Catharanthus Alkaloids XV. Isolation of Vindolinine from *C. lanceus* Leaves

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A continuing study of *Catharanthus lanceus* for antineoplastic alkaloids has resulted in the isolation of vindolinine (as HCl), which is reported for the first time from this plant. Perivine and yohimbine, reported previously, were also obtained in high yield.

In a previous communication (1) the isolation of perivine and yohimbine from the (B) fraction of *C. lanceus* leaves was reported. A reinvestigation of this fraction has resulted in the isolation of vindolinine, as the dihydrochloride salt. This is the first report on the isolation of vindolinine from *C. lanceus*, however it was initially isolated from the related *C. roseus* by two other groups (2, 3).

EXPERIMENTAL

Preparation of Crude Alkaloid Fraction—The alkaloid (B) fraction used in this investigation was derived from 267.5 Kg. of dried *Catharanthus lanceus* leaves, collected in Madagascar in July 1965.[1] Processing of the plant material as previously described (1) yielded 694 Gm. of crude alkaloid (B) fraction.

Isolation of Alkaloids—The crude fraction (694 Gm.) was dissolved in 6 L. of hot benzene, filtered, and cooled to room temperature. The residue on the filter pad was washed with cold benzene to remove brown coloring matter. This resulted in a yield of 43.92 Gm. of benzene-insoluble amorphous alkaloid, which was shown to be one-spot material following thin-layer chromatography (TLC), as previously described (4). The benzene filtrate, on standing at room temperature, formed crystals which were removed by filtration. The crystalline material when washed and dried, yielded 53.16 Gm. of alkaloid which was shown to be identical with the benzene-insoluble isolate. Concentration of the mother liquor to dryness *in vacuo*, and crystallization of the residue from acetone, yielded an additional 86.20 Gm. of alkaloid similar in all respects to the two previous crops. The three crystal crops were combined and an analytical sample was prepared by several crystallizations from hot acetone. After drying *in vacuo* at 50° for 24 hr. the crystals exhibited m.p. 232–233° dec. (cap., uncorr.). An ultraviolet absorption spectrum, in ethanol, showed maxima at 226 mμ (log $E^{1\%}_{1\,cm.}$ 3.20), 281 mμ (log $E^{1\%}_{1\,cm.}$ 2.52), and a shoulder at 290 mμ (log $E^{1\%}_{1\,cm.}$ 2.45). Thin-layer chromatography data in three solvent systems corresponded with that of yohimbine (4). An infrared spectrum (KBr) was identical with that of yohimbine, and the ultraviolet spectrum and melting point data were in agreement with published data for yohimbine (5).

After removal of the three crystal drops, the mother liquor was again taken to dryness and crystallized from hot acetone. After refrigeration, and standing overnight under refrigeration, crystals formed which were removed by filtration, washed with cold acetone, and dried to give a 79.9-Gm. yield. Thin-layer chromatography of this isolate indicated it to be similar, if not identical, with perivine (4). An analytical sample was prepared by several recrystallizations from acetone. The purified alkaloid was dried at 50° *in vacuo* for 24 hr., and was found to have m.p. 178–179° (cap., uncorr.). An ultraviolet absorption spectrum, in ethanol, exhibited a maximum at 316 mμ (log $E^{1\%}_{1\,cm.}$ 2.91) and a shoulder at 240 mμ, as well as an additional maximum being approached near 226 mμ. This spectrum is typical for the 2-acylindole moiety. An infrared spectrum of the isolate was identical with that of reference perivine, and the melting point and ultraviolet absorption data were consistent with literature values for perivine (5).

Following the removal of perivine, the mother liquor was again taken to dryness and 300 Gm. was dissolved in a minimum volume of benzene and the solution was applied to a chromatographic column (12 × 150 cm.) containing a benzene slurry of 14-Kg. Alcoa F-20 alumina, deactivated as previously described, by the addition of 120 ml. of 10% (w/v) acetic acid (1). Elution was initiated with benzene, followed by solvents and solvent mixtures of increasing polarity, and 1,000-ml. fractions were collected. The data for the operation of this column are presented in Table I. Fractions were combined on the basis of chromogenic responses of the resolved components to the ceric ammonium sulfate reagent (CAS), following TLC (4).

Fractions 28–101 (35.46 Gm.) from the column contained a major alkaloid which produced a red-orange color with the CAS reagent, and exhibited an R_f value consistent with that of vindolinine, in three different solvent systems (4). The alkaloid could not be induced to crystallize from the usual solvents, thus the fraction was taken to dryness, dissolved in ether, and HCl gas was passed through the ether solution. After 24 hr. the ether solution was filtered and the crude alkaloid hydrochloride was crystallized three times from methanol to yield 0.12 Gm. of pure vindolinine dihydrochloride. This exhibited m.p. 212–214° and an ultraviolet absorption spectrum having maxima at 245 mμ (log a_m 4.07) and 300 mμ (log a_m 3.63), with a minimum at 274 mμ (log a_m 3.35). An infrared spectrum (KBr) of the isolate was identical in all respects with that of a reference sample of vindolinine·2HCl[2]. The melting point and ultraviolet spectrum were in agreement with litera-

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[1] Supplied by the S. B. Penick and Company, New York, NY 10008. A reference herbarium specimen has been deposited at the above address.

[2] Supplied through the courtesy of G. H. Svoboda, Eli Lilly and Company, Indianapolis, Ind.

TABLE I—CHROMATOGRAPHIC SEPARATION OF *C. lanceus* LEAF ALKALOID (B) FRACTION[a]

Fraction No.[b]	Eluent	Fraction Wt., Gm.	Alkaloid Isolated	Yield, Gm.
1–3	Benzene	0.03		
4–7	Benzene	2.71		
8–18	Benzene	2.40		
19–27	Benzene	1.97		
28–101	Benzene	35.46	Vindolinine·2HCl	0.12
102–458	Benzene	29.50	Perivine	11.17
	Benzene–chloroform (99:1)			
	Benzene–chloroform (97:3)			
	Benzene–chloroform (95:5)			
459–580	Benzene–chloroform (3:1)	35.79	Perivine	13.56
			Yohimbine	9.42
581–608	Benzene–chloroform (3:1)	6.24	Yohimbine	3.46
609–623	Benzene–chloroform (3:1)	2.84	Yohimbine	0.91
624–640	Benzene–chloroform (3:1)	2.93	Yohimbine	1.09
641–735	Benzene–chloroform (3:1)	7.86		
736–788	Benzene–chloroform (2:1)	2.43		
789–806	Benzene–chloroform (1:1)	2.33		
807–842	Benzene–chloroform (1:1)	5.19		
843–901	Benzene–chloroform (1:1)	4.04		
902–1126	Chloroform	27.89		
1127–1151	Chloroform–methanol (99:1)	12.71		
1152–1518	Chloroform–methanol (99:1)	41.10		
	Chloroform–methanol (9:1)			
	Chloroform–methanol (3:1)			
	Chloroform–methanol (2:1)			
1519–1564	Methanol	14.65		

[a] 300 Gm. of alkaloid fraction was chromatographed on 14 Kg. of partially deactivated Alcoa F-20 alumina. [b] Each fraction was 1,000 ml. All fractions contained alkaloids.

ture reports for vindolinine·2HCl (3, 5), and the molecular weight for the alkaloid base was shown to be 336 by mass spectrometry (calcd. mol. wt., 336).

Fractions 102–458 (29.50 Gm.) from the column gave 11.17 Gm. of perivine on crystallization from acetone, benzene, and methanol. An additional 13.56 Gm. of perivine was obtained from fractions 459–580 (25.79 Gm.), from acetone, whereas the mother liquor of these fractions yielded 9.42 Gm. of yohimbine by crystallization from benzene and chloroform. An additional 5.46 Gm. of yohimbine was isolated from fractions 581–640 from the column, from benzene, chloroform, methanol, and acetone (see Table I for details).

This now brings to 19, the number of alkaloids that have been isolated from *C. lanceus* in this laboratory (1, 6–11).

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Keyphrases

Catharanthus lanceus leaves—alkaloids
Vindolinine, yohimbine, perivine—isolation
Column chromatography—separation
TLC—separation, identity
Mass spectrometry—mol. wt.
UV spectrophotometry—structure
IR spectrophotometry—structure