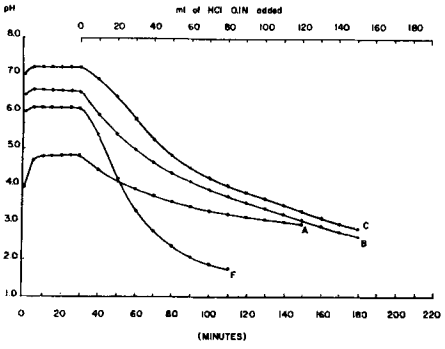

Fig. 1.—Acid consuming activity of four metal complexes of THAM. Key: A, aluminum–gluconic acid–THAM (1:1:1); B, aluminum–PAS acid–THAM (1:2:3); C, aluminum–PAS acid–THAM (1:2:4); F, bismuth–citric acid–THAM (1:1:3).

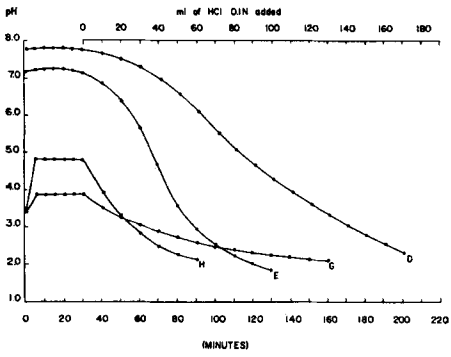

Fig. 2.—Acid consuming activity of four metal complexes of THAM. Key: D, aluminum–salicylic acid–THAM (1:2:4); E, bismuth–citric acid–THAM (1:1:4); G, aluminum–citric acid–THAM (1:1:2); H, bismuth–citric acid–THAM (1:1:2).

In the case of tris(hydroxymethyl)aminomethane–gluconatodihydroxo–aluminate (Al–gluconic acid–THAM, 1:1:1) the pH rose almost instantly to a value of 4.0 and after 5 min., reached a maximum pH of 5.0. The pH remained at about 5.0 for the next 30 min., during which time no additional acid was introduced. This suggests that *in vivo* an overdose of antacid would not increase the pH above 5.0. Moreover, when more acid was reintroduced, the pH did not fall below 3 until after approximately 12 meq. of HCl had been added also indicating that THAM–gluconatodihydroxoaluminate would be an effective soluble buffer antacid compound.

The complex of Bi–citric acid–THAM (1:1:1) had a smaller neutralizing capacity than Al–gluconic acid–THAM within the pH range 3 to 5 and the remaining antacids only have limited buffering capacities.

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Catharanthus Alkaloids X. Isolation of Lanceine and Vinosidine from *Catharanthus lanceus* Roots

By RALPH N. BLOMSTER*, NORMAN R. FARNSWORTH, and DONALD J. ABRAHAM

In a continuing search for new biologically active entities in *Catharanthus lanceus*, additional work on the root (A) fraction has led to the isolation of two alkaloids. One of these, lanceine, was previously isolated from this plant by other workers. Vinosidine, however, although previously reported in a related species, is reported from this species for the first time.

EARLIER STUDIES on *Catharanthus lanceus*, as a part of a continued search for new and active antineoplastic principles from the various alkaloid fractions, and in an effort to elucidate, as completely as possible, the alkaloid composition of this plant, have resulted in the isolation of 12 crystalline alkaloids (1–4). Three of these, cathalanceine, pericyclivine, and periformyline proved to be new entities, with periformyline representing a new alkaloid of novel structure. Of the remaining nine, ajmalicine, yohimbine, and tetrahydroalstonine have been reported previously from *C. lanceus* roots by other workers (5–7). The additional six alkaloids have also been reported from the related *C. roseus* (8–12). Of these (leurosine, perivine, vindoline, pericalline, perimivine, and lochnerinine) leurosine is of major interest because of its high order of activity against the P-1534 leukemia, and because it was isolated initially from a *C. lanceus* crude alkaloid fraction that was devoid of activity against the P-1534 leukemia in DBA/2 mice (1).

This is a report on the isolation of two additional alkaloids, lanceine, previously reported from this plant by other investigators (6), and vinosidine, reported previously only from *C. roseus* (13).

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TABLE I.—COLUMN CHROMATOGRAPHIC SEPARATION OF *C. lanceus* ROOT (A) FRACTION, 210 Gm.

Eluent	Fraction	Fraction, Wt.,[a] Gm.	Alkaloid Isolated	Wt., Gm.
Benzene (fractions 1–40)	1–2	0.02	...	...
Benzene–chloroform (9:1)	3	2.81	...	...
(fractions 41–52)	4–5	32.70	Ajmalicine	0.920
Benzene–chloroform (5:1)	6–17	46.61	Ajmalicine	20.610
(fractions 53–65)	18–23	15.80	Ajmalicine	2.950
Benzene–chloroform (2:1)	24–33	20.40	Lanceine	0.026
(fractions 66–79)				
Benzene–chloroform (1:1)	34–78	6.70	...	...
(fractions 80–147)	79–104	8.80	Vinosidine	0.090
Chloroform	105–174	7.70	...	...
(fractions 148–210)	175–220	1.65	...	...
Chloroform–methanol (99:1)	221–226	2.10	...	...
(fractions 211–258)	227–238	3.20	...	...
Chloroform–methanol (9:1)	239–260	1.50	...	...
(fractions 259–284)	261–278	4.85	...	...
Chloroform–methanol (1:1)	279–316	3.10	...	...
(fractions 285–315)	317–348	11.40	...	...
Methanol				
(fractions 316–348)				

[a] All fractions gave positive tests for alkaloids except fractions 1–2.

EXPERIMENTAL[1]

Chromatographic Separation of C. lanceus Root (A) Fraction.—Two-hundred and forty-five grams of root (A) alkaloid fraction (1) was dissolved in benzene and this solution concentrated *in vacuo* to yield 29.05 Gm. of ajmalicine. Further concentration yielded 6.30 Gm. of benzene-insoluble, non-alkaloid precipitate. The remaining benzene-soluble material was placed at the top of an 8 × 86-cm. glass chromatographic column containing a benzene slurry of 4.0 Kg. of alumina (Alcoa F-20) partially deactivated with 144 ml. of 10% (v/v) acetic acid (1). Elution was accomplished initially with benzene, and then with eluting solvents having an increased polarity to yield 348 one-liter fractions. Each fraction was reduced to dryness, *in vacuo*, the residue dissolved in 20 ml. of the eluting solvent, and monitored by thin-layer chromatography on Silica Gel G plates using an ethyl acetate–absolute ethanol (3:1) eluent and the ceric ammonium sulfate spray reagent (CAS) (14, 15). Shifts in the alkaloid composition of the eluate, as determined by thin-layer chromatographic patterns, as well as differences in chromogenesis of the resolved alkaloids following treatment with the CAS reagent, were the criteria for determining the limits of each fraction (Table I).

Isolation of Lanceine.—Benzene eluate fractions 24–33 from the column chromatographic separation were taken to dryness, *in vacuo*, to yield 20.40 Gm. of residue, from which was obtained 3.60 Gm. of a crystalline material on direct crystallization from benzene. The R_f values of this material as well as its chromogenic reaction to the CAS detecting reagent on Silica Gel G thin-layer chromatography in three solvent systems (16) were identical with a reference sample of lanceine.[2] The melting point and infrared absorption spectrum of this material were identical with those found in the literature for lanceine (17). However, on alumina G thin-layer chromatography, using benzene–ethanol (98:2) as the eluent, the crystalline material proved to be a multicomponent mixture.

Nine grams of the crude alkaloid fraction 24–33 remaining after the removal of the crystals formed from benzene (*vide supra*), was dissolved in benzene and subjected to a gradient pH separation according to the method of Svoboda (18) in an attempt to separate lanceine. The initial pH of the acidic alkaloid solution was 3.3 and it was increased in 0.5 pH increments by the addition of NH_4OH until a final pH of 8.8 was reached. Each fraction was dried with anhydrous sodium sulfate, filtered, and reduced to about 10 ml. *in vacuo*, followed by thin-layer chromatography and treatment of the developed chromatograms with CAS reagent which served to point out those pH fractions to be combined prior to crystallization attempts. The fractions were combined to give two groups corresponding to pH 3.3–4.3 and 4.8–8.8. Fraction pH 3.3–4.3 yielded 0.110 Gm. of crystalline material on direct crystallization from ethanol. Again this material appeared to be identical with lanceine as regards melting point and infrared absorption spectrum, but on alumina G thin-layer chromatography, it proved to be a multicomponent mixture.

Repeated crystallization of the impure materials with several solvents failed to resolve the mixture, as did column chromatography on alumina (Woelm, neutral, activity grade II), utilizing benzene as the initial eluting agent and followed by solvents of increasing polarity. The mixture was finally resolved by repeated preparative thin-layer chromatography, utilizing 1 mm. thick alumina G plates, developed with anhydrous ether in a repetitive manner. In each case the solvent front was allowed to travel 170 mm., the plate air-dried, and rechromatographed. This was repeated 3 times. The area corresponding to lanceine, as determined by chromogenesis with the CAS reagent (16), was removed from the plate, eluted with methanol, filtered, evaporated to dryness *in vacuo*, and the residue was dissolved in a small amount of methanol and placed on the surface of a second

[1] Melting points are uncorrected and were determined using a Thomas-Hoover capillary melting point apparatus. Infrared absorption spectra were determined in a Beckman infrared spectrophotometer model IR-8.

[2] The authors express their appreciation to Dr. Jean LeMen for a sample of crude lanceine which was suitable for thin-layer chromatographic comparison only.

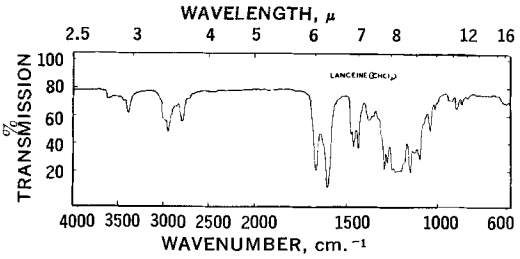

Fig. 1.—Infrared spectrum of isolated lanceine.

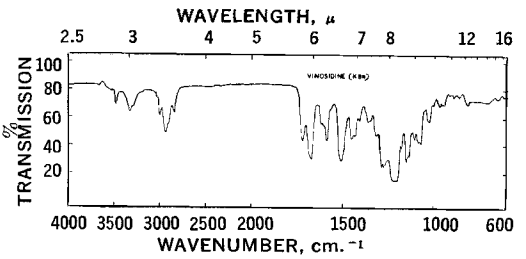

Fig. 2.—Infrared spectrum of isolated vinosidine.

preparative alumina G thin-layer plate. This plate was treated in the same manner as the first plate, and the material obtained on elution of the matrix was crystallized from cyclohexane at room temperature. This yielded 0.013 Gm. of crystalline lanceine, m.p. 143–145°, from each 0.100 Gm. of impure material utilized. The infrared absorption spectrum (Fig. 1) of the isolated lanceine was identical with that found in the literature (17). The R_f values for the isolated lanceine and a reference sample of this alkaloid, as well as chromogenic reactions to the CAS detecting agent after Silica Gel G thin-layer chromatography in three different solvent systems (16), and on alumina G thin-layer chromatography were also identical.

Isolation of Vinosidine.—Treatment of the dried benzene–chloroform (1:1) fractions 79–104 with methanol yielded crystals, which on recrystallization from methanol afforded 0.090 Gm. of vinosidine, m.p. 251–253° dec. The infrared absorption spectrum (Fig. 2) of isolated vinosidine and that of a reference sample[3] were identical, as were their R_f values and chromogenic response to the CAS reagent after thin-layer chromatography in three different solvent systems (16).

SUMMARY

The isolation of two alkaloids is reported. One, lanceine, was previously isolated from *C. lanceus* roots by other workers. Vinosidine was previously isolated from the related species *C. roseus*, but is reported herein for the first time from *C. lanceus*.

[3] A reference sample of vinosidine was supplied through the generosity of Dr. Gordon H. Svoboda.

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