Antiviral Activity of Selected *Catharanthus* Alkaloids

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Thirty-six alkaloids isolated either from *Catharanthus roseus* or *C. lanceus* were evaluated for *in vitro* activity against vaccinia and polio type III viruses. Nine of these alkaloids were effective as antiviral agents, with pericalline being the most effective.

In recent years, investigators have directed their attention toward the plant kingdom as a source of antiviral agents, and a number of plant extracts have been found to inhibit a variety of viruses in tissue culture as well as through *in vivo* experiments (1–11).

Although the authors were primarily interested in *Catharanthus* species as a source of antitumor alkaloids, the presence of compounds with other types of biological activity was not precluded. Thus, all isolates which were available in sufficient quantity were evaluated for *in vitro* antiviral activity against the vaccinia and polio type III viruses.

Prior to this investigation, Johnson had reported briefly on the *in vivo* antiviral activity of leurocristine against the mengo virus (12). To illustrate the specificity of this antiviral agent, it was shown that vincaleukoblastine was completely devoid of activity against the mengo virus *in vivo*, and these two alkaloids differ only slightly in their structures (leurocristine = N—CHO, vincaleukoblastine = N—CH_3).

A search of the literature has revealed that this is the first report to appear on the *in vitro* evaluation of alkaloids as antiviral agents.

EXPERIMENTAL

Materials—The following alkaloids, isolated either from *C. roseus* or *C. lanceus*, were utilized as test compounds in the study: pericalline (hydrochloride) (13, 14), perivine (hydrochloride) (15, 16), periformyline (17), leurosivine (sulfate) (14), leurocristine (sulfate) (18), vincaleukoblastine (sulfate) (16), perividine (19), vindolinine (dihydrochloride) (16, 20), carosine (21), leurosidine (sulfate) (18), desacetyl VLB (sulfate) (22), vindoline (hydrochloride) (15, 23), ajmalicine (13, 16), leurosine (sulfate) (15, 16), neoleurocristine (21), vincarodine (21), vinosidine (14, 23, 24), virosine (16), ammorosine (14), ammocalline (14, 25), catharine (20, 26, 27), carosidine (21), cavincine (sulfate) (14), lochrovicine (28), lochrovidine (28), lochrovine (28), lochnerine (acetate) (16), mitraphylline (14), neoleurosidine (21), perimivine (13, 28), sitsirikine (sulfate) (29), tetrahydroalstonine (16, 17), vinaphamine (22), vincolidine (28), and vincoline (25, 28).

Solutions of each alkaloid were prepared in a suitable solvent (benzene for the free bases and ethanol for the salts), and measured amounts were transferred to suitable filter paper disks, and the solvent was allowed to evaporate spontaneously. Disks were each impregnated with 1000, 500, 250, or 125-mcg. amounts of the separate alkaloids.

Test Procedure—The agar diffusion method based on reports by Herrmann (29) and Siminoff (30) was used. Green monkey kidney cells (BSC_1) were maintained in medium 199, containing 5% calf serum and penicillin (50,000 units/ml.). Vaccinia VI Lindeman and Type III poliomyelitis viruses were separately maintained in the above medium, and when used in the test had a titer at between log 10^5 and 10^7 viral particles/ml.

Interpretation of the Test—Activity (A) values represent the size (mm.) of the zone where protection of the cells from virus damage was observed. Morphology (M) values represent the degree of protection as shown in Table I.

RESULTS AND DISCUSSION

Of 36 alkaloids evaluated, nine showed a degree of antiviral activity which varied from moderate to marked (pericalline, perivine, periformyline, leurosivine, leurocristine, vincaleukoblastine, perividine, vindolinine, carosine) (see Table II). Leurosidine (sulfate), desacetyl VLB(sulfate), and vincarodine exhibited minimal activity. The following alkaloids were inactive at the concentrations tested: vindoline (HCl), ajmalicine, virosine, leurosine (sulfate), neoleurocristine, vinosidine, ammorosine, ammocalline, catharine, carosidine, cavincine, lochrovicine, lochrovidine, lochrovine, lochnerinine, mitraphylline, neoleurosidine, perimivine, sitsirikine, tetrahydroalstonine, vinaphamine, vincathicine, vincolidine, and vincoline.

In all instances, with the exception of perivine, activity was specific for either a DNA virus (vaccinia), or a RNA virus (polio III). It is difficult at this time to draw any conclusions regarding the structure-activity relationship of these alkaloids, since 17 of the 36 evaluated are of unknown structure. Monomeric as well as dimeric alkaloids were active.

Pericalline, the most active of the test compounds, is an unusual indole alkaloid in that it contains no

Table I—Interpretation of Morphology (M) Values

M Value	Description
4+	Dark staining areas that show healthy cells with no visual virus or drug damage upon microscopic examination.
3+	Stained area, not dark, upon microscopic examination show no virus damage but do not appear to be very healthy.
2+	Healthy cells with moderate amount of virus breakthrough.
1+	Healthy cells with an increased virus breakthrough.
—	No viable cells.

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TABLE II—ANTIVIRAL ACTIVITY OF *Catharanthus* ALKALOIDS

Alkaloid	Form	Concn., mcg./ml.	Vaccinia A[a]	Vaccinia M	Polio III A	Polio III M
Pericalline	Hydrochloride	1000	0	0	25	4+
		500	0	0	25	3+
		250	0	0	20	3+
		125	0	0	0	0
Perivine	Hydrochloride	1000	14	2+	23	4+
		500	10	0	15	2+
		250	0	0	0	0
Periformyline	Base	1000	0	0	30	2+
		500	0	0	30	2+
		250	0	0	25	2+
		125	0	0	20	2+
Leurosivine	Sulfate	1000	30	2+	0	0
		500	23	2+	0	0
		250	22	2+	0	0
		125	20	2+	0	0
Leurocristine	Sulfate	1000	>50	2+	0	0
		500	40	2+	0	0
		250	35	2+	0	0
		125	28	2+	0	0
Vincaleukoblastine	Sulfate	1000	33	2+	0	0
		500	30	2+	0	0
		250	27	1+	0	0
		125	24	1+	0	0
Perividine	Base	1000	0	0	22	2+
		500	0	0	0	0
Vindolinine	Dihydrochloride	1000	0	0	20	2+
		500	0	0	20	2+
		250	0	0	15	2+
		125	0	0	0	0
Carosine	Base	1000	0	0	30	2+
		500	0	0	25	2+
		250	0	0	15	2+
		125	0	0	10	2+
Leurosidine	Sulfate	1000	20	1+	0	0
		500	15	1+	0	0
		250	0	0	0	0
Desacetyl VLB	Sulfate	1000	15	1+	0	0
		500	9	1+	0	0
		250	0	0	0	0
Vincarodine	Base	1000	0	0	20	1+
		500	0	0	0	0

[a] A, activity; M, morphology; 0, no activity. See the text and Table I for explanations.

oxygen in the molecule. On the other hand, perivine, periformyline, and perividine are the only representatives of monomeric 2-acylindole bases tested, and all three were active. Leurosivine, leurocristine, vincaleukoblastine, leurosidine, and leurosine, all dimeric indole alkaloids, are active tumor inhibitors, and with the exception of leurosine, they all showed antiviral activity.

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Keyphrases

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