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Pyrrolizidine Alkaloids From *Senecio mulgediifolius*, Two New 13-Membered Macrocyclic 7,9-diesters*

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Abstract. The structure and absolute configuration of the new 13-membered macrocyclic alkaloids mulgediifoline and oxyretroisosenine were determined on the basis of chemical reactions and conventional spectral studies including differential nOe and 2D NMR techniques, COSY, HETCOR, COLOC, HMBC and NOESY. The absolute stereochemistry of the already known compounds retroisosenine and *cis*-nemorensic acid was assigned unambiguously.

INTRODUCTION

Many pyrrolizidine alkaloids (PAs) are toxic to cattle and human beings. The insects of the *Lepidoptera* subfamily[1] accumulate PAs in their bodies for protection against predators or as precursors for pheromone production. Monarch butterflies (*Danaus plexippus*) collected at the overwintering sites in Mexico contain PAs and PAN-oxides[2]. Over 350 PAs have been isolated[3] but only five possess a 13-membered macrocyclic 7,9-diester[4,8]. These alkaloids were isolated from the European species *Senecio nemorensis* L. and *Senecio doronicum* L.[4-7] of the section *Doria* [9] which are related to the Mexican species *S. mulgediifolius* Schauer of the section *Mulgediifolii* [10], according to Jeffrey *et al.*[8] The chemical results reported here support this relationship since we isolated four 13-membered macrocyclic 7,9-diester PAs, bulgarsenine (**2**), retroisosenine (**1b**), mulgediifoline (**1a**) and oxyretroisosenine (**1c**). The structure and absolute configuration of the new PAs (**1a** and **1c**) are determined on the basis of spectral studies and chemical reactions. The absolute stereochemistry of *cis*-nemorensic acid and that of the known alkaloid retroisosenine is assigned unambiguously for the first time.

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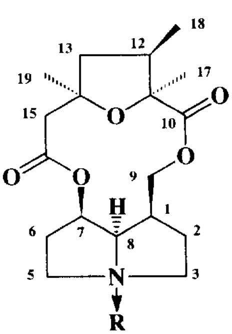
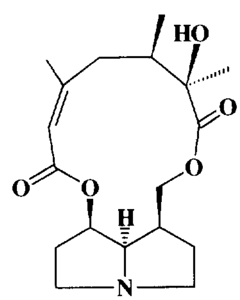
RESULTS AND DISCUSSION

After treatment with H^+/Zn the MeOH extracts of leaves and roots of *S. mulgediifolius* Schauer afforded the 13-membered ring 7,9-diester PAs retroisosenine (**1b**)[5], bulgarsenine (**2**)[5] and the new compound mulgediifoline (**1a**). The known alkaloids were identified by their physical and spectroscopic characteristics. When the extract of the leaves was worked up without Zn treatment yielded exclusively the new PA oxyretroisosenine (**1c**). The remaining aqueous solution treated with H^+/Zn afforded **1a** and **1b**, thus suggesting that these alkaloids are present as N-oxides.

Mulgediifoline (**1a**), mp 104-5°, $[\alpha]_D$ -32.5°, exhibits a strong carbonyl band at 1728 cm^{-1} in its IR spectrum. The MS shows the molecular ion at *m/z* 337 ($C_{18}H_{27}O_5N$) and a peak at *m/z* 122 corresponding to anhydro platynecine base. The ^1H NMR spectrum (Table **1**) resembles that of nemorensine (**3**)[4], with some dissimilarities such as the downfield shifts of H-7 and H-15. An analogous situation is observed in the ^{13}C NMR spectrum for the signals of C-11 and C-12 (Table **2**). The spectral data, the different mp and specific rotation of **1a** and **3** (mp 132-4°, $[\alpha]_D$ -58°)[4] suggest that both PAs differ only in their stereochemistry. Compound **1a** was saponified affording platynecine (**4**) which is the base obtained from nemorensine (**3**). The acid produced was not nemorensic acid (**5**) (mp 173-7°, $[\alpha]_D$ -87°)[4], but *cis*-nemorensic acid (**6**)[5,6] which was identified by its physical (mp 96-100°, $[\alpha]_D$ +49°) and spectroscopic features (Tables **1** and **2**); therefore the difference between **3** and **1a** is in the acid moiety. An HMBC experiment showed that C-10 carbonyl correlates with H-9a, H-9b, H-12 and CH_3-17; and the C-16 carbonyl with H-7, H-15a and H-15b. All of these are in agreement with structure **1a** for mulgediifoline.



1a R = 2e⁻
1b $\Delta^{1,2}$; R = 2e⁻
1c $\Delta^{1,2}$; R = O



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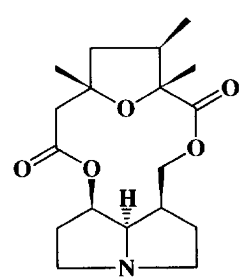

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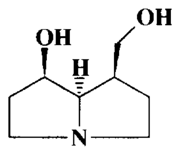

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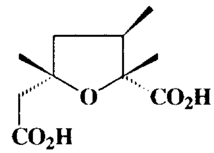

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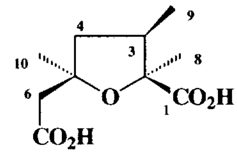

6

In order to establish the stereochemistry of **6**, a NOESY experiment was run (Fig **1**). An nOe effect between CH_3-8 and H-3 indicated a *cis* relationship of these groups. Additional differential nOe experiments showed that when CH_3-8 is irradiated, the H-3 signal is enhanced (8.4 %). Irradiation of CH_3-10 caused enhancement of the H-3, H-4a, H-4b, H-6b and H-6a signals, 29.3, 19.6, 15.1, 15.7 and 13.37 % respectively. The above data are in agreement with the stereochemistry depicted in **6** or its enantiomer.

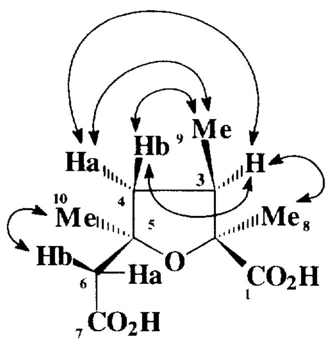

Fig. 1 Results of NOESY experiment of **6**

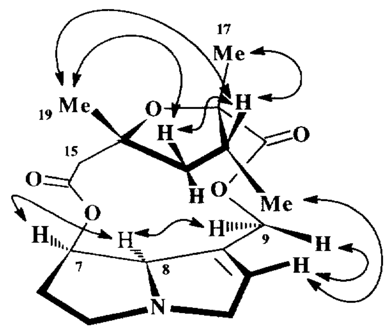

Fig. 2 Results of NOESY experiment of **1b**.

Saponification of retroisosenine (**1b**) yielded (+)-**6**, hence the compounds **1a** and **1b** are esters of the same diacid. In order to determine whether the acid is **6** or its enantiomer, a NOESY spectrum of **1b** was obtained (Fig **2**). Interactions of the vinylic proton (H-2) with the secondary methyl at C-12 was observed. The differential nOe experiments showed that the irradiation of the CH_3-18 signal cause enhancement of the H-2 (3.38 %) and H-9a (2.86 %) signals. Irradiation of H-8 produces a nOe effect only in the H-7 signal (13.47 %). The above results and the fact that the absolute configuration of retronecine base is already known[11] allow us to propose the tridimensional structure depicted in Fig. **2** for retroisosenine, which shows the alkaloid in a sandwich conformation with the tetrahydrofuran plane almost parallel to the retronecine plane. This illustrates that the CH_3-18 is close to H-2 and H-9a. Hence the configurations at C-11, C-12 and C-14 are S, R and R respectively. In this manner the absolute stereochemistry of retroisosenine (**1b**) was established and therefore that of the new alkaloid mulgediifoline (**1a**).

Table 1. ^{1}H NMR data of **1a**, **1b**, **1c**, **3** and **6**, ($CDCl_3$), 300 MHz.

H	1a*	1b	1c	3†	6‡
1	2.57 *m*			3.20-3.50 *m*	
2a	2.04 *dtd* (12.0, 9.0, 3.5)	5.89 *br d* (1.7)	5.96 *br s*		
2b	1.70 *dtd* (12.0, 7.5, 3.0)				
3a	3.08 *ddd* (10.0, 9.0, 7.5)	3.94 *br ddt* (15.8, 3.8, 1.8)	4.57 *br s*		
3b	2.76 *ddd* (10.0, 9.0, 3.0)	3.45 *ddd* (15.8, 6.0, 1.5)			
5a	3.13 *ddd* (10.0, 8.0, 2.0)	3.30 *m*	3.89 *ddd* (11.1, 6.9, 3.2)		
5b	2.70 *td* (10.0, 9.0)	2.60 *m*	3.67 *td* (11.1, 5.4)		
6a	2.07 *m*	2.10 *m*	2.92 *dddd* (14.1, 11.1, 6.7, 4.8)		
6b	1.94 *m*		2.25 *ddt* (14.1, 5.4, 2.7)		
7	5.25 *br t* (4.0)	5.44 *dt* (4.6, 2.4)	5.71 *ddd* (6.3, 4.8, 2.7)	5.03 *m*	
8	3.30 *dd* (7.5, 3.5)	4.37 *m*	4.85 *dd* (6.3, 1.2)	4.32 *dd* (7.0, 3.2)	
9a	4.49 *dd* (12.5, 5.0)	5.09 *d* (11.9)	5.10 *d* (12.5)	4.53 *dd* (12.5, 3.7)	
9b	4.00 dd (12.5, 1.5)	4.16 *dtd* (11.9, 1.9, 1.0)	4.28 *dd* (12.3, 0.9)	4.15 *dd* (12.5, 0.9)	
12/3§	2.30 *ddq* (11.5, 7.0, 6.5)	2.37 *dquint* (10.5, 7.2)	2.37 *quint t* (7.2, 9.3)		2.39 *dquint* (12.5, 6.7)
13a/4a§	2.23 *t* (11.5)	2.11 *dd* (12.0, 10.5)	2.03 *d* (9.3)	2.74 *dd* (11.5, 7.5)	2.08 *dd* (12.4, 6.7)
13b/4b§	1.91 *dd* (11.5, 6.5)	1.96 *dd* (12.0, 7.2)	2.03 *d* (9.3)	1.59 *t* (11.5)	1.87 *t* (12.4)
15a/6a§	2.64 *d* (12.5)	2.66 *d* (12.6)	2.66 *d* (12.8)	2.59 *d* (12.5)	2.92 *d* (15.0)
15b/6b§	2.55 *d* (12.5)	2.59 *d* (12.6)	2.61 *d* (12.6)	2.23 *d* (12.5)	2.68 *d* (15.0)
17/8§	1.40 *s*	1.46 *s*	1.46 *s*	1.36 *s*	1.43 *s*
18/9§	0.94 *d* (7.0)	1.04 *d* (6.6)	1.06 *d* (6.9)	0.98 *d* (6.6)	1.03 *d* (6.9)
19/10§	1.32 s	1.41 s	1.42 *s*	1.27 *s*	1.33 *s*

Values in parenthesis are the coupling constants in Hz. The assigments are based in COSY, HETCOR, COLOC and HMBC experiments. * Run at 500 MHz. † Data from reference[4]. ‡ Run at 200 MHz. § Numbering of **6**.

Oxyretroisosenine (**1c**), mp 128-131°, $[\alpha]_D$ +34°, has a molecular formula $C_{18}H_{25}O_6N$, according to MS. The ^{1}H and ^{13}C NMR data are very similar to those of retroisosenine (**1b**) (Tables **1** and **2**). The downfield shifts of the C-3, C-5 and C-8 signals and the upfield shifts of the C-1, C-2, C-6 and C-7 signals are a consequence of the γ-*anti* effect[12] induced by the N-oxy function, which has been observed for other PAN-oxides[6, 13]. A substance identical in all respects with the natural product was obtained by oxidation of **1b** with MCPBA, thus confirming the structure **1c** for oxyretroisosenine.

The work presented establishes the structure and absolute configuration of the 13-membered macrocyclic 7,9-diester PAs retroisosenine, oxyretroisosenine and mulgediifoline, which produce *cis*-nemorensic acid. The absolute configuration of the 13-membered macrocyclic 7,9-diester PAs which yield nemorensic acid, bulgarsenine and doronenine, was already published[7]. Since nemorensine gives the same acid[5] its stereochemistry is as depicted in **3**.

Table 2. ^{13}C NMR spectral data of **1a-1c**, **3** and **6** (50 MHz, $CDCl_3$)

C	1a	1b	1c	Δδ1b,1c	3*	6†
1	38.9	133.5	131.2	-2.3	40.0	175.1
2	27.6	132.5	126.9	-5.6	35.3	88.1
3	53.4	62.1	78.3	+16.2	57.0	45.0
5	55.0	54.0	69.2	+15.2	54.1	82.9
6	35.9	34.7	32.9	-1.8	43.1	47.0
7	74.6	73.9	72.5	-1.4	70.9	177.3
8	70.9	77.7	97.2	+19.5	75.9	25.2
9	62.3	59.7	59.8		61.5	14.9
10	173.5	169.6	169.2		174.8	27.9
11	87.1	87.0	86.9		85.9	
12	43.9	44.4	44.3		41.5	
13	45.6	45.6	46.1		25.7	
14	81.1	81.6	81.7		83.0	
15	47.2	47.1	47.0		47.6	
16	169.4	173.0	172.5		171.5	
17	24.8	24.9	24.8		32.4	
18	13.9	14.4	14.4		19.4	
19	30.7	30.7	30.4		14.0	

The assigment are based in DEPT pulse sequence, HETCOR, COLOC and HMBC experiments. * Data from reference[6]. † C-4 δ 46.0 ppm.

EXPERIMENTAL

Plant Material. *Senecio mulgediifolius* Schauer was collected along the Mexico-Cuernavaca highway near to Tres Marías in September, 1992 (MEXU 613581). A second sample was collected along the Mexico-Oaxtepec highway near the border line between Federal District and State of Mexico in June, 1994 (MEXU 623377). Voucher specimens are deposited at the Herbario del Instituto de Biología, U. N. A. M.

Isolation of retroisosenine (1b) and bulgarsenine (2). Dried and ground aerial parts of *S. mulgediifolius* (620 g) collected in 1992, were extracted with 2.5 % aq. H_2SO_4. The aqueous extract was stirred with Zn powder (65 g) overnight, then filtered. The acid solution was basified (NH_4OH to pH 10) and extracted with $CHCl_3$. Elimination of the solvent left a pale yellow oil (16.4 g) which gave a Dragendorff positive test. The alkaloid mixture was chromatographed on Kieselgel G (400 g) eluting with $CHCl_3$ and an increasing proportion of MeOH. Fractions eluted with $CHCl_3$-MeOH (9:1) were combined and evaporated to give 4.5 g of residue. 100 mg of the last material were purified by prep. TLC (MeOH-Me_2CO, 7:3) yielding 20 mg of retroisosenine (**1b**), mp 123-125°, $[\alpha]_D$ +110° ($CHCl_3$ c 0.2)[5] and 11 mg of bulgarsenine (**2**), mp 108-110° [5]. Both substances were identified by comparison of their physical and spectroscopic features with those reported in the literature.

Isolation of retroisosenine (1b). The roots of *Senecio mulgediifolius* (158 g) collected in 1994, were extracted with MeOH. The extract was concentrated and stirred overnight at room temperature with 2.5% aq H_2SO_4 (70 ml) and Zn powder (16 g). The mixture was filtered and worked up as above described to give 2.07 g of an alkaloid mixture. The column chromatography over Kieselgel G (30 g) was eluted with MeOH-Me_2CO (7:3) and yielded 1.1 g of **1b**.

Isolation of retroisosenine (1b), mulgediifoline (1a) and oxyretroisosenine (1c). Fresh leaves of *S. mulgediifolius* (1630 g) collected in 1994, were extracted with MeOH. The extract was concentrated and stirred overnight at room temperature with 2.5% aq H_2SO_4 (700 ml) and Zn powder (163 g) and filtered. The usual work up yielded 13.1 g of an alkaloidal mixture. The column chromatography over Kieselgel G (200 g) was eluted with MeOH-Me_2CO (7:3) and gave 7.18 g of **1b** and 191 mg of mulgediifoline (**1a**) as white crystals from hexane, mp 102-104°. $[\alpha]_D$ -32.5° ($CHCl_3$ c 0.28). $\nu^{CHCl_3}_{max}$: 1728. FAB-MS (nitro benzyl alcohol) *m/z* (rel. int.): 338.1971 $[M+1, C_{18}H_{28}O_5N]^+$ (100), 337 $[M]^+$ (14.50), 336 $[M-1]^+$ (26.31), 122 $[C_8H_{12}N]^+$ (10.08), 120 $[C_8H_{10}N]^+$ (6.14), 82 $[C_5H_8N]^+$ (5.26). Obsd. 338.1971 $[M+1]^+$, calcd. for $C_{18}H_{28}O_5N$ 338.1967.

Dried and ground leaves (36.2 g) of the same collection were extracted with MeOH. The extract was acidified with 2.5% aq H_2SO_4, washed with $CHCl_3$, basified (NH_4OH to pH = 10) and extracted with $CHCl_3$. The alkaloidal mixture (500 mg) was chromatographed on Kieselgel G (8.0g) and eluted with MeOH-Me_2CO (7:3) to furnish 150 mg of oxyretroisosenine (**1c**) as a light brown crystalline solid from EtOAc-hexane, mp 128-131°, $[\alpha]_D$ +31.4° ($CHCl_3$ c 0.4), $\nu^{CHCl_3}_{max}$ cm^{-1}: 1765, 1601. FAB-MS (nitro benzyl alcohol) *m/z* (rel. int.): 352.1757 [M+1, $C_{18}H_{26}O_6N]^+$ (100), 154 $[C_8H_{12}O_2N]^+$ (5.0), 136 $[C_8H_{10}ON]^+$ (25), 118 $[C_8H_8N]^+$ (40), 106 $[C_7H_6O]^+$ (11), 43 $[C_3H_7]^+$, (22). Obsd. 352.1757 $[M+1]^+$, calcd. for $C_{18}H_{26}O_6N$ 352.1760.

The aqueous layers were acidified with 2.5% aq H_2SO_4 and stirred overnigth at room temperature with Zn powder (3.7 g). The filtered solution was worked up as above described to give 360 mg of a residue which chromatographed on Kieselgel G (6.0) afforded 249 mg of **1b** and 20 mg of **1a**.

Saponification of mulgediifoline (1a). Mulgediifoline (90.4 mg) and $Ba(OH)_2{\cdot}8H_2O$ (181 mg) in H_2O (5 ml) were refluxed for 2 h, 20 min. The reaction mixture was diluted with H_2O, saturated with CO_2 and filtered. The filtrate was acidified with 5% aq H_2SO_4 and filtered. The acid solution was basified with 5% aq KOH and concentrated *in vacuo*. The residue was extracted with hot $CHCl_3$ (10 x 10 ml), dried and concentrated, yielding 30 mg of platynecine (**4**)[5], mp 142-145°, $[\alpha]_D$ -57.7° ($CHCl_3$ c 0.18). Lit.: mp 145-147°, $[\alpha]_D$ -57±2° ($CHCl_3$). The remaining salts were dissolved in H_2O, acidified with 5% aq H_2SO_4 and extracted with $CHCl_3$. The organic solution was concentrated yielding 31 mg of a colorless oil which was chromatographed over Kieselgel G using as eluent $CHCl_3$-MeOH (9:1) to give 17 mg of *cis*-nemorensic acid (**6**)[5,6], which crystallized after 4 days, mp 96-100°, $[\alpha]_D$ +41° ($CHCl_3$ c 0.2). Lit.: mp 100-104°, $[\alpha]_D$ +49±4° ($CHCl_3$).

Saponification of retroisosenine (1b). A solution of **1b** (1.05 g) and KOH (1.1 g) in MeOH (20 ml) was refluxed for 4 h. The MeOH was evaporated and the residue extracted with $CHCl_3$ (10 x 15 ml). Elimination of the solvent afforded 485 mg of retronecine[11] as a brown oil. The remaining salts were dissolved in H_2O, acidified with 1% aq H_2SO_4, extracted with $CHCl_3$ (10 x 10 ml), dried and concentrated, yielding 303 mg of an oil which after successive column chromatographies over Kieselgel G using as eluents $CHCl_3$-MeOH (9:1) and (19:1) yielded 136 mg of *cis*-nemorensic acid (**6**)[5,6], mp 98-100°.

Oxidation of retroisosenine (1b). 100 mg of retroisosenine (**1b**) in $CHCl_3$ (5 ml) were treated with MCPBA (52 mg) and stirred at room temperature for 1 hr. The solvent was eliminated under reduced pressure and the residue dissolved in H_2O. The aqueous solution was washed with EtOAc and the H_2O evaporated. The residue was chromatographed over Kieselgel G eluting with MeOH-Me_2CO (7:3) to give 8.3 mg of recovered **1b** and 49.6 mg of oxyretroisosenine (**1c**).

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REFERENCES

1. Robin, D. J. *Progress Chem. Natural Products* **1982**, *41*, 115.
2. Kelley, R. B. Seiber, J. N. Jones, A. D. Segall, H. J. Brower, L. P. *Experientia* **1987**, *43*, 943.
3. Logie, C. G. Grue, M. R. Liddell, J. R. *Phytochemistry* **1994**, *37*, 43.
4. Klásek, A. Sedmera, P. Boeva, A. Santavý, F. *Coll. Czech. Chem. Commun.* **1973**, *38*, 2504.
5. Nghia, N. T. Sedmera, P. Klásek, A. Boeva, A. Drjanovska, L. Dolejs, L. Santavý, F. *Coll. Czech. Chem. Commun.* **1976**, *41*, 2952.
6. Klásek, A. Sedmera, P. Vokoun, J. Boeva, A. Dvorácková, S. Santavý, F. *Coll. Czech. Chem. Commun.* **1980**, *45*, 548.
7. Röder, E. Wiedenfeld, H. Frisse, M. *Phytochemistry* **1980**, *19*, 1275.
8. Jeffrey, C. Halliday, P. Wilmont-Dear, M. Jones, S. W. *Kew. Bull.* **1977**, *32*, 4767.
9. Chater, A. O. Walter, S. M. *Flora Europea* **1976**, *4*, 94.
10. Villaseñor, J. L. Ph. D. Thesis, The Claremont Graduate School, Claremont Cal., **1991**.
11. Culvenor, C. C. J. Heffernan, M. L. Woods, W. G. *Aust. J. Chem.* **1965**, *18*, 1605.
12. Wehrli, F. W. Marchand, A. P. Wehrli, S. Interpretation of Carbon 13-NMR Spectra, ed. John Wiley & Sons, **1988**.
13. Were, O. Benn, M. Munavu, R. M. *Phytochemistry* **1993**, *32*, 1595.

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