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Catharanthus Alkaloids XIII

Antineoplastic and Hypotensive Activity of Alkaloid Fractions and Certain Alkaloids from *Catharanthus lanceus*

By NORMAN R. FARNSWORTH, RALPH N. BLOMSTER, and J. P. BUCKLEY

Hypotensive evaluation of 14 alkaloid fractions derived from *C. lanceus* roots and leaves in anesthetized, normotensive rats and dogs revealed that eight of the fractions reduced blood pressure from 22–73 per cent for periods ranging from 38 to more than 378 min., at doses of 8–40 mg./Kg. Yohimbine, a potent α-adrenergic blocking agent, was isolated from three of the eight active fractions. However, removal of the yohimbine from one of these fractions did not result in a loss of hypotensive activity, thus indicating that other hypotensive agents may be present. Leurosine, perivine, and pericyclivine elicited only transient hypotensive activity, whereas vindoline, tetrahydroalstonine, ajmalicine, lochnerinine, and periformyline failed to induce a hypotensive response at several dose levels. The same crude alkaloid fractions were evaluated for antineoplastic activity against the P-1534 leukemia with only one of the 14 fractions being active against this neoplasm. Leurosine, isolated from the active fraction, was shown to be a potent antineoplastic alkaloid with a high degree of cytotoxicity. Lochnerinine, although devoid of activity against the P-1534 leukemia, exhibited reproducible cytotoxicity against the 9 KB cell culture. Vindoline, catharanthine, desacetylvindoline, perivine, perivinol, periformyline, pericyclivine, pericalline, catharine, ajmalicine, tetrahydroalstonine, and yohimbine were devoid of P-1534 leukemia activity, as well as cytotoxicity. Monitoring of column chromatographic cuts of the active fraction with the P-1534 leukemia has shown that at least one additional alkaloid, active against this neoplasm, is present.

THE MADAGASCAN periwinkle, *Catharanthus roseus* (*Vinca rosea, Lochnera rosea*), has yielded at least 66 alkaloids as a result of recent intensive phytochemical investigations. For the most part, these alkaloids have been discovered in the search for new antineoplastic agents, and many of them were obtained in only trace quantities. In certain instances, the small quantities available precluded any determination of their biological effects. However, the authors do know of the antineoplastic activity of vincaleukoblastine, leurocristine, leurosine, and leurosidine (1); of the pronounced oral hypoglycemic effects of vindolinine (hydrochloride), leurosine (sulfate), lochnerine, vindoline, desacetylvindoline, catharanthine (hydrochloride), and tetrahydroalstonine (2); and of the diuretic effect of catharanthine (hydrochloride) and vindolinine (hydrochloride) (3, 4); as well as the antidiuretic action of ajmalicine, lochnerine, and sitsirikine (sulfate) (3, 4). In addition, the antihypertensive and sedative properties of reserpine, also reported as present in *C. roseus*, are well known.

Because of interest in the isolation of biologically active compounds from plants, investigations were initiated on species of *Catharanthus* other than *C. roseus*. The antineoplastic activity of *C. lanceus* alkaloids (5, 6) and the isolation of leurosine, an alkaloid exhibiting a high order of activity against the P-1534 leukemia in DBA/2 mice (5, 6) were previously reported. Also, it has been reported that certain *C. pusillus* alkaloid fractions elicit marked hypotensive activity in anesthetized, normotensive rats (7).

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No reports have appeared in the literature concerned with the biological activity of *C. trichophyllus*, *C. longifolius*, or *C. scitulus*, the remaining three species of the genus *Catharanthus*.

At this time the antineoplastic and hypotensive activity of alkaloid fractions derived from the roots and leaves of *C. lanceus* is reported, in addition to certain alkaloids isolated in our laboratories from this plant.

EXPERIMENTAL

Preparation of Fractions and Isolation of Alkaloids.—Seven alkaloid fractions were prepared according to the method of Svoboda (8) from *C. lanceus* root (9) and seven from leaf material (6) of Madagascan origin. Following column chromatography of certain of these fractions, a number of alkaloids were isolated by direct crystallization. The preparation of these alkaloids has previously been reported and include leurosine (6), perivine (6), yohimbine (6, 9), vindoline (6), pericyclivine (10), ajmalicine (9), perimivine (9), cathalanceine (9), catharanthine (11), pericalline (9), vincoline (11), vinosidine (12), lanceine (12), catharine (13), periformyline (14, 15), tetrahydroalstonine (14), and lochnerinine (14). Prior to these studies, Janot *et al.* had reported on the isolation of ajmalicine, tetrahydroalstonine, yohimbine, and lanceine from the roots of this plant (16–18).

Preparation of Alkaloid Fractions and Alkaloids for Biological Evaluation.—All alkaloid fractions were prepared for use in the hypotensive evaluations by first being dissolved in an appropriate organic solvent, followed by the addition of 0.1 *M* citric acid solution, and removal of the organic solvent by *in vacuo* steam distillation. After filtration, the acidic extract was adjusted to as near neutrality as possible with dilute ammonia solution without precipitation of free alkaloid bases, and then adjusted with distilled water to a known volume. Control solutions were prepared with 0.1 *M* citric acid, and ammonia solution added to a pH identical with the extract to be injected. The details for this phase of the study have been reported previously (7).

Alkaloids were administered either as their salts which were dissolved in distilled water, or as the free base treated in the manner described for the crude fractions, or as the free base dissolved in dimethylacetamide (DMAC) diluted with water.

For the antineoplastic and cytotoxicity evaluations, the extracts and alkaloids were suspended in normal saline solution for the P-1534 leukemia evaluations and either dissolved or suspended in propylene glycol for the 9 KB cell culture assays.

Hypotensive Evaluation of the Alkaloid Fractions and Alkaloids.—The prepared test solutions were administered to albino Wistar, normotensive, anesthetized rats (urethan, 1.25 Gm./Kg. i.p.), and evaluated for hypotensive activity as described by Bickerton *et al.* (19). Initially the dose was 1.0 mg./Kg. i.v., and subsequent doses adjusted accordingly in order to estimate that dose producing approximately a 50% decrease in mean arterial blood pressure. The approximate ED_{50} of each active fraction was administered to a minimum of four rats and the mean time for the blood pressure to return to the predrug levels determined. Each animal received only one dose of extract or alkaloid. Equivalent volumes of control solvent were administered i.v. to determine the effect of the pH and solvent on arterial blood pressure of anesthetized rats.

Certain of the fractions and alkaloids were further investigated for hypotensive activity in dogs anesthetized with sodium pentobarbital, 35 mg./Kg. i.v. Blood pressure was recorded from a femoral artery onto a slow moving kymograph. Pressor responses to a 10-sec. bilateral carotid occlusion (BCO), epinephrine, 1–2 mcg./Kg., and angiotensin II, 1 mcg./Kg., were obtained prior to and after administration of each fraction or alkaloid into an exposed femoral vein.

Antineoplastic Evaluation of Alkaloid Fractions and Alkaloids.—Alkaloid fractions from *C. lanceus* leaves and roots, as well as certain of the alkaloids isolated from this plant, were evaluated for activity against the P-1534 leukemia in DBA/2 mice and for

TABLE I.—HYPOTENSIVE EVALUATION OF *C. lanceus* ALKALOID FRACTIONS[a]

Fraction	Dose, mg./Kg.	Animals, No.	Drop in B.P. % (Range)	Duration of B.P. Drop (min.) (Range)	Alkaloids Isolated from Fraction
Root (A)	20–40	4	33–44	203–310+	Ajmalicine, perimivine, cathalanceine, pericalline, ammocalline, lanceine, vinosidine
Root (A_1)	20	6	28–44	28–67	Ajmalicine
Root (B)	10	2	47–56	350+	Ajmalicine, yohimbine, pericalline[b]
Root (B_1)	20–40	5	32–55	38–147+	Ajmalicine[b]
Root (C)	40	2	27–37	70–120+	Ajmalicine[b]
Root (D)	20	4	13–53	8–70	...
Root (E)	10–20	4	[c]	[c]	Ajmalicine[b]
Leaf (A)	10	6	34–52	147–305+	Ajmalicine,[b] leurosine, yohimbine, perivine, pericyclivine
Leaf (A_1)	5–20	6	[c]	[c]	Ajmalicine,[b] lochnerinine, catharine, periformyline, tetrahydroalstonine
Leaf (B)	8	6	42–73	260–378	Yohimbine, perivine
Leaf (B_1)	8–16	6	22–36	56–222+	...
Leaf (C)	20–40	5	24–36	5–7	...
Leaf (D)	20	6	45–53	112–287+	Perivine
Leaf (E)	10–20	4	0–50	2–3	Vindoline, lochnerinine[b]

[a] In normotensive rats, anesthetized with urethan (1.25 Gm./Kg. i.p.). Extracts were administered intravenously. [b] Unpublished data. [c] No hypotensive activity.

TABLE II.—EFFECTS OF CERTAIN ALKALOID FRACTIONS AND ALKALOIDS FROM *C. lanceus* ON THE BLOOD PRESSURE AND SEVERAL PRESSOR RESPONSES IN THE ANESTHETIZED DOG

Fraction	Animals, No.	Dose, mg./Kg.	Blood Pressure Drop, %	Time to Return to Predrug Levels, min.	% of Control Responses BCO[a]	Epi[b]	Ang[c]
Leaf (A)	1	5.0	[d]	[d]	62	*R*[e]	77
Leaf (B)	1	4.0	42	62	25	*R*[e]	129
Leaf (B)	2	8.0	65	196+	*B*[f]	*R*[e]	81
Leaf (B_1)	1	20.0	[d]	[d]	*B*[f]	No change	No change
Leaf (C)	1	10.0	[d]	[d]	..	...	...
Leaf (D)	1	20.0	70	133+	50	*R*[e]	No change
Root (B)	1	5.0	87	200+	*B*[f]	35	116
Leurosine sulfate	1	2.5	[d]	[d]	*B*[f]	No change	No change
Yohimbine hydrochloride	1	1.0	[d]	[d]	*B*[f]	*R*[e]	80
	2	2.5	30	85	*B*[f]	*R*[e]	67–125

[a] BCO, bilateral carotid occlusion. [b] Epi, epinephrine. [c] Ang, angiotensin II. [d] No effect on blood pressure. [e] *R*, epinephrine reversal, prolonged depressor effect. [f] *B*, response blocked.

TABLE III.—HYPOTENSIVE EVALUATION OF CERTAIN *C. lanceus* ALKALOIDS[a]

Alkaloid	Dose, mg./Kg.	Animals, No.	Drop in B.P., %	Duration of B.P. Drop, min.	Solvent
Yohimbine (HCl)	2.5	6	32–56	217–315	Water
Leurosine (H_2SO_4)	5–10	6	40–60	7–28	Water
Perivine (H_2SO_4)	20–40	6	22–54	21–56	Water
Pericyclivine (citrate)	0.25–2.0	6	11–40	1–2	Water
Pericalline	2.5–5.0	6	[b]	[c]	20% DMAC[d]
Vindoline (citrate)	1.0–20	12	[c]	[c]	Water
Tetrahydroalstonine	25–50	6	[c]	[c]	20% DMAC
Ajmalicine	25–50	6	[c]	[c]	20% DMAC
Lochnerinine (HCl)	10–25	6	[c]	[c]	Water
Periformyline	2.5–20	8	[c]	[c]	50% DMAC

[a] In normotensive rats, anesthetized with urethan (1.25 Gm./Kg. i.p.). Alkaloid solutions were administered intravenously. [b] Analeptic effect was noted. [c] No hypotensive activity. [d] Dimethylacetamide–water.

cytotoxicity against Eagle's human carcinoma of the nasopharynx (9KB) in cell culture (20). These tests were performed through the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md.

RESULTS AND DISCUSSION

Hypotensive Activity.—Initial screening of the 14 *C. lanceus* alkaloid fractions in normotensive, anesthetized rats served to indicate that the root (A), (B), (B_1), and (C), as well as the leaf (A), (B), (B_1), and (D) fractions, elicited moderate to marked hypotensive effects (Table I). This activity was confirmed for the leaf (B) and (D) and the root (B) fractions in the anesthetized normotensive dog (Table II).

Of 10 alkaloids evaluated, only yohimbine was shown to elicit marked hypotensive activity of long duration. Leurosine, perivine, and pericyclivine induced only a transient hypotensive effect; whereas, vindoline, tetrahydroalstonine, lochnerinine, periformyline, ajmalicine, and pericalline were inactive as hypotensive agents. Pericalline also produced marked analeptic effects. (Table III.)

Since yohimbine was found to be present in the leaf (A) and (B) fractions, as well as the root (B) fraction, it most likely contributed to the hypotensive effect of these three fractions. The following experiment was conducted to determine the role of yohimbine in the over-all hypotensive activity of one of the four active crude alkaloid fractions.

The leaf (B) fraction, from which yohimbine was isolated (6) and which elicited marked hypotensive activity of long duration (Table I), was subjected to a column chromatographic separation (6). A total of 270 one-liter fractions were eluted from the column and yohimbine was isolated from cuts 115–164. Examination of the pre-115 and the post-164 cuts by thin-layer chromatography, and use of the ceric ammonium sulfate alkaloid detecting reagent (21), revealed that yohimbine was also present in

TABLE IV.—EFFECT OF YOHIMBINE-FREE *C. lanceus* LEAF ALKALOID (B) FRACTION ON THE BLOOD PRESSURE OF ANESTHETIZED NORMOTENSIVE RATS

Sex	Wt., Gm.	Dose, mg./Kg.	Normal Blood Pressure, mm. Hg	% Change	Time to Return to Normal, min.
M	294	10	80	62	180+[a]
M	265	10	82	58	179+[b]
M	410	10	92	47	166+[c]
F	199	20	84	57	203+[b]
F	165	20	112	33	209
F	153	20	106	39	192+[d]

[a] Blood pressure at termination of experiment was 60 mm. Hg. [b] Blood pressure at termination of experiment was 70 mm. Hg. [c] Blood pressure at termination of experiment was 78 mm. Hg. [d] Blood pressure at termination of experiment was 88 mm. Hg.

cuts 97–114 and 165–192. On the basis of this information, cuts 1–96 and 193–270 were combined and taken to dryness to yield a yohimbine-free alkaloid mixture. The mixture was then prepared for hypotensive evaluation as previously described.

In the normotensive, anesthetized rat, at doses of 10 and 20 mg./Kg., this yohimbine-free alkaloid (B) fraction retained its marked and prolonged hypotensive effects (Table IV). Equivalent activity of shorter duration was observed with the yohimbine-free fraction in the anesthetized dog at 8 mg./Kg. A 33% decrease in arterial blood pressure, which persisted for 32 min., decreasing the bilateral carotid occlusion response by 32%, reversing the epinephrine response (epinephrine produced a depressor effect after administration of the yohimbine-free fraction), was elicited by the fraction. The mechanism of action for the hypotensive alkaloid(s) in this fraction also appeared to be that of α-adrenergic blockade.

Although yohimbine was present in three of the active hypotensive fractions, and it must be considered to be one of the major alkaloids of *C. lanceus*, evidence has been presented suggesting that other hypotensive alkaloid(s) are present in this plant.

Antineoplastic Activity.—The data presented in Table V show that the total antineoplastic activity in *C. lanceus* resides in the leaf (A) alkaloid fraction. Leurosine, previously reported only from *C. roseus* by Svoboda (22, 23), was isolated in our studies

TABLE V.—ACTIVITY OF *C. lanceus* ALKALOID FRACTIONS AGAINST THE P-1534 LEUKEMIA

Source	Fraction	Dose, mg./Kg.	Survivors, ( ) of ( )	Animal Wt. Diff. (T/C)	Survival (Days) (T/C)	% (T/C)[a]
Leaf	A	25.0	6/6	0.5	18.0/14.0	128
		12.5	6/6	−0.5	24.0/14.0	171
		6.25	6/6	0.2	21.0/14.0	150
		3.13	6/6	0.7	20.0/14.0	142
		3.13	6/6	−0.9	17.0/13.0	130
		1.56	6/6	−0.7	15.0/13.0	115
		0.78	6/6	0.1	14.0/13.0	107
		0.39	6/6	−0.9	13.5/13.0	103
Leaf	A_1	Inactive at doses of 3.13–100.0 mg./Kg.				
Leaf	B	Inactive at doses of 3.13–50.0 mg./Kg.				
Leaf	B_1	Inactive at doses of 25.0–200.0 mg./Kg.				
Leaf	C	Inactive at doses of 6.25–50.0 mg./Kg.				
Leaf	D	Inactive at doses of 1.56–50.0 mg./Kg.				
Leaf	E	Inactive at doses of 25.0–200.0 mg./Kg.				
Root	A	Inactive at doses of 3.13–50.0 mg./Kg.				
Root	A_1	Inactive at doses of 3.13–100.0 mg./Kg.				
Root	B	Inactive at doses of 25.0–50.0 mg./Kg.				
Root	B_1	Inactive at doses of 3.13–100.0 mg./Kg.				
Root	C	Inactive at doses of 6.35–50.0 mg./Kg.				
Root	D	Inactive at doses of 3.13–50.0 mg./Kg.				
Root	E	Inactive at doses of 25.0–100.0 mg./Kg.				

[a] Evaluation of assay results by CCNSC is such that a material is considered active if it causes a prolongation of life in excess of 125%.

TABLE VI.—ACTIVITY OF *C. lanceus* ALKALOIDS AND RELATED COMPOUNDS AGAINST THE P-1534 LEUKEMIA AND 9KB CELL CULTURE

Alkaloid	P-1534 Activity: Dose, mg./Kg.	Survivors, ( ) of ( )	Animal Wt. Diff. (T/C)	Survival (Days) (T/C)	% (T/C)[a]	9KB Cell Culture: ED_{50},[b] mcg./ml.
Leurosine sulfate	3.0	5/6	−1.0	36.5/14.0	260	$<1.0 \times 10^{-5}$
	1.5	6/6	−0.1	25.5/14.0	182	
	0.75	6/6	−0.9	20.5/14.0	146	
	0.75	6/6	−0.5	21.5/15.0	143	
	0.375	6/6	−1.0	18.5/15.0	123	
	0.188	6/6	−0.1	15.5/15.0	103	
	0.094	6/6	−0.4	14.5/15.0		
Lochnerinine	Inactive at doses of 3.75–30.0 mg./Kg.					$<1.0 \times 10^{-2}$
Vindoline	Inactive at doses of 7.5–60.0 mg./Kg.					5.9×10^{1}
Desacetylvindoline[c]	Inactive at doses of 3.75–30.0 mg./Kg.					NT[e]
Perivine (H_2SO_4)	Inactive at doses of 12.5–100.0 mg./Kg.					NT[e]
Perivinol[d]	Inactive at doses of 7.5–60.0 mg./Kg.					2.6×10^{1}
Periformyline	Inactive at doses of 3.13–25.0 mg./Kg.					$>1.0 \times 10^{2}$
Pericyclivine	Inactive at doses of 7.5–60.0 mg./Kg.					3.6×10^{1}
Pericalline	Inactive at doses of 0.13–1.0 mg./Kg.					NT[e]
Catharine	Inactive at doses of 50 mg./Kg.					2.7×10^{1}
Ajmalicine	Inactive at doses of 20.0–320.0 mg./Kg.					$>1.0 \times 10^{2}$
Tetrahydroalstonine	Inactive at doses of 100 mg./Kg.					$>1.0 \times 10^{2}$
Yohimbine (HCl)	Inactive at doses of 0.31–40.0 mg./Kg.					2.7×10^{1}
Catharanthine (HCl)	Inactive at doses of 2.5–20.0 mg./Kg.					2.3×10^{1}

[a] A rating of 125% or more is considered active. [b] A rating of ED_{50} = 4.0 mcg./ml. or less is considered active. [c] Prepared from vindoline. [d] Prepared from perivine. [e] Not tested.

TABLE VII.—ACTIVITY OF CHROMATOGRAPHIC CUTS FROM *C. lanceus* LEAF (A) ALKALOIDS AGAINST THE P-1534 LEUKEMIA IN MICE

Fraction[a]	Dose, mg./Kg.	Survivors, ( ) of ( )	Animal Wt. Diff. (T/C)	Survival (Days) (T/C)	% (T/C)[b]	Alkaloids Isolated from Fraction
1–179	NT[c]	...	...	...	...	Ajmalicine
180–355	30.0	5/6	−1.4	14.0/14.0	100	Pericyclivine
356–629	30.0	6/6	−0.8	37.5/15.0	250	Leurosine, perivine yohimbine
	15.0	6/6	−0.7	21.5/15.0	143	
	7.5	6/6	−0.0	22.0/15.0	153	
	3.75	6/6	−0.4	17.0/15.0	113	
630–740	30.0	6/6	−2.0	20.0/15.0	133	None
	15.0	6/6	−1.0	17.0/15.0	113	
	7.5	6/6	−0.6	16.0/15.0	106	
741–824	30.0	6/6	−0.8	33.0/15.0	220	None
	15.0	6/6	−0.5	24.0/15.0	160	
	7.5	6/6	0.4	17.5/15.0	116	
825–915	30.0	6/6	−0.1	14.0/15.0	93	None
	15.0	6/6	−0.1	15.0/15.0	100	
	7.5	6/6	0.4	13.0/15.0	86	

[a] One hundred grams of (A) fraction was chromatographed on 3 Kg. of partially deactivated Alcoa F-20 alumina as previously described (6). Eluate was collected in 1-L. fractions which were combined on the basis of thin-layer chromatographic patterns. [b] Evaluation of assay results by CCNSC is such that a material is considered active if it causes a prolongation of life in excess of 125%. [c] Not tested. Quantity insufficient for proper evaluation.

from this (A) fraction by means of column chromatography and has been shown to have a high order of activity against the P-1534 leukemia in mice (Table VI). In order to establish whether additional active alkaloids might be present in the leaf (A) fraction, selected grouped cuts from a chromatographic separation were monitored for activity against the P-1534 leukemia (Table VII). These results appear to support the conclusion that at least one additional alkaloid is present in cuts 741–824 from the column.

To date, 18 alkaloids have been isolated from *C. lanceus* in our laboratories. Twelve of these have been evaluated for antineoplastic activity against the P-1534 leukemia in mice and only leurosine is considered to be active (Table VI). These alkaloids have also been evaluated for cytotoxicity against Eagle's carcinoma of the nasopharynx (9KB cell culture), and lochnerinine, in addition to leurosine, is considered to be active, although the former alkaloid was found to be inactive against the P-1534 leukemia (Table VI).

SUMMARY

This investigation has been concerned with the evaluation of 14 crude alkaloid fractions derived from *C. lanceus* leaves and roots for hypotensive and antineoplastic activity. Several alkaloids isolated in our laboratories from this plant were similarly evaluated. From these studies, the following conclusions were made.

1. Eight of 14 crude alkaloid fractions elicited moderate to marked hypotensive activity in normotensive, anesthesized rats. Three of the fractions exhibited a similar activity in the anesthetized dog.

2. Of 10 alkaloids evaluated, yohimbine was found to be the most active as a hypotensive agent. Leurosine, perivine, and pericyclivine gave only a transient hypotensive effect, whereas vindoline, tetrahydroalstonine, ajmalicine, lochnerinine, and periformyline were inactive at the dose levels utilized.

3. Evidence has been presented that other highly active hypotensive alkaloids are present in crude alkaloid extracts from this plant. The identity of these alkaloids is presently unknown.

4. Only one of 14 alkaloid fractions from *C. lanceus* was active against the P-1534 leukemia in mice and leurosine was shown to be one of the alkaloids responsible for the activity of the fraction. Monitoring of chromatographic cuts of the active fraction against the P-1534 leukemia has shown that at least one additional active alkaloid remains in this plant.

5. Leurosine and lochnerinine were shown to have reproducible cytotoxic activity against Eagle's 9 KB carcinoma of the nasopharynx.

6. Perivine, periformyline, pericyclivine, vindoline, ajmalicine, tetrahydroalstonine, catharine, catharanthine, yohimbine, pericalline, desacetylvindoline, and perivinol were shown to be devoid of antineoplastic as well as cytotoxic activity.

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