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Alkaloids of *Voacanga Africana*, Stapf. I. Voacafrine and Voacafricine—Two New Alkaloids

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Two new alkaloids, voacafrine and voacafricine, have been isolated from the tree bark of *Voacanga africana*. Their properties are described.

Voacanga africana Stapf is an Apocyanaceous plant and has been under active investigation in recent years. To date, isolation of the following eight alkaloids has been reported: voacangine,[1,2] voacamine[3,4] (same as voacanginine[5]), voacaminine,[6] vobtusine,[3] voacorine[4] (probably same as voacaline[7]), voacamidine,[8] voacristine,[8] and voacangarine.[9] For the greater part, however, isolation procedures have not been published. The present study was undertaken to obtain some of the members of this group for pharmacological studies and to check for the possible presence of new alkaloids.

The crude alkaloid from the tree bark of *V. africana* Stapf was first separated into relatively weak and strong base fractions. Fractional crystallization of the former yielded voacamine and vobtusine as the two major fractions. The strong base fraction was subjected to countercurrent distribution whereby voacorine could be separated from a mixture of minor alkaloids. This mixture could then be separated by partition chromatography into two closely related alkaloids. These did not correspond in their properties to any of the other members of the *Voacanga* alkaloids and appeared to be new. They are designated as voacafrine and voacafricine in the order of their emergence from the column.

Voacafrine, isolated in 0.05% yield was characterized as the crystalline base, hydrochloride and acid oxalate. Voacafricine, isolated in 0.01% yield was characterized as the crystalline base and hydrochloride. Table I compares the properties of these two substances.

TABLE I

PROPERTIES OF VOACAFRINE AND VOACAFRICINE

Property	Voacafrine	Voacafricine
Probable molecular formula	$C_{22}H_{26}O_4N_2$	$C_{22}H_{24-26}O_4N_2$
Formula of the hydrochloride	$C_{22}H_{26}O_4N_2 \cdot HCl$	$C_{22}H_{24-26}O_4N_2 \cdot HCl$
Ultraviolet absorption spectrum (hydrochloride in methanol)	240 mμ, ε = 17,070, 315 mμ, ε = 20,670	238 mμ, ε = 18,570 315 mμ, ε = 22,500
Prominent infrared bands (hydrochloride in KBr)	3.0, 5.80, 6.08, 6.5, 6.82, 7.45, 13.4μ	3.0, 5.75, 5.8, 6.08, 6.48, 6.82, 7.45, 13.3μ
Behavior under ultraviolet light	Bright blue fluorescence	Bright blue fluorescence

Both voacafrine and voacafricine appear to be unstable to aerial oxidation as free bases. They give no characteristic color reactions with alcoholic ferric chloride or aqueous sodium hyroxide. Both gave bright orange red color with concentrated sulfuric acid.

On the basis of the ultraviolet spectrum, three types can be distinguished among the alkaloids of *V. africana*. Voacamine, voacangine, voacorine, voacanginine, voacamidine, voacristine, and voacangarine belong to the first group characterized by their "indole" type spectrum. Vobtusine with its four maxima belongs to the second group. The two new members, voacafrine and voacafricine, represent the third group.

Paper chromatography was used throughout this work for the detection of the various alkaloids of this group. The system used consisted of formamide-impregnated paper developed with solvents of increasing polarity.[10] Table II shows the R_f values of five of the purified alkaloids.

EXPERIMENTAL

Isolation. The powdered tree bark of *V. africana* (1 kg.) was extracted in the cold with 0.5% methanolic acetic acid and the extract (10 l.) concentrated under reduced pressure to about 500 ml. It was then diluted to 2 l. with water, the *p*H brought to 8.0 and the precipitated solid filtered off with Hyflo Supercel. The filtrate and cake were separately extracted with benzene twice and the combined extract concentrated to about 250 ml. This was added to ligroin (1500 ml.) and the precipitated solid filtered; yield, 35–40 g.

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(3) M. M. Janot and R. Goutarel, *Compt. rend.*, **240**, 1719 (1955).
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TABLE II

R_f VALUES OF SOME VOACANGA ALKALOIDS

Alkaloid	System			
	Cyclohexane	Cyclohexane-benzene	Benzene	Benzene-Chloroform
Voacamine	0.7	1.0	1.0	1.0
Vobtusine	0.5–0.7	1.0	1.0	1.0
Voacorine	0.3	0.6–0.7	1.0	1.0
Voacafrine	0	0	0.3–0.4	0.7–0.8
Voacafricine	0	0	0–0.1	0.2–0.3

Voacamine. Concentration of the ligroin filtrate and crystallization from methanol gave a colorless crystalline solid; yield, 2 g. m.p. 220–222°; $[\alpha]_D^{25}$, −48.4 (c = 1 in chloroform). Ultraviolet absorption maxima are at 225, 285, and 295 mμ with $E_{1\,cm.}^{1\%}$ of 771, 270, and 270, respectively. These properties and paper chromatography showed that this fraction is voacamine.[11]

Vobtusine. The crude alkaloid (35 g.) was taken up in benzene (1 l.) and shaken twice with 5% citrate buffer (pH 3.5, 2 × 500 ml.) whereby the material was distributed into the benzene-soluble, buffer-soluble and the insoluble fractions. The insoluble material was separated by filtration and reserved for further studies. The benzene layer on concentration and treatment with methanol gave a crystalline solid which was taken up in ether. The crystals which separated out were filtered and recrystallized from a mixture of methylene chloride and ethanol; yield, 1.2 g.; m.p. 283–285°; $[\alpha]_D^{25}$, −312 (c = 1 in chloroform). Ultraviolet absorption maxima are at 220, 257, 296, and 328 mμ with $E_{1\,cm.}^{1\%}$ of 557, 166, 209, and 210, respectively. These properties and paper chromatography showed that this fraction is vobtusine.[11] Vobtusine is shown to give a characteristic deep blue color with concentrated nitric acid.

The mother liquors from the vobtusine gave additional voacamine (2 g.).

Voacorine. The buffer layer from the previous step was adjusted to pH 8.0 and the alkaloid recovered by extraction with methylene chloride. The product was distributed countercurrently between benzene and 5% citrate (pH 5.1) through 15 transfers. Tubes 12–16 were combined and the alkaloid was recovered and crystallized from methanol; yield, 3.5 g., m.p. 270–273°; $[\alpha]_D^{25}$, −35 (c = 1 in chloroform). Ultraviolet absorption maxima are at 225, 285, and 295 mμ with $E_{1\,cm.}^{1\%}$ of 707, 242, and 242, respectively. These properties and paper chromatography showed that this component is voacorine[11].

Chromatographic separation of voacafrine and voacafricine. The system was prepared by shaking 5% citrate buffer (pH 3.5) and a mixture of ethyl acetate and isopropyl ether (1:1). Celite (40 g.) was impregnated with the equilibrated buffer (20 ml.) and poured into the column as a slurry in the mobile phase. The alkaloid from tubes 1–8 from the countercurrent distribution was recovered by extraction with methylene chloride at pH 8.0. The sample was introduced in and developed with the mobile phase. The fractions were analyzed by optical density at 315 mμ and the two components, voacafrine and voacafricine, tested for purity by paper chromatography.

Fractions which contained voacafrine were combined, concentrated, and taken up in ether. When treated with alcoholic hydrogen chloride, a colorless crystalline solid separated out. It was recrystallized from a mixture of acetone and methanol. Voacafrine hydrochloride separated out as 0.5 g. of colorless rectangular plates which decomposed at 165–167°, $[\alpha]_D^{25}$, −107° (c = 1% in methanol).

Anal. Calcd. for $C_{22}H_{26}O_4N_2 \cdot HCl$: C, 63.09; H, 6.49; N, 6.68; Cl, 8.46; *O*-methyl (1), 7.68; *N*-methyl (1), 6.93 and neutral equivalent, 419.1. Found: C, 63.10; H, 6.74; N, 6.78; Cl, 8.73; *O*-methyl, 7.68; *N*-methyl, 6.72, and neutral equivalent, 413. When titrated in aqueous dioxane, it gives an apparent pK value of 6.56.

Voacafrine. An aqueous solution of the hydrochloride was adjusted to pH 8 and extracted with ether. The base, which crystallized out on standing, was purified by recrystallization from a mixture of methylene chloride and ether. The product separated out as colorless rhombohedral plates which melted with decomposition at 135–137°. Solutions of the base gradually turned dark on exposure to air.

Anal. Calcd. for $C_{22}H_{26}O_4N_2$: C, 69.11; H, 6.84; N, 7.31. Found: C, 68.97; H, 6.74; N, 7.59.

Voacafrine acid oxalate. A solution of voacafrine in ether was treated with an excess of oxalic acid in acetone. After letting the mixture stand for several hours, the crystalline solid was filtered, washed with acetone, and recrystallized from a mixture of acetone and methanol. It separated out as colorless needles, which melted with decomposition at 230–232°.

Anal. Calcd. for $C_{22}H_{26}O_4N_2 \cdot H_2C_2O_4$: C, 61.02; H, 5.97; N, 5.92. Found: C, 61.40; H, 5.91; N, 6.29.

Voacafricine hydrochloride. The fractions which contained voacafricine were combined and the hydrochloride prepared as described under voacafrine. The hydrochloride crystallized from a mixture of acetone and methanol as 0.1 g. of colorless rectangular plates which melted with decomposition at 200–202°.

Anal. Calcd. for $C_{22}H_{24}O_4N_2 \cdot HCl$: C, 63.39; H, 6.04; N, 6.71; and Cl, 8.50. Found: C, 63.25; H, 6.39; N, 7.25; and Cl, 9.31.

Voacafricine, prepared as already described, crystallized from a mixture of methylene chloride and ether as colorless rectangular prisms which melted at 196–198°.

Anal. Calcd. for $C_{22}H_{24}O_4N_2$: C, 69.47; H, 6.35; N, 7.35. Found: C, 69.65; H, 6.68; N, 7.59.

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