PERGAMON

Phytochemistry 50 (1999) 973–976

PHYTOCHEMISTRY

A diterpenoid with antifeedant activity from Scutellaria rubicunda

Maurizio Bruno[a, b, *], Nadia Vassallo[b], Monique S. J. Simmonds[c]

[a]*Dipartimento di Chimica Organica "E. Paternò", Università di Palermo, Archirafi 20, 90123 Palermo, Italy*
[b]*Istituto di Chimica e Tecnologia dei Prodotti Naturali—Consiglio Nazionale delle Ricerche (ICTPN-CNR) (associated with National Institute for the Chemistry of Biological System-CNR), Ugo La Malfa 153, 90146 Palermo, Italy*
[c]*Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*

Received 13 February 1998; received in revised form 13 February 1998

Abstract

Two diterpenoids have been isolated from *Scutellaria rubicunda* subsp. *linneana*: (11*S*,13*S*,15*R* and *S*, 16*R*,19*S*)-6α-acetoxy-19-tigloyloxy-2α,19;4α,18;11,16;15,16-tetraepoxy-neo-clerodan-15-ol (scutecyprol B) and (11*S*,13*S*,15*R* and *S*, 16*R*,19*S*)-6α-acetoxy-2α,19;4α,18;11,16;15,16-tetra-epoxy-neo-cleroda-15,19,diol (scutalbin C). Both compounds were tested for antifeedant activity against larvae of some species of Lepidoptera. Scutecyprol B shows potent activity at 100 ppm. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Scutellaria rubicunda* subsp. *linneana*; Labiatae; Neo-clerodane diterpenes; Scutecyprol B; Scutalbin C; Antifeedant activity

1. Introduction

The neo-clerodane diterpenoids contained in *Scutellaria* species have attracted interest because of their potent antifeedant [1–7] and anti-fungal activities [8]. As part of our continuing studies on *Scutellaria* [9–11] we have now investigated the aerial parts of *Scutellaria rubicunda* Hornem subsp. *linneana* (Carnel) Rech., an endemic species growing in the central part of Sicily.

We report here on the isolation and identification of the two diterpenoids, scutecyprol B (**1**) and scutalbin C (**2**) and the effects of these compounds on the feeding behaviour of larvae from a range of economically important species of Lepidoptera.

2. Result and discussion

Repeated chromatography of the acetone extract of the aerial parts of *S. rubicunda subsp. linneana* gave compound (**1**), isolated from fractions eluted with EtOAc-petrol (7:3) and compound (**2**) eluted with EtOAc-petrol (9:1).

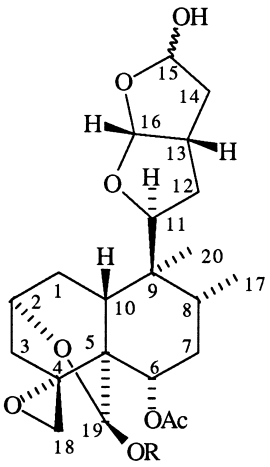
Compound (**1**) ($C_{27}H_{38}0_9$) showed an IR spectrum consistent with the presence of an hydroxyl (3400 cm^{-1}), ester group (1740, 1240 cm^{-1}) and α,β-unsaturated ester (1705 cm^{-1}).

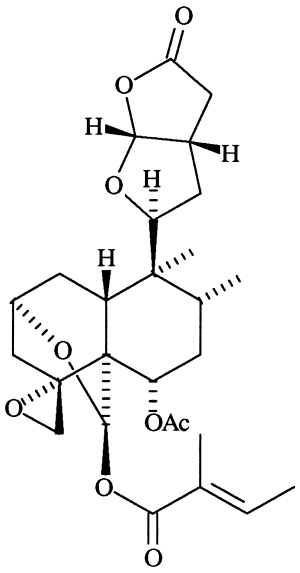
The ^{1}H NMR spectrum of compound (**1**) (Table 1) was in agreement with the presence in a saturated furofuran system of an almost equimolecular mixture of C-15 epimers; in fact H-11, H-15 and H-16 appeared as pairs of signals [12–14] at δ 4.52 (*dd* 0.5 H, J = 10.7 and 6.2 Hz, H-11α in the 15*S* epimer) and δ 3.99 (*dd* 0.5 H, J = 11.4 and 4.8 Hz, H-11α in the 15*R*-epimer), δ 5.62 (*brd* 0.5 H, J = 3.0 Hz, H-15β) and δ 5.53 (*brd* 0.5 H, J = 5.5 Hz, H-15α) and δ 5.80 and 5.78 (both *d*, 0.5 H each, J = 5.3 Hz, H-16β). Other signals indicated the occurrence of the 4α,18-epoxide, the 6α-OAc function, the 2α,19-hemiacetal system esterified with tiglic acid.

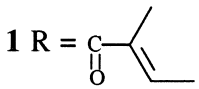
The ^{13}C NMR spectrum of compound **1** (Table 2) was also consistent with the presence of an epimeric mixture since several carbons showed double signals. In a previous paper [15] we reported the identification

* Author to whom correspondence should be addressed.

0031-9422/98 $ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(98)00255-6





1 R = 

2 R = H

3

of a product having structure **1** and named scutecyprol B, which was not possible to isolate as a clean compound because it was not separable from the closely related scutecyprol A. It was characterized as the 15-oxo derivative (**3**) separable from the 15-oxo derivative of scutecyprol A. Oxidation of pure compound **1** with pyridinium chromate in pyridine yielded lactone **3**, identical with that isolated in our previous work [15]. Consequently, compound **1** is scutecyprol B.

The ^{1}H NMR and ^{13}C NMR spectra of compound **2** showed again the presence of a C-15 epimeric mixture. Comparison of spectral data and with an authentic sample allowed us to identify compound **2** as scutalbin C, isolated from *S. albida* [12], *S altissima* [13] (also named there scutaltisin) and *S. discolor* [14]. The ^{13}C NMR spectrum of **2** had been reported [14] in pyridine d_5 solution. We report the data in a $CDCl_3$ solution for comparison with the data of other neoclerodanes, all run in $CDCl_3$. Complete attribution of the ^{1}H NMR signals for the two epimers is reported.

The absolute configuration of compounds **1** and **2** was not ascertained. However, on biogenetic grounds it is reasonable to assume that this new diterpene belongs to the neoclerodane series, like all the clerodanes isolated from Labiatae [16].

Scutecyprol B (**1**) showed significant activity against larvae from all the five species tested at 100 ppm. In contrast, scutalbin C (**2**) showed no potent activity. Table 3

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on Perkin-Elmer 241 MC polarimeter. IR spectra (KBr) were obtained on a Perkin-Elmer 681 spectrophotometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution at 250 MHz using a Bruker AC 250 E apparatus and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25). ^{13}C-NMR spectra were recorded in $CDCl_3$ at 62.7 MHz and chemical shifts are reported with respect to solvent signals (δ $CDCl_3$ 77.00). ^{13}C-NMR assignments were determined by DEPT spectra. MS were recorded on a VG MASSLAB 12–250 instrument. Elemental analyses were made with a Carlo Erba EA 1108 apparatus.

Table 1
^{1}H NMR Spectral Data of Compounds **1** and **2** (250 MHz, $CDCl_3$)

H	**1**(15*R*)	**1**(15*S*)	**2**(15*R*)	**2**(15*S*)
2*β*	4.17*m*	4.17*m*	4.15*m*	4.15*m*
3α	2.55*dt*	2.55*dt*	2.56*dt*	2.56*dt*
6*β*	4.62*dd*	4.62*dd*	4.65*dd*	4.65*dd*
11α	3.99*dd*	4.52*dd*	3.94*dd*	4.48*dd*
13*β*	3.10*m*	2.82*m*	3.00*m*	2.80*m*
15α	5.53*brd*		5.51*brd*	
15*β*		5.64*brd*		5.62*brd*
16*β*	5.78*d*	5.80*d*	5.76*d*	5.77*d*
Me-17	0.90*d*	0.92*d*	0.88*d*	0.90*d*
18A[a]	2.44*d*	2.44*d*	2.42*d*	2.42*d*
18B[b]	2.99*d*	3.00*d*	2.93*d*	2.94*d*
19α	6.81*s*	6.82*s*	5.70*s*	5.71*s*
Me-20	1.16*s*	1.18*s*	1.06*s*	1.07*s*
OAc	1.80*s*	1.80*s*	2.04*s*	2.04*s*
3′	7.08*qq*	7.08*qq*		
Me-4′	1.82*brd*	1.82*brd*		
Me-5′	1.89*brs*	1.89*brs*		
J(Hz)				
1α,3α	2.8	2.8	2.8	2.8
2*β*,3α	2.8	2.8	2.8	2.8
3α,3*β*	14.4	14.4	14.4	14.4
6*β*,7α	4.5	4.5	4.8	4.8
8*β*,17	6.3	6.3	6.1	6.1
11α,12*β*	4.8	6.2	4.9	6.3
11α,12α	11.4	10.7	11.6	10.7
13*β*,16*β*	5.3	5.3	5.3	5.3
14α,15α	5.3		6.1	
14*β*,15*β*		3.0		3.7
18A,18B	4.8	4.8	4.2	4.2
3′,4′	7.1	7.1		
3′,5′	0.9	0.9		

[a] This is the exo hydrogen with respect to ring B.[b] This is the endo hydrogen with respect to ring B.

Table 2
^{13}C NMR Spectral Data of Compounds **1** and **2** (62.7 MHz, $CDCl_3$)

carbon	**1**(15*R*)	**1**(15*S*)	**2**(15*R*)	**2**(15*S*)
C-1	8.5	28.5	28.9	28.9
C-2	67.2	67.2	66.8	66.8
C-3	36.9	36.9	36.7	36.7
C-4	60.6	60.6	60.8	60.8
C-5	41.6	41.6	42.5	42.5
C-6	68.3	68.4	69.8	69.9
C-7	33.0	33.0	32.8	32.8
C-8	35.4	35.6	35.3	35.4
C-9	41.1	41.1	41.0	41.0
C-10	41.0	41.0	40.7	40.7
C-11	84.4	84.6	84.4	84.6
C-12	33.1	33.6	33.0	33.7
C-13	39.7	40.7	39.7	40.5
C-14	38.8	39.9	38.8	39.9
C-15	98.7	98.4	98.7	98.4
C-16	107.9	110.0	107.9	110.0
C-17	16.6	16.5	16.7	16.7
C-18	50.1	50.1	49.6	49.6
C-19	91.5	91.4	93.1	93.1
C-20	14.0	14.1	14.0	14.1
OAc	170.0	170.0	169.3	169.3
	20.9	20.9	21.4	21.4
C-1′	166.3	166.3		
C-2′	128.9	128.9		
C-3′	138.3	138.3		
C-4′	14.5	14.5		
C-5′	11.9	11.9		

3.2. Insect bioassay

The insect bioassay were similar to those described in Simmonds *et al.* [17].

3.3. Plant collection

Plant materials were collected at Rocca Busambra, 80 km south of Palermo (Italy) in July 1995, and voucher specimens were deposited in the Herbarium of the Orto Botanico of Palermo, Italy.

3.4. Extraction and isolation

Dried and finely powdered aerial parts of *Scutellaria rubicunda* Hornem subsp. *linneana* (Carnel) Rech. (740 g) were extracted with Me_2CO (3×5 l.) at room temp. for 1 week. After filtration the solvent was evaporated under red. pres. and low temp. (30°) to give 54 g of crude extract which was subjected to CC (silica gel Merck No. 7734, deactivated with 15% H_2O w/v, 800 g) eluting with petrol, EtOAc-petrol mixtures and MeOH-EtOAc mixtures.

The fractions eluted with EtOAc-petrol (7:3) were rechromatographed [(CC, eluent EtOAc-petrol 3:2) and radial chromatography Chromatotron eluent CH_2Cl_2-MeOH (49:1)] yielding scutecyprol B (**1**, 30 mg).

The fractions eluted with EtOAc-petrol (9:1) were rechromatographed [CC eluent CH_2Cl_2-MeOH (49:1,

Table 3
Effect of Scutecyprol B (**1**) and Scutalbin C (**2**) on the feeding behaviour of final stadium lepidopteran larvae when tested at 100 ppm.

Species	Scutecyprol B	Scutalbin C
Spodoptera littoralis	100 ± 0.0*	32 ± 13.7
Spodoptera frugiperda	85 ± 3.8*	32 ± 18.5
Mamestra brassicae	86 ± 3.5*	41 ± 22.8
Pieris brassicae	75 ± 8.9*	12 ± 14.6
Helicoverpa armigera	65 ± 8.4*	14 ± 13.8

* $P < 0.05$, significant difference in the amount of treated and control disc eaten: Wilcoxon Ranked Pairs test.

47:3)] to give 190 mg of scutalbin C (**2**) which was identified by comparison with an authentic sample.

3.5. Scutecyprol B (**1**)

Amorphous powder; mixture of 15*R* and 15*S*-form. ^{1}H NMR (250 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (62.7 MHz, $CDCl_3$): see Table 2. IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1705, 1210. EI-MS (70 eV, direct inlet) *m/z* (rel. int.): M^+ absent, 407 [M-tiglate]$^+$ (6). Found: C 63.84%; H 7.60%. $C_{27}H_{38}O_9$ requires: C 64.03%; H 7.51%.

3.6. Oxidation of scutecyprol B (**1**) to lactone **3**

20 mg compound **1** of dissolved in pyridine (2 ml) was oxidized with a soln of pyridinium chromate (100 mg) in pyridine (2 ml) at room temp. for 24 hr. After dilution with H_2O, (10 ml) and extraction with Et_2O (8×25 ml), the extract was washed with H_2O, dried and evaporated. The residue was purified [CC, eluent EtOAc-petroleum ether (λ 1:1)] yielding lactone **3** (14 mg) which was identified by comparison of spectral data and with an authentic sample.

Acknowledgements

Spodoptera and *Helicoverpa* were reared under licence from Ministry of Agriculture. We thank Martin Cullum and Paul Green for technical assistance. This work was also supported by MURST-Roma. We thank Prof. Benjamìn Rodrìguez's group, CSIC, Madrid for registration of some spectra and for helpful discussions.

References

Anderson, J. C., Blaney, W. M., Cole, M. D., Fellows, L. E., Ley, S. V., Sheppard, R. N., & Simmonds, M. S. J. (1989). *Tetrahedron Lett., 30,* 4737.

Cole, M. D., Anderson, J. C., Blaney, W. M., Fellows, L. E., Ley, S. V., Sheppard, R. N., & Simmonds, M. S. J. (1990). *Phytochemistry, 29,* 1793.

Rodriguez, B., de la Torre, M. C., Rodriguez, B., Bruno, M., Piozzi, F., Savona, G., Simmonds, M. S. J., Blaney, W., & Perales, A. (1993). *Phytochemistry, 33,* 309.

Cunat, A. C., Dìez-Martìn, D., Ley, S. V., & Montgomery, F. J. (1996). *J. Chem. Soc., Perkin Trans., 1,* 611.

Munoz, D. M., de la Torre, M. C., Rodriguez, B., Simmonds, M. S. J., & Blaney, W. M. (1997). *Phytochemistry, 44,* 593.

Merritt, A. T., & Ley, S. V. (1992). *Nat. Prod. Rep., 9,* 243.

Simmonds, M. S. J. and Blaney, W. M., *Labiatae-Insect Interactions: Effect of Labiatae-Derived Compounds on Insect Behaviour*, in *Advances in Labiatae Science*, eds. R. M. Harley and T. Reynolds. Royal Botanic Gardens: Kew, U.K., 1992, pp. 375–392.

Cole, M. D., Bridge, P. D., Dellar, J. E., Fellows, L. E., Cornish, M. C., & Anderson, J. C. (1991). *Phytochemistry, 30,* 1125.

Hussein, A. A., de la Torre, M. C., Jimeno, MŁL., Rodriguez, B., Bruno, M., Piozzi, F., & Servettaz, O. (1996). *Phytochemistry, 43,* 835.

Rodriguez, B., de la Torre, M. C., Jimeno, MŁL., Bruno, M., Vassallo, N., Bondì, M. L., Piozzi, F., & Servettaz, O. (1997). *J. Nat. Prod., 60,* 348.

de la Torre, M. C., Rodriguez, B., Bruno, M., Vassallo, N., Bondì, M. L., Piozzi, F. and Servettaz, O., *J. Nat. Prod.*, 1997, in the press.

Bruno, M., Piozzi, F., Rodriguez, B., de la Torre, M. C., Vassallo, N., & Servettaz, O. (1996). *Phytochemistry, 42,* 1059.

Malakov, P. Y., Papanov, G. Y., & Boneva, I. M. (1996). *Phytochemistry, 41,* 855.

Ohno, A., Kizu, H., & Tomimori, T. (1996). *Chem. Pharm. Bull., 44,* 1540.

Bruno, M., Fazio, C., & Arnold, N. A. (1996). *Phytochemistry, 42,* 555.

Merrit, A. T., & Ley, S. V. (1992). *Nat. Prod. Rep., 9,* 243.

Simmonds, M. S. J., Blaney, W. M., & Fellows, J. E. (1990). *J. Chem. Ecol., 16,* 3167.