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ISOLATION AND PURIFICATION OF PANARINE, A ALKALOID FROM A VENEZUELAN CURARE

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Key Word Index—Quaternary indole alkaloids; macusine B; panarine; HSCCC; Venezuelan curare; Panare tribe.

Abstract—High Speed Counter Current Chromatography (HSCCC) has been used to isolate and purify two quaternary alkaloids from a sample of curare prepared by the Panare Indians of Estado Bolivar, Venezuela. One of the compounds is the known base macusine B (**1**). The other one is a new but related base named panarine (**2**). Its structure has been established unequivocally by spectral and X-ray crystallographic analysis.

INTRODUCTION

In attempting to isolate and purify quaternary alkaloids from complex mixtures, we have made use of high speed counter current chromatography (HSCCC), a relatively new method that has proven to be efficient, rapid, reliable and inexpensive. Its advantages and disadvantages have been discussed in a number of recent publications [1–4]. We report here its application to the separation and purification of a mixture of two closely related alkaloids isolated from a Venezuelan curare. This sample was prepared by the Panare Indians, a tribe living in the western part of Estado Bolivar, whose subsistence economy has been based on slash-and-burn agriculture, fishing, hunting and gathering. The blowgun with poisoned darts is used for shooting birds and monkeys [5].

RESULTS AND DISCUSSION

After elimination of the tertiary alkaloids, the quaternary fraction, precipitated with picric acid and converted to the chloride form was divided into two fractions by means of their differential solubility in the two phases of a 1:1 mixture of *n*-BuOH–H_2O. The butanol-soluble fraction contained two monomeric alkaloids giving a grey coloration with 1% ceric sulphate in 10% sulphuric acid. This crude fraction was purified by HSCCC to yield two alkaloids, one of which was identified as macusine B (**1**) by comparison (UV, IR, MS and CoTLC) with an authentic sample [6].

The second base, named panarine (**2**), had the same UV spectra as macusine B, indicating an unsubstituted indole chromophore. No shifts were detected in either alkaline or acidic solutions. Its molecular weight was 322, corresponding to the elemental composition $C_{20}H_{22}N_2O_2$. The MS spectrum was quite similar to that of macusine B for the fragments with $m/z < 263$.

A better understanding of the structure of the molecule was gained by examination of its ^1H and ^{13}C NMR spectra (Figs 1 and 2). The assignments were made by comparison with data for related compounds [7, 8] and 2D-experiments. The main differences between macusine B and panarine were the appearance of a signal at 179 ppm and the disappearance of the signal at 64.5 ppm.

X-ray crystallographic analysis is in accord with these findings and indicates the presence of a carboxylate function at C-17 [9]. This explains the absence of any carbonyl band in the IR spectrum and the presence of bands at 1597 and 1380 cm^{-1}. X-Ray crystallography has also established the relative stereochemistry of the molecule, but its absolute stereochemistry has to be deduced from spectral data.

The CD curve, positive at 270 nm and negative at 290 nm, points to a 3*S cis* configuration [10]. The rigidity of the ring system also requires C5-H and C15-H to be *S* [11]. This last configuration agrees with the biogenetic hypothesis [12]. The chemical shifts of C-16, C-14, C-19 and C-20 are in agreement with a 16*R* orientation [13], as in macusine B, and the shifts of C-15 and C-21, diagnostic for the configuration of the ethylidene side-chain, indicate an *E*-configuration as in most sarpagine-type alkaloids [13, 14].

The zwitterion type of structure present in panarine is very unusual in natural products. Only a few alkaloids, e.g. anhydronium bases have a positive and a negative charge in the same molecule. Moreover, it is very rare that those charges are located on atoms situated γ to each other and is evidently due to the sarpagine skeleton present in the alkaloid.

The Panare Indians prepare their curare from the bark

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Table 1. ^{13}C NMR spectra of compounds **1** and **2** (D_2O, 100.8 MHz)

C	1	2
2	139.2	139.6
3	63.1	62.8
5	67.1	66.9
6	26	26.1
7	103.3	103.5
8	128.2*	128.3*
9	120.9	121.1
10	122.6	122.6
11	125.4	125.4
12	114.5	114.5
13	134.2	134.2
14	33.8	33.7
15	28	30.9
16	45.7	51.7
17	64.5	179.8
18	14.7	14.7
19	124.2	123.4
20	128.7*	129.3*
21	67.1	67.3
N_4^+-Me	49.6	49.8

*Interchangeable.
Values in ppm (TMS = 0).

Table 2. ^1H NMR spectra of compounds **1** and **2** (400 MHz, D_2O)

H	1	2
3	4.75 (def)*	4.80 (def)
5	3.32 (*t*)	4.21 (*t*)
6 A	3.10 (*dd*)	3.23 (*dd*)
6 B	2.90 (def)	2.87 (*d*)
9	7.51 (*d*)	7.52 (*d*)
10	7.14 (*t*)	7.15 (*t*)
11	7.23 (*t*)	7.25 (*t*)
12	7.47 (*d*)	7.47 (*d*)
14 A	2.37 (*t*)	2.44 (*t*)
14 B	1.87 (def)	2.08 (*dd*)
15	2.90 (def)	3.40 (*br s*)
16	1.87 (def)	2.50 (*d*)
17	3.46 (*m*)	—
17′	3.46 (*m*)	—
18	1.60 (*d*)	1.58 (*d*)
19	5.58 (*q*)	5.50 (*q*)
21 A	4.05 (*d*)	4.11 (*d*)
21 B	4.25 (*d*)	4.28 (*d*)
N_4^+-Me	2.90 (*s*)	3.01 (*s*)

Values are in ppm (TMS = 0).
*def = deformed.

*Dr Boom has informed the authors that Henley [15] is incorrect in stating that the Panare use *Strychnos fendleri* Sprague et Sandw. This latter species is called '*chirimi yo*" and it is not involved in the making of curare.

of *Strychnos toxifera* Rob. Schomb. ex Lindley, which they call '*mankowa*' [5]*. In addition to the highly potent bis-quaternary muscle-relaxant base toxiferine, it is known to contain the three mono-quaternary sarpagine-type bases macusines A, B, and C as well as many other alkaloids [16].

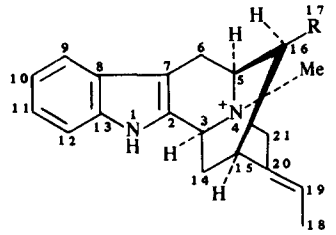
EXPERIMENTAL

Extraction and isolation. The powdered curare (25 g) was extracted by maceration with 1% aq. HOAc. After filtration, the aq. soln was basified to pH 8 by Na_2CO_3 and extracted with $CHCl_3$ to remove the tertiary alkaloids. Following acidification of the aq. soln, the quaternary fraction was pptd with a satd aq. soln of picric acid. The ppt. was then dissolved in MeOH–Me_2CO–H_2O (6:2:1) and the alkaloids converted to the chlorides by passage through a column of Amberlite® IRA 400. The residue, obtained after evaporation of the solvents (1.5 g) was partitioned between the two phases of a 1:1 mixture of *n*-BuOH–H_2O. The crude *n*-BuOH-soluble fraction (0.7 g) contained two major alkaloids. They were purified by HSCCC in the Ito Multilayer-Coil Separator-Extractor (P.C. Inc., Potomac, Maryland, U.S.A.) with 2.6 mm i.d. coiled tubing and *n*-BuOH–Me_2CO–H_2O (8:1:10) as solvent system. The upper (BuOH) phase was used as stationary phase and the mobile (aq.) phase was pumped from the head of the column to the tail. Retention of the stationary phase was *ca* 50% at 800 rpm under a maximum pressure of 2 kg/cm^2. The volume of each fraction was 10 ml.

Fractions 20–28 containing pure macusine B were concd and the alkaloid was precipitated in Et_2O (90 mg). Fractions 56–75 crystallized in hydrated MeOH to give 100 mg of pure panarine.

Macusine B. MS, UV and IR see [5]. CD (MeOH) $\Delta\varepsilon_{220} = -7.92$; $\Delta\varepsilon_{262} = +3.0$; $\Delta\varepsilon_{282} = -0.69$; $\Delta\varepsilon_{284} = -0.16$; $\Delta\varepsilon_{287} = -2.3$. ^1H NMR spectrum (see Table 1) $J_{3-14A} = 11$ Hz; $J_{5-6A} = 5$ Hz; $J_{6A-6B} = 17$ Hz; $J_{9-10} = 8$ Hz; $J_{10-11} = 7.5$ Hz; $J_{11-12} = 8$ Hz; $J_{14A-14B} = 12$ Hz, $J_{14B-15} = 3$ Hz; $J_{18-19} = 6.5$ Hz; $J_{21A-21B} = 15.5$ Hz. ^{13}C NMR spectrum see Table 2.

Panarine. White powder, UV: λ_{max} (nm) (log ε): 221.5 (4.10); 272.5 (3.4); 279 (3.38); 282.5 (3.37); 290 (3.9). CD (MeOH) $\Delta\varepsilon_{220} = -9.6$; $\Delta\varepsilon_{262} = +2.7$; $\Delta\varepsilon_{283} = -1.29$; $\Delta\varepsilon_{284.5} = -0.84$; $\Delta\varepsilon_{288} = -3.03$. IR ν_{max} (cm^{-1}): 3400, 3240, 2940, 1597, 1453, 1380, 1330, 1247, 1190, 1083, 935 and 750. 70 eV EIMS *m/z* (rel. int.): [M + Me]$^+$: 336 (8.5), [M$^+$]: 322 (100), 307 (18.1), 291 (4.5), 263 (11.4), 249 (29.3), 235 (12.1), 207 (5.4), 182 (7.5), 169 (55.2), 168 (46.5), 154 (6.7), 140 (6.1). ^1H NMR spectrum (see Table 1) $J_{3-14A} = 11$ Hz; $J_{5-6A} = 5$ Hz; $J_{5-16} = 7$ Hz; $J_{6A-6B} = 17$ Hz; $J_{9-10} = 8$ Hz; $J_{10-11} = 7.5$ Hz; $J_{11-12} = 8$ Hz; $J_{14A-14B} = 13.3$ Hz; $J_{14B-15} = 4$ Hz; $J_{18-19} = 6.7$ Hz; $J_{21A-21B} = 15.5$ Hz. ^{13}C NMR spectrum see Table 2.



1 R = CH_2OH
2 R = COO^-

Panarine from MeOH–H_2O gave the dihydrate: $C_{20}H_{22}N_2O_2 \cdot 2H_2O$ which crystallized in the orthorhombic system, space group $P2_12_12_1$ with $z = 4$ molecules in a unit cell of dimensions *a*: 9.125(5), *b*: 13.414(8), *c*: 14.953(7)Å.

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ALKALOIDS FROM *PSEUDUVARIA INDOCHINENSIS*

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Key Word Index—*Pseuduvaria indochinensis*; Annonaceae; protoberberine; dehydroscoulerine; liriodenine; atherospermidine; oxoanolobine.

Abstract—Phytochemical investigation of the stem bark of *Pseuduvaria indochinensis* has led to the isolation and identification of a novel quaternary protoberberine alkaloid, dehydroscoulerine, together with three known oxoaporphine alkaloids, liriodenine, atherospermidine and oxoanolobine.

INTRODUCTION

Pseuduvaria indochinensis Merr. (Annonaceae) is a rainforest tree, and the only species of the genus in China [1]. No previous chemical investigation has been reported on this species. The present study has resulted in the isolation of a novel quaternary protoberberine alkaloid, dehydroscoulerine (**1**), and three known oxoaporphine alkaloids, liriodenine (**2**), atherospermidine (**3**) and oxoanolobine (**4**).

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RESULTS AND DISCUSSION

Compound **1** was obtained from the water-soluble part of the extractives and crystallized from methanol as orange-red needles, mp 275–276° (dec). FABMS showed $[M]^+$ at *m/z* 324. UV λ_{max} 229, 279, 350 nm, suggested a quaternary protoberberine type alkaloid [2]. Upon addition of base, the UV spectrum underwent a significant bathochromic shift, which indicated the presence of phenolic groups. This was further supported by IR absorption bands between 3600 to 3200 cm^{-1}. The ^1H NMR (DMSO-d_6) revealed the presence of six aromatic protons that can be assigned to H-4 (δ6.98), H-1