Plant Anticancer Agents III: Isolation of Indole and Bisindole Alkaloids from *Tabernaemontana holstii* Roots

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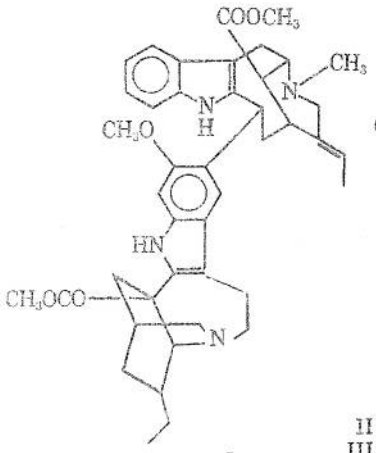
Abstract □ Certain active antileukemic and cytotoxic fractions prepared from *Tabernaemontana holstii* roots were investigated, resulting in the isolation of the known indole alkaloids conoduramine, conodurine, coronaridine, gabunine, 19-oxocoronaridine, pericyclivine, perivine, and vobasine. Two new alkaloids were assigned the structures 19-oxoconodurine and 19-(2-oxopropyl)conodurine. Both gabunine and 19-(2-oxopropyl)conodurine showed significant inhibitory activity against P-388 cell culture. All of the alkaloids are reported for the first time from *T. holstii;* conodurine, conoduramine, gabunine, perivine, and pericyclivine are reported for the first time from any *Tabernaemontana* species.

Keyphrases □ Alkaloids, indole—isolated from *Tabernaemontana holstii* roots, cytotoxic activity evaluated □ *Tabernaemontana holstii*—root extract, various indole alkaloids isolated, cytotoxic activity evaluated □ Cytotoxic activity—evaluated in various indole alkaloids isolated from *Tabernaemontana holstii* roots

A random screening of botanical sources for anticancer activity showed that the aqueous alcoholic extract of the roots of *Tabernaemontana holstii* K. Schum (family Apocynaceae) gave reproducible activity against the cell culture (KB) of a human carcinoma of the nasopharynx and also against P-388 lymphocytic leukemia in the mouse. Systematic fractionation of the extract led to the isolation of the known indole alkaloids conoduramine (I), conodurine (II), gabunine (III), coronaridine (VI), 19-oxocoronaridine (VII), perivine (VIII), vobasine (IX), and pericyclivine (X). In addition, two new alkaloids were isolated, and their structures were determined as 19-oxoconodurine (IV) and 19-(2-oxopropyl)conodurine (V). Both of these compounds, as well as VII, are probably artifacts formed during extraction and purification. Significant cytotoxicity in the P-388 cell culture system was shown by gabunine and 19-(2-oxopropyl)conodurine. No previous work on this plant species has been reported, although several other *Tabernaemontana* species have been investigated and contain many different indole alkaloids (1).



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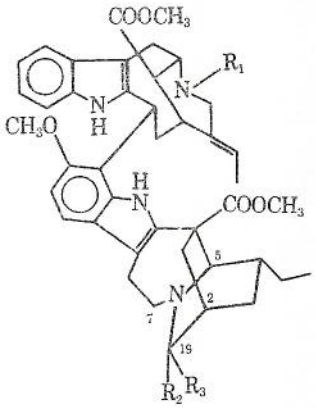
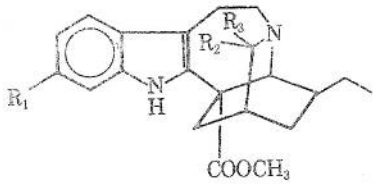
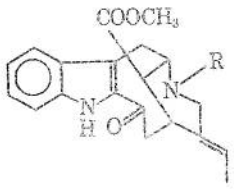


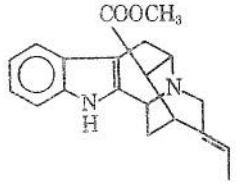
II: $R_1 = CH_3$, $R_2 = H$, $R_3 = H$
III: $R_1 = H$, $R_2 = H$, $R_3 = H$
IV: $R_1 = CH_3$, $R_2 R_3 = O$
V: $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_2COCH_3$



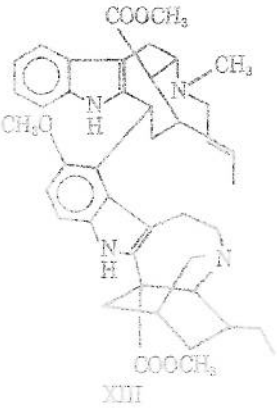
VI: $R_1 = H$, $R_2 = H$, $R_3 = H$
VII: $R_1 = H$, $R_2 R_3 = O$
XIV: $R_1 = OCH_3$, $R_2 = H$, $R_3 = CH_2COCH_3$



VIII: R = H
IX: R = CH_3



X



XIII

EXPERIMENTAL[1]

Plant Material—The air-dried roots[2] of *T. holstii* K. Schum (Apocynaceae) were collected in Kenya during 1971.

Extraction of Alkaloids—Plant material (4 kg) was extracted twice with 95% ethanol by stirring at room temperature for 24 hr, followed by one extraction with ammoniacal ethanol at 60° for 4 hr. The combined ethanol extract (A) (24 liters) was evaporated to 0.4 liter and treated with

[1] Melting points were determined in open capillary tubes or on microscope slides and are uncorrected. UV spectra were taken in ethanol on a Cary model 14 spectrophotometer. IR spectra were measured in chloroform solution or as potassium bromide pellets versus air with a Beckman model IR-20 spectrophotometer. Mass spectra were recorded at 70 ev using a Hitachi Perkin-Elmer RMU-7 mass spectrometer, operated in the direct probe mode. NMR spectra were determined in deuterochloroform, containing tetramethylsilane as the internal standard, using a Jeol PS-100 spectrometer. All concentrations and evaporations were carried out with water pump vacuum at less than 40°.

Column chromatography was carried out on silica gel 60, 0.063–0.200 mm (E. Merck), silica gel, 60–200 mesh (Baker), or Woelm alumina as indicated. Routine TLC was carried out using silica gel GF (Merck) plates, and visualization was by means of UV absorption and by the ceric ammonium sulfate reagent (2). The solvent systems used were: A_1, chloroform–methanol (95:5); and B_1, ethanol–ethyl acetate (1:4). Preparative thick-layer chromatography was carried out on silica gel PF_{254} (Merck) 1-mm layers, resolved components being detected by quenching under 254-nm UV light. Appropriate zones were scraped from the plates, pure components were recovered from the removed zones by elution with methanol–chloroform, followed by filtration and removal of the solvent. High-performance liquid chromatography (HPLC) was carried out on the apparatus previously described (3), using as packing material Porasil C, Porasil B, or Bondapak C_{18}/Porasil B, referred to in the text as C, B, and B, respectively.

[2] Vouchers specimens are on deposit with Dr. R. E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md.

0.5 liter of water, and this brown extract was extracted exhaustively with ethyl acetate in a separator and finally in a liquid–liquid extractor until an aliquot of the aqueous phase gave only a faint positive reaction with Mayer's reagent.

A small quantity of material that formed a suspension in the ethyl acetate was filtered off and put aside, and the ethyl acetate was concentrated to a volume of 1 liter. The concentrated ethyl acetate solution was then extracted six times with 50-ml portions of 5% sulfuric acid, followed by washing of the combined acid extracts with ethyl acetate. Neutralization of the acid with ammonia, followed by extraction with chloroform, yielded 17.6 g (0.44%) of crude tertiary bases after solvent evaporation (Extract B). The residual ethyl acetate after acid extraction was evaporated to yield a nonbasic extract (C).

Chromatographic Separation of Crude Alkaloids on Alumina—The crude alkaloid mixture from two extractions (30 g) was transferred to 300 g of alumina (neutral, activity III), and this material was added to the top of a 5-cm glass column containing 600 g of the same adsorbent. Elution with benzene (4.5 liters) yielded Fraction D (3.1 g), elution with chloroform (1.5 and 2.2 liters) yielded Fractions E (6.2) and F (3.1 g), and elution with methanol (7.2 and 7.3 liters) gave Fractions G (4.3 g) and H (4.0 g).

Separation of Fraction D—Fraction D (7 g) was chromatographed in a 2.5-cm column on 250 g of silica gel, with elution by methanol–chloroform (2:98) and collection of 150-ml fractions. The material eluted in fractions 2–5 (3.5 g) was combined and rechromatographed on a similar column with elution by methanol–dichloromethane (1:99) (33 fractions of 30 ml) and methanol–dichloromethane (2:98) (33 fractions of 30 ml). Fractions 9–16 were combined to yield Fraction I (1.1 g), and fractions 23–42 were combined to yield Fraction J (1.6 g).

Isolation of Coronaridine (VI)—Fraction I was chromatographed in a 2.5-cm column on 100 g of silica gel, with elution by methanol–dichloromethane (1:99) and collection of 30-ml fractions. Fractions 4 and 5 were combined (390 mg) and treated with methanolic hydrogen chloride to yield 100 mg of colorless crystals, mp 221–224° [lit. (4) mp 232–233°]; $[\alpha]_D^{24}$ −8.7° (c 0.32 in methanol). The isolate was homogeneous by TLC and gave a blue color with the ceric ammonium sulfate reagent; it had an R_f value of 0.95 in Solvent System A. The UV absorption spectrum showed λ_{max} (ethanol) 225 (log ε 4.29), 286 (3.73), and 293 (3.67) nm. The IR, UV, and mass spectral data for the compound were identical with those published for coronaridine hydrochloride (5, 6), and the NMR spectrum was consistent with this structure assignment.

Isolation of 19-Oxocoronaridine (VII)—Fraction J was combined with similar material from another batch of Fraction D to yield 3.4 g of material, which was chromatographed on a 2.5-cm column packed with 240 g of silica gel. Elution with dichloromethane (3.8 liters), followed by methanol–dichloromethane (0.5:99.5) (1 liter) and methanol–dichloromethane (1:99) (3 liters), yielded a new component in the fraction eluted with from 2–2.5 liters of the last solvent (390 mg).

Purification of this material by HPLC on Packing E [four 0.61 m × 0.94 cm (four 2 ft × 0.37 in.)] with elution with methanol–dichloromethane (0.25:99.75) yielded homogeneous material as the second peak eluted (100 mg). The material did not crystallize but was homogeneous on HPLC and TLC. It gave a yellow color with ceric ammonium sulfate and had an R_f value of 0.9 in Solvent System A. Its UV absorption spectrum showed λ_{max} 227 (log ε 4.52), 285 (3.81), and 295 (3.86) nm; its IR spectrum showed strong bands at 1730 and 1660 cm^{-1}. Its mass spectrum had major peaks at *m/e* 352 (54), 214 (10), 195 (20), 154 (30), 143 (10), 138 (10), and 124 (100). Comparison of its IR, NMR, and mass spectra with those obtained for 19-oxocoronaridine, recently isolated from *T. heyneana* (7), showed them to be identical, and the compound was identified as 19-oxocoronaridine.

Separation of Fractions E and F—Alkaloid material corresponding to Fractions E and F (12 g) was chromatographed on a 5-cm column on 600 g of silica gel with elution by methanol–chloroform (5:95). The material eluting between 1000 and 1860 ml was combined to yield Fraction K (3.7 g), and that eluting between 2900 and 3840 ml yielded Fraction L (2.2 g).

Isolation of Conodurine (II)—Fraction K was chromatographed on a 2.5-cm column on 170 g of silica gel with elution by ethyl acetate–carbon tetrachloride–ethanol (50:40:10). The resulting fractions were combined into five major fractions on the basis of TLC similarities. The most polar fraction (1.2 g) was chromatographed on a 5 × 75-cm column of silica gel and eluted with methanol–chloroform (3:97); 90-ml fractions were collected. The second fraction yielded crystalline material (40 mg) on evaporation of the solvent but was shown by TLC to consist of two components. Preparative TLC [methanol–chloroform (5:95), multiple development] gave pure material after crystallization from methanol, mp 210° dec. [lit. (8) mp 222–226°]. It had the same R_f values in Solvent Systems A (0.60) and B (0.56) as an authentic sample of conodurine (9) and gave the same yellow-green color with the ceric ammonium sulfate reagent. Its IR, UV, and mass spectra were identical with those of authentic conodurine.

Isolation of Perivine (VIII)—Fraction L was chromatographed on a 2.5-cm column on 200 g of silica gel. Elution with ethyl acetate–carbon tetrachloride–ethanol (50:40:10) and collection of 30-ml fractions gave a major fluorescent product in fractions 16–38 (1.6 g). Combination of this material with similar material from another batch of extract, chromatography of the combined material (2.4 g) on a 2.5-cm column on 200 g of silica gel, elution with methanol–dichloromethane (0.5:99.5), and collection of 30-ml fractions gave homogeneous material in fractions 14–17. Crystallization of this material from methanol yielded off-white crystals (150 mg), mp 179–180°, undepressed in admixture with an authentic sample of perivine (10). The IR and UV spectra of the isolate also were identical with those determined for the authentic sample.

Separation of Fraction E—Fraction E (6 g) from a second batch of crude alkaloid was chromatographed on a 5-cm column on 240 g of silica gel 60. After elution with chloroform (10.7 liters), Fraction M (2.0 g) was eluted with methanol–chloroform (1:99) (2 liters) and Fraction N (1.3 g) was eluted with a further 2.5 liters of the same solvent. A portion of Fraction M (1.75 g) was further chromatographed on a silica gel 60 column, 4 × 25 cm, with elution by methanol–chloroform (1:199) (2.1 liters), methanol–chloroform (1:99) (1.5 liters), methanol–chloroform (2:98) (1.7 liters), and methanol–chloroform (5:95) (2.2 liters). Evaporation of the first 600 ml of the methanol–chloroform (2:98) eluate yielded Fraction O (0.22 g), and evaporation of the final 1200 ml yielded Fraction P (0.45 g).

Isolation of Vobasine (IX)—Fraction O was purified by HPLC on Packing C, 1.8 cm × 1.83 m (0.75 in. × 6 ft), with elution by methanol–chloroform (1:99). The material giving rise to the second major UV absorbing peak in this chromatogram was collected to yield 35 mg of homogeneous alkaloid. This material did not crystallize but was shown to be homogeneous by analytical HPLC and TLC in Solvent Systems A and B. It gave a blue color with the ceric ammonium sulfate reagent, had an R_f value of 0.85 in Solvent System A, and cochromatographed with a sample of vobasine prepared from perivine (11) in Solvent Systems A and B. Its mass spectrum showed peaks at *m/e* 352 (30), 293 (14), 194 (5), 180 (100), and 122 (9); its UV spectrum showed peaks corresponding to those published (5) for vobasine. Its IR spectrum was identical with that published (5) for vobasine.

Isolation of Ketonic Alkaloid V—Fraction P was subjected to HPLC on Packing C, 1.27 cm × 1.22 m (0.5 in. × 4 ft), with elution by methanol–chloroform (1:99) containing 0.1% ammonia. The major fraction was rechromatographed on Packing B, using methanol–water–concentrated ammonia (90:5:5). Evaporation of the fourth fraction yielded a homogeneous material, which was recrystallized from methanol to yield a new alkaloid (20 mg), mp 203–204°. The compound was homogeneous by TLC, having an R_f value of 0.85 in System A, and gave a yellow-green color with the ceric ammonium sulfate reagent.

The UV spectrum of the isolate showed λ_{max} (ethanol) 225 (log ε 4.68), 285 (4.08), and 295 (4.08) nm. An IR spectrum showed a strong absorption band at ν_{max} 1730 cm^{-1}. The mass spectrum showed principal ions at *m/e* 774 (2), 760 (M^+, 4), 704 (2), 703 (2), 702 (2), 682 (4), 565 (5), 509 (4), 194 (23), 192 (6), 182 (50), 181 (74), 180 (100), 178 (3), 136 (32), 122 (96), and 58 (100).

The NMR spectrum showed a triplet at δ 0.90 ppm (3H) and a doublet at δ 1.82 ppm (3H). Five three-proton singlets were observed at δ 2.16, 2.66, 2.84, 3.84, and 4.12 ppm, while signals in the aromatic region showed the presence of eight protons between δ 6.8 and 8.0 ppm. These signals could be assigned to six protons on the indole ring and two protons attached to nitrogen; one of the aromatic protons was clearly visible as a doublet at δ 7.02 ppm. A two-proton multiplet was evident at δ 5.5 ppm, and additional signals were apparent in the δ 4.5–1.5-ppm range.

Isolation of Pericyclivine (X)—Fraction N[3] was purified by HPLC on Packing C to yield a pure fraction as described. On crystallization from methanol, the material had a melting point of 226–228° [lit. (11) mp 226–228° or (12) 232–233°], $[\alpha]_D^{24}$ +2.0° (c 0.40 in chloroform), and an R_f value of 0.60 in Solvent System A; it gave a gray-purple color with the ceric ammonium sulfate reagent. Its UV absorption spectrum showed λ_{max} 226 (log ε 4.29), 284 (3.65), and 292 (3.59) nm; its mass spectrum showed peaks at *m/e* 322 (20), 249 (20), 169 (100), and 168 (100). Finally, a comparison of the material with a sample of pericyclivine prepared from perivine (IR) showed that the two were identical.

[3] Previously designated F 176 (8).

Isolation of Conoduramine (I) and a New Amide (IV)—Material giving rise to the first major peak given by Fraction N on HPLC on Packing C (Fig. 1 of Ref. 3) (518 mg) was rechromatographed on Packing B [0.94 cm × 2.44 m (0.37 in. × 8 ft)] with elution by methanol–water–concentrated ammonia (90:5:5). Collection of the two major fractions and rechromatography under the same conditions yielded homogeneous material from both fractions.

The material from the first fraction (10 mg after crystallization from methanol) was identified as a new bisindole amide. It had a melting point of 217–218° and an R_f value of 0.70 in System A; it gave a yellow-blue color with ceric ammonium sulfate reagent.

The UV spectrum of the isolate showed λ_{max} 224 (log ϵ 4.60), 286 (4.16), and 295 (4.15) nm. An IR spectrum showed strong bands at ν_{max} 1730 and 1670 cm^{-1}. The mass spectrum showed principal ions at *m/e* 732 (5), 718 (M^+, 100), 687 (10), 659 (10), 523 (77), 492 (69), 194 (17), 182 (57), 181 (76), 180 (60), 150 (10), 136 (12), and 122 (43). The NMR spectrum (in acetone-d_6) showed a triplet at δ 0.90 ppm (3H), a doublet at 1.68 ppm (3H), and four three-proton singlets at δ 2.60, 2.68, 3.66, and 3.99 ppm. Signals for two NH protons at δ 7.6 ppm and for six aromatic protons from δ 6.8 to 7.4 ppm were visible, with a clear indication of an *AB* pattern at 6.90 and 7.24 ppm (2H). A broadened two-proton peak at δ 4.30 ppm was clearly visible in addition to a multiplet at δ 5.30 ppm (2H) and other peaks in the 4.0–1.5-ppm range.

The second fraction was identified as conoduramine on the basis of the following evidence. The material was crystallized from methanol to give 20 mg of white crystals, mp 220° dec. [lit. (13) mp 215–218°]. The material had identical TLC behavior in Solvent Systems A and B (R_f 0.75 and 0.44) as an authentic sample of conoduramine (9) and gave a blue color with the ceric ammonium sulfate reagent. Its UV spectrum showed λ_{max} 229 (log ϵ 4.71), 286 (4.22), and 295 (4.20) nm; its IR spectrum was superimposable on that of authentic conoduramine (9).

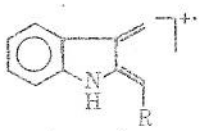
Isolation of Gabunine (III)—Material corresponding to Fractions E and F from a third extraction of plant material (13.8 g) was chromatographed on a 7-cm column on 740 g of silica gel 60. Elution with benzene–dichloromethane–ether (5:4:3) containing 1.5% methanol and collection of 30-ml fractions yielded 13 major combined fractions. Fraction 8 (0.56 g), a combination of tubes 741–1040, was subjected to preparative thick-layer chromatography in benzene–dichloromethane–ether (5:4:3) containing 10% methanol to yield two alkaloids. The slower moving compound (75 mg) was identified as perivine, identical with the sample isolated previously. The faster moving compound (50 mg) was purified by preparative HPLC on a 10-μm octadecylsilyl silica gel packing with elution by methanol–0.1% ammonium carbonate (70:30).

The compound was homogeneous by TLC and had an R_f value of 0.4 in Solvent System A. Its NMR spectrum showed a triplet at δ 0.85 ppm (3H), three methyl singlets at δ 2.54, 3.70, and 3.98 ppm, and a three-proton doublet at δ 1.65 ppm. An aromatic proton signal at δ 6.8 ppm was visible as a doublet (J = 8.5 Hz), corresponding to the similar absorption in conodurine and its derivatives. The mass spectrum showed ions at *m/e* 690 (M^+, 6), 672 (28), 660 (30), 648 (29), 630 (10), 614 (30), 226 (32), 208 (9), 194 (30), 183 (70), 182 (25), 180 (27), 166 (21), 136 (100), 122 (65), and 108 (21). The IR spectrum and TLC behavior of the material were identical with those of an authentic sample of gabunine (9).

Cytotoxic Activities—All isolated compounds were examined for activity against Eagles 9KH carcinoma of the nasopharynx or the P-388 leukemia in cell culture[4]. 19-(2-Oxopropyl)conodurine and gabunine were active in the P-388 system (ED_{50} 2.4 and 3.2 μg/ml, respectively).

DISCUSSION

19-Oxoconodurine (IV)—The mass spectrum of IV indicated clearly that the compound was a bisindole alkaloid consisting of a vobasan unit linked to a modified voacangine or isovoacangine moiety. Peaks at *m/e* 122 and 136 are characteristic of the iboga portion of such an alkaloid, while peaks at *m/e* 182, 180, 181, and 194 indicate the presence of a vobasan moiety (6). The molecular ion of the compound at *m/e* 716 [together with the usual $(M + 14)^+$ ion at *m/e* 732] was 14 mass units higher than that of conodurine and similar bisindole alkaloids, suggesting the presence of an additional oxygen atom in the molecule. Placement of this atom as the carbonyl oxygen of an amide group was demanded by the IR spectrum of IV, which showed a strong amide absorption at 1670 cm^{-1}. In view of the known (14, 15) ready oxidation at the 19-position of the iboga skeleton, it seemed most likely that the new carbonyl group should



XI: R = voacangine or isovoacangine
XII: R = oxovoacangine or oxoisovoacangine

be located at this position, leading to the assignment of Structure IV (or an isomer thereof) to the new alkaloid.

Support for the placement of the additional oxygen atom on the iboga portion of the molecule came from further analysis of its mass spectrum. In bisindole alkaloids of the voacamine type, an abundant ion at *m/e* 509 was assigned Structure XI by Thomas and Biemann (16). In the spectrum of IV, this ion was replaced by an abundant ion at *m/e* 523, which is only explicable if this ion has Structure XII where the additional oxygen atom is retained in the isovoacangine or voacangine moiety.

Finally, the NMR spectrum of the compound offered confirmation that its structure was indeed 19-oxoconodurine (IV). The spectrum showed all peaks characteristic of a molecule of the conodurine type, including signals for the six methyl groups (four singlets, one doublet, and one triplet) and signals for an aromatic *AB* system at 6.90 and 7.24 ppm. The presence of a conodurine system rather than the isomeric voacamidine system (XIII) was indicated by the fact that the downfield methoxycarbonyl group absorbed at 3.66 ppm (3.62 ppm in deuterochloroform), similar to the 3.68 ppm observed for II and quite different from the 3.08 ppm seen for XIII. Since it was unlikely that the presence of a carbonyl group in the 19-position could influence the chemical shift of the methoxycarbonyl group to this extent (VI and VII have nearly identical shifts for their methoxycarbonyl groups), this evidence indicated that the new alkaloid was related to II rather than XIII. The placement of the carbonyl group at C-19 was indicated by the signal at 4.30 ppm, which appeared to be a one-proton singlet superimposed on a broader one-proton signal. This pattern was noted in the spectrum of 19-oxovoacangine and assigned to the protons at C-5 and C-2 (15).

The ready oxidation of the iboga alkaloids at C-19 suggested that IV might well be an artifact of the isolation process.

19-(2-Oxopropyl)conodurine (V)—The mass spectrum of the isolated compound indicated that it had a molecular weight 56 mass units higher than that of II. Apart from the presence of peaks at *m/e* 703 (M − 57), 702 (M − 58), and 58, the remainder of the mass spectrum was very similar to that of II and its isomeric bisindole alkaloids. Thus, peaks at *m/e* 122 and 136 could be identified as deriving from an isovoacangine or modified isovoacangine moiety, while peaks at *m/e* 122, 180, 181, and 194 indicated the presence of a vobasan moiety (6). The peaks at *m/e* 702 and 58 most reasonably arise by a McLafferty rearrangement involving a 2-oxopropyl group attached to the alkaloid at some position (17).

Confirmation of a 2-oxopropyl group in the compound was obtained from its NMR spectrum, which showed a sharp three-proton singlet at δ 2.16 ppm, characteristic of a methyl group bonded to a carbonyl function. In addition, a two-proton doublet at δ 3.04 ppm could be assigned to the group $CHCH_2COCH_3$; the downfield shift of the signal was in accord with similar shifts observed for protons alpha to carbonyl groups in other indole alkaloids (15). The remainder of the NMR spectrum was in accord with the assignment of a modified II structure to the compound. The methyl resonances in particular were characteristic for this structure, while the upfield portion of an *AB* quartet visible at δ 7.02 ppm was evidence for a II rather than a I type of structure. The II structure was favored over the isomeric XIII structure on the same grounds as discussed for VII; the downfield methoxycarbonyl group absorbed at δ 3.64 ppm in V.

The remaining question in the assignment of a structure to the compound concerned position of attachment of the 2-oxopropyl group to the molecule. The observation of two weak peaks in the mass spectrum of the compound at *m/e* 178 (122 + 56) and 192 (136 + 56) suggested that the 2-oxopropyl unit was linked to the isovoacangine portion of the molecule, since ions with *m/e* 122 and 136 are characteristic of the iboga skeleton represented by isovoacangine. The chemical shift of the methylene protons of the 2-oxopropyl unit is only consistent with a linkage of this unit to the isovoacangine moiety at position 7 or 19, and the latter position is favored on the grounds that oxidation of the iboga system occurs much more readily at position 19 than at position 7.

In confirmation of the proposed structure, a recent paper[5] described the properties of 19-(2-oxopropyl)isovoacangine (XIV) (18). This compound was isolated from a crude alkaloid mixture which also contained

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

[5] Published after the conclusions above were reached independently.

19-hydroxyisovoacangine, and it probably was formed by reaction between acetone and the latter alkaloid. The spectral properties of XIV were consistent with those of V. In particular, XIV showed peaks at *m/e* 132, 136, and 122 in its mass spectrum, as did V, and both compounds also showed strong peaks in their IR spectra at about 1720 cm^{-1} for the ester and ketonic carbonyl absorptions. It is concluded, therefore, that the new ketonic alkaloid possesses Structure V, in which the stereochemistry at C-19 is unknown. It is likely that this alkaloid also was formed as an artifact by reaction of 19-hydroxyconodurine with traces of acetone present in the methanol during extraction or chromatography.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 2, 1976, from the *Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.*

Accepted for publication September 27, 1976.

Supported by Research Grant CA-12831 from the National Cancer Institute, U.S. Department of Health, Education, and Welfare, Bethesda, MD 20014.

The authors thank Dr. M. P. Cava for samples of conodurine, conoduramine, and gabunine, Dr. G. H. Svoboda and the Eli Lilly Co. for a sample of perivine, Dr. U. Renner for a sample of voacamidine, Dr. L. Goldman for spectral data of 19-oxocoronaridine, Dr. N. R. Farnsworth and Dr. H. H. S. Fong for the IR spectrum of pericyclivine, and Dr. R. E. Perdue, Jr., for plant material.

Previous paper in this series: D. G. I. Kingston, B. B. Gerhart, and F. Ionescu, *Tetrahedron Lett.*, 1976, 649.

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Physiologically Based Pharmacokinetic Model for Digoxin Distribution and Elimination in the Rat

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Abstract □ A plasma flow rate-limited pharmacokinetic model was developed to describe the distribution of digoxin to the heart, liver, kidneys, skeletal muscle, and GI tract in the rat. The model also provides for renal, hepatic (metabolic and biliary), and GI clearance as well as for biliary and GI secretion and GI reabsorption of digoxin. Predicted concentrations of digoxin in the heart, liver, skeletal muscle, and plasma were consistent with experimental observations in conscious rats after an intravenous dose. The model was extended to describe digoxin concentrations in the plasma of bile duct-ligated rats and ureter-ligated rats, simply by modifying appropriate clearance parameters. Excellent agreement was obtained between predicted and observed urinary excretion rates of digoxin for 12 hr after an intravenous dose to normal and bile duct-ligated rats.

Keyphrases □ Digoxin—pharmacokinetic model for distribution and elimination, effect of ligation of bile duct or ureter, rats □ Pharmacokinetics—digoxin, model for distribution and elimination, effect of ligation of bile duct or ureter, rats □ Distribution, tissue—digoxin, pharmacokinetic model, effect of ligation of bile duct or ureter, rats □ Elimination—digoxin, pharmacokinetic model, effect of ligation of bile duct or ureter, rats □ Cardiotonic agents—digoxin, pharmacokinetic model for distribution and elimination, effect of ligation of bile duct or ureter, rats

Compartment models to describe the pharmacokinetics of drug disposition are usually developed by curve fitting plasma concentration data with multiexponential equations. Due to the limitations of this approach, usually no representation more complex than a two-compartment open model is justified to describe the time course of drug concentrations in plasma. In almost all instances, compartment volumes and transfer constants have no anatomical or physiological reality. Moreover, these models are very species dependent. Although classical pharmacokinetic models have many clinical applications, the amount of basic information they provide is intrinsically limited.

In recent years, there has been considerable interest in the development of anatomically and physiologically realistic pharmacokinetic models for drug disposition based on organ blood or plasma flows and volumes (1). In principle, these models permit the prediction of drug concentrations in any target tissue at any time and may provide considerable insight to drug dynamics. Furthermore, drug distribution in certain pathophysiologic conditions may be simulated by altering estimates of organ blood flow (2, 3). Perhaps most important, physiologically based models can be scaled to apply to several species (4). Thus, the large data base required to develop a physiological pharmacokinetic model may be collected in a laboratory animal