Isolation of an Alkaloid from *Vinca Minor**

By STANLEY SCHEINDLIN and NATHAN RUBIN

The literature concerning the genus *Vinca* has been reviewed. Extraction of *Vinca minor* L. (Fam. *Apocynaceae*) with ammonia and benzene yielded a crude alkaloidal fraction. From the crude alkaloid, by a chromatographic process, a crystalline alkaloid has been isolated. The empirical formula, molecular weight, melting point, ultraviolet and infrared spectra, and color reactions of this alkaloid are reported.

Vinca minor L. (Fam. *Apocynaceae*), the lesser periwinkle, is now considered merely an ornamental plant, useful as a ground-cover especially on steep banks and under trees. Yet this plant has a long, though undistinguished, history of medicinal use. It was mentioned as a drug by both Dioscorides and Galen (1), has enjoyed some use in European folk medicine (2), and is still official in the French Codex under the title of "Pervenche officinale" (3).

There has been no thorough study of the constituents of any of the species of the genus *Vinca*. However, the presence of alkaloids has been reported in several of these species, namely *V. lancea* (4), *V. herbacea* (5), *V. pubescens* (6, 10), *V. rosea* (7, 8), *V. major* (9), and *V. minor* (6). Several alkaloids have been isolated in crystalline form. These together with their physical constants are tabulated below:

V. pubescens: Orechoff, Gurewitch, and Norkina (10).

(*a*) *Vinine*, colorless needles, $C_{19}H_{26}N_2O_4$, m. p. 211–213°, $[\alpha]_D$ −70.12°, (c, 1.6 in absolute alcohol). Hydrochloride, m. p. 212° (decompn.); sulfate, m. p. 229–230° (decompn.); chloroplatinate, m. p. 226–227° (decompn.).

(*b*) *Pubescine*, yellow needles, $C_{20}H_{26}N_2O_4$, m. p. 227–228°, $[\alpha]_D$ −134.2°, (c, 0.298 in absolute alcohol).

(*c*) An unnamed alkaloid, in minute yield: colorless needles, m. p. 194–195°, which strongly depressed the melting point of vinine.

V. rosea: Paris and Moyse-Mignon (8).

(*a*) *Vinceine*, $C_{21}H_{26}N_2O_3$, m. p. 259–260°, $[\alpha]_D$ −56°, (c, 0.5 in chloroform), absorption maxima at 225 and 280 mμ, absorption minimum at 265 mμ. Hydrochloride, m. p. 290° (decompn).

V. major: Janot and LeMen (9).

(*a*) *Reserpinine* (11-methoxy-δ-yohimbine), colorless prisms, $C_{22}H_{26}N_2O_4$, m. p. 242°, $[\alpha]_D$ −109°, (c, 0.5 in pyridine), absorption maxima at 229 and 299 mμ, inflection at 250 mμ.

(*b*) Colorless needles, m. p. 316°, absorption maxima at 240 and 310 mμ. Possibly identical with *serpinine*.

Within the last few years, *Rauwolfia serpentina* and its alkaloids have come into great prominence in the treatment of hypertension. Concurrently, chemical research has shown that the basic ring structures of the alkaloids of various *Apocynaceae* such as *Rauwolfia*, *Iboga*, and *Alstonia*, are essentially similar. The importance of *Vinca minor* as a potential source of related hypotensive alkaloids thus becomes apparent.[1] *Vinca minor*, furthermore, grows in the United States, and can be easily cultivated if necessary, whereas the other drugs mentioned above must be imported from distant places all over the world.

EXPERIMENTAL

The leaves and stems of *Vinca minor* were collected in Jenkintown, Pennsylvania, during the months of August, September, and October. The plant material was washed, dried first in the air and then in an oven at 40°, and finally ground to a coarse powder. Before being ground, each batch was authenticated by Dr. Theodor P. Haas.[2]

Extraction of Crude Alkaloids.—The limitations imposed by the apparatus available made it most convenient to extract the drug in 200-Gm. batches.

Two hundred grams of *Vinca minor* was moistened with 130 cc. of 10% ammonia. The moist drug was packed in a Soxhlet apparatus, covered with benzene, and macerated for twenty-four hours or longer. The drug was then exhausted by continuous extraction. The benzene extract was evaporated to dryness in a current of air, no heat being applied The residue was extracted with several portions of 0.5% hydrochloric acid. The acid solutions were made alkaline with 28% ammonia. The precipitate which formed was allowed

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[1] After the presentation of this paper before the Scientific Section, a personal communication from Dr. Jack Cooper of Ciba Pharmaceutical Products indicated that Schlittler and Furlenmeier [*Helv. Chim. Acta*, **36**, 2017(1953); *Chem. Abstr.*, **49**, 1006(1955)] had isolated from *Vinca minor* an alkaloid which they named *vincamine*. This alkaloid, $C_{21}H_{26}N_2O_3$, m. p. 232–233°, $[\alpha]_D^{23}$ +41°, shows the typical ultraviolet absorption maxima of an indole nucleus. Vincamine appears to be a different alkaloid from the one whose isolation is described in this paper. This conclusion is based on physical constants, elementary composition, and a color reaction mentioned by Schlittler and Furlenmeier. Vincamine gives no red color with nitric acid, whereas the alkaloid reported in this paper does.

[2] Department of Biology, Philadelphia College of Pharmacy and Science.

to stand one hour, then filtered with suction, washed with distilled water, and dried at room temperature or 40°. The crude alkaloidal fraction thus obtained had a faint pink color. The yields ranged from 0.4 to 0.5%, based on the dried drug.

Isolation of a Crystalline Alkaloid.—In a typical experiment, 0.7 Gm. of crude alkaloidal fraction was triturated with five 30-cc. portions of anhydrous ether. A red solution resulted, which was separated by filtration from some brown insoluble matter. The pooled ether extracts were passed through a column, 19 mm. in diameter, containing 10 Gm. of Brockmann alumina. After nearly all the ether had passed through, 20 cc. more anhydrous ether was added to the column. Two additional 20-cc. portions of ether were used in the same way. Passage through the column resulted in adsorption of the red color of the solution in a firmly-held band at the top, while the eluate was colorless or faintly yellow.

The solution which had passed through the column was allowed to evaporate spontaneously in a dark place. Rosettes of white needles, embedded in a yellowish oil, were obtained. Treatment with ice-cold absolute alcohol dissolved the oil, and the crystals were collected on a sintered-glass Buchner funnel, and washed with many portions of cold absolute alcohol. Yields of crystalline alkaloid ranged up to 7%, based on the crude alkaloidal fraction.

The oily material mentioned above is also alkaloidal, but it has thus far resisted all efforts to crystallize it.

Properties of the Crystalline Alkaloid.—The melting point of the white crystals, determined in a capillary using a total immersion thermometer, was 201-202° with decomposition. The melt was deep red. The melting point varied somewhat with the rate of heating of the bath.

Combustion analysis[3] indicated the following percentage composition:

Carbon	69.02	68.77
Hydrogen	6.62	6.63
Nitrogen	7.06	7.05
Oxygen (by difference)	17.30	17.55

Determination of the molecular weight by the Rast method (11) indicated a molecular weight of 386.

Based on the analytical and molecular weight data presented, the empirical formula of the alkaloid was computed to be $C_{22}H_{26}N_2O_4$.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectrum was determined using a Beckman Quartz Spectrophotometer—Model DU. Figure 1 shows the curve obtained at a concentration of 8.03 mg. per liter in 95% ethanol. The alkaloid exhibited absorption maxima at 229 and 274 mμ, and an inflection at 290 mμ. Its absorption minima were at 251 and 288 mμ.

The absorption spectrum in 0.05 *N* hydrochloric acid showed a hypsochromic shift, with maxima at 222 and 269.5 mμ respectively, an inflection at 289 mμ, and minima at 246.5 and 287 mμ, respectively.

The spectra obtained were similar to those given by alkaloids having an α,β-substituted indole nucleus (12, 13).

[3] Clark Microanalytical Laboratory, Urbana, Ill.

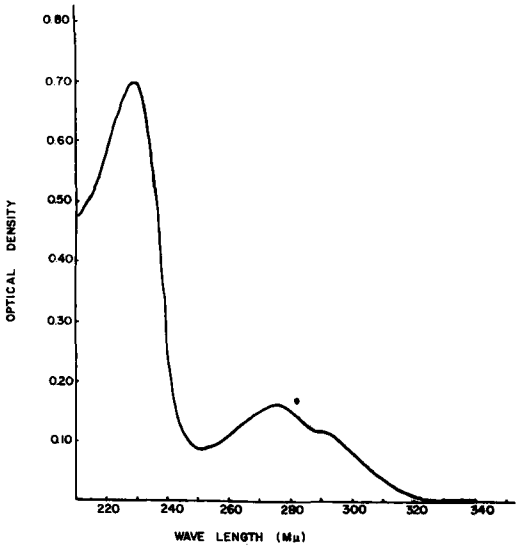

Figure 1.—Ultraviolet spectrum.

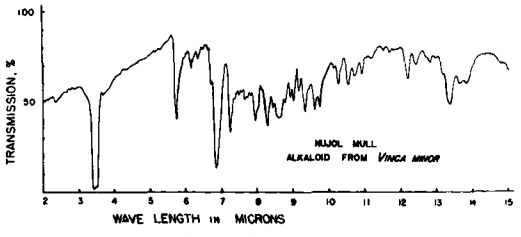

Figure 2.—Infrared spectrum.

Infrared Absorption Spectrum.[4]—The infrared absorption spectrum was determined in a Nujol mull, using a Perkin-Elmer Model 21 instrument. The curve obtained is shown in Fig. 2.

The strong bands at 3.4, 6.8, and 7.25 μ are due to the Nujol. No band was found around 3 μ. This would indicate the absence of the NH group. However, decarboxycorynantheine and dihydrocorynantheane, which are known to have an indole nucleus also fail to show the NH band in the infrared (12).

The strong band at 5.75μ is indicative of the ester group. The bands found between 6.15 and 6.70μ can be attributed to the benzenoid ring (14, 15) and have been stated to be typical of indoles (16). A strong band at 7.95μ may be attributable to a methoxyl group attached to an unsaturated carbon atom (12). A band at 9μ has been attributed to the group C—O—C (12). Bands at 13.35 and 13.65μ have been stated to be characteristic of the 1,2-disubstituted benzene ring, which is present also in indoles (12). Bands were found at 12.15 and 12.4μ. Bands in the region of 11.8–12.6μ have been stated to indicate a 1,2,4-substituted benzene ring (17).

Saponification Equivalent.—To confirm the indication of the presence of an ester group, the hydrox-

[4] We wish to thank A. Turner of Smith, Kline and French Laboratories for carrying out the infrared determination, and S. Rump, of the same Laboratories, for aid in interpreting the infrared data.

amate test as outlined by Buckles and Thelen (18) was scaled down and applied to a 2.3-mg. sample of alkaloid. A positive test was obtained.

The saponification equivalent of the alkaloid was determined by a method described by Shriner and Fuson (19) scaled to accommodate a sample weighing 0.0207 Gm. The reagent used was alcoholic potassium hydroxide, and the reflux time was two hours. The saponification equivalent was 395.1, which agrees very closely with the calculated molecular weight (394.46), indicating the presence of one ester group in the molecule.

Test for Methylenedioxy Group.—Labat's test (20) was applied to 0.5 mg. of the alkaloid. A negative result was obtained. The same quantity of hydrastine hydrochloride gave a strongly positive test under the same conditions.

Color Test for Indole Alkaloids.—The sample was dissolved without heating in 1 cc. of concentrated hydrochloric acid. Four drops of 2% alcoholic *p*-dimethylaminobenzaldehyde were added, followed by 2 drops of 0.05% sodium nitrite solution. Results follow:

Control (no alkaloid)—colorless
Hydrastine hydrochloride—colorless
Yohimbine hydrochloride—pale greenish color
Vinca alkaloid—deep yellow color

Yohimbine has been reported to give a yellow to green color with this reagent. Other alkaloids containing the indole nucleus also give various colors (21).

Effect of Light.—The crystalline alkaloid, and also solutions of the alkaloid in alcohol or chloroform, gradually develop a yellow color when exposed to light. This phenomenon is being investigated further.

SUMMARY

1. The literature pertaining to the alkaloids of the genus *Vinca* (Fam. *Apocynaceae*) has been reviewed.

2. From *Vinca minor* L. a crystalline alkaloid has been isolated. Some physical and chemical properties of this alkaloid have been determined.

3. Since its properties are not identical with those of any other alkaloid isolated from the family *Apocynaceae*, the name *perivincine* is suggested for the crystalline alkaloid from *Vinca minor*.

REFERENCES

(1) Wehmer, C., "Die Pflanzenstoffe," 2nd ed., Gustav Fischer, Jena, 1929, vol. 2, p. 983.
(2) Hegi, G., "Illustrierte Flora von Mittel-Europa," J. F. Lehmann, Vienna, 1907–1931, vol. 5, p. 2055.
(3) "Codex Medicamentarius Gallicus," 7th ed., 1949, p. 559.
(4) Githens, T. S., "Drug Plants of Africa," University of Pennsylvania Press, Philadelphia, 1949, p. 109.
(5) Vintilesco, J., and Ioanid, N. I., *Bull. soc. chim. Romania*, **14**, 12(1932); through *Chem. Abstr.*, **27**, 1029 (1933).
(6) Orechoff, A., *Arch. Pharm.*, **272**, 673(1934).
(7) Cowley, R. C., and Bennett, F. C., *Australasian J. Pharm.*, **9**, 61(1928).
(8) Paris, R. A., and Moyse-Mignon, H., *Compt. rend.*, **236**, 1993(1953).
(9) Janot, M. M., and LeMen, J., *ibid.*, **238**, 2550(1954).
(10) Orechoff, A., Gurewitch, H., and Norkina, S., *Arch. Pharm.*, **272**, 70(1934).
(11) Niederl, J. B., and Niederl, V., "Organic Quantitative Microanalysis," 2nd ed., John Wiley and Sons, Inc., New York, 1946, pp. 218–219.
(12) Janot, M. M., and Goutarel, R., *Bull. soc. chim.*, **18**, 588(1951).
(13) Klohs, M. W., Draper, M. D., Keller, F., Malesh, W., and Petracek, F. J., *J. Am. Chem. Soc.*, **76**, 1332(1954).
(14) Marion, L., Ramsay, D. A., and Jones, R. N., *ibid.*, **73**, 305(1951).
(15) Goutarel, R., Janot, M. M., Prelog, V., Sneeden, R. P. A., and Taylor, W. I., *Helv. Chim. Acta*, **34**, 1139 (1951).
(16) Gellert, E., and Witkop, B., *ibid* **35**, 114 (1952).
(17) Janot, M. M., Goutarel, R., and Sneeden, R. P. A., *ibid.*, **34**, 1205(1951).
(18) Buckles, R. E., and Thelen, C. J., *Anal. Chem.*, **22**, 676(1950).
(19) Shriner, R. L., and Fuson, R. C., "Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, 1948, p. 134.
(20) "Merck Index," 5th ed., Merck and Co., Rahway, N. J., 1940, p. 801.
(21) Mesnard, P., *Bull. soc. pharm. Bordeaux*, **89**, 20(1951); through *Chem. Abstr.*, **45**, 9798(1951).

The Behavior of Organic Bases in Nitromethane*

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When organic bases are dissolved in nitromethane they react with the solvent to form salts. This reaction occurs only if the pK_b (H_2O) value of the base is less than 11. Experimental evidence for such interaction is presented by isolation of a typical reaction product and by a study of the behavior of these compounds during nonaqueous titration.

IT WAS NOTED by Fritz and Fulda (1) who reported the titration of weak bases in acetic anhydride-nitromethane solvent systems, that a number of nitrogen heterocyclics could not be titrated successfully because of solvent interference which was attributed to the presence of acetic anhydride. Their solvent system offered optimum conditions for the quantitative determination of caffeine and attempts were made, in this laboratory, to extend the method to the differential analyses of codeine and caffeine in pharmaceutical preparations containing acetylsalicylic acid, phenacetin, caffeine, and codeine. However, the two alkaloids could not be recovered stoichiometrically. It is the purpose of this paper to explain these findings and to extend the investigation to a number of other organic bases.

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