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Obuya Were, Michael Benn, and Raphael M. Munavu

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PYRROLIZIDINE ALKALOIDS FROM *SENECIO HADIENSIS*

OBUYA WERE, MICHAEL BENN,*

Department of Chemistry, The University, Calgary, Alberta T2N 1N4, Canada

and RAPHAEL M. MUNAVU

Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

ABSTRACT.—Rosmarinine, 12-*O*-acetylrosmarinine [**6**], neorosmarinine [**3**], hadiensine (1α-hydroxyplatyphylline) [**2**], 12-*O*-acetylhadiensine [**4**], 12-*O*-acetylneohadiensine [**5**], and petitianine (2α-hydroxy-1,2-dihydroretrorsine) [**7**] were isolated from *Senecio hadiensis*. The alkaloids **2–7** have not been described before.

Senecio hadiensis Forsk. (syn. *Senecio petitianus* A. Rich., Compositae) is a trailing climber, common along the edges of the forests of the interior of Kenya. Some use of the plant has been made in folk medicine (1), and local belief has associated ingestion of the leaves with liver-fluke in cattle (2). As the genus *Senecio* is well known as a source of pyrrolizidine alkaloids, several of which are hepatotoxic (3), we wondered if such substances were present in *S. hadiensis*, i.e., whether the fancied association with liver-fluke was a distortion of a genuine hepatotoxicity. We therefore undertook an examination of *S. hadiensis* with the following results.

RESULTS AND DISCUSSION

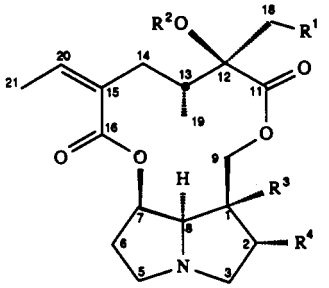
Conventional processing of the air-dried epigeal parts of *S. hadiensis*, including a reductive workup to convert any *N*-oxides to tertiary bases, resulted in the isolation of a mixture of alkaloids (ca. 1.1%) which was shown by glc and tlc analysis to consist of at least nine components. Fractionation of this mixture largely by vacuum cc and preparative tlc (see Experimental) resulted in the isolation of seven alkaloids **1–7**, homogeneous or very nearly so by tlc and glc.

One of the major alkaloids **1**, (ca. 34% of the total alkaloids) was identified as rosmarinine on the basis of eims, ^{1}H- and ^{13}C-nmr data (see Experimental and Tables 1 and 2), and its mp and [α]D, but the others do not appear to have been described before.

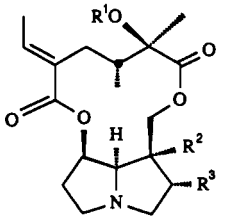
Alkaloid **2** (ca. 52% of the total alkaloids) was obtained as a gum, homogeneous by tlc and glc analysis, from which a crystalline perchlorate salt was prepared. The eims of the base revealed an apparent molecular ion at *m/z* 353 with a composition $C_{18}H_{27}NO_6$, isomeric with rosmarinine, as established by a high resolution measurement. Of particular value were the ^{1}H- and ^{13}C-nmr spectra of **2** (Tables 1 and 2). Inter alia, the former contained prominent absorptions which could be attributed to the structural units Me—$\underset{|}{\overset{|}{C}}$H—, (*Z*)MeCH=$\underset{|}{\overset{|}{C}}$—$CO_2$—, Me—$\underset{|}{\overset{|}{C}}$—O—, $\underset{|}{\overset{|}{C}}$H—OOC—, —C—$CH_AH_B$—OOC—, while being devoid of the vinylic-H absorption (at ca. 6 ppm) which characterizes the 1,2-didehydronecines, such as senecionine [**8**]. The ^{13}C-nmr spectrum contained signals corresponding to 18 magnetically non-equivalent atoms, of which two appeared to be ester carbonyls, two belonged to a trisubstituted ethylene, two to methyls, one to a methine attached to a nitrogen, and three to carbons attached to oxygen (these being a methylene, a methine, and quaternary carbon).

Given the traditional association of *Senecio* with pyrrolizidine alkaloids (3,4), these data suggested to us that **2** had the structure shown, in which the stereochemistry at C-1, -7, -8, -12, and -13 remained to be decided. Saponification of **2** yielded a di-acid which was found to be identical to senecic acid [**9**], as well as the necine **10** which we

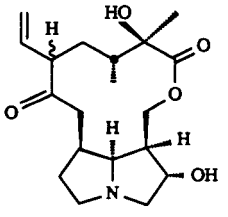
have called hadienecine. This established the stereochemistry at C-12 and -13, and confirmed the *Z* stereochemistry of the ethylidene unit in **2**. Furthermore, dehydration of **2** gave senecionine [**8**]: a transformation which additionally yielded the stereochemistry at C-7 and -8 and left only that at C-1 undecided. As we were unable to obtain crystals of salts of **2** suitable for X-ray crystallographic analysis, we attempted to determine this by nOe measurements using **11**, the *O*-acetyl derivative of **2**. Irradiation of the methyl protons of **11** gave no observed enhancement of the signals due to H-7 or -8. However, in the ^{1}H-nmr spectrum of **11** the signal due to H-7 is shifted downfield by 0.25 ppm, as compared to its position in **2**. We attribute this to a deshielding effect of the acetate carbonyl, which requires a syn relationship between H-7 and the acetate (the carbonyl being oriented into the fold of the bicyclo system), and thus indicates the 1α oxygenation shown in **2**.



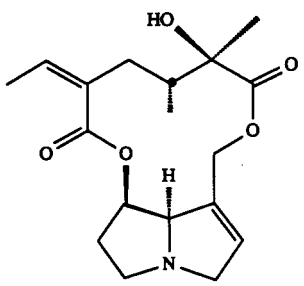
1 $R^1=R^2=R^3=H$, $R^4=OH$
2 $R^1=R^2=R^4=H$, $R^3=OH$
4 $R^1=R^4=H$, $R^2=Ac$, $R^3=OH$
6 $R^1=R^3=H$, $R^2=Ac$, $R^4=OH$
7 $R^1=OH$, $R^2=R^3=H$, $R^4=OH$
11 $R^1=R^2=R^4=H$, $R^3=OAc$
14 $R^1=R^4=H$, $R^2=Ac$, $R^3=OAc$
15 $R^1=R^2=R^3=H$, $R^4=OAc$
16 $R^1=R^3=H$, $R^2=Ac$, $R^4=OAc$



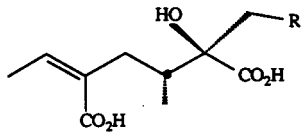
3 $R^1=R^2=H$, $R^3=OH$
5 $R^1=Ac$, $R^2=OH$, $R^3=H$



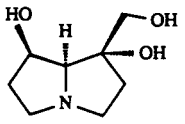
13



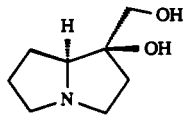
8



9 R=H
17 R=OH



10



12

Among the multitude of pyrrolizidine alkaloids described to date (3,4) the only other 1-hydroxypyrrolizidine alkaloid which appears to have been reported before is curassanecine, with the suggested but unproven stereochemistry shown in **12** (5). Given this rarity, we were concerned that **2** might be an artifact produced by a reductive ring-opening of a 1,2-α-epoxy precursor during the isolation of the alkaloids. We therefore repeated the isolation of the alkaloids from *S. hadiensis* omitting the reductive step. Alkaloid **2** was again obtained as a major component of the mixed bases, and accordingly we conclude that it is a genuine natural product which we have named hadiensine.

Alkaloid **3** (ca. 5% of the total bases) was also isomeric with rosmarinine, and the only striking difference between the two alkaloids was that in the ^{1}H-nmr spectra of **3** the resonance for the vinylic proton was at δ 6.51 ppm, as compared to δ 5.78 ppm in the spectrum of **1**. This suggested (6) that **3** was the *E* isomer of **1**. Consistent with this, in **3** the ^{13}C resonance for C-14 (δ 30.8) was shifted upfield by 8.8 ppm as compared to its position in the ^{13}C-nmr spectrum of rosmarinine [**1**] (δ 39.6). Compare the corresponding positions of this signal in senecionine [**8**] (δ 38.3) and its *E* isomer, intergerrimine (δ 29.6) (7,8). Indeed, photolysis of rosmarinine generated a mixture of **1**, **3**, and a third substance which appeared to be another expected intramolecular rearrangement product **13**. In accord with the terminology used for other pairs of geometrically isomeric pyrrolizidine alkaloids, we have named **3** neorosmarinine.

Alkaloid **4** (ca. 3% of the total bases) was quickly recognized to be 12-*O*-acetylhadiensine. Thus, the apparent molecular ion in the eims of **4** was established by high-resolution measurements to have the composition $C_{20}H_{29}NO_7$, while the ^{1}H-nmr spectrum of **4** closely resembled that of hadiensine except for an additional resonance attributable to an acetate (Table 1), and the ^{13}C-nmr spectrum similarly resembled that of **2**, with additional resonances for the acetate and shifts for C-12 and -13 corresponding to attachment of the acetoxy function to C-12 (Table 2). Acetylation of **4** gave the same diacetate as that obtained from **11**, i.e., 1,12-di-*O*-acetylhadiensine [**14**].

Alkaloid **5** (ca. 1% of the total bases) appeared to be isomeric with **4**, but in its ^{1}H-nmr spectrum the vinylic H resonance appeared at δ 6.78 ppm (as compared to δ 5.89 in **4**) while in its ^{13}C-nmr spectrum the C-14 resonance appeared at δ 29.6 ppm as compared to δ 38.1 in **4**, suggesting that **5** was the *E* isomer. This was confirmed by photolysis of **4** to give a ca. (1:1) mixture of **4** and **5**. We have named **5** 12-*O*-acetylneohadiensine.

Alkaloid **6** (ca. 0.5% of the bases) corresponded in composition and spectroscopic properties to an acetate of rosmarinine. Comparison of its properties with those of 2-*O*-acetylrosmarinine [**15**] revealed that these were different compounds and thus required that **6** be the 12-*O*-acetyl compound, a deduction which was also indicated by a comparison of the ^{13}C-nmr spectra of **1** and **6** (see Table 2, and note the shift differences for C-12 and -13). When acetylated, both **1** and **6** gave the same diacetate **16**. Thus **6** was 12-*O*-acetylrosmarinine.

Alkaloid **7** (ca. 0.5% of the total bases) was as before (hreims and ^{1}H- and ^{13}C-nmr data) deduced to have the composition $C_{18}H_{27}NO_7$. In contrast with the other alkaloids **1–6** there was no signal in the ^{1}H-nmr spectrum of **7** corresponding to a methyl group attached to a quaternary carbon carrying an oxygen substituent. We therefore surmised that C-18 was hydroxylated, and this was supported by methylene ^{13}C- and ^{1}H-nmr absorptions, at δ 68.5 and ca. 3.62 (AB quartet) respectively. Assuming that the necic acid portion of the alkaloid was not further hydroxylated, the necine was therefore deduced to be saturated, and a triol, perhaps rosmarinecine. This tentative identification of the alkaloid as **7** was confirmed by its saponfication, which yielded

TABLE 1. ^{1}H-nmr Data for Rosmarinine [1], Hadiensine [2], Petitianine [7], and Derivatives.[a]

Proton	Compound					
	1	2	3	4	5	6
H-1	2.50 m	—	2.47 dq (1.1, ca. 7.5)	—	—	2.45 m
H-2a	4.23 m	2.21 m	4.20 q (7.3)	ca. 2.08 m	1.95 m	4.31 q (ca. 8)
H-2b	—	2.19 m	—		1.85 m	—
H-3a	3.07 dd (7.6, 11.4)	3.27 m	3.01 dd (7.2, 11.2)	3.15 m	3.15 ddd (6.5, 6.5, 10.6)	3.09 dd (7.4, 11.1)
H-3b	2.92 dd (8.1, 11.1)	2.78 m	2.92 dd (7.3, 11.2)	2.84 m	2.87 ddd (2.6, 7.2, 10.7)	2.91 dd (8, 11.1)
H-5a	3.27 m	3.11 m	3.20 ddd (2.5, 8, 10)	3.02 m	3.01 m	3.30 t (8.7)
H-5b	2.58 m	2.72 m	2.59 ddd (6.6, 10, 10)	2.78 m	2.77 ddd (4.1, 10, 11.5)	ca. 2.5 m
H-6a	ca. 2.0 m	2.19 m	2.09 m	2.26 m	2.24 m	2.30 dd (6.4, 14)
H-6b	2.26 m		2.26 m	2.08 m	ca. 1.90 m	2.05 m
H-7	5.05 m	5.30 q (4.9)	5.10 m	5.30 q (ca. 5)	5.45 q (ca. 6.3)	4.98 m
H-8	3.55 dd (3.2, 7.9)	3.41 d (4.9)	3.65 dd (3.9, 7.6)	3.31 d (4.9)	3.38 d (6.1)	3.56 dd (3.1, 7.6)
H-9a	4.90 dd (5.2, 12.6)	4.57 d (11.6)	4.75 dd (5.9, 12.3)	4.41 d (11.6)	4.41 (11.4)	4.88 dd (5, 12)
H-9b	4.12 d (12.6)	4.04 d (11.6)	4.09 dd (1.1, 12.3)	4.17 d (11.6)	4.15 (11.4)	4.10 dd (ca. 1, 12)
H-13	ca. 1.8 m	ca. 1.95 m	ca. 2.0 m	1.83 m	ca. 2.10 m	ca. 1.72 m
H-14a	1.94 dd (9.6, 13.2)	ca. 1.97 m	2.32 dd (8.4, 13.9)	2.53 dd (4.5, 14.3)	2.41 dd (6.2, 14.2)	ca. 2.5 m
H-14b	2.26 m		2.18 dd (4.2, 13.9)	ca. 2.05 m	2.28 dd (7.2, 14.2)	1.91 dd (9.8, 14)
H-18	1.34 s	1.32 s	1.33 s	1.72 s	1.76 s	1.72 s
H-19	0.97 d (6.7)	0.97 d (6.8)	0.99 d (6.8)	1.01 d (6.8)	1.05 d (6.8)	0.99 d (6.8)
H-20	5.78 q (7.1)	5.88 q (7.1)	6.51 q (7.1)	5.89 q (7.1)	6.78 q (7.1)	5.79 dq (1.1, 7.1)
H-21	1.84 d (7.1)	1.83 d (7.1)	1.78 d (7.1)	1.89 d (7.1)	1.80 d (7.1)	1.86 dd (1.5, 7.1)
Ac				2.10 s	2.13 s	2.11 s

TABLE 1. (Continued)

Proton	Compound					
	11	**14**	**15**	**16**	**7**[b]	**10**[c]
H-1 . . .	—	—	2.68 m	ca. 2.7 m	2.43 m	—
H-2a . . .	2.49 m	ca. 2.40 m	5.06 ddd (5,6.8,6.8)	5.19 m	4.23 m	2.70 ddd (9,12,12)
H-2b . . .	2.35 m		—	—	—	ca. 2.10 m
H-3a . . .	ca. 2.46 m	ca. 2.83 m	3.08 dd (5.1,11.3)	3.16 m	ca. 2.94 m	4.21 ddd (7.2,11,11)
H-3b . . .	ca. 2.8 m		2.91 (6.8,11.3)	2.92 dd (5.3,11.2)		3.23 t (10)
H-5a . . .	ca. 2.46 m	ca. 2.83 m	ca. 3.14 m	ca. 3.18 m	3.19 m	4.10 m
H-5b . . .	ca. 2.80 m		ca. 2.69 m	2.94 m	2.63 m	3.37 m
H-6a . . .	2.13 m	ca. 2.39 m	ca. 2.23 m	ca. 2.22 m	2.68 m	
H-6b . . .	ca. 2.47 m	ca. 2.08 m			2.21 m	ca. 2.05 m
H-7 . . .	5.55 ddd (3.3,4.9,4.9)	5.45 m	5.19 q (ca. 4)	5.19 m	5.23 m	4.85 brs
H-8 . . .	3.38 d (4.9)	3.51 d (4.1)	3.60 dd (4.2,7.2)	3.59 dd (4.2,7.9)	3.72 dd (4.7,7.8)	4.50 d (3.2)
H-9a . . .	4.63 d (11.7)	4.63 d (12.2)	4.69 dd (7.6,11.8)	4.49 dd (7.8,12)	4.78 dd (7.5,12)	4.56 d (11)
H-9b . . .	4.42 d (11.7)	4.35 d (12.2)	4.00 dd (1.7,11.8)	4.21 dd (1.5,12)	4.10 dd (1.9,12)	4.37 d (11)
H-13 . . .	ca. 2.0 m	ca. 1.84 m	1.90 m	ca. 1.7 m	2.02 m	
H-14a . .	ca. 2.30 m	2.63 dd (5.2,14.4)	2.26 m	ca. 2.7 m	2.34 dd (4.1,13)	
H-14b . .	ca. 2.26 m	ca. 2.08 m	2.12 dd (7.1,13.9)	2.01 dd (7.3,14.4)	2.07 dd (7.7,13)	
H-18 . . .	1.25 s	1.65 s	1.29 s	1.66 s	3.64 d (11.2); 3.60 d (11.2)	
H-19 . . .	0.98 d (6.8)	1.03 d (6.8)	0.96 d (6.8)	1.01 d (6.8)	0.89 d (6.6)	
H-20 . . .	5.85 q (7.2)	5.87 q (7.1)	5.83 q (7.1)	5.85 q (7.1)	5.91 q (7.2)	
H-21 . . .	1.83 d (7.2)	1.88 d (7.1)	1.82 d (7.1)	1.87 d (7.1)	1.83 d (7.2)	
Ac	2.01 s	2.09 s, 2.02 s	2.08 s	2.08 s × 2		

[a]Chemical shifts (δ) are in ppm, the coupling constants in parentheses are in Hz (± 0.2).
[b]In CD_3OD (reference CHD_2OD δ 3.31).
[c]Reference pyridine-H δ 7.19.

TABLE 2. ^{13}C-nmr Data for Rosmarinine [**1**], Hadiensine [**2**], Petitianine [**7**], and Derivatives.[a]

Carbon	Compound											
	1	**2**	**3**	**4**	**5**	**6**	**11**	**14**	**15**	**16**	**7**[b]	**10**[c]
C-1	49.1	79.0	49.0	78.7	79.1	49.3	83.8	84.6	45.0	44.3	50.0	81.9
C-2	69.1	39.2	68.8	39.5	39.1	69.1	40.0	36.1	74.3	74.8[d]	72.8	36.9
C-3	61.3	53.6	60.6	52.7	53.0	61.2	50.4	50.9[d]	59.5	60.0	63.0	54.3
C-5	53.4	52.1	53.1	51.5	51.8	53.5	50.4	51.0[d]	52.3	52.4	53.6	54.3
C-6	34.4	34.5	34.1	35.0	33.4	34.3	36.4	38.9	35.2	34.6	34.9	35.7
C-7	75.1	73.3	75.0	73.3	73.3	75.6	71.5	73.0	75.6	74.5[d]	76.4	69.7
C-8	69.3	77.0	71.5	77.7	76.9	69.4	76.2	75.9	68.3	68.2	69.1	80.7
C-9	62.1	67.7	63.2	67.9	67.5	61.7	66.2	66.4	63.1	61.6	63.6	65.2
C-11	180.6	178.4	180.2	170.7	170.5	173.4	177.9	170.5	178.5	171.4	177.2	
C-12	77.5	76.2	76.8	83.2	83.1	84.5	75.2	82.5	76.2	83.0	82.2	
C-13	37.9	37.2	38.3	40.6	41.0	40.7	37.3	40.7	37.3	40.5	36.2	
C-14	39.6	38.4	30.8	38.1	29.6	38.7	39.0	37.4	39.2	37.8	40.2	
C-15	132.7	131.6	133.3	131.6	131.4	132.7	131.6	131.8	132.1	132.2	133.2	
C-16	167.5	167.5	168.5	167.5	167.7	167.4	167.6	167.3	167.6	167.5	169.0	
C-18	25.7	26.2	26.1	22.7	22.6	22.1	26.0	22.3	26.0	22.2	68.5	
C-19	11.7	13.0	12.9	13.5	14.0	12.0	14.2	14.4	13.0	13.5	13.2	
C-20	134.7	136.2	135.9	136.9	139.1	134.7	135.7	136.2	135.2	135.7	136.7	
C-21	15.1	15.5	14.2	15.5	14.5	15.1	15.6	15.6	15.3	15.4	15.5	
CO				169.8		170.0	169.6	169.8 × 2	170.7	170.8,170.0		
Me				21.5	21.6	21.6	21.4	21.5,21.4	20.9	21.4,20.9		

[a]Chemical Shifts (δ) are in ppm.
[b]In CD_3OD (reference δ 49.0).
[c]In pyridine-d_5 (reference δ 123.5).
[d]May be interchanged.

isatinecic acid [**17**] and rosmarinecine. So **7** is indeed 2α-hydroxy-1,2-dihydroretrorsine, which we have called petitianine.

The most striking feature of these new alkaloids is the presence of the 1-hydroxy functionality in **2**, **4**, and **5**. In the apparent absence of any pyrrolizidin-1-enes among the alkaloids, it is tempting to speculate that the 1α-hydroxy system arises via monooxygenase hydroxylation of the corresponding platynecine esters. It will be interesting to see if hadiensine can serve as a precursor of senecionine in plants capable of forming that alkaloid.

The hepatotoxicity of pyrrolizidine alkaloids appears to be due to esters of unsaturated necines (3), none of which were detected in our study of *S. hadiensis*. Thus we conclude that the alkaloids of this plant are unlikely to be responsible for liver damage to cattle.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler apparatus and are uncorrected. The eims were measured with Kratos MS-80 and VG-7070 instruments. Infrared spectra were recorded with a Nicolet DX-1 system, with the samples dispersed in KBr discs or as solutions in $CHCl_3$. The ^{1}H- and ^{13}C-nmr spectra were collected using Bruker AM-400 and ACE-200 spectrometers. Unless otherwise indicated, the samples were dissolved in $CDCl_3$, and for the ^{13}C spectra the solvent resonance (δ 77.0 ppm) was used as an internal reference, while for the ^{1}H spectra the residual $CHCl_3$ signal (δ 7.27 ppm) of the solvent absorption was used for the same purpose. The numbers of H atoms attached to individual carbons were determined using the Bruker Instrument DEPT microprogram, and the same company's COSY and XHCORR software were used for ^{1}H,^{1}H and ^{1}H,^{13}C 2D-correlation spectroscopy. Optical rotations were measured with a Rudolph Instrument Autopol III polarimeter. The gc analyses were performed with an HP-5890 chromatograph fitted with a flame ionization detector (fid) and DB-1 megabore capillary column (30 m × 0.53 mm i.d. × 1.5 μ film thickness). A temperature program was used which consisted of 1 min at 200° followed by a 10°/min increase to 240°, which was then maintained for the duration of the analyses. Nitrogen was used as the carrier gas at a flow rate of 24 ml/min. The percent values given after Rt's are those obtained from the fid, i.e., from the detector uncalibrated for variations in sensitivity to individual alkaloids. Centrifugally accelerated radial chromatography (carc) was carried out with a Harrison Research Chromatotron using a rotor coated with Si gel 60 containing $CaSO_4$

(Merck 7749). For both tlc and preparative tlc Si gel 60 F254 plates (Merck) were used, with a layer thickness of 0.25 mm. Vacuum short column chromatography (vscc) (9) was also carried out on tlc grade Si gel 60 F254 (Merck). Photolyses were carried out using a Southern New England UV Rayonet Reactor fitted with 254 nm lamps, using quartz tubes to contain the solutions.

PLANT MATERIAL.—The epigeal parts of flowering *S. hadiensis* were collected at the forest margin along the top of the Ngong Hills, near Karen, Kajiado district, Kenya in June 1988 and July 1989. A voucher specimen is deposited in the Herbarium of the University of Calgary. The plants were air-dried, and pulverized.

ISOLATION OF THE ALKALOIDS.—*With reductive processing.*—The powdered plant material (1.24 kg) was first defatted by soaking in hexanes (4 liters) and, after filtration and brief drying in air, was transferred to a Waring blender (1 gal) and extracted by repeated maceration in 95% EtOH (10 × 4 liters). The combined EtOH extracts were then processed as described previously (10) to yield the alkaloids as a light brown glass (14.1 g, 1.1%). A tlc analysis [$CHCl_3$-MeOH-NH_4OH (50:10:1)] of this revealed five components, R_f = 0.37, 0.33, 0.27, 0.12, 0.07, while gc showed nine Rt's: 8.7 (**2**, 52%), 9.2 (3%), 10.0 (1%), 10.6 (**1**, 34%), 11.8 (**3**, 5%), 12.4 (**4**, 3%), 13.8 (**5**, 1%), 14.5 (**6**, 0.5%), 17.5 min (**7**, 0.5%).

Without reductive processing.—Powdered plant (1.08 kg) was defatted with hexanes and extracted with 95% EtOH as above. The processing of the EtOH extracts differed from that previously described (10) only in that the treatment with aqueous H_2SO_4 and Zn dust was omitted. Analysis of the alkaloids so obtained, a light brown glass (2.3 g, 0.2%), by tlc and gc again revealed nine components chromatographically indistinguishable from those described above but with some quantitative variations in their relative amounts: e.g., Rt's 8.7 (**2**, 45%), 9.2 (3%), 10 (1%), 10.6 (**1**, 40%), 11.8 (**3**, 3%), 12.4 (**4**, 6%), 13.8 (**5**, 1%), 14.5 (**6**, 1%), 17.5 min (**7**, 0.5%).

SEPARATION OF THE INDIVIDUAL ALKALOIDS.—The mixed alkaloids were resolved into the individual components by preliminary fractionation using vscc [$CHCl_3$-MeOH-NH_4OH, 10:50:1)→(20:50:1)→(30:50:1) and finally MeOH] or carc [$CHCl_3$-MeOH-NH_4OH (100:20:1)]. The fractions obtained were analyzed by tlc and gc and pooled accordingly before being subjected to preparative tlc to yield the alkaloids.

Rosmarinine [**1**].—Mp 208–209° from Me_2CO [lit. (11) 203–204°, lit. (12) 209°]; $[\alpha]_D$ −121° (c = 4, $CHCl_3$) [lit. (11) −120° (c = 1, $CHCl_3$)]; ^1H nmr see Table 1, ^{13}C nmr see Table 2 and (13); ir ν max (KBr) 1719, 1743, 3408 (br s) cm^{-1}; eims *m/z* (rel. int. %) $[M]^+$ 353 (1), 156 (18), 154 (28), 138 (38), 98 (16), 83 (12), 82 (43), 81 (18), 44 (100), 43 (68).

Hadiensine [**2**].—Obtained as a colorless gum: perchlorate salt mp 279–280° from EtOH/MeOH; $[\alpha]_D$ ($HClO_4$ salt) −83 ± 1° (c = 1.6, MeOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2; ν max ($HClO_4$ salt) 3483 (br s), 3081 (m), 2987 (m), 2852 (m), 1732 (s), 1640 (w), 1455 (m), 1378 (m), 1343 (w), 1326 (w), 1320 (w), 1301 (w), 1250 (m), 1227 (m), 1146 (s), 1110 (s), 1053 (m), 1020 (m), 1002 (s), 996 (w), 930 (m), 907 (w), 853 (w), 876 (w), 833 (w), 811 (w), 796 (w), 782 (w), 763 (w), 626 (m); eims *m/z* (%) $[M]^+$ 353.1829 (13) ($C_{18}H_{27}NO_6$ requires 353.1839), 338 (10), 336 (12), 282 (18), 180 (15), 156 (8), 154 (12), 138 (22), 137 (82), 98 (18), 82 (100), 68 (14), 55 (26), 53 (15), 44 (32), 43 (66), 41 (30).

SAPONIFICATION OF HADIENSINE.—A solution of **2** (67 mg), and $Ba(OH)_2 \cdot 8H_2O$ (300 mg) in H_2O (10 ml) was boiled under reflux for 5 h. After the solution had cooled to room temperature, CO_2 was bubbled in until no further precipitation occurred. The mixture was filtered, and the filtrate was acidified with aqueous H_2SO_4 and extracted continuously with Et_2O. Evaporation of the Et_2O extract afforded a solid (30 mg) which was recrystallized from Et_2O to give a product, mp 147–149°, whose ir, ^1H- and ^{13}C-nmr spectra, and $[\alpha]_D$ were the same as those of a sample of senecic acid [**9**] prepared by a similar saponification of rosmarinine.

The aqueous solution remaining after the Et_2O extraction was passed through a column of Dowex-3X anion exchange resin (20–50 mesh, OH form), and the column was washed with H_2O. Evaporation of the eluates under reduced pressure gave a solid which was extracted with Me_2CO/MeOH. Evaporation of these extracts gave a crystalline product which was recrystallized (Me_2CO/MeOH) to give **10** (1.2 mg) as colorless crystals: mp 156–158°; ν max (KBr) 3360 (br s), 2934 (s), 2792 (w), 2657 (w), 1419 (m), 1407 (m), 1395 (m), 1261 (m), 1229 (m), 1206 (m), 1157 (m), 1143 (m), 1100 (s), 1041 (s), 1025 (s), 1000 (m), 977 (m), 960 (m), 884 (m), 803 (br m); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 173.1058 (5) (calcd for $C_8H_{15}NO_3$, 173.1051), 155 (11), 129 (6), 112 (7), 99 (66), 98 (100), 82 (80), 70 (10), 68 (8), 57 (8), 56 (12), 55 (11), 42 (23), 41 (25).

CONVERSION OF HADIENSINE TO SENECIONINE.—To a solution of **2** (15 mg) in pyridine (2 ml) was added MsCl (40 μl), and the mixture was boiled under reflux for 30 min. After removing excess re-

agents under reduced pressure the residue was partitioned between aqueous Na_2CO_3 (ca. 5 ml) and $CHCl_3$ (10 × 5 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield a solid residue judged, by tlc and gc analysis, to be a mixture of **2** and **8**. Separation by preparative tlc [$CHCl_3$-MeOH-NH_4OH, (50:10:1)] resulted in the isolation of recovered **2** (5.6 mg) and **8** (3.9 mg). The latter was obtained as colorless crystals, mp 229–230° from EtOH, whose ir spectrum was superimposable upon that of authentic senecionine. The eims and ^1H and ^{13}C spectra were also identical with those of senecionine.

1-*O*-ACETYLHADIENSINE [**11**].—A solution of **2** (30 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was stirred for 14 h at room temperature and worked up in the usual way to afford **11** as colorless crystals (22.5 mg): mp 142–144°, $[\alpha]D$ −67° (c = 0.5, EtOH); ν max (KBr) 3423 (br w), 2968 (m), 1740 (s), 1729 (s), 1463 (m), 1457 (m), 1376 (m), 1367 (m), 1251 (s), 1242 (s), 1217 (s), 1163 (m), 1148 (s), 1115 (s), 1097 (m), 1058 (m), 1021 (m); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 395.1948 (0.5) (calcd for $C_{20}H_{29}NO_7$, 395.1945), 380 (0.3), 378 (0.6), 220 (10), 180 (18), 138 (35), 136 (30), 121 (100), 99 (47), 82 (70), 55 (27), 43 (72), 41 (30).

12-*O*-ACETYLHADIENSINE [**4**].—A colorless gum: ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 395.1938 (0.2) (calcd for $C_{20}H_{29}NO_7$, 395.1945), 336 (0.6), 308 (0.5), 290 (0.8), 270 (1), 252 (2), 226 (3), 180 (20), 138 (23), 137 (72), 136 (20), 125 (17), 117 (70), 106 (31), 104 (26), 90 (56), 89 (43), 82 (50), 67 (31), 60 (62), 55 (45), 45 (75), 44 (60), 43 (100), 41 (57).

1,12-DI-*O*-ACETYLHADIENSINE [**14**].—*From* **2**.—A mixture of hadiensine (30 mg), anhydrous NaOAc (15 mg), and Ac_2O was boiled under reflux overnight and evaporated under reduced pressure. The residue was partitioned between aqueous Na_2CO_3 [4 ml, pH ca. 10 (indicator paper)] and $CHCl_3$ (5 × 5 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was subjected to preparative tlc [$CHCl_3$-MeOH-NH_4OH (10:50:1)] to afford **14** (4.9 mg) with ^1H- and ^{13}C-nmr as in Tables 1 and 2.

From hadiensine 12-O-acetate.—A solution of **4** (ca. 5 mg) in pyridine (0.1 ml) and Ac_2O (0.1 ml) was stirred overnight at room temperature. The reaction mixture was then processed in the usual way to afford the diacetate **14** (ca. 5 mg), with spectroscopic properties identical to those of the material obtained from compound **2** as described above.

NEOROSMARININE [**3**].—A gum: $HClO_4$ salt mp 205–207° from EtOH, $[\alpha]D$ ($HClO_4$ salt) −12° (c = 0.6, EtOH), ν max (KBr) ($HClO_4$ salt) 3425 (br s), 3072 (w), 2973 (m), 2931 (m), 1727 (s), 1637 (w), 1280 (s), 1225 (s), 1147 (s), 1111 (s), 1089 (4), 627 (s) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 353.1829 (2) (calcd for $C_{18}H_{27}NO_6$, 353.1838), 282 (1), 242 (1.5), 227 (6), 156 (30), 154 (40), 138 (60), 122 (20), 112 (15), 111 (17), 98 (25), 82 (75), 68 (15), 55 (27), 54 (15), 53 (20), 43 (100), 41 (39).

PHOTOLYSIS OF ROSMARININE.—Rosmarinine (15.9 mg) in EtOH (1.5 ml) was irradiated (Rayonet reactor) for 2.75 h. Analysis of the reaction product by gc revealed three components (two of which corresponded to rosmarinine and neorosmarinine), while two were discernible by tlc. Separation by preparative tlc [Et_2O-MeOH-NH_4OH (160:19:5)] gave rosmarinine (2 mg) and a mixture (10 mg) whose ^1H and ^{13}C were consistent with its being a ca. 3:2 mixture of neorosmarinine [**3**] and **13** (14).

12-*O*-ACETYLNEOHADIENSINE [**5**].—This compound was obtained as a pale yellow oil: eims *m/z* (%) $[M]^+$ 395.1948 (1), 336 (3), 308 (2), 234 (31), 180 (37), 154 (23), 153 (22), 138 (45), 137 (89), 136 (20), 117 (28), 83 (23), 82 (73), 81 (20), 67 (15), 43 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

PHOTOLYSIS OF 12-*O*-ACETYLHADIENSIENE.—A solution of **4** (10 mg) in EtOH (1.3 ml) contained in a quartz tube was irradiated with 254 nm light for 150 min. The solution was then concentrated under reduced pressure and the residue subjected to preparative tlc on alumina [Merck Type E, F254, $CHCl_3$-MeOH-NH_4OH (95:4:1)] to give a substance (2 mg), with ^1H and ^{13}C nmr spectroscopic properties identical with those of **5**.

2-*O*-ACETYLROSMARININE [**15**].—A solution of **1** (20 mg) in Ac_2O (0.2 ml) and pyridine (0.2 ml) was stirred and kept at room temperature for 24 h. Excess reagents were removed under high vacuum and the residue was positioned between aqueous Na_2CO_3 (3 ml) and $CHCl_3$ (5 × 5 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield colorless crystals of **15** (17 mg): mp 153–154°, $[\alpha]D$ −113° (c = 1.8, MeOH); ν max (KBr) 3452 (br w), 2964 (m), 2935 (m), 2896 (w), 1741 (s), 1713 (s), 1465 (w), 1449 (w), 1370 (m), 1250 (s), 1217 (s), 1160 (s), 1149 (s), 1115 (s), 1102 (m), 1050 (m), 1042 (m), 1019 (w), 975 (w), 752 (w); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 395.1948 (18) (calcd for $C_{20}H_{29}NO_7$, 395.1945), 380 (3), 378 (3), 336 (12), 335 (10), 269 (22), 220 (20), 198 (30), 180 (32), 153 (25), 138 (32), 137 (28), 136 (40), 119 (100), 108 (30), 80 (38), 67 (30), 43 (50).

12-*O*-ACETYLROSMARININE [**6**].—A pale amber-colored oil: $[\alpha]_D$ −36° (*c* = 1.8, EtOH), *v* max ($CHCl_3$) 3543 (br m), 2981 (m), 2966 (m), 1750 (s), 1729 (s), 1708 (s), 1448 (m), 1370 (m), 1188 (s), 1164 (s), 1110 (s); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 395.1939 (2) (calcd for $C_{20}H_{29}NO_7$, 395.1945), 336 (1), 270 (1), 226 (18), 154 (40), 138 (28), 117 (10), 111 (10), 98 (12), 86 (15), 84 (25), 82 (22), 55 (18), 44 (100), 43 (78), 41 (20).

2,12-DI-*O*-ACETYLROSMARININE [**16**].—*By acetylation of rosmarinine.*—A stirred solution of **1** (22 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) containing DMAP (5 mg) was kept at 90 ± 10° for 48 h. The dark reaction mixture was cooled to room temperature, poured into excess aqueous Na_2CO_3, and extracted with $CHCl_3$ (5 × 10 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield a dark oil from which **16** (1.5 mg) was isolated by preparative tlc [Et_2O-MeOH-NH_4OH (160:9:5)] as a pale yellow oil: ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 437.2069 (11) (calcd for $C_{22}H_{31}NO_8$, 437.2050), 378 (5), 377 (4), 342 (3), 311 (15), 268 (38), 180 (30), 153 (20), 138 (18), 137 (25), 136 (59), 122 (20), 121 (55), 119 (60), 118 (20), 117 (48), 106 (30), 95 (25), 94 (35), 93 (38), 90 (25), 83 (34), 82 (50), 81 (36), 80 (37), 79 (15), 77 (10), 71 (25), 69 (35), 68 (21), 67 (23), 60 (31), 57 (43), 55 (50), 53 (22), 51 (15), 46 (32), 45 (85), 44 (45), 43 (100), 42 (38), 41 (100).

By acetylation of **6**.—A solution of **6** (2 mg) in pyridine (0.1 ml) and Ac_2O (0.1 ml) was stirred for 8.5 h at room temperature. Workup by addition of aqueous Na_2CO_3 and extraction with $CHCl_3$ (6 × 2 ml) followed by removal of solvent from the dried ($MgSO_4$) extracts gave **16** (1.2 mg) as a pale yellow oil with ^{1}H- and ^{13}C-nmr spectra identical to those of the product obtained by acetylation of rosmarinine.

PETITIANINE [**7**].—A white powder: mp 207–209° from MeOH, $[\alpha]_D$ −60° (*c* = 0.5, MeOH); *v* max (KBr) 3491 (br m), 3259 (br m), 2970 (m), 2939 (m), 2874 (2), 1745 (s), 1717 (s), 1654 (w), 1445 (m), 1373 (w), 1346 (w), 1325 (w), 1305 (w), 1263 (m), 1229 (s), 1206 (s), 1165 (s), 1148 (s), 1126 (s), 1089 (s), 1055 (s), 1000 (m), 966 (w), 926 (w), 907 (w), 750 (w), 572 (w); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 369.1802 (5) (calcd for $C_{18}H_{27}NO_7$, 369.1788), 354 (0.5), 352 (1), 333 (5), 243 (8), 156 (40), 154 (88), 138 (100), 122 (25), 112 (22), 111 (21), 98 (37), 82 (85), 68 (25), 67 (20), 55 (33), 53 (28), 43 (50), 41 (48).

SAPONIFICATION OF PETITIANINE.—Petitianine (10 mg) was added to a solution of $Ba(OH)_2 \cdot 8H_2O$ (115 mg) in H_2O (10 ml), and the mixture was boiled under reflux for 5 h and worked up as described for the saponification of **2**. This resulted in the isolation of isatinecic acid [**17**] (3.9 mg), indistinguishable by ^{1}H and ^{13}C nmr from an authentic sample of that acid obtained from retrorsine (Sigma), and rosmarinecine, similarly identical with the necine from **1**.

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