Structure Elucidation and Chemistry of *Catharanthus* Alkaloids II. Isolation and Partial Structure of Catharine, a Dimeric Indole Alkaloid from *C. lanceus* and *C. roseus*

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A continuing study of *Catharanthus lanceus* alkaloid fractions, in a search for new antineoplastic alkaloids, has led to the isolation of catharine, a dimeric indole alkaloid found previously only in the related *C. roseus*. High resolution mass spectrometric measurements have shown that catharine has the formula $C_{46}H_{54}N_4O_{10}$ and is made up of a vindoline moiety and an alkaloid moiety with a molecular formula of $C_{21}H_{23}N_2O_4$.

PREVIOUS STUDIES in these laboratories on *Catharanthus lanceus* have led to the isolation of the monomeric alkaloids lanceine (1), tetrahydroalstonine (2), ajmalicine (3), and yohimbine (4), which were previously reported from this plant by Janot *et al.* (5–7). In addition, monomeric alkaloids previously unreported from this plant, but reported present in related genera or species, were found to be vinosidine (1), perivine (4), perimivine (3), pericalline (tabernoschizine, apparicine, gomezine) (3), vindoline (4), and lochnerinine (2). Three new and hitherto unreported monomeric alkaloids, catha-lanceine (3), pericyclivine (8), and periformyline (2, 9), were similarly isolated in the authors' studies. On the other hand, only one dimeric alkaloid, leurosine (4), had been encountered in this investigation of *C. lanceus* alkaloids. This alkaloid was previously isolated from *C. roseus* by Svoboda *et al.* (10), and has been subsequently shown to be highly active against the P-1534 leukemia in DBA/2 mice (11–13).

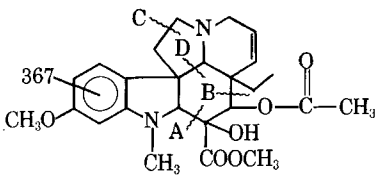
A continued investigation of alkaloid fractions obtained from *C. lanceus* has resulted in the isolation of a second dimeric alkaloid, catharine, from this plant. The isolation, characterization, and partial structure elucidation of this alkaloid is reported here.

EXPERIMENTAL

Isolation of Catharine.—In a previous paper the authors investigated the leaf alkaloid (A_1) fraction from *C. lanceus* and isolated the alkaloids tetrahydroalstonine, lochnerinine, and a new alkaloid periformyline (2). The structure for periformyline was subsequently shown by us to be $N_{(b)}$-formyl perivine, the first example of an $N_{(b)}$-substituted formyl iodole alkaloid to be found in nature (9). Additional studies on this leaf (A_1) fraction have resulted in the isolation of catharine, previously isolated only by Svoboda *et al.* from *C. roseus* (14).

Work-up of the chloroform eluted fractions 175–181 from the column chromatographic separation of the (A_1) alkaloids (100 Gm.), as previously described (Table I), resulted in the formation of 0.730 Gm. of crystals from benzene after several weeks of refrigeration. Recrystallization of these fine crystals from methanol–anhydrous ether afforded an analytical sample of catharine, m.p. 257–258° dec. A mixed melting point with reference to catharine showed no depression,[1] and an infrared absorption spectrum (Fig. 1) was superimposible with that of a reference sample, as was that of a comparison ultraviolet absorption spectrum. The molecular weight of catharine, as determined by mass spectrometry, was found to be 822.

Partial Structure Elucidation of Catharine.—The mass spectrum[2] of catharine showed major peaks at *m/e* 822, 763, 735, 733, 622, 555, 554, 158, 144, 136, 135, 130, 122, 121, 108, and 107. From the above data it became apparent that vindoline made up one-half of this dimeric alkaloid. If one considers the partial structure (I) for catharine, the mass spectral data can be interpreted reasonably. The 367 in I



I

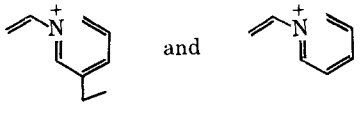
represents the molecular weight of the other half of the dimeric alkaloid. The *m/e* 763 peak represents a loss of 59 mass units, or a carbomethoxy group. Previous mass spectral degradations of vindoline (16, 17) are also evident here. The *m/e* 822 to *m/e* 662 (II) transition (Scheme I), with loss of 160 mass units, indicates ABD cleavage to give the fragment II*a*. A strong metastable ion for this transition is observed at *m*/e* 533.1. The *m/e* 554 peak can possibly be represented as the following ion (III) (Scheme II), from cleavage at E in II, which is similar to that basic ion formed by vindoline at *m/e* 188. The *m/e* 282 (IV) to *m/e* 222 (V) transition has a weak metastable ion at *m/e** 174.8 and is pictured as similar to that particular degradation in vindoline (Scheme III).

Other characteristic vindoline peaks (17) are at *m/e* 135 (VI) and *m/e* 107 (VII)[3] with a metastable

[1] Although the literature (11, 14) states 271–275° as the m.p. for catharine, an authentic sample, found to be homogeneous when examined by thin-layer chromatography using three different solvent systems (15), and supplied by G. H. Svoboda, gave m.p. 257–258° dec.

[2] The AEI MS 9 high resolution mass spectrometer was used in this work.

[3] These structures are designated in *Reference 16* as the open chain analogs, *i.e.*,



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Previous paper: Abraham, D. J., Farnsworth, N. R., Blomster, R. N., and Sharkey, A. J., Jr., *Tetrahedron Letters*, 1965, 317.

TABLE 1.—COLUMN CHROMATOGRAPHIC SEPARATION OF *C. lanceus* LEAF (A_1) FRACTION, 100 GM.

Eluent	Fraction[a]	Fraction Wt., Gm.[b]	Alkaloid Isolated	Wt., Gm.
Benzene (fractions 1–87)	1–2	0.29	...	...
	3	2.10	...	...
Benzene–chloroform (9:1)	4	1.33	...	...
(fractions 88–97)	5–6	8.55	...	...
Benzene–chloroform (3:1)	7–15	10.67	Lochnerinine	1.950
(fractions 98–118)	16–28	10.57	Tetrahydroalstonine	7.415
Benzene–chloroform (2:1)	29–40	1.69	Tetrahydroalstonine	0.760
(fractions 119–160)	41–52	0.29	...	...
Benzene–chloroform (1:1)	53–89	0.58	...	...
(fractions 161–173)	90–112	0.54	...	...
Chloroform	113–120	5.79	...	...
(fractions 174–243)	121–125	1.17	...	...
Chloroform–methanol (99:1)	126–133	1.29	...	...
(fractions 244–280)	134–140	0.86	...	...
Chloroform–methanol (4:1)	141–149	1.12	...	...
(fractions 281–418)	150–162	1.65	Periformyline	0.220
Chloroform–methanol (2:1)	163–174	1.31	...	...
(fractions 419–439)	175–181	6.56	Catharine	0.730
Chloroform–methanol (1:1)	182–186	0.55	...	...
(fractions 440–453)	187–214	1.61	...	...
Methanol	215–247	0.65	...	...
(fractions 454–481)	248–357	3.38	...	...
	358–438	1.28	...	...
	439–454	0.57	...	...
	455–474	7.40	...	...
	475–481	...	...	...

[a] All fractions collected were 1000 ml. [b] All fractions collected were alkaloidal.

peak at *m/e** 84.8, and at *m/e* 122 (VIII), resulting from B,C,D cleavage (Scheme IV).

High resolution mass spectrometry measurements on several of these peaks showed them to be in agreement with the postulated formulas (Table II). However, the site of the attachment of the 367 group to vindoline has not as yet been determined.

Some possible molecular formulas for catharine are listed in Table III and it can be seen from high resolution data that the formula for catharine is in agreement with $C_{46}H_{54}N_4O_{10}$.

If $C_{46}H_{54}N_4O_{10}$ is the correct molecular formula for catharine, it follows that with the vindoline moiety having a molecular formula of $C_{25}H_{31}N_2O_6$, then the other half of the dimer has the formula $C_{21}H_{23}N_2O_4$. The authors are at present investigating the nature of this second half of the dimer.

Preliminary results on the acid cleavage of catharine show desacetylvindoline, by thin-layer chromatography, to be one of the products. This would be expected, and attempts are in progress to isolate the cleavage products and rigorously establish their structures.

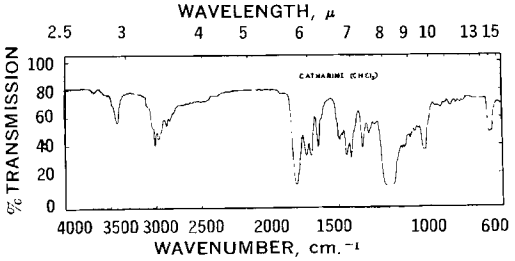
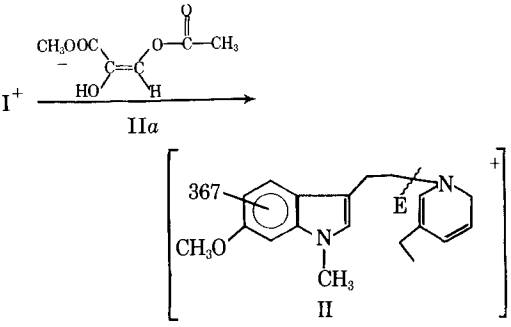
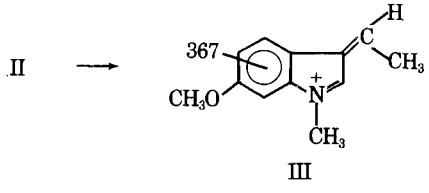


Fig. 1.—Infrared spectrum of catharine.

It was previously reported (14) by other workers, that the formula of catharine was $C_{46}H_{52}N_4O_9 \cdot CH_3OH$; however, they only reported an elemental analysis as their proof for the proposed structure. Their first elemental analysis of catharine is also in agreement with our proposed formula of $C_{46}H_{54}N_4O_{10}$.[4]



Scheme I

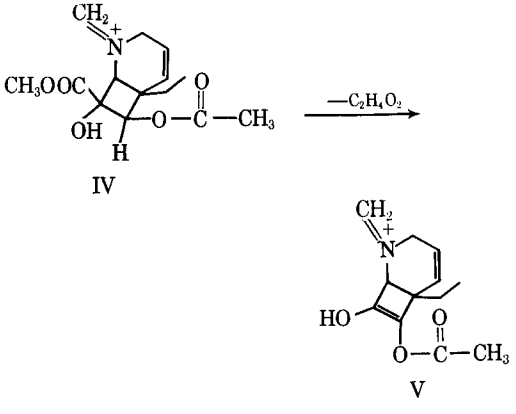


Scheme II

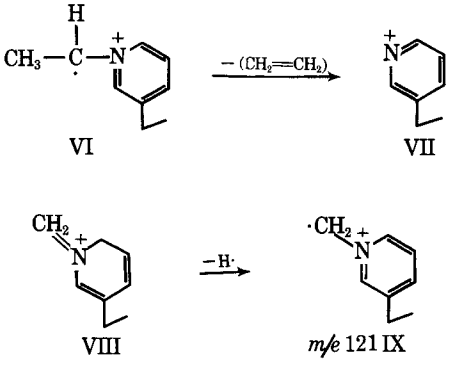
[4] *Anal.*—Calcd. for $C_{46}H_{54}N_4O_{10}$: C, 67.15; H, 6.57; N, 6.81; O, 19.46; 4-OCH_3, 15.08; OAc, 7.18. Found (14): C, 67.45; H, 6.95; N, 6.97; O, 19.68; 4-OCH_3, 15.34; OAc, 7.81.

TABLE II.—HIGH RESOLUTION DATA OF FRAGMENTED IONS FROM CATHARINE

Ion	Formula	Calcd. Wt. *m/e*	Observed Wt. *m/e*	Difference in Mass Units
I-($C_2H_3O_2$)	$C_{44}H_{51}N_4O_8$	763.3707	763.3691	−0.0016
II	$C_{40}H_{46}N_4O_5$	662.3468	662.3433	−0.0035
III	$C_{33}H_{36}N_3O_5$	554.2655	554.2635	−0.0020
V	$C_{12}H_{16}NO_3$	222.1130	222.1123	−0.0007
VI	$C_9H_{13}N$	135.1048	135.1047	−0.0001
VII	C_7H_9N	107.0735	107.0734	−0.0001
VIII	$C_8H_{12}N$	122.0970	122.0962	−0.0008
IX	$C_8H_{11}N$	121.0892	121.0882	−0.0010



Scheme III



Scheme IV

TABLE III.—HIGH RESOLUTION MOLECULAR WEIGHT OF CATHARINE

Proposed Formulas	Calcd. Wt. *m/e*	Observed Wt. *m/e*	Difference
$C_{45}H_{50}N_4O_{11}$	822.3476	822.3793	+0.0317
$C_{46}H_{54}N_4O_{10}$	822.3840	822.3793	−0.0047
$C_{47}H_{58}N_4O_9$	822.4203	822.3793	−0.0410
$C_{48}H_{62}N_4O_8$	822.4567	822.3793	−0.0774

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