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Problems in Chemotaxonomy I

Alkaloids of *Peschiera affinis*

By JERRY A. WEISBACH, ROBERT F. RAFFAUF, OSCAR RIBEIRO,† EDWARD MACKO, and BRYCE DOUGLAS

A phytochemical study of this plant has yielded three indole alkaloids, two of them being of the α-ketoindole type and the third containing a simple nonconjugated indole moiety. One of the α-ketoindole alkaloids has been identified as vobasine, whose recently described structure is found compatible with NMR spectral data. The isolation procedures are described for the second α-ketoindole alkaloid, affinine, and for the indole alkaloid, affinisine.

THE HISTORY of the genus *Tabernaemontana* L. (family *Apocynaceae*) is exceedingly complex. At various times, botanists have split other genera from it (*e.g.*, *Voacanga*, *Ervatamia*, *Gabunia*, *Conopharyngia*) leaving a residue of synonymy of genera and/or species within the group. With regard to South American representatives, botanical opinion concerning their classification has varied widely. Markgraf has separated and readjusted the genus *Tabernaemontana* L. into nine genera, preserving that name for species found in the Antilles, Central America, and parts of northwestern South America, while maintaining *Peschiera* A. DC. for those found in Brazil (1). Woodson does not share this opinion (2).

There is reason to believe that even in closely related genera sufficient differences in alkaloid composition exist to serve as a basis for chemotaxonomic distinctions. In Farnsworth's excellent review of the periwinkles (3) one notes that of the 48 characterized alkaloids isolated from *Vinca* and *Catharanthus* species, only one, akuammine (vincamajoridine) is common to both genera. Obviously, it will be necessary to know more about the actual structures before detailed comparisons can be made between the chemical types found in each genus. As of now, however, it can be stated that the dimeric C_{46} bases have been found only in the genus *Catharanthus*.

From the chemical point of view, the genera in the subtribe Tabernaemontainae have been found to contain two major structural types of alkaloids: the predominant ibogaine, I, type (4, 5) and the tabernaemontanine,[1] II, type (6, 7).

In the course of a broad screening program, about thirty plants which were identified as either *Tabernaemontana* or *Peschiera* species by South American botanists have been encountered. In order to detect any chemical differences which may exist between, and perhaps thus differentiate, these closely related genera, we have chosen a number of them for detailed study. The first of these, reported here, is *Peschiera affinis* (Meull.-Arg.) Miers.

DISCUSSION

A 5.8-Kg. sample of *P. affinis*[2] collected in northeastern Brazil was extracted with alcohol until removal of alkaloidal material was complete (Mayer's reagent). The extract was concentrated under reduced pressure and the residue was treated with dilute phosphoric acid. The filtered, aqueous

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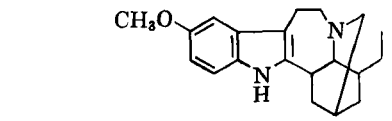
[1] It is of interest to note that the source of tabernaemontanine is said to be *Tabernaemontana coronaria* (8) = *Ervatamia coronaria* (7), possibly synonymous with *E. divaricata* (7).

[2] A specimen of this plant has been deposited with Prof. R. E. Schultes, Curator, Botanical Museum, Harvard University, Cambridge, Mass.



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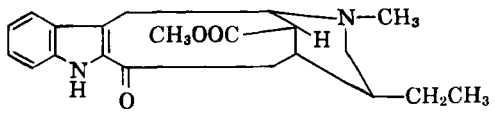
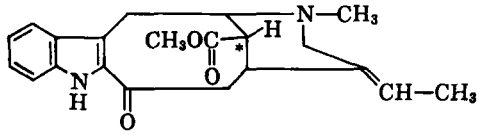


II

solution (pH 1) was subjected to continuous exhaustive extraction with ether. The pH of the aqueous phase was adjusted to 7 and exhaustive extraction with ether was repeated. Finally, extraction with chloroform, after further adjustment of pH to 10, removed residual non-quaternary alkaloidal materials. The quaternary constituents of the aqueous solution were investigated separately and will be the subject of a future communication. The bulk of the crude alkaloids was obtained in the ether extract made at pH 7. Much smaller amounts were found in the chloroform fraction, and the ether extract made at pH 1 was devoid of materials giving a positive alkaloid test.

The ether (pH 7) fraction was chromatographed on 100–200 mesh Florisil. Elution with chloroform gave (in the early fractions) a viscous oil which yielded a crystalline hydrochloride on treatment with ethyl acetate-ethereal hydrogen chloride solution. The regenerated base, obtained in *ca.* 0.45% overall yield, was found identical to the known alkaloid vobasine, III (6, 9, 10), by a mixed melting point determination, and by a comparison of its infrared spectrum and X-ray diffraction pattern with those of an authentic sample.[3]

The nuclear magnetic resonance spectrum[4] of vobasine was found to be in good accord with the elegant structure III, arrived at by Renner and Prins (6) on the basis of chemical as well as electronic and vibrational spectral results. The NMR data are described and interpreted as



III

δ (11)	Assignment
1.70	Vinyl-CH_3 split into doublet by adjacent proton
2.58, 2.65	Ester OCH_3 and NCH_3
3.50	A two-proton singlet from methylene adjacent to the basic nitrogen
5.43	Vinyl proton split into quartet by adjacent methyl
~7.3	Aromatic protons
9.37	N—H hydrogen bonded to carbonyl

[3] We are grateful to Dr. U. Renner, Geigy A. G., Basel, Switzerland, for an authentic sample of vobasine.

[4] The NMR spectrum was determined and interpreted by Dr. G. O. Dudek, Chemistry Department, Harvard University.

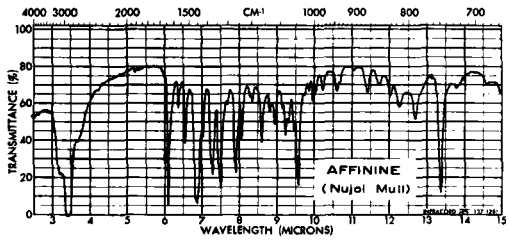


Fig. 1

Some commentary is required with regard to the signals assigned to methoxyl and tertiary aliphatic N-methyl. The expected shifts (as compared to tetramethyl silane) for the two groups are *ca.* 3.7–3.8 and 2.2–2.6 p.p.m., respectively (11, 12, 13). Thus, either signal may be assigned to the N-methyl. The striking shift of O-methyl to higher fields can be explained on the basis of the stereochemistry at C* (as written in III) by an interaction with the diamagnetic portion of the induced field from the aromatic π electrons leading to the observed increased shielding effects (14). If the alternative stereochemistry does in fact

obtain, an $N:\rightarrow C(=O)-OCH_3$ interaction would be expected which also leads to increased shielding of methoxyl, but this time accompanied by a diminished shielding of the N-methyl. In either event there will be a small (~0.2–0.3 p.p.m.) diamagnetic effect from the adjacent double bond on the latter grouping. This situation is also compatible with the observed signals. The resolution of this point is presently under investigation and will be the subject of a future communication.

The above bands represent the majority of the substituents of the molecule and are located and spin-spin coupled in conformity with III. The remaining signals from protons located in the fused ring system cannot be assigned with any certainty as the expected signals cover a small portion of the spectrum. In addition, both their position and spin-spin couplings are dependent upon ring geometry and conformation.

Further elution of the column yielded two new alkaloids, affinisine, $C_{19}H_{24}N_2O$, (5.5 Gm., 0.095%, isolated as hydrochloride) and affinine, $C_{20}H_{24}N_2O_2$ (0.85 Gm.[5]).

Affinisine has an ultraviolet absorption spectrum indicating the presence of a simple nonconjugated indole. Its infrared spectrum (Nujol mull) shows no bands in the 5.5 to 6.5 μ region corresponding to a carbonyl function and did not afford a decision as to the presence or absence of a hydroxyl group on the basis of the 3 μ region. A negative methoxyl determination requires that the single oxygen be present as in either an internal ether or a hydrogen bonded hydroxyl group.

The ultraviolet spectrum of affinine in 95% ethanol indicates the presence of an α-keto indole moiety similar to that found in tabernaemontanine, II, vobasine, III, dregamine (7, 10, 15), voacafrine (16), voacafricine (16), and perivine (17). The infrared spectra of these alkaloids are all reported to

[5] Additional affinine was isolated from the chloroform extract at pH 10 to bring the total yield to *ca.* 0.07%.

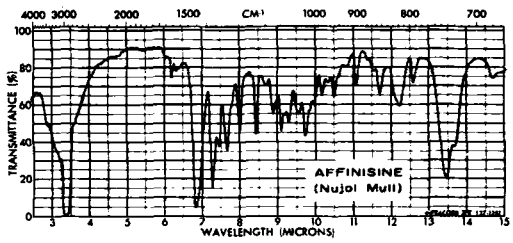


Fig. 2

contain a band at *ca.* 6.06–6.08 μ ascribable to the keto indole grouping, accompanied by a band at 5.79–5.80 μ, probably due to an ester grouping. The infrared spectrum of affinine contains only one band, at 6.08 μ, making it the first example of an alkaloid of this type with a single carbonyl functionality. Its empirical formula, $C_{20}H_{24}N_2O_2$, corresponds to a des-carbomethoxylated voacafrine ($C_{22}H_{26}N_2O_4$) to which it may be directly related.

Isolation of two α-keto indole alkaloids as well as a third containing a simple indole chromophore is in good accord with the predicted general botanical-chemical relationship with *Tabernaemontana*, *Ervatamia*, *Voacanga*, etc. The absence of any of the known alkaloids of the ibogaine type is suggestive of differences between *Peschiera* and the other genera. However, the structure determination of affinisine as well as additional chemical studies in this area are necessary before a firm generalization can be made.

PHARMACOLOGY OF VOBASINE, AFFININE, AND AFFINISINE

Vobasine hydrochloride exhibited weak, but significant, central nervous system depression and ataxia after oral doses of 200 mg./Kg. in mice. Doses of 300 mg./Kg. caused lacrimation, mydriasis, and respiratory depression as well as a progressive increase in the central depressant activity. This compound failed to produce overt effects in cats receiving doses of 15 mg./Kg. intraperitoneally, but was found to produce vocalization, mydriasis, and short-lived, tonic-clonic convulsions following doses of 35 mg./Kg. intraperitoneally. When injected intravenously into the ether-chloralose anesthetized cat at doses ranging from 0.5 to 5.0 mg./Kg. it produced transient depressor activity. A dose of 10 mg./Kg. proved lethal as a result of respiratory depression. No significant changes were observed in the standard test agents (epinephrine, norepinephrine, DMPP, or furfuryl trimethyl ammonium iodide) which measure alterations in the autonomic nervous system after drug treatment. No diuretic activity was observed in the saline hydrated rat receiving doses of 25 mg./Kg. of vobasine orally. Utilizing a modification of the method of Randall and Selitto (18) as presumptive evidence for anti-inflammatory action, we found that vobasine in doses of 25 mg./Kg. orally produced very weak analgesia and moderate antipyretic action, but failed to reduce edema in the rat paw. In the rat, doses of 100 mg./Kg. orally produced signs of toxicity.

Affinine produced gross behavioral changes in the mouse similar to those observed with vobasine. Orally, doses of 200 mg./Kg. caused only slight CNS depressant action, whereas doses of 300 mg./Kg. produced delayed intention tremors, marked CNS depressant activity, ataxia, hypothermia, and bradypnea. No overt effects were observed in the unanesthetized cat receiving 25 mg./Kg. of the compound intraperitoneally. In the anesthetized cat, affinine produced only transient lowering of the mean arterial blood pressure after a cumulative dose as high as 19 mg./Kg. intravenously. Some evidence of toxicity was uncovered since bradycardia, respiratory depression, and cardiac arrhythmias were observed as the dose levels were increased. Affinine was practically inactive when tested for analgetic, antipyretic, or antiedema activity in the Randall and Selitto anti-inflammatory test. No diuretic action was produced in the saline hydrated rat.

Affinisine produced central nervous system depression, lacrimation, and tremors after doses of 50 mg./Kg. orally in the mouse. However, no side effects were observed in the unanesthetized cat following a dose of 10 mg./Kg. intraperitoneally. In the ether-chloralose anesthetized cat, affinisine caused respiratory toxicity after a dose of 1.0 mg./Kg. and proved lethal after an acute dose of 10 mg./Kg. intravenously. Affinisine possesses moderate analgetic activity in the rat after doses as low as 25 mg./Kg. orally. On the other hand, this activity was not accompanied by an effect on skin temperature or on edema. There was no evidence of diuretic activity when affinisine was tested in doses of 25 mg./Kg. orally in the saline-loaded rat.

EXPERIMENTAL[6, 7]

Isolation

The ground whole plant of *P. affinis* (5.8 Kg.) was extracted in a Soxhlet type apparatus with hot 95% ethanol until the marc gave a negative alkaloid test (Mayer's reagent). The extract was concentrated *in vacuo* to a syrupy consistency and the residual solvent was removed at 25° in a stream of air.

The residue was extracted with phosphoric acid (0.2 *M*, total of 14 L.) and the resultant mixture was filtered with Celite. The aqueous solution was treated with ether in a liquid-liquid continuous extractor to yield *ca.* 7 Gm. of nonalkaloidal material. An aliquot (*ca.* two-thirds) of the aqueous layer was neutralized (pH 7) with concentrated sodium bicarbonate solution and extracted to completion with ether. The organic layer yielded 43 Gm. of alkaloid as a brown, crispy foam, which was further processed as described below. The aqueous phase was made alkaline with ammonia and extracted with chloroform to yield an additional 2.3 Gm. of an alkaloidal precipitate and *ca.* 3.5 Gm. of viscous bases.

Forty grams of the alkaloids, extracted at pH 7,

[6] Melting points were determined on a Thomas-Hoover Uni-Melt apparatus. Ultraviolet absorption spectra were obtained using a Cary model 14 spectrometer, and infrared spectra were obtained using a Perkin-Elmer model 173B.

[7] We are indebted to Mr. Irving Eisdorfer of our laboratories for the following paper chromatographic procedure used throughout this study. The mixture *iso*-amyl alcohol: *tert*-amyl alcohol:water:88% formic acid–5:5:10:2 was shaken well. The separated lower layer was used as standing phase and the upper layer as the moving phase on 3 mm. Whatman paper (12 in. × 18 in. sheet) arranged for circular development. The spray reagent used was potassium iodoplatinate.

was dissolved in chloroform and chromatographed on a Florisil column (1 Kg. adsorbent, 3 in. diameter) made up with chloroform. The initial chloroform eluate (*ca.* 3 L.) on combination and evaporation yielded a pale yellow, viscous liquid. Treatment with ethyl acetate-ethereal hydrogen chloride yielded a white crystalline product (17.9 Gm.). Recrystallization from ethyl acetate-methanol gave an analytical sample, m.p. 247° (decompn.), R_f 0.65.

Anal.—Calcd. for $C_{21}H_{24}N_2O_3 \cdot HCl$: C, 64.84; H, 6.48; N, 7.21; Cl, 9.12; O—CH_3, 8.05; N—CH_3 (calcd. CH_3), 3.89; C—CH_3, 6.98. Found: C, 64.42; H, 6.37; N, 7.10; Cl, 8.84, O—CH_3, 8.06; N—CH_3, 3.48; C—CH_3, 5.01.

The free base was obtained by treatment of the hydrochloride with ammoniacal chloroform and isolation of the alkaloid in the usual manner. Recrystallization from ether yielded an analytical sample, m.p. 112.5–115°.

Anal.—Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.85. Found: C, 71.32; H, 6.98; N, 7.94.

A mixed melting point determination with authentic vobasine,[3] m.p. 112.5–115° gave m.p. 112.5–115°. The infrared spectra (Nujol mull) of this base and of authentic vobasine were essentially identical and their X-ray diffraction patterns were superimposable.

Affinisine

Further elution with chloroform (9 L.) gave an eluate which on combination and evaporation yielded a viscous oil. On treatment with ethereal hydrogen chloride-ethyl acetate a white crystalline product (5.55 Gm.) was obtained. Recrystallization from methanol gave fine needles, m.p. 287° (decompn.) $[\alpha]_D^{25}$ +40.3 (C = 0.5, MeOH), R_f 0.76.

Anal.—Calcd. for $C_{19}H_{24}N_2O \cdot HCl$: C, 68.57; H, 7.52; N, 8.42; Cl, 10.65; O—CH_3, 9.31; N—CH_3 (calcd. CH_3), 4.50; C—CH_3, 8.11. Found: C, 68.78; H, 7.36; N, 8.30; Cl, 10.44; O—CH_3, negligible; N—CH_3, 3.53; C—CH_3, 3.58. $\lambda_{max.}^{EtOH}$ 224 mμ (log ε 4.65), 282 mμ (log ε 3.92), 292 mμ (log ε 3.82).

The free base was obtained in the usual manner and was recrystallized from methylene chloride-petroleum ether, m.p. 115–118° (insertion at 100°).

Anal.—Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 7.53; N, 9.45. Found: C, 77.07; H, 7.79; N, 9.05, negligible O—CH_3.

Affinine

Elution of the chromatographic column with 15% methanol in chloroform (2 L.) yielded a viscous oil which readily crystallized (0.85 Gm.) from ethyl acetate. Recrystallization from methanol-ethyl acetate yielded an analytical sample as a white crystalline powder, m.p. 265° (decompn.), R_f 0.60.

Anal.—Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 74.19; H, 7.49; N, 8.64. $\lambda_{max.}^{EtOH}$ 238 mμ (log ε 4.18), 318 mμ (log ε 4.34).

The hydrochloride salt was prepared by solution in methanol, addition of ethereal hydrogen chloride, and removal of solvent *in vacuo*. An analytical sample, m.p. 267–269° (decompn.) was obtained by recrystallization from methanol-ethyl acetate $[\alpha]_D^{25}$ −105.4 (C = 0.5, MeOH).

Anal.—Calcd. for $C_{20}H_{24}N_2O_2 \cdot HCl$: C, 66.56; H, 6.98; N, 7.76. Found: C, 66.21; H, 7.03; N, 7.88, negligible O—CH_3.

Further elution of the column with 15% methanol in chloroform (5 L.) yielded only dark viscous oils (9.9 Gm.), which were not further characterized.

Infrared examination of the alkaloidal precipitate obtained at pH 10 with chloroform showed it to be essentially pure affinine.

No additional non-quaternary crystalline compounds were obtained upon chromatography of any other materials from this plant.

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