1,2,10-Trimethoxydibenzo[*de,g*]quinolin-7-one (VIII).—Fraction E (19 mg) was subjected to thick layer chromatography on silica gel H_{254} (one plate, 20 × 20 cm, 0.5 mm thick) with 10% ethanol–chloroform. The principal band was transferred to a column and washed with chloroform. The chloroform eluate yielded a red solid residue (11 mg) which was recrystallized from acetone–ethanol to yield red microneedles (VIII, 5 mg): mp 256–258°; λ_{max}^{MeOH} 234 mμ (ε 33,330), 246.5 (41,050), 322 (7420), 470 (5830); λ_{max}^{KBr} 5.99 μ (conjugated ketone); nmr signals at τ 6.27 (3 H, OCH_3), 5.97 (6 H, two OCH_3), 1.75–2.82 (6 H, aromatic *H*).

Anal. Calcd for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71. Found: C, 70.95; H, 4.76.

6′-Hydroxylaudanosine (III).—Fraction F (312 mg) was dissolved in 10% acetic acid (5 ml) and the solution was filtered. The filtrate was treated with aqueous potassium iodide to complete precipitation. The precipitate was collected, washed with water, and recrystallized from ethanol to yield needles (118 mg), mp 184–186°. The melting point was not depressed by admixture with an authentic sample of 6′-hydroxylaudanosine hydroiodide, and the ultraviolet and infrared (Nujol) spectra and tlc mobility were identical with those of the authentic sample.[10]

Fraction B, shown by tlc to contain III as a major component, yielded 25 mg of III hydriodide.

Catalytic Reduction of Dehydrothalicarpine (I).—A suspension of platinum oxide (50 mg) in glacial acetic acid (15 ml) was saturated with hydrogen, and dehydrothalicarpine (50 mg) in acetic acid (2 ml) was added. The mixture was subjected to catalytic hydrogenation at 26° and atmospheric pressure. The reaction was terminated after 18 hr (hydrogen consumption, 10.0 ml) and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in 2.5% hydrochloric acid (10 ml). The solution was made alkaline with dilute ammonium hydroxide and extracted with chloroform. The chloroform was dried over anhydrous sodium sulfate and evaporated to dryness (residue, 41 mg). The residue was dissolved in the upper phase of the system Skellysolve B–ethylene chloride–methanol–water (10:2:2:0.16) and chromatographed on a column of Celite 545 impregnated with chlorophenol red and lower phase of the solvent system.[17] The fractions containing thalicarpine (tlc) were combined and crystallized from ether to yield thalicarpine (II, 17 mg), mp 156–158°, $[\alpha]^{29}D$ +133° (*c* 0.31, methanol). The melting point was not depressed by admixture with an authentic sample of thalicarpine, and the infrared spectrum and mixed tlc were identical with those of the authentic sample.

Oxidation of Thalicarpine (II) with DDQ.—To a stirred solution of thalicarpine (II, 1.00 g) in benzene (65 ml) was added a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (320 mg) in benzene (10 ml), and the solution was heated in an oil bath at 62° for 5 hr. A black powdery solid (855 mg) was separated by filtration, and the benzene solution was extracted with 2.5% hydrochloric acid. The solution was made alkaline with 10% ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure to dryness (residue, 502 mg). The residue was subjected to partition chromatography as described for thalicarpine above, and two bands were separated. The first band eluted corresponded to dehydrothalicarpine (364 mg) and the second to thalicarpine (26 mg). Crystallization of the first band from acetone yielded dehydrothalicarpine (338 mg), mp 180–183°, characterized by mixture melting point determination, mixed tlc, and ir, uv, and nmr spectral comparison with the authentic sample. The black solid (855 mg) separated from the original reaction mixture was shaken for 3 hr with 2.5% sodium hydroxide solution (25 ml) and benzene (100 ml). The benzene layer was filtered through a bed of anhydrous sodium sulfate, and concentrated to dryness under reduced pressure. The residue (460 mg) was subjected to partition chromatography, as described above, to yield 23 mg of dehydrothalicarpine, mp 180–183°, and 206 mg of thalicarpine, mp 150–155°. The yield of dehydrothalicarpine, based on unrecovered starting material, was 47%.

Registry No.—I, 7224-94-4; I dimethiodide, 15569-53-6; IV, 15562-38-6; IV hydrochloride, 15562-39-7; (±)-VI hydroiodide, 15562-40-0; thalidasine oxalate, 11040-48-5; VII, 15562-41-1; VIII, 15562-42-2.

The Alkaloids of *Tabernaemontana riedelii* and *T. rigida*

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The alkaloids of two Amazonian species of *Tabernaemontana* have been studied; each species yielded indole alkaloids of only one structural type. Thus, *T. riedelii* contained the new alkaloids (+)-8-oxominovincine and (+)-minovincine, as well as a mixture of (±)- and (+)-vincadifformine. *T. rigida* contained (±)-vincamine, (+)-vincamine, (+)-apovincamine, and a substance believed to be a mixture of (±)- and (−)-14-epivincamine.

The genus *Tabernaemontana* of the family Apocynaceae has been the subject of continued controversy with regard to problems of botanical classification. Even recent authorites such as Markgraf[3] and Woodson[4] have disagreed on the question of whether a considerable number of *Tabernaemontana* species should be retained within the genus or should be reclassified as members of related genera. At the present time, we are engaged in a broad study of the alkaloids of the genus *Tabernaemontana*, in the expectation that the results will have chemotaxonomic value in a future reclassification of the genus.

Indole alkaloids have been isolated and identified from a number of *Tabernaemontana* species.[5] These include *Tabernaemontana australis*,[6] *T. psychotrifolia*,[6] *T. oppositifolia*,[6] *T. alba*,[7] *T. pachysiphon*,[8] *T. pandacaqui*,[9] *T. mucronata*,[10] *T. laurifolia*,[11] *T. heyneana*,[12]

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(2) Instituto da Pesquisas da Amazonia, Manaus, Brazil.

(3) F. Markgraf, *Notizbl.* **14**, No. 121, 151 (1938).

(4) R. E. Woodson, Jr., "North American Flora: Asclepiadales-Apocynaceae," Vol. 29, Part II, 1938, p 103.

(5) The related genera *Voacanga*, *Ervatamia*, *Gabunia*, and *Conopharyngia* are not included in this listing.

(6) M. Gorman, N. Neuss, N. J. Cone, and J. A. Deyrup, *J. Amer. Chem. Soc.*, **82**, 1142 (1960).

(7) O. Collera, F. Walls, A. Sandoval, F. García, J. Herrán, and M. C. Perezamador, *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.*, **14**, 3 (1962).

(8) J. Thomas and G. A. Starmer, *J. Pharm. Pharmacol.*, **15**, 487 (1963).

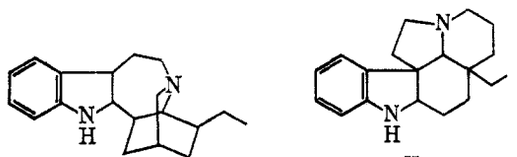
(9) G. Aguilar-Santos, A. C. Santos, and L. M. Joson, *J. Phillipine Pharm. Assoc.*, **50**, 321, 333 (1964).

(10) A. C. Santos, G. Aguilar-Santos, and L. L. Tibayan, *An. Real Acad. Farm.*, **31**, 3 (1965).

(11) M. P. Cava, S. K. Mowdood, and J. L. Beal, *Chem. Ind.* (London), 2064 (1965).

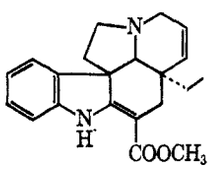
(12) T. R. Govindachari, B. S. Joshi, A. K. Saksena, S. S. Sathe, and N. Viswanathan, *Chem. Commun.*, **97** (1966).

and *T. rupicola*,[13] as well as the ambiguous species *Peschiera affinis* (*T. affinis*).[14,15] Almost all of these alkaloids have the ibogamine-type skeleton (I); a few[13] are derived from this skeleton by rearrangement.[16] It seems significant that the large and important group of alkaloids having the aspidospermine-type skeleton (II) has been represented up to this time among *Tabernaemontana* alkaloids only by tabersonine (III).[7]



I

II

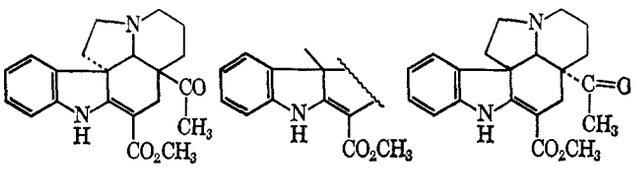


III

We now report the isolation and identification of a number of alkaloids, none of which have the ibogamine skeleton I, from the Amazonian species *Tabernaemontana riedelii* and *T. rigida*.

The Alkaloids of *T. riedelii*.—Since *T. riedelii* is a small shrub, the entire above-ground portion of the plant was examined without separation of the plant parts. Isolation of the crude tertiary base mixture was followed by chromatographic purification to give a mixture consisting mainly of three components; the latter were separated by partitioning between benzene and aqueous acid solutions of increasing acidity. One major constituent and two minor ones were thus obtained.

The amorphous major alkaloid of *T. riedelii* was assigned the structure of (+)-minovincine (IV) on the basis of the following evidence. Its ultraviolet spectrum shows maxima at 300 and 330 mμ, suggesting the presence of the chromophore V, present in a number of dihydroindole alkaloids.[17] Its nmr spectrum shows, in addition to four aromatic protons and a hydrogen-bonded NH at low field, a methyl singlet at δ 3.71 and at 1.82; the latter may be assigned to a carbomethoxy methyl and a C-acetyl methyl, respectively. Its infrared spectrum in chloroform solution shows carbonyls at 1670 and 1700 cm^{-1}, attributable to a hydrogen-bond ester group and an unconjugated ketone, respectively. Finally, direct comparison of the infrared spectrum of IV with the published spectrum of (−)-minovincine (VI)[18] show the two to be essentially identical.

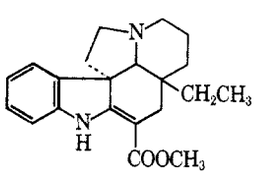


IV V VI

The mass spectrum of (+)-minovincine (IV) confirmed its molecular weight, and showed additional significant peaks at 309, 214, 138 (base peak), and 43. The breakdown pattern leading to the 138 and 214 fragments is quite typical for the aspidospermine-type skeleton.[19]

(+)-Minovincine (IV) has not been previously described, although it is the major alkaloid of *T. riedelii*. In contrast, the previously reported (−)-minovincine (VI) occurs only as a minor alkaloid in *Vinca minor* L.[18]

The first of the two minor bases of *T. riedelii* is, like minovincine, amorphous though homogeneous by thin layer chromatography. Its ultraviolet spectrum is similar to that of (+)-minovincine, suggesting the presence of the same chromophore V. Its infrared spectrum indicates the absence of a ketonic carbonyl, although the remainder of the spectrum in the 1600–1700-cm^{-1} region is quite similar to that of (+)-minovincine. It seemed very likely, therefore, that this base might be the desoxo analog of (+)-minovincine, namely, (+)-vincadifformine (VII). Support for this structure was obtained by mass spectrometry, which confirms the molecular weight (338); details of the spectrum are the same as those previously recorded for vincadifformine.[19]



VII

The amorphous (−)-vincadifformine, $[\alpha]D$ −540°, has been isolated from *Vinca minor* L.,[18] whereas the crystalline (±)-vincadifformine, mp 124–125°, has been isolated from *Vinca difformis* Pourr.[20] Since the rotation of our amorphous base is only +185°, it must be a mixture of (+)-vincadifformine (VII) and (±)-vincadifformine. Vincadifformine occurs also in *Rhazya stricta* as a mixture of the (+) and (±) forms.[21]

The second of the minor bases of *T. riedelii* forms colorless crystals: mp 257.5–259.5°; $[\alpha]D$ +268°. Its ultraviolet spectrum shows maxima at 298 and 330 mμ, suggesting the presence of chromophore V. Its infrared spectrum in potassium bromide is quite similar to that of (+)-minovincine, but it exhibits a carbonyl band at 1620 cm^{-1} in addition to the two minovincine carbonyls. The position of this third carbonyl band, together with the very weakly basic nature of the compound, suggested the structure of (+)-8-oxominovincine (VIII). Elemental analysis supported the compo-

(13) C. Niemann and J. W. Kessel, *J. Org. Chem.*, **31**, 2265 (1966).

(14) J. A. Weisbach, R. F. Raffauf, O. Ribeiro, E. Macko, and B. Douglas, *J. Pharm. Sci.*, **52**, 350 (1963).

(15) M. P. Cava, S. K. Talapatra, J. A. Weisbach, D. Douglas, R. F. Raffauf, and O. Ribeiro, *Chem. Ind.* (London), 1193 (1964).

(16) *Peschiera affinis* is a significant exception to this rule, since only alkaloids derived from the sarpagine-type skeleton have been isolated from it.[14,15] This fact lends chemotaxonomic support to its classification outside of the genus *Tabernaemontana*.

(17) K. Aghoramurthy and R. Robinson, *Tetrahedron*, **1**, 172 (1957); G. F. Smith and J. T. Wrobel, *J. Chem. Soc.*, 739 (1960); K. Bernauer, W. Arnold, C. Weissmann, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **43**, 717 (1960); J. Lévy, J. LeMen, and M. M. Janot, *Bull. Soc. Chim. Fr.*, 979 (1960).

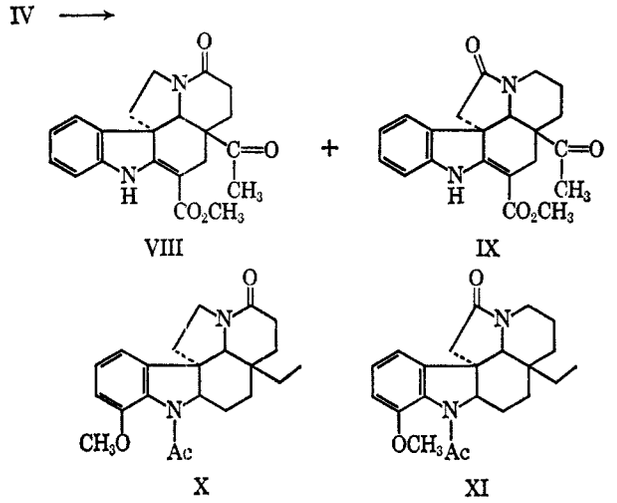
(18) M. Plat, J. LeMen, M. M. Janot, H. Budzikiewicz, J. M. Wilson, L. J. Durham, and C. Djerassi, *Bull. Soc. Chim. Fr.*, 2237 (1962).

(19) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I (Alkaloids), Holden-Day, Inc., San Francisco, Calif., 1964, p 98 ff.

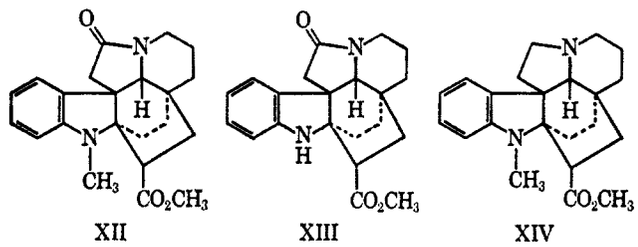
(20) J. Gosset, J. LeMen, and M. M. Janot, *Ann. Pharm. Fr.*, **20**, 448 (1962); C. Djerassi, H. Budzikiewicz, J. M. Wilson, J. Gosset, J. LeMen, and M. M. Janot, *Tetrahedron Lett.*, 235 (1962).

(21) G. F. Smith and M. A. Wahid, *J. Chem. Soc.*, 4002 (1963).

sition $C_{21}H_{22}N_2O_4$; the corresponding molecular weight (366) was confirmed by mass spectrometry.[22] Final proof of the structure of VIII was obtained by a study of the controlled permanganate oxidation of (+)-minovincine, which afforded two weakly basic lactams. The first product was the crystalline δ-lactam, (+)-8-oxominovincine (VIII), identical in all respects with the compound isolated from *T. riedelii*. The second product was the amorphous γ-lactam, 10-oxominovincine (IX). The lactam carbonyl frequencies of VIII and IX in chloroform solution are 1630 and 1670 cm^{-1}, respectively, as expected on the basis of the different sizes of the lactam rings. These values are in good accord with the corresponding reported values of 1625 and 1680 cm^{-1} for the lactam functions of compounds X and XI, respectively.[23]



(+)-8-Oxominovincine (VIII) is the first example of a naturally occurring aspidospermine-type alkaloid which contains a δ-lactam function. On the other hand, pleiocarpinilam (XII) and kopsinilam (XIII) are examples of related naturally occurring γ-lactam alkaloids.[24] It is of interest to note that an attempt to effect the air oxidation of pleiocarpine (XIV) to γ-lactam XII (or its δ-lactam isomer) was unsuccessful.[24] Consequently, the possibility that 8-oxominovincine may be an artifact arising from minovincine by air oxidation seems quite unlikely.



(+)-Vincadifformine, (+)-minovincine, and (+)-8-oxominovincine all have the same absolute configuration, namely, that shown in structures VII, IV, and VIII, respectively. This assignment follows from the known stereochemistry of (−)-vincadifformine and (−)-minovincine.[25]

The Alkaloids of *T. rigida*.—*Tabernaemontana rigida* Miers has a curious history of repeated botanical reclassification which is worthy of brief review in the light of the unexpected alkaloid type which it contains. The plant was first classified as *Tabernaemontana macrophylla* in 1860 by Miers,[26] who was unfortunately unaware of the earlier use of this name to describe a different species.[27] In 1878, Miers reassigned the plant to another genus, calling it *Phrissocarpus rigidus*.[28] Many years later, Markgraf assigned it to still a different genus, calling it *Anacampta rigida*.[29] Most recently, R. E. Woodson transferred it back to the genus *Tabernaemontana*, calling it *T. rigida* Miers.[30]

Since *T. rigida* is a tree of medium height (up to 5 m), only the rather thick bark was chosen for phytochemical examination. Conventional work-up of the bark for tertiary bases yielded a chloroform solution from which a considerable quantity of white powdery material separated on concentration. The solid fraction and the amorphous chloroform residue were investigated separately as fraction A and fraction B, respectively.

Fraction A was purified readily by crystallization from chloroform–methanol to give substance A_1, mp 227–228°, $[\alpha]D$ +4°. Elemental analysis indicated that A_1 had the empirical composition $C_{21}H_{26}N_2O_3$; its infrared spectrum indicated the presence of a carbonyl function (1757 cm^{-1}, in Nujol), and its ultraviolet spectrum showed maxima (226 and 279 mμ) consistent with the presence of an unsubstituted indole chromophore. The above data suggested that A_1 might be vincamine in the form of a mixture of (±)-vincamine and (+)-vincamine (XV), since the reported rotation of the latter is $[\alpha]D$ +41°.[31] Repeated crystallization of A_1 from tetrahydrofuran–methanol provided an efficient separation of the material into (±)-vincamine, mp 235–236°, and (+)-vincamine (XV), mp 232–233°, $[\alpha]D$ +40°; the infrared spectra of the two forms were identical (even in potassium bromide), and the identity of the (+)-vincamine was proven by direct comparison with an authentic sample.[32]

(+)-Vincamine (XV) has been isolated previously only from various species of *Vinca*, *i.e.*, *Vinca minor L.*,[33]

(22) The mass spectrum of VIII differs considerably from that of IV owing to the presence of the 8-oxo function. Although details of the spectrum are not given here, the major peaks are recorded in the Experimental Section.

(23) R. H. F. Manske, "The Alkaloids," Vol. VIII, Academic Press Inc., New York, N. Y., 1965, p 361.

(24) C. Kump and H. Schmid, *Helv. Chim. Acta*, **45**, 1090 (1962).

(25) W. Klyne, R. J. Swan, B. W. Bycroft, D. Schumann, and H. Schmid, *Helv. Chim. Acta*, **48**, 443 (1965).

(26) J. Mueller, *Fl. Bras.*, **6** [1], 75 (1860).

(27) J. L. M. Poiret in *Encyclopédie Meth. Bot.*, Suppl. V [1], 276 (1817).

(28) J. Miers in *Apocyn. S. Amer.*, 72 (1878).

(29) F. Markgraf, *Notizbl.*, **14**, 146 (1938).

(30) We are grateful to Dr. W. Rodriguez (Instituto da Pesquisas da Amazonia) for this information. The latest reclassification of this plant does not appear to have been published, presumably owing to the untimely death of Dr. Woodson.

(31) J. Trojánek, O. Štrouf, J. Holubek, and Z. Čekan, *Tetrahedron Lett.*, 702 (1961).

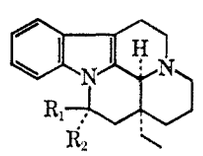
(32) We are indebted to Dr. I. Kompiš, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, for a generous sample of (+)-vincamine.

(33) E. Schlittler and A. Furlenmeier, *Helv. Chim. Acta*, **36**, 2017 (1953); M. Pailer and L. Belohlav, *Monatsh.*, **85**, 1055 (1954); F. E. King, J. H. Gilks, and M. W. Partridge, *J. Chem. Soc.*, 4206 (1955); K. Szasz, L. Szporny, E. Bittner, I. Gyenes, E. Havel, and E. Mago, *Magy. Kém. Foly.*, **64**, 296 (1958); J. Trojánek, J. Hoffmannová, O. Štrouf, and Z. Čekan, *Collect. Czech. Chem. Commun.*, **24**, 526 (1959); J. Trojánek, K. Kavková, O. Štrouf, and Z. Čekan, *ibid.*, **26**, 867 (1961); J. Trojánek, O. Štrouf, K. Kavková, and Z. Čekan, *ibid.*, **27**, 2801 (1962); Z. Čekan, J. Trojánek, O. Štrouf, and K. Kavková, *Pharm. Acta Helv.*, **35**, 96 (1960); O. Bauerová, J. Mokry, I. Kompiš, S. Bauer, and J. Tomko, *Chem. Zvesti*, **15**, 523 (1961); J. Trojánek, O. Štrouf, K. Kavková, and Z. Čekan, *Chem. Ind.* (London), 790 (1961).

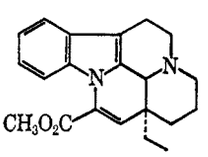
V. major L.,[34] *V. difformis* Pourr.,[35] and *V. erecta* R. and L.[36] (±)-Vincamine has not been encountered previously in nature.

Fraction B of the crude alkaloid mixture was partially resolved chromatographically. In addition to a further amount of (±)- and (+)-vincamine mixture, a fair yield of (+)-apovincamine (XVI) was isolated. The (+)-apovincamine was optically pure, indicating that it is probably not an artifact formed during the isolation process by the action of acid on vincamine.

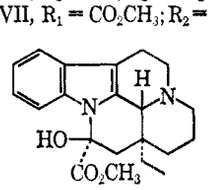
Further work-up of fraction B yielded small amounts of two additional bases, designated here as TR-1 and TR-2. Base TR-1, mp 186–187°, is isomeric with vincamine and is weakly levorotatory, $[\alpha]_D$ −5°. Its mass spectrum is virtually identical with that of vincamine,[19] suggesting that it is a diastereomer of the latter alkaloid. (−)-14-Epivincamine (XVII), $[\alpha]_D$ −36.4°, has been described as melting at 181–185° and as having a mass spectrum nearly identical with that of vincamine.[37] It is quite likely, therefore, that base TR-1 is a mixture of (−)-14-epivincamine (XVII) and (±)-14-epivincamine. Base TR-2, mp 209–211°, is also isomeric with vincamine but weakly dextrorotatory, $[\alpha]_D$ +1.8°; its infrared spectrum is distinctly different from that of vincamine in the fingerprint region. Like base TR-1, base TR-2 shows a mass spectrum which is virtually identical with that of vincamine. Base TR-2 would appear, therefore, to be a stereoisomer of vincamine (or possibly 14-epivincamine) in which the D–E ring juncture is *trans* rather than *cis;* 8-epivincamine (XVIII, as a mixture of + and ± forms) is the biogenetically most probable structure for this substance. Unfortunately, the chemical degradation of bases TR-1 and TR-2 could not be studied due to lack of material.



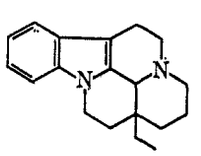
XV, R_1 = OH; R_2 = CO_2CH_3
XVII, R_1 = CO_2CH_3; R_2 = OH



XVI



XVIII



XIX

The apparently exclusive production of alkaloids having the eburnamine-type skeleton (XIX) by *T. rigida* is quite interesting from the chemotaxonomic point of view, since no alkaloids of this structural type have been found in any species of *Tabernaemontana* previously examined. Perhaps the earlier botanical classification of *T. rigida* as *Anacampta rigida*[29] should be reconsidered, and a phytochemical study of other *Anacampta* species should be undertaken in order to determine whether they also produce alkaloids of the vincamine type.

(34) N. R. Farnsworth, F. J. Draus, R. W. Sager, and J. A. Bianculli, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 589 (1960).

(35) M. M. Janot, J. LeMen, and Ch. Fan, *Ann. Pharm. Fr.*, **15**, 513 (1957).

(36) P. Ch. Yuldasev, *Izv. Akad. Nauk SSSR*, 188 (1953); S. Yu. Yunusov, P. Ch. Yuldasev, and N. V. Plechanova, *Dokl. Akad. Nauk Uzb. SSR*, **7**, 13 (1956); S. Yu. Yunusov and P. Ch. Yuldasev, *Zh. Obshch. Khim.*, **27**, 2015 (1957).

(37) J. Mokry and I. Kompiš, *Tetrahedron Lett.*, 1917 (1963).

Experimental Section[38]

Tabernaemontana riedelii. **Plant Material and Crude Tertiary Bases.**—The leaves and twigs of *T. riedelii* were collected by Mr. Luis Coelho in the vicinity of Manaus, Brazil, in February 1965. The dried and ground plant material (10 kg) was extracted exhaustively with 95% ethanol for several days. The concentrated alcoholic extract was extracted with ethyl acetate and the ethyl acetate solution was subsequently shaken with 10% aqueous sulfuric acid to remove tertiary bases. The aqueous acid solution was washed with benzene, and was then made alkaline with ammonia and extracted with chloroform to give, after solvent evaporation, 33.7 g of crude tertiary bases.

Separation and Characterization of the Tertiary Bases.—A major aliquot (26.7 g) of the crude tertiary bases was dissolved in chloroform (200 ml) and mixed with alumina (130 g), and the mixture was dried (*in vacuo*) and then mixed thoroughly with fresh alumina (390 g). The resulting powder was then eluted exhaustively in succession with (a) benzene, (b) chloroform, (c) chloroform–methanol 97:3, and (d) methanol; the weights of nonvolatile eluted bases were (a) 16.0 g, (b) 3.0 g, (c) 1.4 g, and (d) 2.0 g, respectively. The work described in this paper was carried out exclusively with the major benzene fraction; preliminary attempts to resolve the components of the more polar fractions were unrewarding.

The major portion (15.0 g) of the bases eluted by benzene was dissolved in benzene (100 ml) and the solution was extracted successively with aqueous acid of increasing acidity (see Table I). In each case the bases were liberated from the aqueous acid by ammonia and extracted with methylene chloride.

TABLE I

Acid used	Fraction	Wt, g
pH 4 phosphate–citrate buffer	F-1	1.76
pH 2.6 phosphate–citrate buffer	F-2	0.306
1 *N* hydrochloric acid	F-3	12.01
(Not extracted by 1 *N* HCl)	F-4	0.810

Fraction F-1 was not identified. Most of the material was lost during attempted chromatography on alumina, since the material was not recovered from the column even by polar solvents; it may have consisted of hydroxylic oxidation products formed during work-up.

Fraction F-2 was crude (+)-*vincadifformine* (VII). An aliquot (100 mg) of this fraction was purified by extraction with hot chloroform, the insoluble residue being discarded. The resulting amorphous (+)-vincadifformine (31 mg) was homogeneous by tlc (neutral alumina, 1:1 chloroform–benzene). The following physical data were recorded: $[\alpha]^{26}D$ +185° (*c* 1.0, ethanol); λ_{max} mμ (log ε) 220 (4.20), 297 (3.80), and 330 (3.86); $\nu_{max}^{CHCl_3}$ 3490, 1670, 1610, and 720 cm^{-1}. The mass spectrum shows a molecular ion peak at 338, and a base peak at 124.

Fraction F-3 was homogeneous by tlc (neutral alumina, 1:1 chloroform–benzene), and consisted entirely of (+)-minovincine (IV). The following physical data were recorded: $[\alpha]^{26}D$ +340 (*c* 1.0, ethanol); λ_{max} mμ (log ε) 228 (4.08), 300 (3.99), and 330 (4.17); γ_{max} 3400, 2800, 1700, 1670, 1600, and 742 cm^{-1}; $\nu_{max}^{CHCl_3}$ 3390, 2800, 1690, 1670, and 1600 cm^{-1}; δ 8.78 (NH, singlet), 7.30–6.67 (aromatic multiplet, 4 H), 3.71 (singlet, $-COOCH_3$, 3 H), 1.82 (singlet, $-COCH_3$, 3 H). The mass spectrum shows important peaks at 352 (M^+), 309, 214, 138, and 43.

Fraction F-4 crystallized slowly from a concentrated benzene solution to give 300 mg of (+)-8-oxominovincine (VIII), mp 252–253°; recrystallization from methanol gave fine colorless needles of pure VIII, mp 257.5–259.5°.

Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.73; H, 6.47; N, 7.55.

(38) Melting points are uncorrected. Analyses were carried out by Midwest Microlab Inc., Indianapolis, Ind. Mass spectra were determined using an Atlas CH-4 mass spectrometer. Unless otherwise specified, infrared spectra were determined in potassium bromide disks, ultraviolet spectra in 95% ethanol solution, and nmr spectra in deuteriochloroform solution. Column chromatography was carried out using Woelm neutral alumina, activity grade II, unless otherwise stated.

The following physical data were recorded: $[\alpha]^{26}D$ +268° (*c* 0.1, ethanol); λ_{max} mμ (log ε) 229 (4.30), 298 (4.32), and 330 (4.48); γ_{max} 3160, 1680, 1660, 1620, 1580; $\nu_{max}^{CHCl_3}$ 3390, 1670, 1630, 1580 cm^{-1}; nmr, δ 8.88 (NH, singlet, 7.34–6.73 (aromatic multiplet, 4 H), 3.75 (singlet, $-COOCH_3$, 3 H), 1.93 (singlet, $-COCH_3$, 3 H). The mass spectrum shows important peaks at 366 (M^+), 335, 308, 307, 214 (base peak), 154, 110, and 43.

Oxidation of (+)-Minovincine (IV) with Potassium Permanganate.—Into a solution of 250 mg of (+)-minovincine (IV) in 25 ml of acetone (reagent grade) cooled to 20°, was added all at once 250 mg of potassium permanganate. The mixture was stirred for 3 hr, an additional 100 mg of potassium permanganate was added, and the mixture was stirred for a further 2 hr at 20°. The excess of potassium permanganate was then removed by addition of ethanol until the purple color disappeared. The manganese dioxide was filtered off and the solvent evaporated *in vacuo;* the residue, after redissolving in benzene, was extracted with aqueous hydrochloric acid to recover unchanged starting material. (The reaction was not carried out until the starting material had completely reacted as the formation of degradation products was faster than the disappearance of the starting material.)

The aqueous acid solution was neutralized with aqueous ammonia and extracted with methylene chloride to give 122.2 mg of unchanged starting material. The benzene solution was dried over potassium carbonate and evaporated *in vacuo*, yielding 90 mg of a residue which showed two spots by tlc (neutral alumina, $CHCl_3$). Both compounds were separated by preparative tlc on a neutral alumina plate using chloroform as the developing solvent.

The alumina containing the less polar product was extracted with chloroform to give, after crystallization from methanol, fine needles of lactam VIII, mp 253–255° (5 mg), $[\alpha]^{26}D$ +269.0 (*c* 0.1, ethanol).

The infrared spectrum of this material ($CHCl_3$ solution) was identical with that of naturally occurring lactam VIII.

The alumina containing the more polar product was worked up by the same procedure as described above for lactam VIII. The resulting lactam IX (30 mg) was amorphous; infrared bands were at $\nu_{max}^{CHCl_3}$ 3480 (-NH), 1720 ($-COOCH_3$), 1695 ($-COCH_3$) 1670 (γ lactam), 1625, and 730 cm^{-1}.

Tabernaemontana rigida. **Plant Material and Crude Tertiary Bases.**—The bark of *T. rigida* was collected by Mr. Luis Coelho in the vicinity of Manaus, Brazil, in February 1965. The dried and ground bark (23 kg) was extracted exhaustively with 95% ethanol for several days. The concentrated alcoholic extract was extracted with ethyl acetate, and the ethyl acetate solution was subsequently shaken with 5% aqueous sulfuric acid to remove tertiary bases. The aqueous acid solution was washed with benzene, and was then made alkaline with ammonia and extracted with chloroform. Concentration of the chloroform extract to a small volume caused the separation of a gray powder (20.0 g), which was removed by filtration and designated as fraction A. Evaporation of the filtrate yielded fraction B as a gum (67.5 g).

Separation and Characterization of the Tertiary Bases. Examination of Fraction A.—Crystallization of fraction A from chloroform–methanol (1:1) yielded substance A_1 (12.85 g), mp 227–228°, which was homogeneous by tlc (alumina, $CHCl_3$–CH_3OH, 1:1). A small sample was recrystallized for analysis.

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.93; N, 7.90. Found: C, 71.18; H, 7.39; N, 7.90.

The following physical data were recorded: $[\alpha]^{26}D$ +4° (*c* 1.0, pyridine); λ_{max} mμ (log ε) 226 (4.13) and 279 (3.59); ν_{max} 1730 and 742 cm^{-1}.

Substance A_1 (12.0 g) was subjected to fractional crystallization from tetrahydrofuran–methanol (3:1), when it was separated into pure (±)-vincamine (9.0 g), mp 235–236°, and pure (+)-vincamine (XV, 1.8 g), mp 232–233°, $[\alpha]D$ +40° (*c* 1.0, pyridine). The (+)-vincamine obtained was identical (infrared spectrum, mixture melting point) with authentic material.[32]

Examination of Fraction B.—Fraction B was dissolved in warm 8% aqueous tartaric acid (1200 ml). The cooled brown solution was filtered through glass wool and extracted with five 500-ml portions of ether to remove neutral material (3.00 g). Basification of the aqueous acid solution with ammonia followed by extraction with five 500-ml portions of ether removed the major portion of the alkaloids, designated as fraction B_1 (33.8 g). Further extraction of the ammoniacal solution with chloroform (1000 ml) removed the remaining bases, designated as fraction B_2 (15.4 g). An extensive effort to resolve fraction B_2 by a combination of buffer separation and alumina chromatography failed; it will not be referred to further in this discussion.

Fraction B_1 was chromatographed on alumina (grade III, 860 g). A total of 71 fractions was obtained, which were recombined into six fractions on the basis of tlc similarities (see Table II).

TABLE II

Fraction	Eluent	Wt, g
B_{2a}	Benzene	9.5
B_{2b}	Benzene	3.0
B_{2c}	Benzene–Et_2O (2:1)	1.5
B_{2d}	Benzene–Et_2O (1:1)	3.0
B_{2e}	Chloroform	8.0
B_{2f}	Methanol	2.7

Fraction B_{2a} yielded, by direct crystallization from acetone, (+)-apovincamine (1.0 g), mp 158–159°; an additional amount of the same compound (0.50 g) was obtained from the mother liquors by rechromatography: $[\alpha]^{26}D$ +118° (*c* 2.11, chloroform). The following constants have been recorded for (+)-apovincamine:[39] mp 159–161°; $[\alpha]^{26}D$ +121° (chloroform). The ultraviolet spectrum and the infrared spectrum (chloroform solution) of the (+)-apovincamine isolated as described above were identical with those of (±)-apovincamine prepared from (±)-vincamine by acid dehydration.[31]

Fractions B_{2b} and B_{2c} yielded only a further quantity (3.10 g) of the crystalline mixture of (+)- and (±)-vincamine: mp 227–228°; $[\alpha]^{26}D$ +4°.

Fraction B_{2e} was rechromatographed on alumina, the column being eluted with benzene, 1:1 benzene–chloroform, and chloroform. The material eluted by benzene–chloroform was crystallized from acetone and petroleum ether to yield a small amount of crystalline material, designated as TR-2. Recrystallization from ethanol–methylenechloride yielded the pure base (0.015 g): mp 209–211°; $[\alpha]^{26}D$ +1.8° (*c* 1.45, pyridine); λ_{max} mμ (log ε) 225 (4.69) and 278 (4.14).

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.05; H, 7.51; N, 7.35.

Concentration of the mother liquors from TR-2 afforded crystals (0.070 g) of base TR-1: mp 186–187°; $[\alpha]^{26}D$ −5° (*c* 2.69, pyridine); λ_{max} mμ (log ε) 226 (3.58) and 277 (3.03).

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 70.82; H, 7.51; N, 7.46.

Registry No.—(+)-IV, 15622-69-2; (+)-VII, 15539-10-3; (+)-VIII, 15539-11-4; IX, 11040-85-0; (+)-XV, 15539-08-9; (±)-XV, 2122-39-6; (±)-vincadifformine, 11040-87-2.

(39) O. Clauser, K. Gesztes, and K. Szasz, *Tetrahedron Lett.*, 1147 (1962).