Pergamon

PII: S0031-9422(98)00358-6

*Phytochemistry* Vol. 49, No. 8, pp. 2449–2452, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

# NEO-CLERODANE DITERPENOIDS FROM *SCUTELLARIA ALPINA*

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(*Received in revised form* 7 *April* 1998)

**Key Word Index**—*Scutellaria alpina*; Labiatae; neo-clerodane diterpenes; scutalpins N and O.

**Abstract**—Two new *neo*-clerodane diterpenoids, scutalpins N and O, have been isolated from *Scutellaria alpina*, together with six previously known *neo*-clerodanes. The structures of scutalpin N (19-acetoxy-6 α-benzoyloxy, 4α, 18-epoxy-8 β hydroxy-neo-clerod-13-en-15,16-olide) and scutalpin O (11*S*,13*S*, 15*R* and *S*, 16*R*)-6 α-acetoxy-19-isobutyroyloxy-neo-cleroda-15,16-hemiacetal) were established by chemical and spectroscopic means and by comparison with related compounds. 

## INTRODUCTION

In previous communications [1, 2] we have described the isolation of three *neo*-clerodane diterpenoids from the aerial parts of *Scutellaria alpina*. In our search for new *neo*-clerodane diterpenoids [3–7], we have re-investigated the aerial parts of this species and isolated the previously isolated scutalpins A,E and F, together with minor quantities of scutalpin L (**1**) [8], scutecyprol A (**2**) [9], scutorientalin E [7] and the new diterpenoids scutalpin N (**3**) and scutalpin O (**4**). We report here on the isolation and structure elucidation of these compounds.

## RESULTS AND DISCUSSION

Scutalpin N (**3**) was assigned the molecular formula $C_{29}H_{36}O_8$. Its IR spectrum showed bands for hydroxyl (3475 $cm^{-1}$), α, β-unsaturated γ-lactone (1782, 1636 $cm^{-1}$), acetoxyl (1742, 1241 $cm^{-1}$) and benzoate groups (3058, 1728, 1600, 1587, 714 $cm^{-1}$). The $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) revealed the existence of an acetoxyl group (δ 2.11 *s*, 3H, $\delta_C$ 171.0 *s*, 21.6q) and a benzoyloxy substituent [(δ 7.99 *dd*, 2′, 3′; 7.39 *t* 3′,5′ and 7.52 *tt*, 4′; OCOPh δ 130.6 *s* (C-1′) 129.6*d* (C-2′,6′),128.0 *d*(C-3′,5′) and 132.7 *d* (C-4′)], together with the characteristic signals of a *neo*-clerodane diterpene [(δ 0.92 *s* Me-20; 20.7 *q* C-20), having a 4α, 18-oxirane (δ 3.06 *dd*, $J_{gem}$ = 4.0 Hz; $J_{18B,\ 3\alpha}$ = 2.2 Hz, and 2.26 *d*, H2-18; $\delta_C$ 64.6 *s* (C-4) and 48.5 *t* (C-18)] [3, 5, 7, 8, 13]. The tertiary hydroxyl group of **3** must be at the C-8 β position, because Me-17 appeared as a singlet and was paramagnetically shifted to $\delta_H$ 1.12 *s* [$\delta_C$ 21.3 *q* (C-17) and $\delta_C$ 76.5 *s* (C-8)] [3, 7, 8]. The above spectral data were almost identical with those of scutalpin L (**1**), a *neo*-clerodane diterpene recently isolated from *S. alpina* collected in Italy [8]. In fact the observed differences were consistent with the presence in the former of a C-7 methylene group instead of the benzoyloxy substituent of the latter [8].

The relative stereochemistry of all the asymmetric centres of **3** was firmly established from the NOESY spectrum. The H-6 β proton (δ 5.17) showed NOE cross-peaks with $H_B$-18 and H-10β. Moreover the Me-20 protons showed NOEs with Me-17 and $H_B$-19, consequently scutalpin N (**3**) possessed the same stereochemistry as scutalpin L (**1**) [8] and had the structure depicted in **3**.

The second diterpenoid, scutalpin O (**4**, $C_{26}H_{40}O_8$), was homogeneous on TLC. Its $^1H$ NMR spectrum showed essentially the same signals as those present in the $^1H$ NMR spectrum of the scutecyprol A (**2**), a *neo*-clerodane diterpene recently isolated from *Scutellaria cypria var. cypria* [9] and in the present study (see Experimental). The observed differences in the $^1H$ NMR spectra of **2** and **4** were consistent with the presence in **4** of an isobutyroyloxy group [$\delta_H$ 1.19 *d*, 1.23 *d* and 2.57 *sept*. $J_{2',3'} = J_{2',4'} = 7.0$ Hz] and only one OAc group (δ 1.95 *s*, 3H), instead of the two OAc groups of **2**. Oxidation of the C-15

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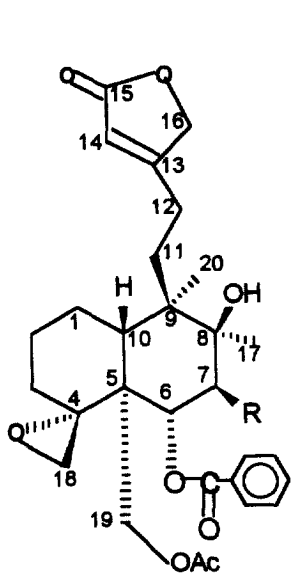
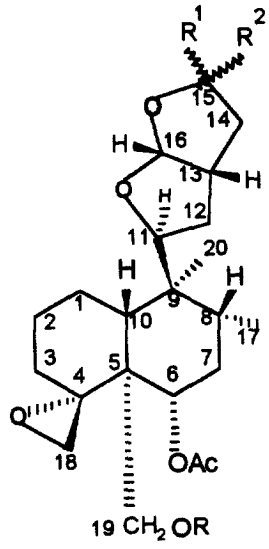
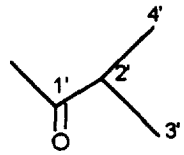
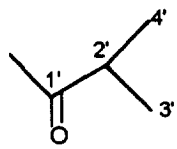




1. R = OBz

3. R= H

| | R | $R^1$ | $R^2$ |
|---|---|---|---|
| 2 | Ac | H | OH ( *R* and *S* forms ) |
| 4. |  | H | OH ( *R* and *S* forms ) |
| 5. |  | O | |
| 6. | Ac | O | |

hemiacetal function of **4** (see Experimental) yielded the derivative **5** ($C_{26}H_{38}O_8$).

The IR and $^1H$ NMR spectra of this substance revealed the presence of a $\gamma$-lactone moiety [$\nu_{CO}$ 1786 $cm^{-1}$, downfield resonance of the H-16 proton ($\delta$ 6.04*d*), C-14 methylene protons as the AB part of an ABX system (Table 1)] [2, 4, 10]. The location of the acetyl and isobutyroyloxy substituents in scutalpin O (**4**) was established by comparison of the $^1H$ NMR spectrum of **5** with the derivative (**6**) of scutecyprol A (**2**). A careful analysis showed that the $H_A$-19 proton of **5** was paramagnetically shifted ($\Delta$ppm +0.11), whereas 19 $H_B$ was diamagnetically shifted ($\Delta$ppm −0.28) in comparison with those of **6**. The remaining signals resonated at an almost identical field in both compounds (Table 1), thus suggesting that the isobutyroyloxy substituent of the new diterpenoid (**4**) was attached to the C-19 carbon.

The absolute configuration of **3** and **4** was not ascertained. However, on biogenetic grounds it may be supposed that **3** and **4** belong to the *neo*-clerodane series [11] like the other diterpenoids isolated from *Scutellaria* species whose absolute configuration has been established from X-ray diffraction analysis [1, 12].

It is noteworthy that from *S. alpina* growing in Bulgaria, we isolated several 13-spiro *neo*-clerodane

Table 1. $^1$H NMR spectral data of compounds **3**, **5** and **6** (250 MHz, $CDCl_3$, TMS as int. standard)*

| H | 3 | | 5 | 6[+−] | Δppm | J (Hz) | 3 | 5 |
|---|---|---|---|---|---|---|---|---|
| 6β | 5.17 *dd* | | 4.69 *dd* | 4.67 *dd* | −0.02 | 6β,7α | 10.2 | 11.1 |
| 10β | 2.42 *m* | | a | — | | 6β,7β | 4.8 | 5.8 |
| 11α | — | | 4.12 *dd* | 4.11 *dd* | −0.01 | 11α12A | — | 11.3 |
| 12B | 3.02 *m*[−] | | a | | | 11α,12B | — | 5.1 |
| 13 (β in 5) | 5.81 *m* | | 3.17 *m* | 3.18 *m* | +0.01 | 13β,14A | — | 3.9 |
| 14A | — | | 2.38 *dd* | 2.39 *dd* | +0.01 | 13β,14B | — | 10.6 |
| 14B | — | | 2.88 *dd* | 2.89 *dd* | +0.01 | 14A,14B | — | 18.7 |
| 16A | | | | | | 13β,16 | — | 5.6 |
| (β in 5) | 4.76 *s br* | | 6.04 *d* | 6.04 *d* | 0 | | | |
| 16B | | | | | | 19A,6β | — | <0.4 |
| Me-17 | 1.12 *s* | | 0.86 *d* | 0.86 *d* | 0 | 17,8β | — | 6.2 |
| 18A[II] | 2.26 *d* | | 2.20 *d* | 2.20 *d* | 0 | 18A,18B | 4.0 | 4.1 |
| 18B[#] | 3.06 *dd* | | 2.98 *dd* | 2.97 *d* | −0.01 | 18B,3α | 2.2 | 2.3 |
| 19A | 4.49 *d* | | 4.47 *d* br | 4.36 *d* | −0.11 | 19A,19B | 12.2 | 11.8 |
| 19B | 4.73 *d* | | 4.69 *d* | 4.97 *d* | +0.28 | 2′(6′),4′ | 8.5 | — |
| Me-20 | 0.92 *s* | | 0.94 *s* | 0.94 *s* | 0 | 2′(6′),4′ | 1.4 | — |
| OAc | 2.11 *s* | | 1.95 *s* | 1.93 *s* | −0.02 | 4′,3′(5′) | 7.7 | — |
| OAc | — | | — | 2.09 *s* | — | 2′3′ | — | 7.0 |
| 2′/6′ | 7.99 *dd* | 2′ | 2.59 *sept* | — | | 2′4′ | — | 7.0 |
| 3′/5 | 7.39 *t* | 3′ | 1.19 *d* | — | | | | |
| 4′ | 7.52 *tt* | 4′ | 1.23 *d* | — | | | | |

* Spectral parameters were obtained by first order approximation. All these assignments were confirmed by double resonance experiments.
[II] *Exo* hydrogen with respect to ring B
[#] *Endo* hydrogen with respect to ring B
[−] Overlapped signal
[+ −] Taken from Ref. [9]
a not observed.

diterpenoids with 13*S* and 13*R* configuration [1, 2], and all these compounds were without oxidation at C-11, in contrast to those isolated from *S. alpina* collected in Japan [13], *S. alpina subsp. javalambrensis* growing in Spain [3, 14] and *S. alpina* growing in Italy [8].

## EXPERIMENTAL

### *General*

Mps: uncorr. Plant materials were collected in August 1996 at Pirin Mountains near Bansko, Bulgaria, and voucher specimens (No. 33308) are deposited in the Herbarium of the Department of Botany of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

Table 2. $^{13}$C NMR spectral data of scutalpin N (**3**) (62.9 MHz, $CDCl_3$, TMS as int. standard)*

| C | 3 | C | 3 |
|---|---|---|---|
| 1 | 21.0 *t* | 15 | 175.7 *s* |
| 2 | 25.2 *t*[−] | 16 | 73.2 *t* |
| 3 | 32.6 *t*[−] | 17 | 21.1 *q* |
| 4 | 64.6 *s* | 18 | 48.5 *t* |
| 5 | 45.1 *s* | 19 | 61.8 *t* |
| 6 | 68.8 *d* | 20 | 20.7 *q* |
| 7 | 39.9 *t* | OAc | 171.0 *s* |
| 8 | 76.5 *s* | | 21.6 *q* |
| 9 | 41.7 *s* | OCOPh | 165.9 *s* |
| 10 | 41.3 *d* | 1′ | 130.6 *s* |
| 11 | 34.2 *t*[+] | 2′6′ | 129.6 *d* |
| 12 | 26.3 *t*[+] | 3′, 5′ | 128.0 *d* |
| 13 | 171.7 *s* | 4′ | 132.7 *d* |
| 14 | 114.3 *d* | | |

* Multiplicities determined from DEPT-135 experiments
[+] These assignments may be reversed.

### *Extraction and isolation of the diterpenoids*

Dried and finely powdered aerial parts of *S. alpina L.* (1120 g) were extracted with $Me_2CO$ (3 × 6 L) as described previously [1]. The $CHCl_3$-soluble fraction (5.7 g, bitter fr.) was subjected to CC (Silica gel Merck No. 7734, deactivated with 10% $H_2O$, w/v, 100 g). Elution with petrol–EtOAc (2:1) gave crude scutalpin A (1.2 g) and scutalpin E (18 mg), and elution with petrol–EtOAc (7:3) gave scutalpin L (**1**, 12 mg), scutorientalin E (18 mg) and scutalpin N (**3**, 36 mg). Compound **3** was rechromatographed [CC, Silica gel, petrol–$CHCl_3$ (1:1)] yielding pure **3** (27 mg). Further elution with petrol–EtOAc (3:2) yielded crude scutalpin F (71 mg), scutecyprol A (**2**, 15 mg) and scutalpin O (**4**, 16 mg).

The previously known compounds, scutalpin A [1], and scutorientalin E [7], scutalpins E and F [2], scutalpin L (**1**) [8], were identified by their physical (mp, $[\alpha]_D$) and spectroscopic ($^1$H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

### *Scutalpin N (**3**)*

Amorphous solid, mp 78–84°, $[\alpha]_D$ 20 −21.8° ($CHCl_3$, *c* 0.297). IR $\nu_{max}$ KBr cm$^{-1}$: 3475, 3130, 3058, 2967, 2878, 1782, 1742, 1728, 1636, 1600, 1587, 1452, 1376, 1241, 1186, 1155, 1121, 1089, 1029, 912, 888, 849, 731, 714; $^1$H NMR: Table 1; $^{13}$C NMR; Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 512 $[M]^+$ (0.1), 452 (0.6), 407 $[M^+ - Bz]^+$(4), 390

$[M^+ - BzOH]^+$ (8), 330 $[M^+ - BzOH - AcOH]^+$ (17), 312 $[M^+ - H_2O - BzOH - AcOH]^+$ (75), 257 (7), 167 (10), 122 (90), 105 (100), 83 (64), 85 (21), 69 (48), 43 (41). (Found C 67.71; H 6.98, $C_{29}H_{36}O_8$ requires C 67.95, H 7.08%).

*Scutalpin O (**4**)*

Mp 86–92°, amorphous solid: mixture (1:1) of the 15*R* and 15*S* forms: $^1$H NMR (250 MHz, $CDCl_3$): $\delta$ 5.80 and 5.78 (0.5 H each, both *d*, $J$ = 5.3 Hz, H-16), 5.63 (0.5 H, *dd*, $J_1$ = 1.2 Hz and $J_2$ = 4.8 Hz, H-15), 5.53 (0.5 H *d br*, $J$ = 5.3 Hz, H-15), 4.48 and 4.69 (2H, both *d*, *br*, $J$ = 12.1 Hz, $H_A$-19 and $H_B$-19), 4.59 (1H, *dd*, $J_1$ = 6.0 Hz and $J_2$ = 11.0 Hz, H-6), 4.00 (1H, *dd*, $J_1$ = 4.6 Hz and $J_2$ = 11.6 Hz, H-11 $\alpha$), 3.10 (0.5 H, *m*, $W_{1/2}$ = 21 Hz, H-13 $\beta$), 2.85 (0.5 H, *m*, $W_{1/2}$ = 20 Hz, H-13$\beta$), 2.98 (1H, *dd*, $J_1$ = 2.8 Hz and $J_2$ = 6.4 Hz, $H_B$-18), 2.19 (1H, *d*, $J$ = 4.0 Hz, $H_A$-18), 2.57 (1H, *sept.* $J$ = 7.0 Hz, H-2′), 1.95 (3H, *s*, OAc), 1.22 (3H, *d*, $J$ = 7.0 Hz, Me-3′), 1.19 (3H, *d*, $J$ = 7.0 Hz, Me-4′), 0.94 and 0.93 (1.5 H each, both *s*, Me-20), 0.88 and 0.86 (1.5 H each, both *d*, $J$ = 6.3 Hz, Me-17).

*Scutecyprol A (**2**)*

Mp 91–97°, amorphous solid: mixture (1:1) of the 15*R* and 15*S* forms $^1$H NMR (250 MHz, $CDCl_3$): $\delta$ 5.78 and 5.76 (0.5 H each, both *d*, $J$ = 5.2 Hz, H-16), 5.61 (0.5 H, *dd*, $J_1$ = 1.8 Hz and $J_2$ = 4.6 Hz, H-15), 5.50 (1.5 H, *br d*, $J$ = 5.7 Hz, H-15), 4.36 and 4.86 (2H, both *d*, $J$ = 12.2 Hz, $H_A$-19 and $H_B$-19), 4.64 (1H, *dd*, $J_1$ = 6.0 Hz and $J_2$ = 11.0 Hz, H-6$\beta$), 4.0 (1H, *dd*, $J_1$ = 4.6 Hz and $J_2$ = 11.4 Hz, H-11$\alpha$), 2.96 (1H, *dd*, $J_1$ = 2.1 Hz and $J_2$ = 4.2 Hz, $H_B$-18), 2.18 (1H, *d*, $J$ = 4.2 Hz, $H_A$-18), 2.80 (1H, *m*, $W_{1/2}$ = 21 Hz, H-13$\beta$), 2.07 (3H, *s*, OAc), 1.92 (3H, *s*, OAc), 0.93 and 0.92 (1.5H each, both, *s*, Me-20), 0.84 and 0.83 (1.5 H each, both, *d*, $J$ = 6.2 Hz, Me-17).

*Oxidation of **4** to **5***

To a soln of **4** (10 mg) in $Me_2CO$ (2 ml) was added an excess of Jones' reagent [15] at 10–12° with stirring. After 20 min., excess Jones's reagent was destroyed by addition of EtOH, and then the reaction mixture was diluted with $H_2O$ (10 ml). Extraction with $CHCl_3$ (4 × 8 ml) and work-up as usual gave **5** (6 mg) as an amorphous solid, mp 88–94°, $[\alpha]_D$ 20° 0° ($CHCl_3$, *c*, 0.211) IR $\nu_{max}$, KBr, $cm^{-1}$: 3053, 2963, 2930, 2856, 1786, 1729, 1468, 1373, 1262, 1099, 1024, 966, 802, 710; EIMS (70 eV, direct inlet) *m/z* 478 $[M^+]\cdot C_{26}H_{38}O_8$, *Mr* 478; $^1$H NMR: Table 1.

*Acknowledgements*—This work was supported by the Bulgarian Ministry of Education, Science and Technology (Funds for Scientific Research).

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