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Cytotoxicity of Modified Indole Alkaloids

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Abstract □ Indole alkaloids of the iboga series were structurally modified by incorporation of a 3,4-dimethoxybenzyl or -benzoyl unit so that they contained the N-O-O triangle required for antileukemic activity according to the triangulation hypothesis. The cytotoxicities of the modified alkaloids in the *in vitro* P-388 system were not significantly increased over the unmodified alkaloids, suggesting that the triangulation hypothesis does not apply in this series at least.

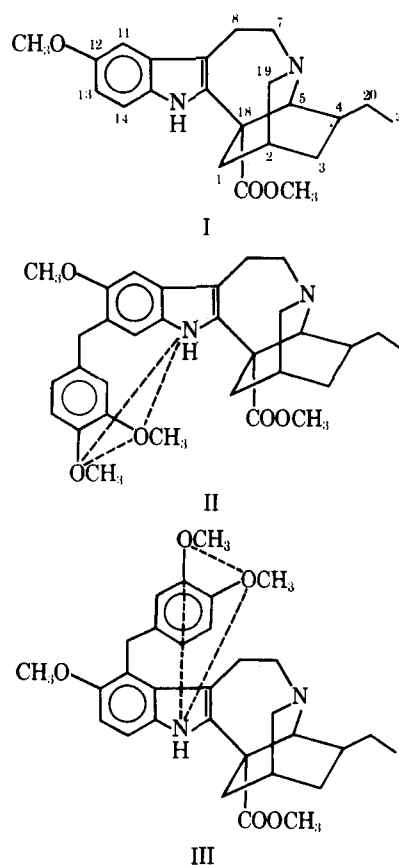
Keyphrases □ Indole alkaloids—semisynthetic, cytotoxicity, structure-activity relationships □ Antineoplastic agents, potential—modified indole alkaloids, cytotoxicity, structure-activity relationships □ Structure-activity relationships—modified indole alkaloids, antineoplastic activity □ Voacangine—derivatives, cytotoxicity, structure-activity relationships □ Catharanthine—derivatives, cytotoxicity, structure-activity relationships

In an earlier paper (1), the cytotoxicity of various bis-indole alkaloids of the voacamine type was shown to be strikingly dependent on the detailed structure of the alkaloid and, in particular, on the position of the vobasane unit attachment to the iboga unit of the bisalkaloid. The antileukemic activity of various compounds, including the bisindole alkaloids vincristine and vinblastine, can be correlated with the structural feature of an N-O-O triangle of defined dimensions in these molecules (2). It was possible that indole alkaloids of modest antileukemic activity could be modified to contain the N-O-O triangle and might then show an increased antileukemic activity. This paper presents the results of some studies directed toward this question.

RESULTS AND DISCUSSION

The iboga alkaloids voacangine (I) and catharanthine were selected as the parent alkaloids for structural modification. Voacangine, which was available from previous isolation studies on *Tabernaemontana arborea* (3), shows a weak cytotoxicity in the P-388 cell culture system. This system, using a leukemia-derived cell line, was selected for the initial bioassay, with *in vivo* testing available at a later stage for promising candidates.

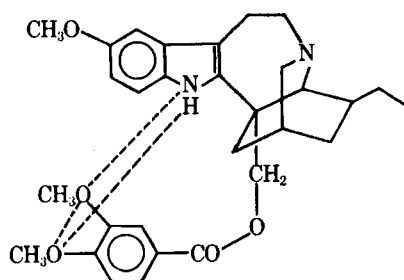
Reaction of I with 3,4-dimethoxybenzyl alcohol yielded the dimethoxybenzyl derivatives II and III, together with unreacted I and more highly substituted products. Assignment of Structures II and III to the two major substitution products was done primarily on the basis of PMR and mass spectra. Compound II showed singlets in its PMR spectrum at δ 6.89 and 6.84 ppm, assignable to H-14 and H-11, respectively. In III, the relevant signals appeared as doublets at δ 7.14 and 6.90 ppm, corresponding to H-14 and H-13, respectively. The mass spectra of both



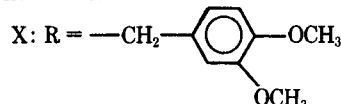
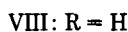
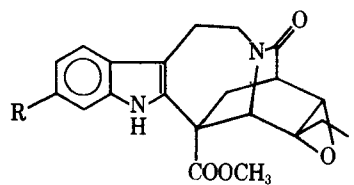
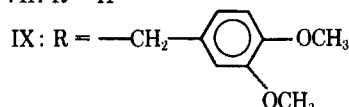
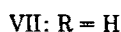
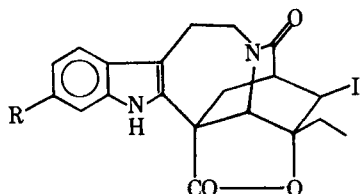
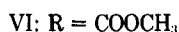
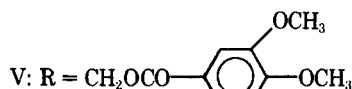
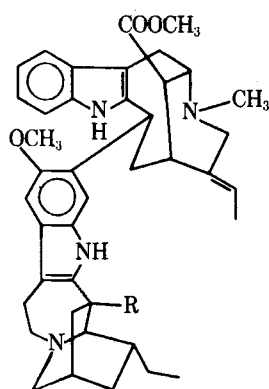
compounds showed molecular ion peaks at *m/e* 518 and typical peaks for iboga alkaloids at *m/e* 136 and 124 (4).

Reduction of I with lithium aluminum hydride yielded the known compound voacanginol (5). Esterification of voacanginol with 3,4-dimethoxybenzoyl chloride yielded the ester IV, which had NMR and mass spectra consistent with its assigned structure. Coupling of IV with vobasanol yielded the bisalkaloid V; its PMR spectrum was essentially a superimposition of the vobasane spectrum on that of IV. Coupling of the vobasane molecule to the 13'-position of IV (rather than the 11'-position) was indicated by the singlet resonances assignable to the 11'- and 14'-protons and by the broadening of the resonance of the I methoxyl group at δ 3.93 ppm due to hydrogen bonding (6).

Conversion of catharanthine to its epoxy lactam (VIII) was effected by published procedures (7, 8). Coupling of the intermediate iodolactone (VII) with 3,4-dimethoxybenzyl alcohol yielded the 3,4-dimethoxybenzyl derivative (IX). The assignment of Structure IX to the coupled product



IV



was based on an analysis of its PMR spectrum, which showed a doublet at δ 7.30 ppm assignable to the 11-proton, and by analogy to the synthesis of tabernamine, in which coupling also took place at the 13-position of an unsubstituted iboga alkaloid (9). Conversion of the iodolactone IX to the epoxide X was effected with base and gave a product with spectral data in accord with the assigned structure.

According to the triangulation hypothesis (2), antileukemic activity can be correlated with the presence of an N-O-O triangle in the molecule with the following dimensions: N-O₁, 652-764 pm; N-O₂, 804-920 pm; and O₁-O₂, 270-400 pm. The crucial triangle does not have to be rigidly fixed since the activity of molecules such as daunorubicin, streptovictin, and vinblastine, which contain the triangle in one particular conformation, is explained by the hypothesis. An examination of Dreiding models

Table I—Interatomic Distance Measurements (Picometers) of Some Semisynthetic Indole Alkaloids in Their Optimum Conformations

Alkaloid	N-O ₁	N-O ₂	O ₁ -O ₂
II	740	860	303
III	760	840	303
IV, V	708	840	303
X	740	860	303

of the modified alkaloids II-V and X showed that each contained an N-O-O triangle of appropriate dimensions in at least one conformation; the dimensions of the conformation that gave the best fit in each case are given in Table I.

The P-388 *in vitro* cytotoxicities of synthetic II-V and X are given in Table II, together with the corresponding data for the natural alkaloids voacangine (I) and voacamine (VI). Inspection of these data indicates that the synthesized compounds appear not to have any additional activity due to the triangulation effect. Thus, although II and III both have N-O-O triangles of comparable size, one is slightly more active than the parent alkaloid and one is slightly less active. The modified bisindole alkaloid V, which contains the N-O-O triangle, is considerably less active than VI, which does not. Neither modified alkaloid VIII nor X shows any significant activity, although both contain an alkylating (epoxy) function, and one contains the N-O-O triangle.

The four compounds II-V also were tested for activity in the P-388 *in vivo* test system. None showed significant activity at dose levels in the 0.5-25-mg/kg range.

In interpreting the results, it must be remembered that the N-O-O triangular arrangement was never envisioned as a necessary and sufficient criterion for antileukemic activity (2). Clearly, it cannot be applied in any simple way to improving the activity of marginally active compounds such as the iboga alkaloids. Further experiments are required to test its applicability in other systems.

EXPERIMENTAL

Materials, methods, and procedures were identical to those described previously (9).

13-(3,4-Dimethoxybenzyl)voacangine (II) and 11-(3,4-Dimethoxybenzyl)voacangine (III)—A mixture of voacangine (I) (1.22 g) and 3,4-dimethoxybenzyl alcohol (0.57 g) was heated under reflux in 1.5% methanolic hydrochloric acid for 10 days. Usual workup yielded a crude product, which gave six major spots on TLC. Purification of the crude product by preparative TLC (hexane-ether-methanol, 5:4:1) yielded unreacted I (0.23 g), II (0.22 g), and III (0.31 g) from the first, second, and third bands, respectively. The remaining bands consisted of mixtures of oxidized and *N*-benzylated products and were not investigated further.

Compound II showed major ions in its mass spectrum at *m/e* 518 (M⁺, 100), 503 (12), 433 (20), 394 (14), 323 (12), 311 (17), 310 (43), 260 (11), 259 (20), 208 (16), 151 (39), 148 (24), 137 (18), 136 (99), 135 (43), 124 (38), 123 (12), and 122 (43). Its PMR spectrum showed peaks at δ 7.66 (1H, s, NH), 6.89 (1H, s, H-14), 6.84 (1H, s, H-11), 6.80-6.75 (3H, m, H-2',5',6'), 3.96 (2H, s, ArCH₂Ar'), 3.86, 3.82, 3.80 (3H each, s, -OCH₃), and 3.66 (3H, s, COOCH₃) ppm, together with signals for 18 protons in the 3.5-0.9-ppm region, which closely matched signals in the corresponding region in the spectrum of I. Its IR spectrum showed ν_{\max} 3350 (NH) and 1710 (COOCH₃) cm⁻¹, and it had λ_{\max} (ethanol) 296 (sh) (log ϵ 4.00), 283 (4.09), and 224 (4.49) nm.

Anal.—Calc. for C₃₁H₃₈N₂O₅: *m/e* 518.2778. Found: *m/e* 518.2785.

Compound III had a similar mass spectrum to II, showing major peaks of *m/e* 518 (M⁺, 100), 503 (10), 433 (11), 384 (10), 367 (15), 311 (11), 151 (21), 136 (87), 135 (37), 124 (15), and 122 (26). Its PMR spectrum (CDCl₃) showed peaks at δ 7.84 (1H, s, NH), 7.14 (1H, d, *J* = 8 Hz, H-14), 6.90 (1H, d, *J* = 8 Hz, H-13), 6.8-6.6 (3H, m, H-2',5',6'), 4.42 (2H, s, ArCH₂Ar'), 3.80, 3.78, 3.76 (3H each, s, -OCH₃), and 3.68 (3H, s, COOCH₃) ppm, together with signals for 18 protons in the 3.5-0.9-ppm region. In this case, the signals for the eight protons in the 3.5-2.5-ppm region did not match the corresponding signals in the spectrum of I, indicating some shielding of the seven, eight, and, possibly, 19 protons by the dimethoxybenzyl ring system. Signals in the 2.2-0.9-ppm region matched the corresponding signals in I quite well. Its IR spectrum showed ν_{\max} 3370 (NH) and 1725 (COOCH₃) cm⁻¹.

Anal.—Calc. for C₃₁H₃₈N₂O₅·H₂O: C, 69.36; H, 7.51; N, 5.27. Found: C, 68.96; H, 7.13; N, 5.13.

Table II—Cytotoxicity of Various Semisynthetic Indole Alkaloids

Alkaloid	Cytotoxicity (ED ₅₀ in P-388 Cell Culture), μg/ml
I Voacangine	6.8
II 13-(3,4-Dimethoxybenzyl)voacangine	10
III 11-(3,4-Dimethoxybenzyl)voacangine	3.8
IV O-(3,4-Dimethoxybenzyl)voacanginol	16
V 13'-Vobasi-O-(3,4-dimethoxybenzyl)voacanginol	16
VI Voacamine	2.6
VIII 3α,4α-Epoxy-19-oxodihydrocatharanthine	>100
X 13-(3,4-Dimethoxybenzyl)-3α,4α-epoxy-19-oxodihydrocatharanthine	31

O-(3,4-Dimethoxybenzyl)voacanginol (IV)—A mixture of voacanginol (5) (0.28 g), 3,4-dimethoxybenzyl chloride (0.22 g), and pyridine (2 ml) was allowed to stand for 2 days at room temperature. The reaction mixture was poured into water, and the crude product was recovered by filtration. Purification by preparative TLC in hexane-ether-methanol (6:3:1) yielded IV as an amorphous white powder. The compound showed major mass spectral peaks at *m/e* 504 (*M*⁺, 74), 489 (6), 323 (100), 322 (53), 307 (23), 239 (13), 237 (23), 215 (98), 200 (24), 198 (15), 182 (9), 174 (12), 165 (50), 162 (18), 136 (76), 135 (99), 124 (8), and 122 (56).

Its PMR spectrum (CDCl₃) showed peaks at δ 8.35 (1H, s, NH), 7.45 (1H, dd, *J* = 8 Hz, H-6'), 7.25 (1H, d, *J* = 2 Hz, H-2'), 7.13 (1H, d, *J* = 8 Hz, H-14), 6.89 (1H, d, *J* = 2 Hz, H-11), 6.71 (1H, d, *J* = 8 Hz, H-5'), 6.71 (1H, dd, *J* = 8 and 2 Hz, H-13), 4.71 (1H, d, *J* = 12 Hz, H-22a), 4.33 (1H, d, *J* = 12 Hz, H-22b), and 3.83, 3.83, 3.65 (3H each, s, 3 × -OCH₃) ppm, together with signals for 18 protons in the region 3.5–0.9 ppm corresponding to the signals in this region in the spectrum of voacanginol. Its IR spectrum showed *ν*_{max} 3370 (NH) and 1700 (ArCOOR') cm⁻¹, and it had *λ*_{max} (ethanol) 288 (log *ε* 4.21), 262 (4.32), and 217 (4.73) nm.

Anal.—Calc. for C₃₀H₃₆N₃O₅·H₂O: C, 68.94; H, 7.24; N, 5.44. Found: C, 69.43; H, 7.67; N, 5.44.

13'-Vobasi-O-(3,4-dimethoxybenzyl)voacanginol (V)—A solution of vobasinol (0.177 g) and IV (0.252 g) in 1.5% methanolic hydrogen chloride (10 ml) was heated under reflux for 48 hr. Usual workup yielded a crude product (0.40 g), which was purified by preparative TLC in hexane-ether-methanol (4:5:1) to yield unreacted IV (0.055 g) and V (0.172 g). After a second preparative TLC, V was obtained as a white solid, homogeneous on TLC in two systems (0.110 g).

The isolated material had a PMR spectrum (CDCl₃) with peaks at δ 7.88 (1H, s, NH), 7.70 (1H, s, NH), 7.5 (2H, C, H-12 and H-6'), 7.24 (1H, d, *J* = 2 Hz, H-2'), 7.0 (3H, c, H-9,10,11), 6.89 (1H, s, H-14'), 6.77 (1H, s, H-11'), 6.72 (1H, d, *J* = 8 Hz, H-5'), 5.2 (2H, c, H-3 and H-19), 4.54 (1H, d, *J* = 12 Hz, H-22'), 4.28 (1H, d, *J* = 12 Hz, H-22'), 3.93 (3H, bd s, -OCH₃), 3.85 (3H, s, -OCH₃), 3.69 (3H, s, -OCH₃), 3.52 (3H, s, -COOCH₃), 2.43 (3H, s, -NCH₃), 1.64 (3H, d, *J* = 7 Hz, -CHCH₃), and 0.88 (3H, t, *J* = 7 Hz, CH₂CH₃), together with peaks in the 3.5–1.0-ppm region assignable to the voacangine or vobasine portions of the molecule. Its IR spectrum showed *ν*_{max} 3380 (NH) and 1710 (broad peak,

-COOCH₃ and ArCOOR') cm⁻¹, and it had *λ*_{max} (ethanol) 288 (log *ε* 4.29), 263 (4.31), and 218 (4.77) nm.

Anal.—Calc. for C₆₁H₆₀N₄O₇: N, 6.66. Found: N, 6.65.

3α,4α-Epoxy-19-oxodihydrocatharanthine (VIII)—Compound VIII was prepared by treatment of 4α-hydroxy-3β-iodo-19-oxodihydrocatharanthine acid lactone (VII) with base as previously described (7, 8). The material had identical properties to those described.

13-(3,4-Dimethoxybenzyl)-4α-hydroxy-3β-iodo-19-oxodihydrocatharanthine Acid Lactone (IX)—Treatment of VII (200 mg) with 3,4-dimethoxybenzyl alcohol (85 mg) in 1.5% methanolic hydrogen chloride under reflux for 48 hr yielded a mixture of six major components after the usual workup. Separation of the mixture by preparative TLC (acetone-hexane, 1:2) yielded the benzyl iodolactone IX (63 mg); IR: *ν*_{max} (KBr) 1770 and 1690 cm⁻¹; PMR (CDCl₃): δ 9.32 (1H, bd s, NH), 7.30 (1H, d, *J* = 8 Hz, 11-H), 7.2–6.9 (2H, complex), 6.68 (3H, bd s), 4.65–4.36 (2H, m, 2-H and 3-H), 4.30 (1H, s, 5-H), 3.96 (2H, s, ArCH₂Ar'), 3.79 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), and 1.00 (3H, t, 21-CH₃); mass spectrum: *m/e* 612 (*M*⁺, 3), 384 (4), 319 (11), 253 (100), 169 (8), 168 (11), 167 (6), 151 (20), 142 (25), and 127 (90).

13-(3,4-Dimethoxybenzyl)-3α,4α-epoxy-19-oxodihydrocatharanthine (X)—The benzyl iodolactone IX (40 mg) was added to sodium methoxide (4 mg) in methanol (2 ml), and the mixture was allowed to stand at room temperature for 2 hr. The resulting solution was poured into dilute acid; the aqueous layer was extracted with chloroform to yield a crude product, which was purified by preparative TLC on silica gel (acetone-hexane, 1:2). Elution of the major band yielded X as a homogeneous powder, *R*_f 0.44; PMR (CDCl₃): δ 8.16 (1H, bd s, NH), 7.4–6.8 (3H, complex), 6.64 (3H, bd s), 4.74 (1H, s), 3.92 (2H, s, ArCH₂Ar'), 3.76 (3H, s, OCH₃), 3.74 (3H, s, OCH₃), 3.68 (3H, s, OCH₃), and 1.00 (3H, t, 21-CH₃); mass spectrum: *m/e* 516 (*M*⁺, 65), 457 (10), 413 (9), 377 (11), 345 (15), 320 (17), 294 (23), 164 (80), 151 (100), 137 (25), and 136 (31).

Anal.—Calc. for C₃₀H₃₂N₂O₆: *m/e* 516.2259. Found: *m/e* 516.2267.

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