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VENECURINE, AN INDOLE ALKALOID FROM CURARE

J. QUETIN-LECLERCQ, R. WARIN,* N. G. BISSET† and L. ANGENOT‡

Institut de Pharmacie, Université de Liège, rue Fusch, 5, B-4000 Liège, Belgium; *Institut de Chimie, Université de Liège, Sart Tilman, B-4000 Liège, Belgium; †Chelsea Department of Pharmacy, King's College London, University of London, Manresa Road, London SW3 6LX, U.K.

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Key Word Index—Venezuelan curare; quaternary indole alkaloid; venecurine; 2D-NMR.

Abstract—A new quaternary indole alkaloid, venecurine, has been isolated by chromatographic techniques from a curare obtained from the Hoti tribe of Venezuela. Elucidation of its structure is based mainly on 2D-NMR studies.

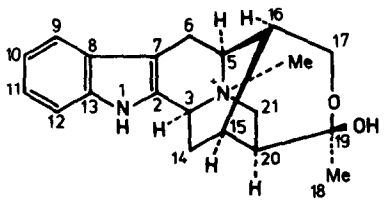
INTRODUCTION

In a preliminary TLC screening of some South American curares we encountered a sample that appeared not to contain any of the known dimeric curarizing alkaloids. The sample originated from the Hoti, a small tribe who inhabit the mountainous border region between the Venezuelan States of Bolivar and Amazonas north of the Rio Ventuari region where curares are prepared from the *Strychnos* genus of the *Loganiaceae* family [1]. They do not use the bow and arrow, but for hunting small animals rely on the blowpipe and unpoisoned or curare-poisoned darts. The southern Hoti make their own curare, part of which is destined for external trade, while the northern Hoti obtain theirs from another tribe, the E'niepa (= Panare) [2].

The present communication reports the isolation and structure determination of a new quaternary monomeric indole alkaloid (**1**), to which we have given the trivial name venecurine, from the above-mentioned sample of curare.

RESULTS AND DISCUSSION

The major alkaloid afforded a grey coloration with 1% ceric sulphate in 10% aqueous sulphuric acid. Its UV spectrum showed the characteristic chromophore of an unsubstituted indole alkaloid and was similar to that of macusine B. No shifts were detected in either basic or acidic solution. The FAB mass spectrum showed a $[M]^+$ at *m/z* 325, corresponding to the elemental composition $C_{20}H_{25}N_2O_2$, which gives an unsaturation number of 10. A strong peak at *m/z* 324 $[M-1]^+$, of the elemental composition $C_{20}H_{24}N_2O_2$ (calc.: 324.1837; found: 324.1823), was observed in the EI mass spectrum. Characteristic fragments of a sarpagan skeleton were observed at



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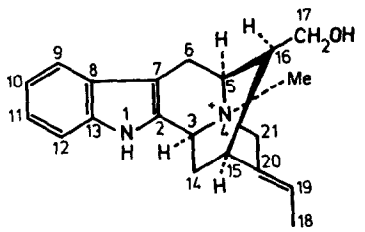

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‡ Author to whom correspondence should be addressed.

m/z 310 $[M-Me]^+$, 307 $[M-H_2O]^+$, 291, 249, 169 and 168 [3, 4]. The absence in the mass spectrum of a peak at $[M-COOMe]^+$ and in the IR spectrum of a carbonyl band suggested that the oxygen atoms might be present either as a hydroxyl group or in an ether linkage.

A better understanding of the structure of the molecule was gained by examination of the ^{1}H NMR spectrum (Table 1). This confirmed the occurrence of an unsubstituted indole moiety and the signal at 3.07 ppm revealed the presence of a $[N-Me]^+$ group. Comparison with the spectrum of macusine B (**2**) showed striking differences: a three-proton singlet at δ 1.48, indicating attachment to a quaternary carbon atom, and the absence of an ethylidene side-chain or other double bond. Because of the unsaturation number, the presence of a sixth ring must be assumed. The chemical shift of one of the quaternary carbon atoms, at δ 98.5, calls to mind a hemiketal function—such as is found in diaboline [5]—which is plausibly involved in the sixth ring. Acetylation at room temperature was unsuccessful, but treatment for 8 hr at 40° gave the diacetylated derivative (acetylation of both –OH and –NH). The difficulty in effecting this acetylation suggests that a tertiary alcohol function is present.

On the basis of these considerations structure **1** is proposed for venecurine. All the chemical shifts and couplings in the ^{1}H and ^{13}C, 2D COSY and 2D COSY-RCT spectra, as well as the NOE experiments, fully support this structure (Tables 2 and 3). The stereochemistry remains to be considered. The CD curve, positive at 270 nm and negative at 290 nm, points to a 3*S* *cis*-configuration [6]. The rigidity of the ring system also requires that H-5, H-15, H-16, and H-20 be, respectively, *S*, *S*, *R* and *R*. The indicated H-15 orientation also agrees with a biogenetic hypothesis [7]. The C-16, C-14 and C-17 chemical shifts likewise correspond with a 16*R* orientation [8]. The stereochemistry at C-19 was deduced from the 2D NOE (NOESY) experiments, in which NOE connectivities were observed between Me-18 and H-20, H-21A and H-21B—hence C-19 has the *R*-configuration (Table 3). A closely related quaternary alkaloid, macrosalhine, has previously been found in *Alstonia macrophylla* [11]. The absolute configuration of its skeleton was established by X-ray analysis [12].

Table 1. ^{1}H NMR spectra of **1** and **2*** (D_2O, 400 MHz, ext. ref. D_2O-DSS)

H	1	2	H	1	2
3	4.79 (*dd*)	4.8 (*def*)	15	2.47 (*m*)	2.90 (*def*)
5	3.86 (*m*)	3.32 (*t*)	16	1.77 (*m*)	1.87 (*def*)
6A	3.34 (*dd*)	3.1 (*dd*)	17A	4.04 (*d*)	3.46 (*m*)
6B	3.03 (*d*)	2.9 (*def*)	17B	3.60 (*m*)	3.46 (*m*)
9	7.51 (*d*)	7.51 (*d*)	18	1.48 (*s*)	1.60 (*d*)
10	7.11 (*t*)	7.14 (*t*)	19	—	5.58 (*q*)
11	7.19 (*t*)	7.23 (*t*)	20	2.28 (*m*)	—
12	7.47 (*d*)	7.47 (*d*)	21A	3.86 (*m*)	4.05 (*d*)
14A	2.43 (*m*)	2.37 (*t*)	21B	3.56 (*m*)	4.25 (*d*)
14B	2.02 (*dd*)	1.87 (*def*)	N_4^+-Me	3.07 (*s*)	2.90 (*s*)

Values are in ppm, def: deformed.
*Data obtained from ref. [10].

Table 2. ^{13}C NMR spectra of **1** and **2*** (D_2O 100 MHz)

C	1	2	C	1	2
2	139.3	139.2	13	134.4	134.2
3	60.9	63.1	14	34.1	33.8
5	65.5	67.1	15	23.5	28.0
6	26.0	26.0	16	37.4	45.7
7	102.2	103.3†	17	65.3	64.5
8	128.2	128.2	18	27.1	14.7
9	121.1	120.9	19	98.5	124.2
10	122.5	122.6	20	41.4	128.7†
11	125.4	125.4	21	63.1	67.1
12	114.4	114.5	N_4^+-Me	50.1	49.6

*Data obtained from ref. [10].
†Values can be reversed.

Table 3. Selected ^{1}H 2D-NMR data for venecurine (D_2O 400 MHz)

COSY	COSY RCT	NOESY	
H-3–H-14A	H-3–H-5	H-3–N^+-Me	Me-18–H-20
H-3–H-14B	H-5–H-6B	H-3–H-14A	H-20–H-21A
H-5–H-16	H-5–H-17A	H-5–H-6A	H-21A–H-21B
H-5–H-6A	H-5–H-17B	H-6A–H-6B	H-21A–N^+-Me
H-6A–H-6B	H-6A–H-16	H-6B–H-16	H-21B–N^+-Me
H-14A–H-14B	H-14A–H-15	H-14A–H-14B	
H-15–H-16	H-14B–H-15	H-15–H-16	
H-15–H-20	H-14B–H-16	H-15–H-20	
H-16–H-17B	H-14B–H-20	H-15–H-17A	
H-17A–H-17B	H-15–H-21A	H-16–H-17A	
H-20–H-21A	H-16–H-20	H-16–H-17B	
H-20–H-21B	H-16–H-17A	H-17A–H-17B	
H-21A–H-21B	+ regular couplings	Me-18–H-21A	
		Me-18–H-21B	

Concurrently with the above structural work, frog bioassays were carried out. The curare itself had paralysing activity at a dose of *ca* 20 mg/kg; total recovery was observed a few hr later. The purified alkaloid venecurine (**1**) exhibited the same type of activity but at a dose level of *ca* 0.5 mg/kg. This activity, relatively low as compared with the dimeric curarizing alkaloids, may be an indication that the sample of curare investigated was a weak one intended to be used for capturing rather than killing animals.

EXPERIMENTAL

The curare (7.5 g), a sample of which is kept in the collections of the Pharmaceutical Institute of the University of Liège (Belgium) and at the Chelsea Department of Pharmacy of the University of London (U.K.), was extracted by maceration with 1% aq. HOAc. After partial clean-up of the soln by extraction with Et_2O and $CHCl_3$, Na_2CO_3 was added to give pH 8, and traces of tertiary alkaloids were removed by extn with $CHCl_3$. The quaternary alkaloids were then pptd by a satd soln of picric acid and the picrates converted to the chlorides by passage through a column of Amberlite® IRA 400. The crude fraction (757 mg) was sepd firstly with MeOH on a Fractogel® TSK 40*S* column and secondly at pH 2.5 with the mixture 0.02 M tetramethylammonium hydroxide, 0.02 M camphorsulphonic acid, and H_3PO_4 in H_2O–MeOH (6:4) on a reversed-phase Lobar® column (LichroPrep RP 8). Frs containing the major alkaloid were then purified by high-speed counter current chromatography [9] using *n*-BuOH–Me_2CO–H_2O (8:1:10) to afford *ca* 80 mg of pure venecurine.

Venecurine (1). UV λ_{max} nm (log ε): 221.5 (4.1), 268 (3.27), 271 (3.27), 278 (3.26), 281 (3.24), 288.5 (3.11). CD (MeOH) $\Delta\varepsilon_{220} = -5.5$; $\Delta\varepsilon_{262} = +1.3$; $\Delta\varepsilon_{281} = -0.65$; $\Delta\varepsilon_{284} = -0.52$; $\Delta\varepsilon_{287.5} = -1.95$; $\Delta\varepsilon_{297} = +0.2$. IR ν_{max} cm^{-1}: 3360, 3240, 2940, 2880, 1620, 1467, 1451, 1375, 1320, 1306, 1115, 1105, 1073, 1054, 960, 929, 900, 753. 70 eV EIMS *m/z* (rel. int.) 324 $[M-1]^+$ (12), 309 (23), 291 (100), 280 (19), 263 (9), 249 (11), 238 (9), 219 (37), 204 (28), 194 (12), 186 (57), 183 (60), 169 (66), 159 (47), 140 (12), 129 (14), 117 (62). ^{13}C and ^1H NMR spectra: see Tables 1, 2 and 3. $J_{3-14A} = 10$ Hz; $J_{6A-5} = 5$ Hz; $J_{6A-6B} = 17$ Hz; $J_{9-10} = 7.7$ Hz; $J_{10-11} = 7.5$ Hz; $J_{11-12} = 8$ Hz; $J_{14A-14B} = 10.4$ Hz; $J_{17A-17B} = 11$ Hz; $J_{20-21A} = 10.5$ Hz; $J_{21A-21B} = ca$ 15 Hz.

Acetylation. To 1 mg of venecurine was added 1 ml pyridine and 0.2 ml Ac_2O. After standing for 8 hr at 40°, the reagents were removed by evapn to afford the diacetylated compound which showed a $[M]^+$ at *m/z* 411 (FAB MS).

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