

procedure used for III. Recrystallization from 95% ethanol yielded 5.0 Gm. (66%) of XV as white cottonlike crystals, m.p. 258–259°; $[\alpha]_D -81^\circ$; λ_{\max} . 6.13 μ with no inflection at 6.03 μ .

Anal.—Calcd. for $C_{21}H_{57}N_2O_2$: C, 60.57; H, 9.35; I, 20.65; N, 4.56. Found: C, 60.79; H, 9.09; I, 20.62; N, 4.42.

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Catharanthus lanceus VII

Isolation of Tetrahydroalstonine, Lochnerinine, and Periformyline

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A continuing study of *Catharanthus lanceus* leaf alkaloids for antineoplastic compounds has led to the isolation of tetrahydroalstonine, lochnerinine, and a new alkaloid, periformyline. Details concerning the isolation of these alkaloids and the structure elucidation of periformyline, the first example of an N_(b)-substituted formyl indole alkaloid to be found in nature, are presented.

AS PART of a continuing search for new and active antineoplastic agents from the various alkaloid fractions of *Catharanthus lanceus*, nine crystalline compounds have been isolated from both the leaf and root alkaloid fractions. One of these is the antineoplastic alkaloid leuroisine (1–3).

Of the three alkaloids reported herein, tetrahydroalstonine and lochnerinine represent alkaloids previously reported in *C. roseus* (4, 5) while the third, periformyline, represents a new alkaloid of novel structure.

The infrared absorption spectrum (Fig. 1) and ultraviolet absorption spectrum of isolated tetrahydroalstonine and a reference sample were identical. Their R_f values and chromogenic reactions to the ceric ammonium sulfate (CAS) detecting reagent (6, 7) in three different solvent systems were also identical (8).

The identity of lochnerinine was shown by comparison of our data with that found in the

literature and is presented in Table I (5, 9). The infrared absorption spectrum (Fig. 2) seemed identical with that in the literature and the mass spectrum of lochnerinine furnished by Bose (10) was in good agreement with that of our

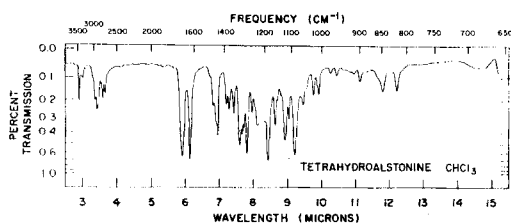


Fig. 1.—Infrared absorption spectrum of tetrahydroalstonine.

TABLE I.—IDENTIFICATION OF LOCHNERININE

	Lit. (5, 9)	Observed Data
M.p., °C.	168–169°	164–166°
Specific rotation (chloroform)	–424° (16°C.)	–442° (26°C.)
U.V. max.	247 μ (log ϵ 4.19)	247 μ (log ϵ 4.22)
(in ethanol)	326 μ (log ϵ 4.33)	326 μ (log ϵ 4.41)
Mass spectrum		
mol. wt.	382	382
Base peak	138	138

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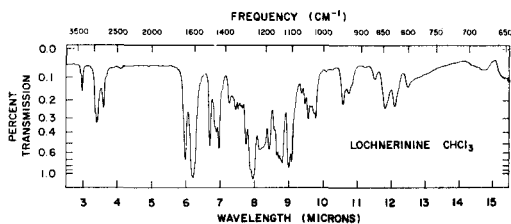


Fig. 2.—Infrared absorption spectrum of lochnerinine.

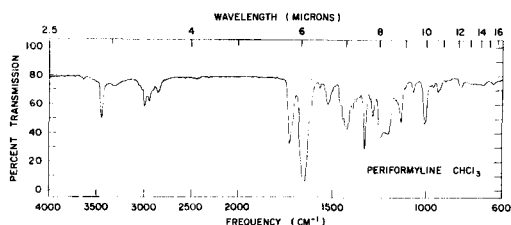


Fig. 3.—Infrared absorption spectrum of periformyline.

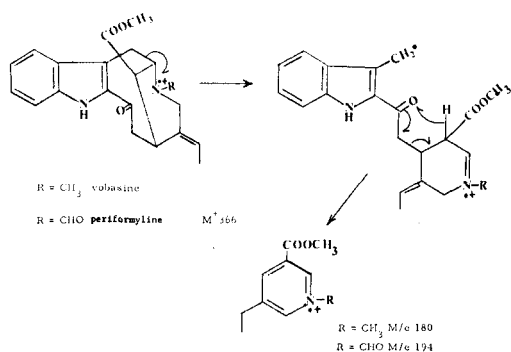
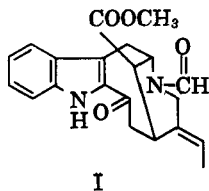


Fig. 4.—Mass spectral fragmentation pattern for periformyline and vobasine.

isolated material. Cochromatography of the two alkaloids (only an impure sample of lochnerinine was available) in three different solvent systems showed them to be identical. The chromogenic reaction of the isolated lochnerinine with the CAS detecting reagent was also consistent with that produced by authentic lochnerinine (8).

Periformyline (I) was established as N_(b)-formylperivine (11), and to our knowledge, is the



first N_(b)-substituted formyl indole alkaloid to be found in nature.

The ultraviolet absorption spectrum showed

the compound to be a 2-acylindole with absorption maxima at 240 m μ (log ϵ 4.22) and 315 m μ (log ϵ 4.32), and the infrared absorption spectrum (Fig. 3) was very similar to that of perivine, except that the absorption of the 2-acylindole carbonyl was overlapped by the strong amide absorption at 1650 cm⁻¹. In perivine the absorption of the carbomethoxyl carbonyl was stronger than that of the 2-acylindole carbonyl. Analysis for the presence of the N—CH₃ group was negative.

The mass spectrum of periformyline gave 366 as the molecular weight compared to the calculated value of 366. The intense *m/e* peak at 194 suggested an increase of 14 mass units to the basic ion formed in the spectrum of vobasine (Fig. 4) (12, 13). This could possibly be explained by the exchange of a formyl group for the N_(b)-methyl group in vobasine to form the ion.

The NMR spectrum agrees with that of the proposed structure (11).

Finally, perivine was formylated at room temperature using a previously prepared solution of acetic anhydride in excess formic acid. The synthetic product was recrystallized from methanol and melted at 206–209°. The mixed melting point of synthetic periformyline and isolated periformyline showed no depression. The infrared absorption spectra of the two were identical. Their *R_f* values and chromogenic response to the CAS detecting reagent following cochromatography in three different solvent systems were also identical.

EXPERIMENTAL¹

Chromatographic Separation of *C. lanceus* Leaf (A₁) Fraction.—One hundred grams of leaf (A₁) fraction (1) was dissolved in benzene and placed at the top of an 8 × 86 cm. glass chromatographic column containing a benzene slurry of 3.6 Kg. of Alcoa F-20 alumina partially deactivated with 112 ml. of 10% acetic acid (1). Elution was accomplished initially with benzene and then with eluting solvents having an increased polarity to yield 481 one-liter fractions. Each fraction was reduced to dryness, *in vacuo*, dissolved in 20 ml. of the eluting solvent, and monitored by thin-layer chromatography on Silica Gel G plates utilizing an ethyl acetate–absolute ethanol (3:1) eluent with a ceric ammonium sulfate detecting reagent. Grouping of the fractions was accomplished on the basis of their thin-layer chromatographic patterns as well as the chromogenic reaction of the resolved alkaloids to the CAS detecting reagent (Table II).

Isolation of Tetrahydroalstonine.—Benzene fractions 16–40 from the column chromatographic separation yielded 8.175 Gm. of a crystalline material on direct crystallization with methanol. This material, on recrystallization from methanol, gave

¹ Melting points are uncorrected and were determined using a Thomas-Hoover capillary melting point apparatus. Ultraviolet absorption spectra were determined in a Beckman DK-2 recording spectrophotometer and infrared absorption spectra in a Beckman infrared spectrophotometer model IR-8.

TABLE II.—COLUMN CHROMATOGRAPHIC SEPARATION OF *C. lanceus* LEAF (A_1) FRACTION (100 Gm.)

Fraction No.	Eluent	Fraction Wt., ^a Gm.	Alkaloid Isolated	Wt., Gm.
1-2	Benzene (fractions 1-87)	0.29
3	...	2.10
4	...	1.33
5-6	...	8.55
7-15	...	10.67	Lochnerinine	1.950
16-28	...	10.57	Tetrahydroalstonine	7.415
29-40	...	1.69	Tetrahydroalstonine	0.760
41-52	...	0.29
53-89	Benzene-chloroform (9:1) (fractions 88-97)	0.58
90-112	...	0.54
113-120	Benzene-chloroform (3:1) (fractions 98-118)	5.79
121-125	...	1.17
126-133	Benzene-chloroform (2:1) (fractions 119-160)	1.29
134-140	...	0.86
141-149	...	1.12
150-162	...	1.65	Periformyline	0.220
163-174	Benzene-chloroform (1:1) (fractions 161-173)	1.31
175-181	Chloroform (fractions 174-243)	6.56
182-186	...	0.55
187-214	...	1.61
215-247	Chloroform-methanol (99:1) (fractions 244-280)	0.65
248-357	...	3.38
358-438	Chloroform-methanol (1:2) (fractions 419-439)	1.28
439-454	...	0.57
455-474	Methanol (fractions 440-481)	7.40
475-481

^a All fractions were alkaloidal.

m.p. 227-227.5° dec. which was not depressed on mixture melting point with reference tetrahydroalstonine.

Isolation of Lochnerinine.—Treatment of the dried benzene fractions 10-12 with methanol yielded a white crystalline material, which on recrystallization from methanol afforded 1.95 Gm. of crystals, m.p. 164-166° and was shown to be lochnerinine (*vide supra*).

Isolation of Periformyline.—Dried benzene-chloroform (2:1) eluted fractions 150-162 from the column afforded a compound on crystallization from methanol that yielded, on recrystallization, 0.22 Gm. of an alkaloid, m.p. 206-209° dec. This alkaloid, periformyline, could not be identified with any previously reported from the genus *Catharanthus*. The pK'a for periformyline was indeterminate and is low—less than 4. Thin-layer chromatography of periformyline on Silica Gel G in three different solvent systems gave the following data:² solvent A, R_f 0.69; solvent B, R_f 0.73; solvent C, R_f 0.69. And on the basis of its chromogenic reaction to the CAS detecting reagent it was classified as a class VIII *Catharanthus* alkaloid, as defined by Farnsworth *et al.* (8).

Synthesis of Periformyline.—Into a 100-ml. flask under N_2 were placed 50 ml. of 97+ % formic acid and 10 ml. of acetic anhydride. The solution was left at room temperature for 4 hr., then 500 mg. of pervine was added and the resulting solution left overnight at room temperature. The solvent was

² Solvent A, ethyl acetate-absolute ethanol (3:1); solvent B, *n*-butanol-glacial acetic acid-water (4:1:1); solvent C, absolute methanol.

removed *in vacuo* and the resulting oil treated with methanol. Removal of the methanol, *in vacuo*, gave a white solid, m.p. 208-213°. Two recrystallizations from methanol gave 250 mg. of periformyline, m.p. 206-209°. A mixed melting point with the isolated periformyline showed no depression, and the infrared spectra were superimposable.

SUMMARY

The isolation of three alkaloids is reported. Two, tetrahydroalstonine and lochnerinine, were previously isolated from the related species *C. roseus*, but are reported for the first time from *C. lanceus*.

The new alkaloid, periformyline, represents a novel compound and, to our knowledge, is the first $N_{(10)}$ -substituted formyl indole alkaloid to be found in nature.

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