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.

REFERENCES

(1) D. G. I. Kingston, B. B. Gerhart, and F. Ionescu, *Tetrahedron Lett.*, **1976**, 649.

(2) D. G. I. Kingston, B. T. Li, and F. Ionescu, *J. Pharm. Sci.*, **66**, 1135 (1977).

(3) D. G. I. Kingston, F. Ionescu, and B. T. Li, *Lloydia*, **40**, 215 (1977).

(4) D. G. I. Kingston, B. B. Gerhart, F. Ionescu, M. M. Mangino, and S. M. Sami, *J. Pharm Sci.*, **67**, 249 (1978).

(5) D. F. Dickel, C. L. Holden, R. C. Maxfield, L. E. Paszek, and W. I. Taylor, *J. Am. Chem. Soc.*, **80**, 123 (1958).

(6) N. Neuss, "Physical Data of Indole and Dihydroindole Alkaloids," Lilly Research Laboratories, Indianapolis, Ind., vol. I, 1964, and vol. II, 1964–1968.

(7) H. Budzikiewicz, C. Djerassi, F. Pusieux, F. Percheron, and J. Poisson, *Bull. Soc. Chim. Fr.*, **1963**, 1899.

(8) G. Büchi, R. E. Manning, and S. A. Monti, *J. Am. Chem. Soc.*, **85**, 1893 (1963).

(9) *Ibid.*, **86**, 4631 (1964).

(10) J. Poisson, F. Puisieux, C. Miet, and M. B. Patel, *Bull. Soc. Chim. Fr.*, **1965**, 3549.

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

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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-

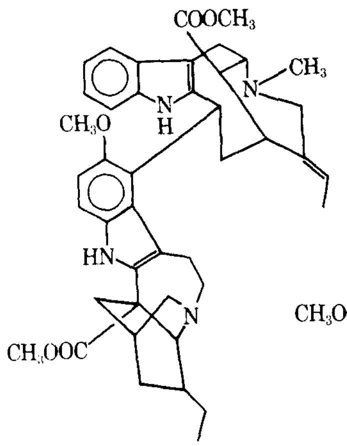
Table III—Cytotoxicity of Bisindole Alkaloids as a Function of the Position of Attachment of the Vobasane Unit to the Iboga Moiety

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

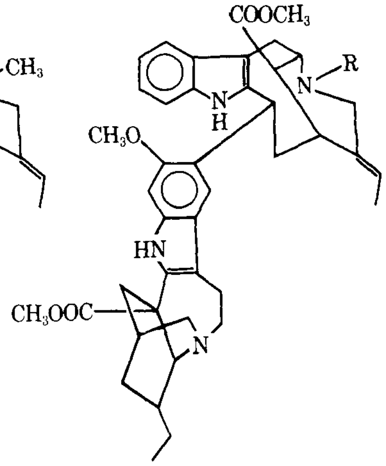
Table IV—Cytotoxicity of 13′-Perivoacangine (IV) and 13′-Peribogamine (V)

Alkaloid	Cytotoxicity (ED_{50}), µg/ml P-388	KB
IV	0.39	0.35
V	0.44	2.1

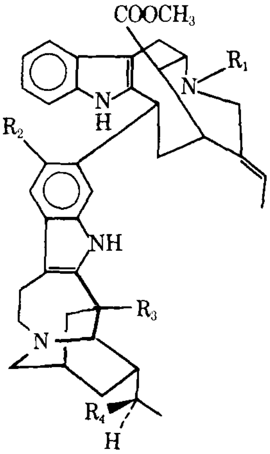
mon cell culture systems, the P-388 lymphocytic leukemia and Eagle's carcinoma of the nasopharynx (KB). Inspection of the data in Tables I–IV indicates that the P-388 system was slightly more sensitive in most cases to the alkaloids tested, giving cytotoxicities that were greater by a factor between 1 and 5 but usually close to 1.



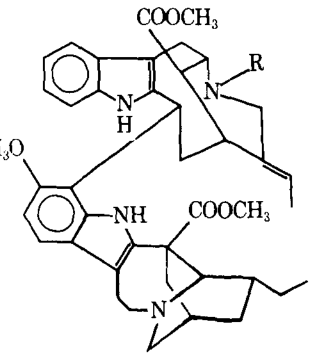
I



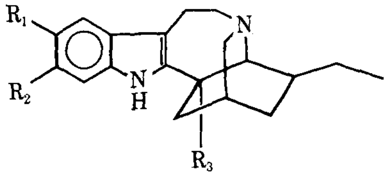
II: R = H
III: R = CH_3



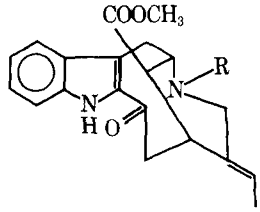
IV: R_1 = H, R_2 = OCH_3, R_3 = $COOCH_3$, R_4 = H
V: R_1 = R_2 = R_3 = R_4 = H
VI: R_1 = CH_3, R_2 = OCH_3, R_3 = $COOCH_3$, R_4 = H
VII: R_1 = CH_3, R_2 = OCH_3, R_3 = $COOCH_3$, R_4 = OH
VIII: R_1 = CH_3, R_2 = R_3 = R_4 = H



IX: R = H
X: R = CH_3



XI: R_1 = OCH_3, R_2 = H, R_3 = $COOCH_3$
XII: R_1 = H, R_2 = OCH_3, R_3 = $COOCH_3$
XIII: R_1 = R_2 = R_3 = H



XIV: R = H
XV: R = CH_3

Table I lists the cytotoxicities of XI–XV. These alkaloids may be considered to be the components from which the bisindole alkaloids are formed, although it is recognized that perivine and vobasine are not exact models for the vobasane part of a voacamine-type bisalkaloid. Nevertheless, these simple alkaloids were largely inactive in both systems, and voacangine was the only alkaloid showing activity approaching the arbitrary figure of 4 µg/ml that justifies further screening by the National Cancer Institute[1].

The cytotoxicities of the bisindole alkaloids gabunamine (II), conoduramine (III), gabunine (IX), and conodurine (X) are presented in Table II. The first conclusion that may be drawn from these data is that the bisindole alkaloids show significantly greater cytotoxicity than would be expected from the cytotoxicities of the component parts. Thus, a simple mixture of vobasine and isovoacangine would be expected to have an ED_{50} in the KB system no less than 80 µg/ml, while conoduramine and conodurine had ED_{50} values of 19 and 31 µg/ml, respectively, in this system. Similar results were found with the P-388 system; thus a mixture of perivine and isovoacangine would be exected to have an ED_{50} of about 19 µg/ml, while gabunamine and gabunine had ED_{50} values of 1.3 and 3.2 µg/ml, respectively.

A second conclusion is based on a comparison of the cytotoxicities of conoduramine and conodurine derived from vobasine with those of the alkaloids gabunamine and gabunine derived from perivine. In each case, the perivine-derived alkaloid, lacking an *N*-methyl group in the vobasane moiety, was significantly more active than the corresponding vobasine-derived alkaloid. This finding suggests that the presence of a free alicyclic NH group in the vobasane moiety of a bisindole alkaloid of this type is necessary for significant cytotoxicity.

Cytotoxicities are presented in Table III for bisindole alkaloids that differ in the point of attachment of the vobasane unit to the iboga moiety. In each case, the vobasane unit in question bears an *N*-methyl group so as to eliminate the effects due to this group. Of the six alkaloids tested, only those linked between the 13′-position of the iboga moiety and the vobasane system showed significant cytotoxicity, and all alkaloids linked at the 13′-position showed about the same level of cytotoxicity in the P-388 system. These findings are the more striking when consideration is given to the fact that the iboga moieties of VI–VIII linked at the 13′-position are quite different in many respects. Thus, voacangine (XI) showed modest cytotoxicity, while ibogamine (XIII) did not; yet the bisalkaloids derived from these simple iboga alkaloids, voacamine (VI) and tabernamine (VIII), respectively, showed very similar cytotoxicities.

The presence or absence of methoxyl, methoxycarbonyl, and hydroxyl substituents on various parts of the iboga moiety seems to make little difference to the cytotoxicity of these bisindole alkaloids, and the dominant feature giving rise to activity appears to be the position of linkage between the vobasane and iboga units. It would be interesting to see whether this finding carried over into other classes of bisindole alkaloids, particularly those related to vincaleukoblastine, since recent synthetic work may make such systems accessible (6–8).

[1] Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute.

The major conclusion regarding the naturally occurring bisalkaloids shown in Tables II and III is that activity is increased both by the absence of the *N*-methyl group of the vobasane unit and by the presence of a linkage to the 13′-position of the iboga unit. To test this conclusion and to determine whether both effects operate together, two new alkaloids, 13′-perivoacangine (IV) and 13′-peribogamine (V), were prepared from perivinol and voacangine or ibogamine, respectively.

The structure of IV follows from its method of synthesis by analogy with the synthesis of voacamine from vobasinol and voacangine (9). The spectroscopic properties of the isolated material fully support the assigned structure: in particular, the NMR spectrum of IV differed from that of VI only in that the latter showed a three-proton singlet at δ 2.62 ppm, which was absent in the former. The mass spectrum of IV also corroborated its structure, being completely in accord with expectations for this class of compound (10).

The structure of V could also be assigned by analogy with that of tabernamine (4). The NMR spectrum of V showed a pair of doublets centered at δ 6.88 and 7.31 ppm, which could be assigned to the 12′- and 11′-protons of the ibogamine moiety, respectively, thus indicating that coupling had occurred in the 13′-position as with tabernamine. The mass spectrum of V also supported the structural assignment proposed, being entirely consistent with expectations (10).

After this work was completed, the isolation of *N*-demethylvoacamine from *Tabernaemontana accedens* was reported (11); its physical properties appear to be identical with those of perivoacangine within normal experimental error.

The cytotoxicity data for IV and V are given in Table IV. The expected synergistic effect of the two structural variations did, in fact, occur, and both alkaloids showed a greater cytotoxicity than any other alkaloid of this class tested. The cytotoxicity of IV is particularly noteworthy because it is of approximately equal potency in both the P-388 and KB systems, thus giving an increased chance that this substance or related compounds will be active in other tumor systems, including *in vivo* systems.

In summary, the results presented provide a first attempt to correlate the structure and cytotoxicity of a well-defined group of bisindole alkaloids. Some initial correlations were made and tested successfully by the synthesis of compounds with increased cytotoxicity. Further studies along these lines should eventually lead to compounds with significant *in vivo* activity. Such studies are currently in progresss.

EXPERIMENTAL

Materials, methods, and bioassays were identical to those described previously (2, 4).

13′-Perivoacangine (IV)—Perivinol (250 mg) and voacangine (170 mg) were heated under reflux in 20 ml of 1.5% hydrogen chloride in methanol for 12 hr under nitrogen. Workup as previously described (4), followed by purification of the major product by preparative TLC [ethyl acetate and then benzene–ether–methanol (10:10:2)], yielded IV as an off-white amorphous powder, homogeneous on TLC (two systems). The isolated material had R_f 0.45 in chloroform–methanol (95:5) and 0.37 in benzene–ether–methanol (10:10:2). It had $[\alpha]_D - 83°$ (c 2.3, $CHCl_3$); UV: λ_{max} 227 (log ϵ 4.62), 286 (4.18), and 294 (4.18) nm. Its IR spectrum showed ν_{max} 1725 cm^{-1}.

Its PMR spectrum was almost identical to that of voacamine (VI), except that it lacked one three-proton singlet at δ 2.62 ppm, attributable to the *N*-methyl group of the vobasane ring in voacamine. Its mass spectrum showed ions at *m/e* 718 ($M^+ + 28$, 2), 704 ($M^+ + 14$, 8), 690 (M^+, 2), 646 (16), 614 (5), 451 (13), 225 (43), 194 (42), 183 (26), 182 (47), 181 (12), 180 (28), 166 (22), 149 (24), 148 (12), 139 (11), 136 (82), 135 (45), 124 (13), 123 (10), 122 (145), and 43 (100).

13′-Peribogamine (V)—Perivinol (164 mg) and ibogamine (179 mg) were condensed as described for IV; workup followed by preparative TLC (dichloromethane–methanol, 88:12) yielded peribogamine (V, 57 mg) as an amorphous white powder, homogeneous on TLC (three systems) and high-pressure liquid chromatography. It had λ_{max} 235 (log ϵ 4.55), 287 (4.08), and 295 (4.03) nm; its IR spectrum showed ν_{max} 1715 cm^{-1}.

Its PMR spectrum (methanol) showed peaks at δ 0.98 (3H, t), 1.75 (3H, d), 2.52 (3H, s), 5.45 (1H, m), 6.88 (1H, d, J = 8 Hz), and 7.31 (1H, d, J = 8 Hz) ppm. Its mass spectrum showed ions at *m/e* 616 ($M^+ + 14$, 2), 602 (M^+, 10), 181 (30), 169 (45), 149 (42), 136 (100), 122 (42), and 119 (55).

Anal.—Calc. for $C_{39}H_{46}N_4O_2$: C, 77.74; H, 7.64; N, 9.30. Found: C, 78.31; H, 7.74; N, 9.53.

REFERENCES

(1) D. G. I. Kingston, B. B. Gerhart, and F. Ionescu, *Tetrahedron Lett.*, **1976**, 649.

(2) D. G. I. Kingston, B. T. Li, and F. Ionescu, *J. Pharm. Sci.*, **66**, 1135 (1977).

(3) D. G. I. Kingston, F. Ionescu, and B. T. Li, *Lloydia*, **40**, 215 (1977).

(4) D. G. I. Kingston, B. B. Gerhart, F. Ionescu, M. M. Mangino, and S. M. Sami, *J. Pharm. Sci.*, **67**, 249 (1978).

(5) D. G. I. Kingston, *ibid.*, **67**, 271 (1978).

(6) N. Langlois, F. Guéritte, Y. Langlois, and P. Potier, *J. Am. Chem. Soc.*, **98**, 7017 (1976).

(7) J. P. Kutney, A. H. Ratcliffe, A. M. Treasurywala, and S. Wunderly, *Heterocycles*, **3**, 639 (1975).

(8) G. H. Büchi (unpublished results), cited by G. A. Cordell and N. R. Farnsworth in *Heterocycles*, **4**, 393 (Ref. 30) (1976).

(9) G. Büchi, R. E. Manning, and S. A. Monti, *J. Am. Chem. Soc.*, **86**, 4631 (1964).

(10) M. Hesse, "Indolalkaloide," Verlag Chemie, Weinheim, West Germany, 1974.

(11) H. Achenbach and E. Schaller, *Chem. Ber.*, **109**, 3527 (1976).

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