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PYRROLIZIDINE ALKALOIDS FROM *SENECIO GALLICUS* AND *S. ADONIDIFOLIUS*

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Abstract—Four alkaloids, ligularizine, senkirkine and senecionine *N*-oxide, were isolated from *S gallicus* Apart from florosenine, a new alkaloid was isolated from *S. adonidifolius* and identified as 12,13,19-trihydroxy-15,20-epoxy-15,20-dihydro(12*S*,15*R*,20*R*) senecionan-11,16-dione.

Two dimensional spectroscopic correlation experiments (^{1}H–^{1}H and ^{13}C–^{1}H) were performed, permitting the unequivocal assignation of the ^{1}H and ^{13}C NMR spectra of the alkaloids and enabling us to correct some of the data appearing in the literature.

INTRODUCTION

In recent years considerable attention has been directed towards the study of pyrrolizidine alkaloids [1] mainly in view of their hepatotoxicity and to losses in liverstock numbers owing to the ingestion of plants containing such alkaloids Certain earlier studies relating to *S. gallicus* involved the study of flavonoids [2], alkaloids [3], acetophenones and terpenoids [4] The present work reports on the isolation and identification of four pyrrolizidine alkaloids. Concerning *S adonidifolius*, a previous study [5] examined polyphenols and another [6] reported on alkaloids Here, we describe the isolation and identification of two pyrrolizidine alkaloids, one of which is described for the first time.

RESULTS AND DISCUSSION

The aerial part of *S. gallicus* was extracted with ethanol at room temperature and chromatography of the alkaloid fraction afforded **1**–**4**.

The ^{1}H NMR spectrum of **1** (Table 1) showed signals that were characteristic of a pyrrolizidine alkaloid with a senecionane skeleton. The complete assignment of the signals appearing in its ^{1}H NMR spectrum was conducted by ^{1}H–^{1}H homonuclear two-dimensional correlation experiments. The physical and spectroscopic properties of the compound coincide with those described in the literature [7] for senecionine. We also performed ^{13}C–^{1}H heteronuclear two-dimensional correlation experiments which allowed us to assign inequivocally the signals of the ^{13}C NMR spectrum.

The alkaloid **2** had a ^{1}H NMR (Table 1) showing signals at 6 21 (*t*, *J* = 1.46 Hz, H-2), 5.10 (*t*, *J* = 2 9 Hz, H-7), 5.26 (*d*, *J* = 11.2 Hz, H-9a), 4 29 (*d*, *J* = 11.2 Hz, H-9b), similar to those of **1** though the signal corresponding to H-8 did not appear However, there was a signal at δ2.01 (*s*, 3H) of a N-Me grouping, which suggested the presence of *seco*-senecionane skeleton. There were also signals of a proton geminal to an oxyranic ring at δ3.02(1H, *q*, *J* = 5.4 Hz, H-20), 1.10(3H, *d*, *J* = 6.3 Hz, Me-18), 1 48(3H, *d*, *J* = 5.4 Hz, Me-21), 1 38(3H, *s*, Me-19) and 2.03 (3H, *s*, acetoxyl).

Assignment of the signals was performed by ^{1}H–^{1}H homonuclear correlation experiments. The shift of Me-21 and H-20 in the ^{1}H NMR spectrum allowed us to assign a β-stereochemistry to the epoxide [8]. Furthermore, the shift of Me-18 was in agreement with a (12*S*)-stereochemistry [8], and hence **2** was identified as ligularizine.

Compound **3** was also an alkaloid with a *seco*-senecionane skeleton. The physical and spectroscopic properties of **3** coincided with those reported for senkirkine [9] However, homonuclear and heteronuclear (normal and long-range) two dimensional experiments were conducted and these permitted the complete and unequivocal assignation of the signals of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) and allowed us to correct data in the literature for this compound [9].

The more polar compound **4** had a ^{1}H NMR spectrum very similar to that corresponding to **1** except in the shift of H-8 (**1** δ4.27 and **4** δ4.83) and C-8 (**1** δ77 67 and **4** δ97 15). This suggested that **4** was the *N*-oxide of senecionine. Oxidation of **1** with *m*-chloroperbenzoic acid affords **4**.

Two alkaloids **5** and **6** were isolated from the ethanol extract of *S adonidifolius* Compound **5** had a 4,8-*seco*-senecionane skeleton as may be deduced from its ^{1}H and ^{13}C NMR spectra. There were also signals that are characteristic of an oxyranic ring at C-15, C-20, at δ2.95 (1H, *q*, *J* = 5.4 Hz, H-20), 1.17(3H, *d*, *J* = 5.4 Hz, Me-21) and acetoxylic group 2 0 (3H, *s*).

According to the shifts of H-20 and Me-21, an α stereochemistry was inferred for the epoxide [8] and from the shift of Me-18 a (12*R*)-stereochemistry was deduced [8] such that compound **5** was identified as florosenine [10]

Assignment of the signals in the ^{1}H and ^{13}C NMR spectra was performed by two dimensional experiments. These allowed an unequivocal assignment and correction of the data in the literature (11) Compound **6** was purified by crystallization in acetone; its ^{1}H NMR and

Table 1 ^{1}H NMR data of pyrrolizidine alkaloids

H	1	2	3	4	5	6
2	6 19 *br s*	6 21 *t* (1 46)	6 07 *t* (2 4)	6 22 *br s*	6 11 *t* (2 44)	6 11 *m*
3a	3 94 *ddd* (15 63, 4 39, 2 44)	3 54 *br d* (19 04)	3 39 *dd* (18 55, 1 9)	4 62 *d* (6 2)	3 37 *d* (18 0)	3 97 *d* (15 6)
3b	3 40 *ddd* (15 6, 6 4, 1 6)	3 31 *br d* (19 04)	3 17 *ddd* (18 55, 1 9, 2 9)	4 52 *dd* (6 2, 2 4)	3 23 *d* (18 0)	3 22 *m*
5a	3 26 *t* (8 31)	2 90 *m*	2 81 *ddd* (2 9, 5 86, 12 2)	3 92 *ddd* (10 7, 7 3 1 9)	2 87 *m*	3 3 *m*
5b	2 53 *m*	2 90 *m*	2 68 *ddd* (3 91, 12 2, 12 2)	3 64 *m*	2 56 *m*	2 61 *dd* (6 4, 9 3, 16)
6a	2 37 *dd* (14 1, 5 8)	2 65 *m*	2 45 *m*	2 96 *m*	2 60 *m*	2 12 *m*
6b	2 13 *m*	2 05 *m*	2 45 *m*	2 45 *m*	2 10 *dd* (10 7, 2 4)	2 12 *m*
7	5 02 *dd* (5 8, 3 4)	5 10 *t* (2 93)	4 91 *t* (2 3)	5 45 *m*	4 92 *m*	5 52 *t* (3 42)
8	4 27 *m*			4 83*d* (4 9)		4 25 *m*
9a	5 50 *d* (11 7)	5 26 *d* (11 23)	5 35 *d* (11 2)	5 49 *d* (12 2)	5 22 *d* (11 23)	5 29 *d* (11 72)
9b	4 04 *d* (11 07)	4 29 *d* (11 23)	4 28 *d* (11 2)	4 14 *d* (12 2)	4 31 *d* (11 23)	4 25 *d* (11 72)
14a	2 16 *m*	2 21 *br d* (13 2)	2 33 *m*	2 14 *d* (13 2)	2 39 *d* (14 6)	2 12 *m*
14b	1 75 *m*	0 82 *dd* (11 72, 13 08)	2 00 *m*	1 78 *m*	1 21 *m*	2 12 *m*
Me-18	1 32 *s*	1 38 *s*	1 27 *s*	1 30 *s*	1 63 *s*	1 56 *s*
Me-19	0 93 *d* (6 4)	1 10 *d* (6 35)	0 84 *d* (6 37)	0 88 *d* (6 56)	1 10 *d* (6 35)	*
20	5 72 *dq* (7 20, 1 32)	3 02 *q* (5 37)	5 79 *dq* (1 5, 7 3)	5 81 *q* (7 32)	2 95 *q* (5 37)	3 47 *q* (6 35)
Me-21	1 84 *dd* (7 2, 1 6)	1 48 *d* (5 37)	1 84 *dd* (1 7, 7 3)	1 84 *dd* (7 2, 4 1)	1 17 *d* (5 37)	1 36 *d* (6 35)
Me-C=O		2 01 *s*			2 00 *s*	
Me-4		2 10 *s*	2 02 *s*		2 01 *s*	

*6 shows H-19a 3 75, *dd* (J = 12 21 and 1 46 Hz) H-19b 3 71, *d* (J = 12 21 Hz)

^{13}C NMR spectra (Tables 1 and 2) showed signals characteristic of an alkaloid of the retrocenine type

Apart from the signals corresponding to the retrocenine ring, its ^{1}H NMR spectrum showed signals of the following a proton centred at δ3 47 (*q*, J = 6 35 Hz) that was coupled to a methyl doublet at 1 36 (J = 6 35 Hz), suggesting an epoxide grouping at C-15/C-20, a methyl geminal to an oxygenated function (δ1 56, 3H, *s*) and a -CH_2OH group (AB system, δ3.75, *dd*, J = 12 21 and 1 46 Hz and 3 71, *d*, J = 12 21 Hz)

The remaining signals of its ^{13}C NMR spectrum corresponded to five quaternary carbons (two carbonylic and three bound to an oxygenated function), one methyne (δ73 70), two methylenes (one of them at 63 17 bound to a hydroxyl group) and two methyl groups Comparison of the shifts of H-20 (^{1}H NMR) and of Me-21 (^{1}H and ^{13}C NMR) with the values found for **5** and with the data appearing in the literature [8] showed that the stereochemistry of the epoxide was β Also by comparison with the ^{1}H and ^{13}C NMR data for Me-18 with those appearing in the literature [8] and with those corresponding to compound **1**, it was possible to conclude that the stereochemistry at C-12 is *S* The hydroxymethylene group is located at C-13 according to the data found for ^{1}H- ^{1}H homonuclear two-dimensional correlations carried out for **6** in which it was seen that one of the H-19 protons was correlated with H-14a and H-14b

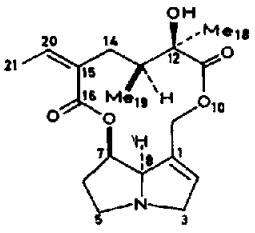
The data of the ^{13}C NMR spectrum were assigned by ^{13}C ^{1}H heteronuclear two-dimensional correlation experiments With these data it was possible to assign to compound **6** the structure 12,13,19-trihydroxy-15,20-epoxy-15,20-dihydro (12*S*,15*R*,20*R*)-senecionan-11, 16-dione, though the stereochemistry at C-13 remains unknown

EXPERIMENTAL

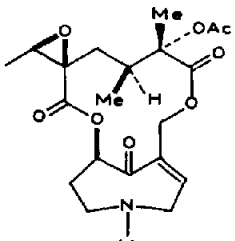
Mps uncorr, ^{1}H NMR 200 MHz, $CDCl_3$ TMS as int standard, ^{13}C NMR 50 3 MHz

Extraction and isolation of the alkaloids Dry and ground portions of the plant were immersed in EtOH for 4 weeks, they were then filtered and EtOH evapd off The ethanolic extract was divided into 2 parts, one of them was fractionated without reduction and the other was reduced with Zn powder

(i) *Fractionation of the non-reduced extract* The hexane-sol-



1



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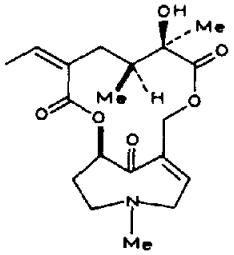

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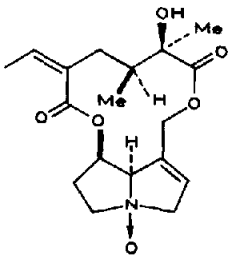

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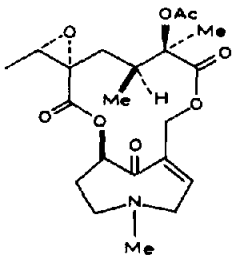

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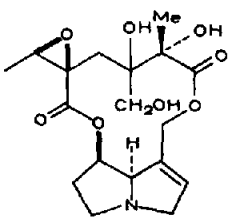

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Table 2 ^{13}C NMR data of pyrrolizidine alkaloids

C	1	3	4	5	6
1	133 28	134 39	129 23	133 79	131 29
2	136 55	137 37	136 84	137 98	135 97
3	62 89	58 52	79 07	59 15	60 55
4		40 51		40.35	
5	53 04	53 18	69 45	53 18	53 74
6	34 84	36 29	33 18	36 85	35 20
7	75 00	78 11	73 53	78 95	77 92
8	77 67	192 41	97 15	189 78	78.80
9	60 68	64 45	60 23	64 32	67 35
11	178 18	177 97	177 98	170 91	169 91
12	76 72	77 79	76 86	83 72	74 06
13	38 42	38 60	38 64	40 16	65 39
14	38 37	37 74	38 20	35 11	40 84
15	131.66	131 94	131 87	63 77	61 57
16	167 50	166 41	166 58	167 84	167 48
18	25 01	24 59	24 98	21 30	15 44
19	11 14	10 15	11 02	12 93	63 17
20	133 95	136.99	131 87	55 90	73 70
21	14 96	15 23	15 16	13.53	14.49
C–Me ‖ O				169 69 21 30	

uble part was removed from the EtOH extract and suspended in 1 M H_2SO_4. The acid soln was extracted with $CHCl_3$ and the remaining H_2SO_4-soluble part was made alkaline with NH_4OH to pH 9, it was then extracted with $CHCl_3$, thus obtaining the free bases

(ii) *Fractionation of the reduced extract* The hexane-soluble part was removed from the EtOH extract and suspended in 1 M H_2SO_4. This acid soln was stirred for 12 hr with an excess of Zn powder Following this it was filtered, made alkaline to pH 9 with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was dried and evapd, thus obtaining a mixture of alkaloids (free bases plus reduced *N*-oxides)

Senecio gallicus was collected at Valparaiso (Zamora, Spain), and was extracted with EtOH at room temp. (14% extract with respect to the weight of the plant) The extract was fractionated, to isolate the alkaloids, using the above method to obtain the following free bases 1 73% (with respect to the weight of the EtOH extract) and 2 70% of the reduction product (with respect to the weight of the EtOH extract)

The free-base fraction was chromatographed on silica gel, obtaining **1** (10%) ($CHCl_3$–MeOH, 19 1 and crystallization in Me_2CO), **2** (4 mg) ($CHCl_3$–MeOH, 19 1), **3** (70%) ($CHCl_3$–MeOH, 9 1 and crystallization in Me_2CO) and **4** (10%) ($CHCl_3$–MeOH, 4 1) The product of reduction with Zn was chromatographed on silica gel, obtaining **1** (31 1% $CHCl_3$–MeOH, 19 1) and **3** (67, 1%, $CHCl_3$–MeOH, 9 1) showing that **1** is found free and in the form of *N*-oxide **4**

S adonidifolius was collected at Puerto de Villatoro close to the municipality of Villanueva del Campillo (Avila, Spain). It was allowed to dry in air and was extracted with EtOH at room temp. The EtOH extract (7 03%) was fractionated by the above procedure, obtaining a fraction of free bases (0 56% with respect to the ethanolic extract) and the product of reduction with Zn (0 60% with respect to the EtOH extract)

The free-base fraction was chromatographed on silica gel, obtaining **5** (10 4%) ($CHCl_3$–MeOH, 19 1 and later chromatography on neutral alumina of act I), and **6** (52 0%) ($CHCl_3$–MeOH, 9 1 and crystallization in Me_2CO) The product of reduction with Zn was chromatographed on silica gel, isolating **5** and **6** in approximately the same proportions as in the free-base fraction, from this it is inferred that they are not found in the form of the *N*-oxide

Oxidation of **1** *with* meta-*chloroperbenzoic acid.* A soln of 0 029 g of *m*-chloroperbenzoic acid in 1 ml of $CHCl_3$ was slowly added to 56 4 mg of **1** in 5 ml of $CHCl_3$, cooled with ice The mixture was stirred for 3 hr, during which it warmed to room temp Following this, it was passed through a column of basic alumina (20 fold the weight of the product) and eluted with a mixture of $CHCl_3$–MeOH (9·1), thus providing a mixture of **1** and **4** which was separated by chromatography on silica gel, obtaining 30 mg of **4** ($CHCl_3$–MeOH, 4 1)

12,13,19-*Trihydroxy*-15,20-*epoxy*-15,20-*dihydro,* (12S,15R,20R) *senecionan*-11,16-*dione* (**6**) Mp = 200° $[\alpha]_D = +84\,74°$ (MeOH, *c* 0 78%) IR ν_{max}^{KBr} cm^{-1} 1750, 1260, 1140, 1005, 990, 962, 836 and 820 ^{1}H NMR and ^{13}C NMR (Tables 1 and 2) (Found C, 56.37, H, 6 43; N, 3 58, $C_{18}H_{25}NO_8$, requires C, 56 39, H, 6 53, N, 3 65%)

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