Studies on *Vinca major* (*Apocynaceae*) II

Phytochemical Investigation

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Vinca major L. (*Apocynaceae*), commonly known as "Pervenche Grande" or the "Greater Periwinkle," has been mentioned frequently as a folk remedy. This plant, collected in Yugoslavia, has been subjected to a phytochemical screening and found to contain alkaloids, saponins, unsaturated sterols, organic acids, and phenols. Tannins, flavanols, and cardiac glycosides were not detected by the micromethods used. The results of a proximate analysis are also reported together with a method for the chromatographic separation of the total alkaloids. Paper partition and thin layer chromatographic data of the separated fractions are presented to indicate the complex character of the total alkaloids of this plant. An aqueous extract of the leaves and stems, as well as the total alkaloids, were shown to have no leukopenic activity in rats.

VINCA MAJOR[1] has been used for centuries as a folk remedy in the treatment of diabetes (2), as an abortifacient (2, 3), vulnerary, and in the treatment of menorrhagia (2). The plant has a wide distribution, having been introduced to the United States (4), England, Portugal, Spain, Malta, and Algeria (1). It is reported indigenous to France, Italy, Switzerland, Sicily, Madeira, Canary Islands, Crete, Rhodes, Chios, and the south and east coasts of the Black Sea (1). There is a great deal of current interest in the plants of the genus *Vinca*, not only because they are members of the alkaloid-rich *Apocynaceae*, but because of the recent isolation of the antileukemic alkaloid vincaleukoblastine from a related plant, *Catharanthus roseus* (L.) G. Don (*Vinca rosea* L.) (5).

To date, nine crystalline alkaloids have been isolated from *V. major* (Table I). They include reserpinine (6), vincamajoridine (akuammine) (7), vincamajoreine (8), vincamajine (9), perivincine (10), pubescine, vinine, and an alkaloid with m.p. 194–195° (11), as well as an alkaloid thought to be serpinine (6). Djerassi, *et al.* (12), have suggested that serpinine is identical with tetraphyllicine. In addition to these alkaloids, ursolic acid (13), dambonitol (14), and robinoside (15) have been isolated, and *o*-pyrocatechuic acid has been found to be present in acid hydrolysates of this plant (16).

TABLE I.—ALKALOIDS FROM *Vinca major* L.

No.	Name	Formula
1	Reserpinine	$C_{22}H_{26}N_2O_4$
2	Vincamajoridine	$C_{22}H_{26}N_2O_4$
3	Vincamajoreine	$C_{21}H_{26-28}N_2O_2$
4	Vincamajine	$C_{22}H_{26}N_2O_3$
5	Perivincine	$C_{22}H_{28}N_2O_4$
6	Pubescine	$C_{20}H_{26}N_2O_4$
7	Vinine	$C_{19}H_{26}N_2O_4$
8	Alkaloid m.p. 194–195°	
9	Serpinine (?) m.p. 316°	

The total alkaloids of *V. major* have been found to be ganglioplegics as well as spasmolytics, but only exert a transient hypotensive effect (17). Reserpinine (18) and perivincine (10) have been reported to produce only a transient hypotensive action in animals but vinine exerts a pronounced and prolonged blood pressure depression (11).

In this communication we wish to report the results of a phytochemical study of the leaves and stems of *V. major* and the effects of extracts of this plant on the leukocyte counts of rats.

EXPERIMENTAL

Phytochemical Screening.—The plant material used in this investigation was collected in Yugoslavia during the summer of 1957 and consisted of the dried leaves and stems.[2] The air-dried material was ground to a coarse powder and screened for alkaloids, saponins, flavanols, tannins, unsaturated sterols, organic acids, and phenols by the micromethod of Wall, *et al.* (19). In addition, cardiac glycosides were tested for by the method of Krider, *et al.* (20). The results of these tests are listed in Table II. There is some conflict of these results with those obtained by Wall, *et al.* (21), who used *V. major* leaves and twigs collected in Athens, Georgia, during 1951 and recorded negative alkaloid, organic acid, and phenol tests for this plant. The

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[1] Synonymous with *Vinca major* L. var. *major* Pich., *V. pubescens* Urv., *V. grandiflora* Salisb., *V. ovatifolia* Stokes, and *V. obliqua* Porta (1).
[2] Generously supplied as botanically authenticated *Vinca major* L. leaves and stems by the S. B. Penick & Co., Inc., 100 Church Street, New York 8, N. Y.

TABLE II.—PHYTOCHEMICAL SCREENING

Alkaloids	+
Saponins	+
Sterols, unsaturated	+
Organic acids	+
Phenols	+
Flavanols	−
Tannins	−
Cardiac glycosides	−

negative alkaloid test by these workers can be explained on the basis of their extracts being too dilute. Their current revised alkaloid testing procedure has been modified so that instead of 1 ml. being equivalent to 0.2 Gm. of dry plant sample, it is now equivalent to 4.0 Gm. (22). The positive hemolysis test for saponins is due to the ursolic acid content of the leaves.

Proximate Analysis.—A proximate analysis of the air-dried leaves and stems of *V. major* was conducted according to standard U.S.P. and A.O.A.C. methods (23–30). The results are shown in Table III and represent averages for four determinations expressed in terms of the air-dried material.

TABLE III.—PROXIMATE ANALYSIS

Determination	Per Cent[a]	Method (Ref.)
Total ash	3.76	(23)
Acid-insoluble ash	1.03	(23)
Crude fiber	10.49	(23)
Moisture		
By toluene distillation	6.99	(24)
By method for drugs containing no constituents volatile at 100°	6.74	(24)
Extractives		
Alcohol-soluble	36.31	(25)
Diluted alcohol-soluble	23.07	(25)
Petroleum ether-soluble	2.75	(25)
Volatile ether-soluble	0.73	(25)
Nonvolatile ether-soluble	4.96	(25)
Water-soluble	22.07	(26)
Starch	3.72	(27)
Pentosans	12.54	(28)
Reducing sugars	13.72	(29)
Organic and ammoniacal nitrogen	1.39	(30)

[a] Average per cent for four determinations.

Alkaloid Isolation and Fractionation.—The method used for the isolation of total alkaloids and separation into crude fractions I, II, and III has been previously reported (10). Figures 1 and 2 represent schematic outlines of this work.

Chromatographic Separation of Alkaloid Fractions.—*Fraction I.*—Three grams of fraction I (petroleum ether-insoluble) crude alkaloids was dissolved in 10 ml. of methanol with the aid of gentle heat. Fisher adsorption alumina, 10 Gm. (80–200 mesh, pH 9.95), which had been previously heated at 200° for 4 hours, was added to the solution of alkaloids and the mixture stirred on a water bath until the methanol was removed. A glass column (36 × 3/4 in.) was tightly packed with 230 Gm. of alumina, activated as described, and the alkaloid-adsorbed alumina added to the column in the same manner. A small pledget of glass wool was added to prevent disruption of the adsorbent on addition of the eluent. Elution of the alkaloids was effected by means of the following series of gradient eluents: benzene, benzene-methanol (99.5:0.5), benzene-methanol (99:1), benzene-methanol (98:2), benzene-methanol (95:5), benzene-methanol (90:10), benzene-methanol (50:50), and methanol.

Fractions were collected utilizing an automatic fraction collector equipped with a 20-ml. siphon.[3] Each fifth fraction was tested for alkaloids in the usual manner with Mayer's reagent. By observing the density of precipitation with this reagent it was possible to follow the relative concentration of alkaloids throughout the separation. Consecutive fractions were pooled according to the results of this estimation, evaporated to dryness, and weighed. When negative fractions could not be found for cut-off points, the low point between any two peaks was selected. The results of the separation of fraction I crude alkaloids are shown in Table IV.

TABLE IV.—CHROMATOGRAPHY OF FRACTION I (3 GM.)

Fraction No.	Volume, ml.	Eluent	Weight, Gm.	Test for Alkaloids
I-A	2,500	Benzene	...	..
I-B	2,000	Benzene-methanol (99.5–0.5)	...	..
I-C	2,000	Benzene-methanol (99:1)	0.420	+
I-D	2,100	Benzene-methanol (98:2)	0.249	+
I-E	2,000	Benzene-methanol (95:5)	0.223	+
I-F	1,500	Benzene-methanol (90:10)	0.119	+
I-G	2,000	Benzene-methanol (50:50)	0.132	+
I-H	3,600	Methanol	0.245	+

Fraction II.—This fraction (benzene-petroleum ether-soluble) of alkaloids was treated in the same manner as fraction I except that 4 Gm. of crude alkaloids was dissolved in 10 ml. of chloroform and mixed with 10 Gm. of alumina prior to application on a 230-Gm. column of alumina, as previously described. The same series of gradient eluents was used (Table V).

Fraction III.—Four grams of fraction III (benzene-insoluble) crude alkaloids was extracted with five successive 20-ml. portions of methanol by trituration in a mortar, followed by filtration. The nonalkaloidal residue weighed 1 Gm. The methanol filtrates containing 3 Gm. of crude alkaloids were mixed with 23 Gm. of alumina and dried as previously described. This mixture was then placed on top of a packed column containing 200 Gm. of alumina and the same series of eluents added. The results are summarized in Table VI.

[3] Rinco chromatographic fraction collector, Rinco Instrument Co., Greenville, Ill.

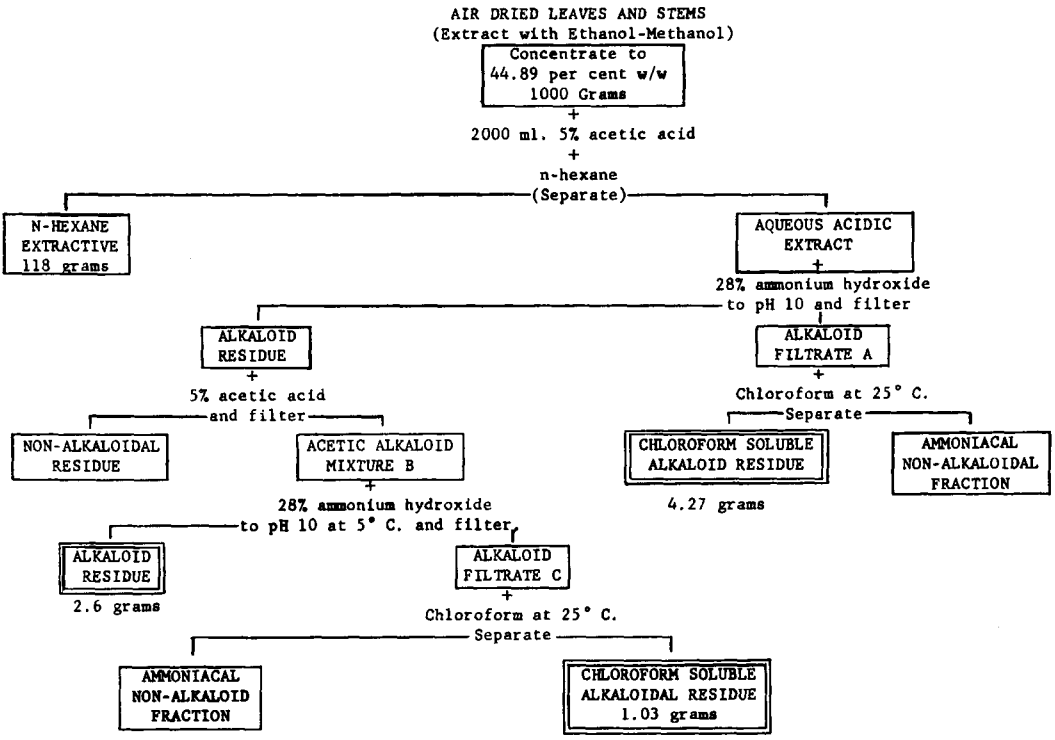

Fig. 1.—Flowsheet for the removal of the total crude alkaloids from the leaves and stems of *Vinca major*.

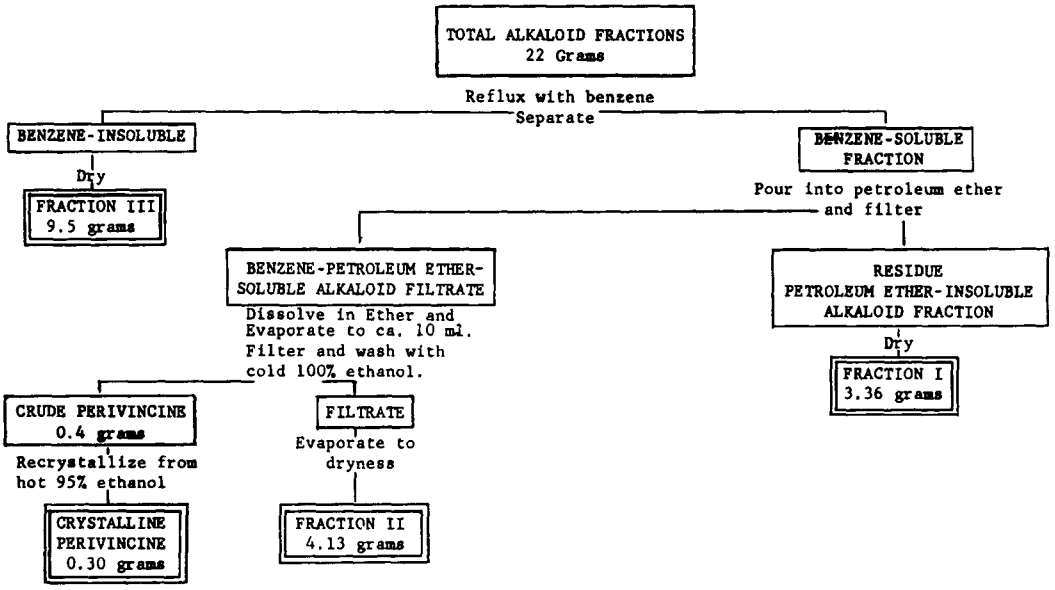

Fig. 2.—Flowsheet for the preliminary fractionation of the total crude alkaloids from the leaves and stems of *Vinca major*.

Alkaloid Composition of Separated Fractions.—The alkaloid composition of the separated fractions was determined by paper as well as by thin-layer chromatography. It was possible to determine alkaloid spill-over in this manner by the comparison of R_f values as well as by the color and intensity of the alkaloid spots, as viewed under ultraviolet illumination, and by the use of Dragendorff's reagent.

Paper Partition Chromatography.—The most efficient paper chromatographic method for the separation of *Vinca* alkaloids was found to be that of Korzun, *et al.* (31), with the modification of pH adjustment of the formamide with formic acid to

TABLE V.—CHROMATOGRAPHY OF FRACTION II (4 GM.)

Fraction No.	Volume, ml.	Eluent	Weight, Gm.	Test for Alkaloids
II-A	4,000	Benzene	0.193	+
II-B	2,200	Benzene-methanol (99.5-0.5)	1.823	+
II-C	900	Benzene-methanol (99:1)	0.173	+
II-D	2,040	Benzene-methanol (98:2)	0.370	+
II-E	1,700	Benzene-methanol (95:5)	0.239	+
II-F	1,280	Benzene-methanol (95:5)	0.051	+
II-G	2,100	Benzene-methanol (95:5)	0.069	+
II-H	3,400	Benzene-methanol (90:10)	0.191	+
II-J	1,900	Benzene-methanol (50:50)	0.142	+
II-K	2,760	Methanol	0.135	+

TABLE VI.—CHROMATOGRAPHY OF FRACTION III (3 GM.)

Fraction No.	Volume, ml.	Eluent	Weight, Gm.	Test for Alkaloids
III-A	1,300	Benzene	...	..
III-B	1,900	Benzene-methanol (99.5:0.5)	0.011	+
III-C	1,110	Benzene-methanol (99.5:0.5)	0.058	+
III-D	1,300	Benzene-methanol (99:1)	0.033	+
III-E	3,100	Benzene-methanol (98:2)	0.140	+
III-F	2,600	Benzene-methanol (95:5)	0.173	+
III-G	1,280	Benzene-methanol (95:5)	0.066	+
III-H	1,780	Benzene-methanol (95:5)	0.059	+
III-J	3,400	Benzene-methanol (90:10)	0.134	+
III-K	1,900	Benzene-methanol (50:50)	0.276	+
III-L	2,100	Methanol	0.324	+

either pH 5.2 or pH 8.0. Samples of commercial formamide were found to vary in pH from 10.1 to 11.7 which led to variation in results. Solutions of the alkaloid fractions were prepared in either methanol or chloroform at 1% (w/v) concentrations and 50 λ of each applied. An equilibration period of 4 hours was found necessary in order to obtain reproducible results. The completed chromatograms were dried at 90–95° for one-half hour and then observed under long and short wave ultraviolet light. Fluorescent areas were outlined and the colors noted. After spraying with a modified Dragendorff reagent (32), the R_f values were calculated. In some instances areas giving a positive Dragendorff reaction were not distinct and appeared to run together. In these instances, the more sensitive fluorescent areas which had been marked, aided in the differentiation of distinct areas for R_f calculation. The results of chromatographing all of the alkaloid fractions in a system utilizing formamide adjusted to pH 8.0 are listed in Table VII. The R_f values tabulated represent averages of duplicate samples of each fraction.

TABLE VII.—R_f VALUES FOR SEPARATED FRACTIONS BY PAPER PARTITION CHROMATOGRAPHY AT pH 8.0[a]

	Fraction I	Fraction II	Fraction III
A	...	0 o	...
	...	0.32b	...
	...	0.70y	...
	...	0.85oy	...
B	...	0 b	0 b
	...	0.15b	...
	...	0.22b	...
	...	0.30b	...
	...	0.37b	...
	...	0.49b	...
	...	0.77b	...
C	0 o	0 y	0 b
	0.03b	0.08g	0.09o
	0.17o	0.16y	0.16b
	0.33y	0.26b	0.27y
	0.45p	0.32b	0.37b
	0.55b	0.41y	0.49b
D	0 b	0 y	0 y
	0.07b	0.06g	0.17y
	...	...	0.72y
E	0 b	0 y	0 y
	...	0.33y	0.07b
F	0 b	0 o	0 bb
	0.07b	...	...
G	0 b	0 g	0 b
	0.23b	...	0.20b
	0.38b	...	0.82b
	0.50b	...	...
	0.90b	...	...
H	0 b	0 b	0 b
	0.03b	0.29b	...
J	...	0 b	0 b
	...	0.23b	0.18b
	...	0.39b	...
	...	0.50b	...
	...	0.90b	...
K	...	0 b	0 b
	...	0.13b	...
	...	0.75y	...
L	...	...	0 b

[a] Each R_f represents the average from two chromatograms. Letters following each R_f refer to fluorescent color under long wave ultraviolet light: o = orange, b = blue, bb = brilliant blue, p = pink, y = yellow, g = green, oy = orange-yellow.

Thin-Layer Chromatography.—A rapid and relatively simple method for detecting the alkaloids in each fraction was thin-layer chromatography. Plates were prepared using silica gel G and the

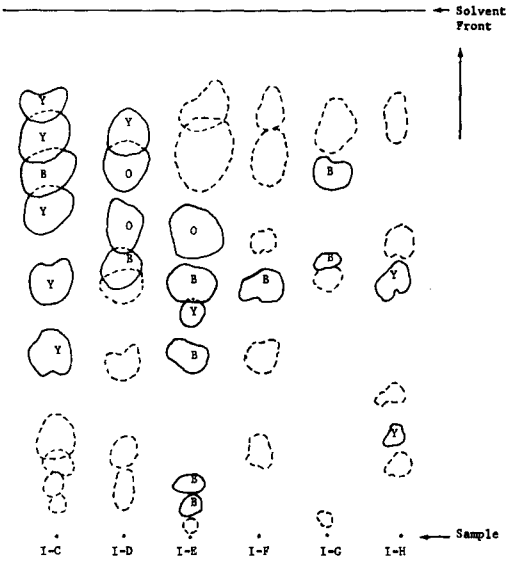

Fig. 3.—Thin-layer chromatogram of fraction I alkaloids using silica gel G adsorbent and chloroform-methanol (95:5) as eluent. Key: (under long wave U.V. light) B = blue, O = orange, Y = yellow fluorescence.

Desaga[4] apparatus. The adsorbent was mixed by slowly adding 40 ml. of distilled water, with stirring, to 30 Gm. of silica gel G. An additional 20 ml. of distilled water was then added, mixed thoroughly, and poured into the spreading applicator and applied to 200 × 200-mm. glass plates in the usual manner. The glass plates were then placed in a preheated oven at 105° for 30 minutes, allowed to cool to room temperature, and then spotted with the various alkaloid fractions. Solutions of the alkaloid fractions were prepared at 10% (w/v) concentrations in either methanol or chloroform. Ten-lambda increments were spotted on the prepared plates, allowed to air-dry, and then the plates were placed into equilibrated glass chambers lined with filter paper. Several eluents were used, with chloroform-methanol (95:5) giving the best separations. Normal development time was found to be about 1 hour. The results of the chromatographic separations using this method are shown in Figs. 3–5. Interpretation of the chromatograms was accomplished as previously described. The relative concentration of alkaloids, as indicated by the intensity of fluorescence under ultraviolet illumination, has been shown in the figures as follows: (*a*) spots of definite color and intensity are circled with a solid line enclosing a letter indicating the fluorescent color, (*b*) spots which could definitely be determined but were of low intensity so that colors could not be assigned to them are circled with broken lines.

Interpretation of Chromatograms.—The results of both the paper partition and the thin-layer methods of chromatography were compared to chromatograms of the following alkaloids:[5] reserpinine, yohimbine, α-yohimbine, lochnerine, vincamine, sarpagine, serpentine, vincamajoridine, ajmaline, ajmalicine, vincamajine, perivincine, reserpiline, tetrahydroalstonine, reserpine, deserpidine, and rescinnamine. The presence of serpentine, vincamine, sarpagine, and tetrahydroalstonine is strongly suspected from these results and work is now in progress to evaluate all of the alkaloid spots against a large number of reference alkaloids from the *Apocynaceae*. Although there is little doubt from our data that perhaps 40 or more distinct alkaloids are present in this plant, more work must be conducted to determine the extent of alkaloid spill-over from one fraction to another.

Screening of Extracts for Leukopenic Activity.—Noble, *et al.* (5), were the first to report on the leukopenic activity of *Vinca rosea* in experimental animals. Their study resulted in the isolation of vincaleukoblastine, the alkaloid responsible for the major portion of the leukopenic activity of crude extracts of *V. rosea*. Vincaleukoblastine is now available as an aid in the treatment of Hodgkin's disease and choriocarcinoma in humans.[6] Although these workers indicated that extracts of *V. major* as well as *V. minor* did not affect the blood picture of rats in their studies, it seemed advisable to confirm these results with studies using *V. major* from Yugoslavia, especially since Noble, *et al.* (5), reported that *V. rosea* plants grown as annuals in England possessed only about one-fourth of the leukopenic activity of plants grown as perennials in the West Indies. For testing purposes, two extracts were prepared; extract A was essentially an aqueous concentrate, containing all of the alkaloids of the plant in addition to other water-soluble materials, while extract B was a propylene glycol solution of total alkaloids as free bases.

Preparation of Extract A.—A Soxhlet extraction apparatus was packed with 380 Gm. of powdered air-dried leaves and stems and continuously extracted with methanol until free from alkaloids. The methanol extract was cooled to room temperature, filtered, and concentrated *in vacuo* at 40° to *ca.* 500 ml., mixed with 500 ml. of distilled water, shaken with Celite filter aid, and filtered. The filtrate was again concentrated *in vacuo* to 225 ml. which, after filtration, resulted in an alcohol-free aqueous extract containing the equivalent of 1.7 Gm. of air-dried plant material in each 1 ml.

Preparation of Extract B.—A portion of extract A was treated with 28% ammonium hydroxide solution to pH 10 with constant stirring. The precipitated alkaloids were removed by several extractions with chloroform. The combined chloroform extracts were dried over sodium sulfate, filtered, and evaporated to dryness *in vacuo*. The alkaloid residue was dissolved in propylene glycol to make a solution containing 0.155 Gm. in each 1 ml.

Leukocyte Counting.—Doses of 8.25 ml./Kg. of extract A and 0.82 ml./Kg. of extract B were selected after toxicity tests indicated that these doses were the highest that could be tolerated with 100% survival of all animals injected. Both total and differential leukocyte counts were performed in the usual manner utilizing blood from the tail of male albino rats.[7] All counts were made on consecutive

[4] Desaga apparatus for thin-layer chromatography, Brinkmann Instruments Inc., Great Neck, Long Island, N. Y.

[5] The authors wish to thank the S. B. Penick & Co., Abbott Laboratories, Charles Pfizer & Co., Ciba Pharmaceutical Products Inc., and Eli Lilly and Co., for generous samples of alkaloids as well as Drs. M. M. Janot, N. A. Rubin, S. Goodwin, C. Djerassi, and A. Stoll.

[6] Velban, trademarked name of Eli Lilly & Co., Indianapolis, Ind., for vinblastine sulfate.

[7] Holtzman Co., Madison 4, Wis.

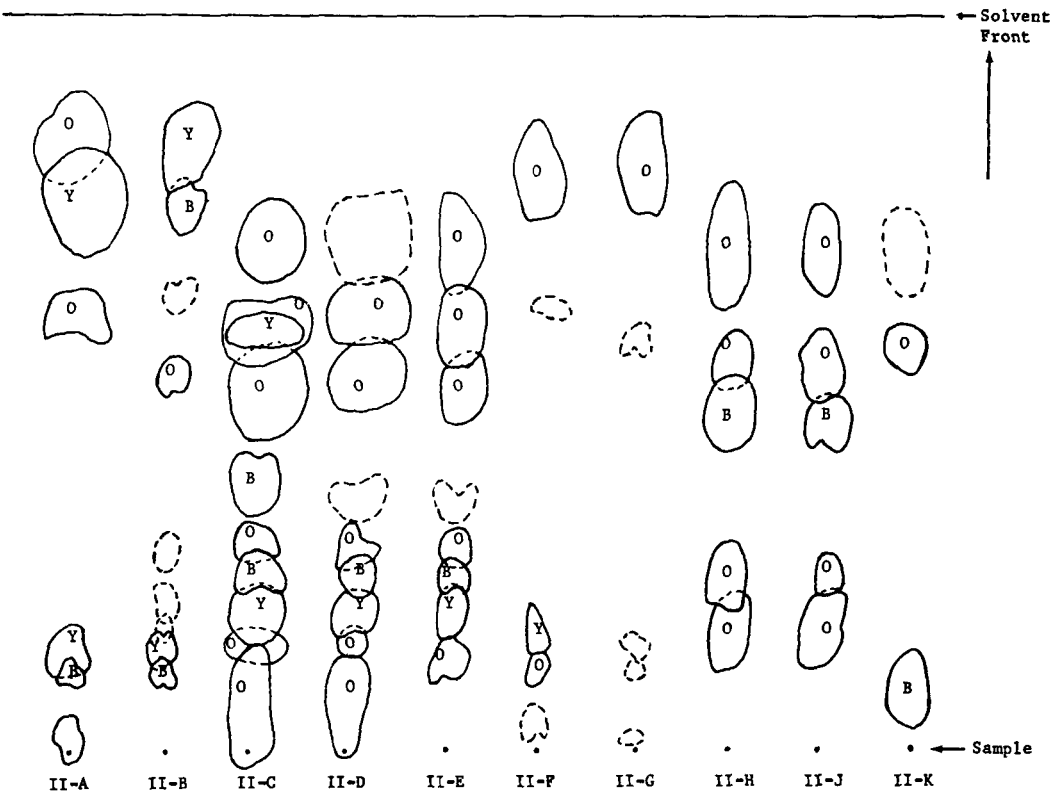

Fig. 4.—Thin-layer chromatogram of fraction II alkaloids using silica gel G adsorbent and chloroform-methanol (95:5) as eluent. Key: (under long wave U.V. light) B = blue, O = orange, Y = yellow fluorescence.

days and at approximately the same time each day. The rats were fed a normal diet *ad libitum*. The rats were divided into four groups of six animals each. In addition to daily blood counts, rat weight was tabulated since, according to Noble, *et al.* (5), leukopenia due to extracts of *V. rosea* was always accompanied by a drop in weight one to two days after i.p. injection. After 7 consecutive days of control normal blood counts from all animals, single i.p. injections were given. Group 1 received 8.25 ml./Kg. of extract A and group 2 received 8.25 ml. of distilled water as a control. Group 3 received 0.82 ml./Kg. of extract B, and group 4 received 0.82 ml./Kg. of propylene glycol as a control. Daily blood counts and weight recordings were continued for at least 6 days. The results as shown in Figs. 6 and 7 indicate that extract A and extract B do not depress the leukocyte counts under these experimental conditions. Differential counts in all groups remained within normal limits when compared to the control animals. These results are consistent with those reported by Noble, *et al.* (5), for their extracts of *V. major*.

DISCUSSION

This investigation has pointed out the complex nature of the total alkaloids of *V. major*. Through an interpretation of our chromatographic data, which will be reported at a later date, we can show the presence of at least 40 alkaloids in this plant. Although this is a rather large number it is not unusual since a related plant, *V. rosea*, has yielded at least 21 well described alkaloids at the time of this writing, in addition to four more which appear, from available data, to be additions to this list. *V. lancea* (*Catharanthus lanceus*) total alkaloids appear to include at least 70 separate alkaloids which we have been able to detect in some preliminary work by two-dimensional thin-layer chromatography. Although we have proposed that tetrahydroalstonine and sarpagine are present in the *V. major* alkaloids, it will be interesting, after additional work, to confirm this since these two alkaloids have not been reported to date as being present in any of the *Vinca* species which have been investigated, although they do appear in the *Catharanthus* group. Interestingly enough, of the 21 alkaloids isolated to date from species of *Catharanthus*, only vincamajoridine (akuammine) has been reported to be present in any of the *Vinca* species.

It has become evident to us during this investigation that thin-layer chromatography will prove to be an invaluable tool in phytochemistry and especially in chemotaxonomic studies. The method is simple, rapid, and accurate for the tentative identification of substances which are chromatographed on the same plate with known reference compounds.

The chromatographic separation of alkaloid fractions from *V. major* on a pilot scale has indicated that reasonable separation of alkaloids has been accomplished and that by inspection of paper and

Fig. 5.—Thin-layer chromatogram of fraction III alkaloids using silica gel G adsorbent and chloroform-methanol (95:5) as eluent. Key: (under long wave U.V. light) B = blue fluorescence, O = orange fluorescence, W = white fluorescence, Y = yellow fluorescence.

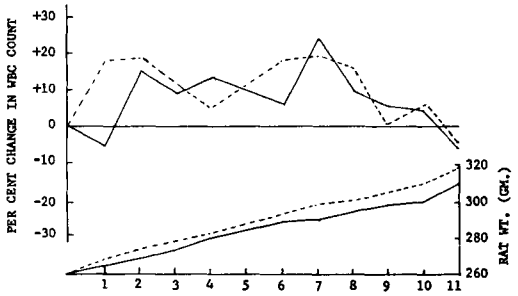

Fig. 6.—The effect of *Vinca major* extract A on rat wbc counts and weight after a single i.p. injection (8.25 ml./Kg.) *vs.* distilled water as a control. Mean values for groups of six rats following a 7-day control counting period. —— extract A, - - - - distilled water.

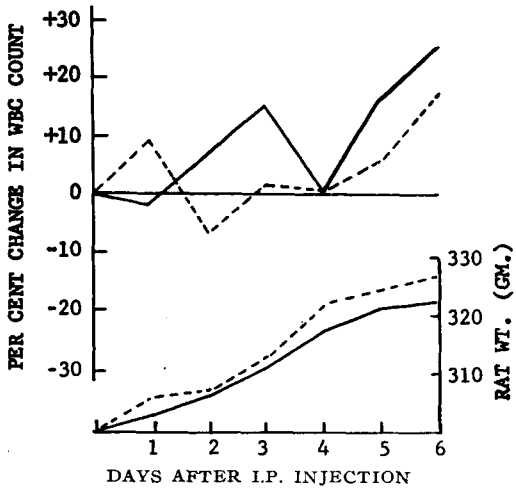

Fig. 7.—The effect of *Vinca major* extract B on rat wbc counts after a single i.p. injection (0.82 ml./Kg.) *vs.* propylene glycol as a control. Mean values for groups of six rats following a 7-day control counting period. —— extract B, - - - - propylene glycol.

thin-layer chromatograms, we can group certain eluates prior to attempting crystallizations from them.

SUMMARY

1. A phytochemical screening of the leaves and stems of *Vinca major* L. collected in Yugoslavia has shown the presence of alkaloids, saponins, unsaturated sterols, organic acids, and phenols. Tannins, flavanols, and cardiac glycosides were not detected.

2. A proximate analysis of the plant material is reported.

3. Methods for the separation of total alkaloids as well as their fractionation by gradient elution chromatography on alumina and detec-

tion by paper partition and thin-layer chromatography are described.

4. Screening of an aqueous extract as well as the total alkaloids of *V. major* in rats for possible leukopenic activity has confirmed a previous report of the absence of such activity.

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Stability of Antibacterial Preservatives in Parenteral Solutions I

Factors Influencing the Loss of Antimicrobial Agents from Solutions in Rubber-Stoppered Containers

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Losses of preservative due to degradation and absorption in rubber-closed multiple-dose vials were investigated. The preservatives evaluated were benzyl alcohol, phenylethyl alcohol, *p*-chloro-β-phenylethyl alcohol, chlorobutanol, and methylparaben. The elastomer closures used in this study were intended to be representative of those most commonly employed for pharmaceuticals and were natural, neoprene, and butyl rubbers. The temperature dependency of the degradation and diffusion processes were studied. An attempt was made to correlate preservative loss and microbiological activity.

RUBBER CLOSURES are used extensively by pharmaceutical manufacturers to seal vials containing injectable solutions. It is well known that rubber may react with, absorb, or even dissolve substances in contact with it. Thus, incompatibilities between elastomer closures and injectable solutions occur frequently, thereby presenting many problems. One such very important problem is the interaction between rubber closures and bacteriostatic agents present in the solutions. These agents are added to multiple-dose parenteral preparations to insure bacteriostasis for the life of the product. Loss of preservative from solution by interaction with rubber closures could result in bacterial contamination of the injectable preparation if bacteria were accidently introduced.

The 1953 edition of the British Pharmacopoeia (1) recognizes the tendency of rubber to absorb preservatives from injectable solutions. It therefore directs that closures for parenteral solutions should be boiled in several changes of distilled

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