

A NEW PEPTIDE ALKALOID FROM *CANTHIUM ANORLDIANUM*

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ABSTRACT.—A new peptide alkaloid designated as anorldianine [1] has been isolated from the  $\text{CHCl}_3$  extract of the stem bark of *Canthium anorldianum* and characterized by spectroscopic methods.

*Canthium anorldianum* De Wild & Th. Dur (1) (Rubiaceae) is one of 230 *Canthium* species (2) widely distributed throughout the world. The genus is known for its folkloric applications against a number of ailments such as cough, diabetes, and hypertension (3,4). Previous studies (5–10) on the genus reported the isolation of triterpenes, coumarins, iridoids, and a peptide alkaloid. As part of our contribution to the study of the family Rubiaceae, we now report the isolation and characterization of a new peptide alkaloid named anorldianine [1] from *C. anorldianum*.

## RESULTS AND DISCUSSION

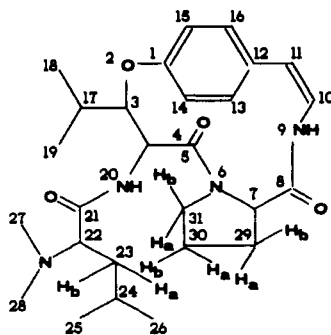
The  $\text{CHCl}_3$  extract of the powdered stem bark of *C. anorldianum* was fractionated by Si gel cc.

Fractions eluted with  $\text{CHCl}_3$  yielded, among other compounds, anorldianine [1] obtained as pinkish crystals from  $\text{Et}_2\text{O}$ . Compound 1 gave a positive reaction with Mayer's reagent, showing that

it was an alkaloid. Elemental analysis and the eims indicated that it had the composition  $\text{C}_{27}\text{H}_{40}\text{N}_4\text{O}_4$ . This structure implied the presence of 10 unsaturations. The ir spectrum displayed strong amide absorption bands at  $\nu_{\text{max}}$  3400 and  $1720\text{ cm}^{-1}$  and  $\text{C}=\text{C}$  bonds at  $1625\text{ cm}^{-1}$ . The uv  $\lambda_{\text{max}}$  (EtOH) 228 nm  $\epsilon$  62400 is consistent with related peptide alkaloids (12).

The  $^{13}\text{C}$ -nmr spectrum (Table 1) of anorldianine [1], which is similar in parts to spectra of peptide alkaloids reported in the literature (11, 12), was well resolved and confirmed the presence of 27 carbon atoms distributed as follows: three carbonyl carbons; eight  $\text{sp}^2$  carbons with one bearing an oxygen atom, one disubstituted and six monosubstituted; and sixteen  $\text{sp}^3$  carbons comprised of six methines, four methylenes, and six methyls.

The  $^1\text{H}$ -nmr spectrum (Table 2) analyzed by the COSY technique led to the presence of two isolated methyl



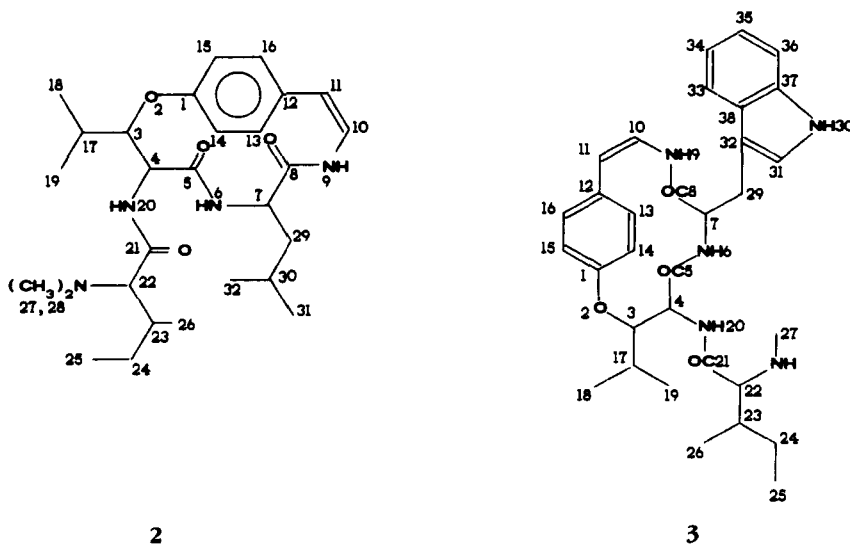


TABLE 1.  $^{13}\text{C}$ -nmr Spectral Data of Anorldianine [1] ( $\text{CDCl}_3$ ), Frangulanine [2] ( $\text{CDCl}_3$ ), and Discarine-I [3] ( $\text{CDCl}_3 + 1\% \text{CD}_3\text{OD}$ ).

Carbon	Compound		
	1	2 <sup>a</sup>	3 <sup>b</sup>
	$^{13}\text{C}$ (50.32 MHz)	$^{13}\text{C}$ (100 MHz)	$^{13}\text{C}$ (100 MHz)
1	157.1 s	156.2	156.2
3	83.9 d	81.6	81.5
4	62.5 d	55.4	55.3
5	172.0 s	171.5	171.6
7	52.3 d	52.7	54.5
8	166.8 s	168.0	168.1
10	131.8 s	131.2	131.2
11	131.4 d	130.2	129.7
12	130.4 d	131.8	136.1
13	120.9 d	122.7	—
14	115.2 d	117.9	—
15	120.8 d	122.0	—
16	125.8 d	125.9	—
17	28.9 d	29.2	29.1
18	23.4 q	20.3	14.7
19	21.8 q	15.1	20.2
21	172.4 q	172.4	172.4
22	68.2 d	73.0	69.0
23	28.3 t	33.9	35.0
24	28.9 d	28.4	25.1
25	14.8 q	12.3	11.6
26	20.4 q	15.3	15.6
27,28	42.3 q	43.0	—
29	23.8 t	40.2	29.6
30	38.5 t	24.5	—
31	46.7 t	22.9	—

<sup>a</sup>Values are from Hennig *et al.* (12).

<sup>b</sup>Values are from Païs *et al.* (13).

TABLE 2.  $^1\text{H}$ -nmr Spectral Data of Compound **1**.<sup>a</sup>

Proton	Chemical shift and multiplicity	Coupling constants (Hz)
3,4 . . . . .	4.85 d	0.7
7 . . . . .	4.24 d	7.4
9 . . . . .	6.13 d	10.7
10 . . . . .	6.66 dd	7.6, 10.4
11 . . . . .	6.36 d	7.6
13 . . . . .	7.11 dd	2.5, 8.2
14 . . . . .	7.00 dd	1.9, 8.2
15 . . . . .	7.07 dd	1.9, 8.5
16 . . . . .	7.18 dd	2.5, 8.5
17 . . . . .	2.00 m	
23a . . . . .	3.90 dt	7.0, 10.1
23b . . . . .	3.32 ddd	8.2, 2.1, 10.1
27,28 . . . . .	2.12 s	
29a . . . . .	2.26 dd	12.0, 5.7
31 . . . . .	2.69 dd	7.8, 6.6

<sup>a</sup>The spectrum was obtained in  $\text{D}_2\text{O}$  at 360 MHz. The  $\delta$  values are in ppm downfield from TMS.

groups borne by a nitrogen atom, one proline moiety and a para disubstituted benzene ring.

Further analysis of the  $^1\text{H}$ -nmr spectrum of **1** by COSY afforded the substructures and fragments shown in Figure 1. These elements, along with the fact that anorldianine [**1**] is an alkaloid having a proline moiety and 10 unsaturations, and comparison with data published for peptide alkaloids (10–12) are consistent with structure **1**.

This formulation was supported by the eims diagnostic fragments A, B, C, D, and E, respectively, at  $m/z$  190, 135, 114, and 97, as shown in Scheme 1. This fragmentation pattern is consistent with the one reported by Hennig *et al.* (12).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting point was taken on a Kofler hot stage equipped with a microscope and is uncorrected. Ir spectrum was recorded on a Perkin-Elmer model 727 B spectrometer.  $^1\text{H}$ -nmr measurement, made in  $\text{D}_2\text{O}$  on a Perkin-Elmer R 12 spectrometer with TMS as internal standard, was determined in  $\text{CDCl}_3$  solution which also provided the lock signal on a Varian XL-100 spectrometer with VFT-100 accessory; the chemical shifts are reported in ppm downfield from TMS.  $^1\text{H}$ - $^1\text{H}$  2D measurement was recorded on a Bruker 200SY instrument ( $^1\text{H}$  200 MHz,  $^{13}\text{C}$  50.32 MHz).

Tlc of compound **1** was run on Merck Si gel 60H. Cc was conducted on Merck silica 60H-254 (70–230 mesh).

PLANT MATERIAL.—Stem bark of *C. anorldianum* was collected on Mount Bokwa Kenden, South West Cameroon, in January 1986.

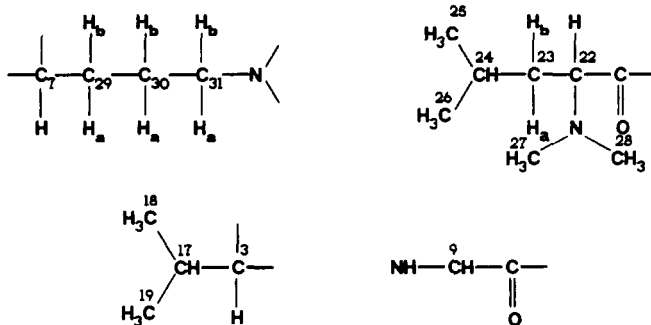
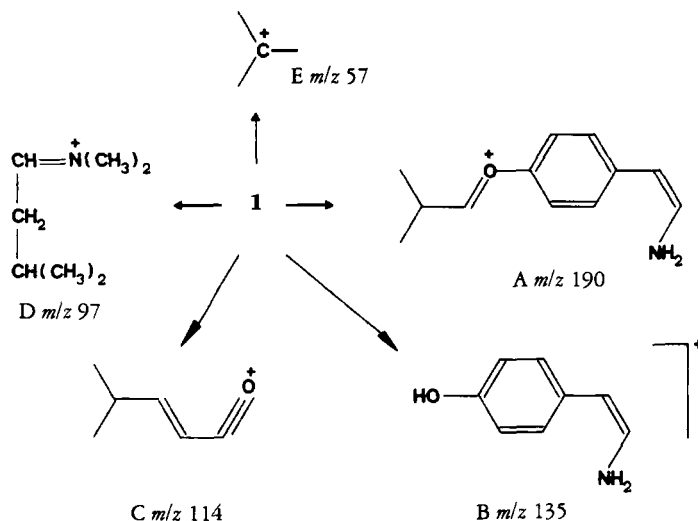


FIGURE 1. Substructures and fragments of **1** obtained from the  $^1\text{H}$ -nmr spectrum.



Voucher specimens documenting this collection has been deposited in the National Herbarium, Yaounde.

**EXTRACTION AND ISOLATION OF 1.**—The sun-dried, milled stem bark of *C. anoroldianum* (6 kg) was successively extracted with *n*-hexane,  $\text{CHCl}_3$ , and  $\text{EtOAc}$ . The  $\text{CHCl}_3$  extract (30 g) was chromatographed over a Si gel column. Fractions eluted with  $\text{CHCl}_3$ -MeOH (9:1) yielded a simplified mixture (300 mg) which was further purified over a small Si gel column in a similar way. The fractions eluted with pure  $\text{CHCl}_3$  provided one major component which on crystallization in  $\text{Et}_2\text{O}$  gave 0.1 g of anoroldianine [**1**]: pinkish crystals, mp  $160^\circ$  ( $\text{Et}_2\text{O}$ );  $\text{ir } \nu_{\text{max}} \text{ cm}^{-1}$  3400, 2925, 2850, 1720, 1625, 1270, 1040, 970, 700;  $\text{uv } \lambda_{\text{max}}$  (EtOH) 228 nm ( $\epsilon$  max 62400); eims (70 eV  $200^\circ$ ),  $m/z$  (rel. int.)  $[\text{M} + \text{H}]^+$  485 (85),  $[\text{M}]^+$  484 (1), 328 (8.5), 190 (8.5), 166 (17), 134 (34.5), 114 (100), 97 (4), 57 (8.5); Found C 66.90, H 8.34, N 11.54;  $\text{C}_{27}\text{H}_{40}\text{N}_4\text{O}_4$  requires C 66.91, H 8.32, N 11.56%.

#### ACKNOWLEDGMENTS

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