Plant Anticancer Agents VI: Isolation of Voacangine, Voacamine, and Epivoacorine from *Tabernaemontana arborea* Sap

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Received December 22, 1976, from the *Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.* Accepted for publication May 13, 1977.

Abstract □ Fractionation of the sap of *Tabernaemontana arborea*, guided by cytotoxicity against the P-388 lymphocytic leukemia, yielded the known indole alkaloids voacangine, voacamine, and epivoacorine as the major cytotoxic constituents.

Keyphrases □ Alkaloids, various—isolated from *Tabernaemontana arborea* sap, cytotoxic activity evaluated □ *Tabernaemontana arborea*—sap, various alkaloids isolated, cytotoxic activity evaluated □ Cytotoxic activity—various alkaloids isolated from *Tabernaemontana arborea* sap evaluated

Previously (1–4), the isolation of various indole and bisindole alkaloids from *Tabernaemontana johnstonii* and *T. holstii* was described. In all cases, the bisindole alkaloids isolated were derived from isovoacangine coupled to a vobasane moiety, and no evidence for the presence of voacangine-derived bisalkaloids was found.

During a systematic search for novel anticancer agents of plant origin, dried extracts of the sap of *T. arborea* J. N. Rose ex J. D. Smith became available. In this investigation, the major cytotoxic components were isolated.

EXPERIMENTAL

Methods and materials were identical to those described previously (2, 4).

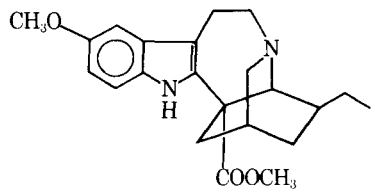
Plant Material—Samples of dried water-displaced extracts of the trunk sections of two trees of the species *T. arborea* J. N. Rose ex J. D. Smith were obtained from Costa Rica.

Initial Fractionation and Chromatography—A 1000-g sample of the dried extract was subjected to partition between water (2.5 liters) and chloroform (4 × 3 liters). The cytotoxic chloroform fraction (107 g) was separated from the inactive water fraction and subjected to a further partition between 90% aqueous methanol (3 liters) and hexane (3 liters). The cytotoxic aqueous methanol extract (45 g) was separated from the hexane fraction and from some insoluble material and subjected to chromatography on 2.5 kg of TLC grade silica gel. Elution was carried out with ethyl acetate containing increasing amounts of ethanol; 10 fractions, A–J, were collected.

Voacangine (I)—Fraction A (6.3 g) was recrystallized from methanol to yield I as colorless needles, mp 136–137° [lit. (5) mp 136–137°]. The sample had identical IR and UV spectra to those reported for I (6) and had an $[\alpha]_D^{22}$ of −42° (c 3.7, chloroform) [lit. $[\alpha]_D^{22}$ −42° (6) and −37° (5)]. The mass spectrum was identical with that reported (7), except for minor variations in peak intensities, and the NMR spectrum was entirely consistent with a voacangine structural formulation.

Voacamine (II)—A portion of Fraction E (1.2 g) was subjected to preparative TLC with benzene–dichloromethane–ether–methanol (38:30:22:10) with three developments. The UV-absorbing central band was scraped and eluted to yield 260 mg of a fraction which was crystallized from methanol to yield II (35 mg), mp 222–237° dec. [lit. (6) mp 223°], $[\alpha]_D^{22}$−46° (c 1.4, chloroform). Its UV spectrum showed λ_{max} 226 (log ϵ 4.60), 288 (4.13), and 295 (4.15) nm; its IR spectrum in chloroform was identical with that published for II (6).

Its mass spectrum also was identical with that published for II (7), except for minor variations in peak intensities, and its NMR spectrum was in agreement with published spectral data for II (8, 9). The observation of one-proton aromatic singlets at δ 6.82 and 6.96 ppm established the structure as being either of the conoduramine or voacamine types.



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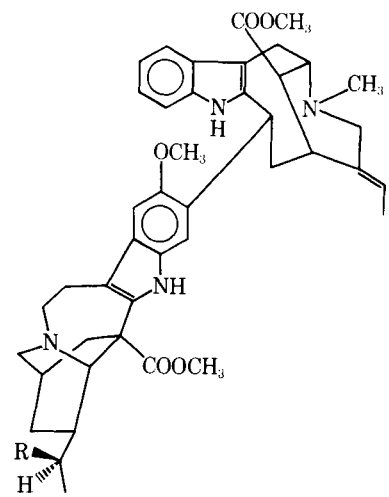

II: R = H
III: R = OH

The remote possibility that the compound could be conoduramine was excluded by a direct high-pressure liquid chromatographic (HPLC) comparison of samples, which showed that they were different.

Epivoacorine (III)—A portion of Fraction I (0.1 g) was subjected to preparative TLC with benzene–dichloromethane–ether–methanol (37:29:22:12) with three developments. The UV absorbing band, R_f ~0.25, was scraped and eluted to yield a fraction from which III was obtained

Table I—Cytotoxicity of Certain Fractions and Pure Alkaloids from *T. arborea*[a]

Fraction or Alkaloid	P-388 ED_{50}, μg/ml
Aqueous methanol extract	8.0
A	6.8
B	14
C	8.6
D	7.8
E	9.0
F	23
G	18
H	7.1
I	2.7
J	9.0
Voacangine	6.8
Voacamine	2.6
Epivoacorine	1.7

[a] Bioassays were performed by the A. D. Little Co., Cambridge, Mass., using established protocols.

by crystallization from methanol, mp 260–270° dec. [lit. (10) mp 265°], $[\alpha]_D^{22}$ −51° (c 2.2, chloroform). Its UV absorption spectrum showed λ_{max} 226 (log ε 4.63), 288 (4.17), and 295 (4.18) nm, and its IR spectrum was identical to that published for III (6).

Its NMR spectrum was consistent with this structural formulation; in particular, the aromatic region and the methyl signals were essentially identical to those recorded for II, except that the triplet at δ 0.92 ppm in II was replaced by a doublet at δ 1.26 ppm. Its mass spectrum also was consistent with this structural formulation (7). Finally, chromatographic comparison with an authentic sample established the identity of the isolated material as III.

Cytotoxic Activities—The cytotoxicities of Fractions A–J and of the three isolated alkaloids are given in Table I.

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Plant Anticancer Agents VII: Structural Effects on Cytotoxicity of Bisindole Alkaloids of Voacamine Type

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Received December 22, 1976, from the *Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.* Accepted for publication May 26, 1977.

Abstract □ Structural effects on the cytotoxicity of bisindole alkaloids of the voacamine series were investigated with compounds isolated from various *Tabernaemontana* species and compounds synthesized specifically for this purpose. Activity is sensitive both to the position of attachment of the vobasane unit on the iboga moiety and to the presence of an *N*-methyl group on the vobasane unit.

Keyphrases □ Alkaloids—isolated from various *Tabernaemontana* species or synthesized, structural effects on cytotoxicity evaluated □ *Tabernaemontana* alkaloids, various—structural effects on cytotoxicity evaluated □ Cytotoxicity—alkaloids isolated from various *Tabernaemontana* species or synthesized, structural effects evaluated □ Structure-activity relationships—alkaloids isolated from various *Tabernaemontana* species or synthesized, cytotoxicity evaluated

During studies on the isolation and structure elucidation of naturally occurring anticancer agents, some bisindole alkaloids of the voacamine type were isolated from various species of the genus *Tabernaemontana* and their structure and cytotoxicity were determined (1–5). This paper presents a preliminary account of the structure–activity relationships observed.

Table I—Cytotoxicity of the Simple Indole Alkaloids from which the Bisindoles Are Formed

Alkaloid		Cytotoxicity (ED_{50}), μg/ml P-388	KB
XI	Voacangine	6.8	>100
XII	Isovoacangine	18	59
XIII	Ibogamine	—	>100
XIV	Perivine	20	70
XV	Vobasine	—	>100

Table II—Cytotoxicity of Bisindole Alkaloids as a Function of the Presence of a Methyl Group on the Vobasane Ring

Alkaloid	R	Cytotoxicity (ED_{50}), μg/ml P-388	KB
II	H	1.3	5.8
III	CH_3	20	19
IX	H	3.2	—
X	CH_3	26	31

RESULTS AND DISCUSSION

Cytotoxicity data for the bisindole alkaloids I–X and for the simple indole alkaloids XI–XV are given in Tables I–IV. All bisindole alkaloids tested were of the voacamine type and may be considered to be composed of an iboga unit coupled to a vobasane unit. Data are given for two com-

Table III—Cytotoxicity of Bisindole Alkaloids as a Function of the Position of Attachment of the Vobasane Unit to the Iboga Moiety

Alkaloid		Position of Attachment	Cytotoxicity (ED_{50}), μg/ml P-388	KB
I	Voacamidine	11′	14	—
III	Conoduramine	12′	20	19
VI	Voacamine	13′	2.6	—
VII	Epivoacorine	13′	1.7	—
VIII	Tabernamine	13′	2.1	—
X	Conodurine	14′	26	31