11a), 3.12 (d, 1H, J₁₈,₁₈B = 4.4 Hz, H-18B), 2.22 (d, 1H, J₁₈,₁₈A = 4.4 Hz, H-18A), 2.15, 2.03 (s, each 3H, OAc x 2), 0.95 (s, 3H, Me-20), 0.82 (d, 3H, J₁₇,₈b = 6.5 Hz, Me-17). 13C NMR (62.9 MHz, CDCl₃): 22.47 (C-1), 25.28 (C-2), 33.23 (C-3), 65.34 (C-4), 46.12 (C-5), 72.39 (C-6), 34.04 (C-7), 36.31 (C-8), 40.54 (C-9), 48.72 (C-10), 83.41 (C-11), 32.58 (C-12), 41.02 (C-13), 39.85 (C-14), 99.32 (C-15), 107.61 (C-16), 16.57 (C-17), 48.29 (C-18), 61.90 (C-19), 14.21 (C-20), 170.58 (OCoCH₃), 169.71 (OCoCH₃), 21.30 (OCoCH₃).

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REFERENCES


**ДИТЕРПЕНОИДИ С нео-КЛЕРОДАНОВ СКЕЛЕТ ОТ SCUTELLARIA ALTISSIMA**

ПЕТКО И. БОЗОВ, ЙОАНА П. ГЕОРГИЕВА, РУМЕН Д. МЛАДЕНОВ

**Резюме.** Четири нео-клероданови дитерпеноиди, scutecyprin, scupolin H, clerodin and scutecyprol A, са изолирани от ацетоновия екстракт от надземните части на *S. altissima*.

Структурата на съединенията са установени чрез физични константи, спектроскопски анализи и сравнение с литературните данни.

**Ключови думи.** *S. altissima*; Labiatae; нео-клероданови дитерпеноиди; scutecyprin, scupolin H, clerodin and scutecyprol A.

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