Aggregation of Certain Medicinal Amines in Aqueous Solutions of Their Salts

By BAHRAM FARHADIEH*, NATHAN A. HALL, and E. R. HAMMARLUND

The osmotic coefficient and the equivalent conductance of aqueous solutions of certain medicinal amine salts were determined in order to examine their aggregating properties. Procaine HCl and tripelennamine HCl formed small ionic micelles. Partially ionized micelles of approximately constant size were the main constituent of solutions of dibucaine HCl and bromodiphenhydramine HCl. Tetracaine HCl and diphenhydramine HCl formed aggregates of different sizes in their solutions. No definite conclusion could be drawn about the types of aggregates that were present in solutions of pyrilamine maleate. An apparent critical micelle concentration (CMC) was obtained only for dibucaine HCl and bromodiphenhydramine HCl. From the CMC values at 0° and 25° it was concluded that the standard enthalpy change associated with formation of micelles in solutions of these two salts was practically zero. This finding together with a negative change in the standard free energy of micellization indicated that the process of micelle formation was mainly an entropy effect.

AQUEOUS SOLUTIONS of many long chain amine salts, sulfonates, sulfates, etc., exhibit an abrupt change in physical properties over a relatively short concentration range (1). This phenomenon which is common to aqueous solutions of association colloids has been attributed to the formation of aggregates, and the concentration at which it occurs has been termed "the critical concentration for the formation of micelles" (CMC). The nature of the transition taking place at the critical concentration is as yet unknown and a controversy still exists concerning the possible existence of aggregates at lower concentrations (2).

The freezing point depression, ΔT_f, of aqueous solutions of many substituted amine salts of pharmaceutical interest has been measured by Hammarlund and co-workers (3). It was shown that in dilute solutions these amine salts behave as simple electrolytes. At higher concentrations, however, a number of them appear to form molecular aggregates as evidenced by a sharp change in slope when ΔT_f is plotted against the salt concentration. Recently Johnson *et al.* (4) measured the vapor pressure of aqueous solutions of several of these amine salts using a vapor pressure osmometer of their own design. They also concluded that these salts tend to form molecular aggregates above a certain concentration.

Generally, measurements of several physicochemical properties of a solution are needed in order to ascertain the formation of aggregates by a solute. The work presented here was designed to investigate the aggregating tendency of several amine salts by determining the molal osmotic coefficient as well as the equivalent conductance of their aqueous solutions.

EXPERIMENTAL

Materials.[1]—The amine salts used in this study were procaine HCl, tetracaine HCl, dibucaine HCl, tripelennamine HCl, pyrilamine maleate, diphenhydramine HCl, and bromodiphenhydramine HCl. Since they were of medicinal grade, no further purification was attempted. Only tetracaine HCl and dibucaine HCl lost weight upon drying in vacuum and in the presence of phosphorus pentoxide. These were dried to constant weight before use.

Preparation of Solutions.—Solutions employed for the determination of the osmotic coefficients were prepared on the molal basis in double distilled

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TABLE I.—MOLAL OSMOTIC COEFFICIENT OF AQUEOUS SOLUTIONS OF POTASSIUM CHLORIDE AND AMINE SALTS

Concn., Molal	Compd.[a] 1	2	3	4	5	6	7	8
0.01	0.965	0.943	0.943	0.942	0.952	0.952	0.933	0.924
	0.967[b]							
0.03	0.950	0.918	0.896	0.839	0.918	0.911	0.867	0.816
0.05	0.941		0.850	0.774			0.789	
	0.941[b]							
0.06		0.884			0.868	0.864	0.543[c]	0.724
0.100	0.929	0.835	0.754	0.611	0.816	0.798		0.625
	0.930[b]							
0.150	0.920		0.645	0.452	0.752	0.728		0.505
0.160		0.780					0.361	
0.200	0.915	0.754	0.554	0.367	0.697	0.650	0.306	0.448
	0.915[b]							
0.250		0.722	0.483	0.317	0.648	0.579	0.275	
0.260								0.381
0.300		0.687	0.428	0.282	0.599	0.522	0.250	0.349
0.350			0.389			0.472		0.315
0.360							0.230	

[a] The numbers represent: *1*, potassium chloride; *2*, procaine HCl; *3*, tetracaine HCl; *4*, dibucaine HCl; *5*, tripelennamine HCl; *6*, diphenhydramine HCl; *7*, bromodiphenhydramine HCl; *8*, pyrilamine maleate. [b] Data obtained from literature. [c] 0.09 molal solution.

water. For the study of electrical conductivity solutions were made on molar basis in conductivity water (specific conductance, 1–1.5 $\times$ 10^{-6} ohms^{-1} cm.$^{-1}$).

Apparatus and Measurement Procedures.—A Mechrolab[2] vapor pressure osmometer was used to determine the molal osmotic coefficients of the solutions. Since preliminary experiments with potassium chloride solutions showed stable readings within 6 min., all readings were taken 6 min. after the solution drop was placed on the sample thermistor bead. All measurements were made at 25.000 $\pm$ 0.001°.

A Dike-Jones conductivity bridge[3] was used to determine the electrical resistance of the solutions. The frequency of the bridge current was 1000 c.p.s. and the null point was detected by a cathode ray oscilloscope. Three different conductivity cells were employed. The cell constants were *A*, 1.0449; *B*, 1.0241; and *C*, 13.0078. Cell *A* had unplatinized electrodes and was used for the determination of resistance of the very dilute solutions. The polarization resistance introduced by the bright platinum electrodes of this cell amounted to less than 0.10% of the total resistance of the solutions at 1000 c.p.s. Therefore, the error due to polarization was considered to be negligible and no attempt was made to correct the electrical resistance of the very dilute solutions for this effect. The measurements were obtained at 25.00° in an oil bath which was maintained at 25.00° $\pm$ 0.02°. Usually readings were taken 45 min. after the cells containing the solutions were immersed in the oil bath.

The molal osmotic coefficients of the aqueous solutions of the samples were calculated from the following relationship (5):

$$\Delta R = \nu\alpha\phi m \qquad \text{(Eq. 1)}$$

where ΔR is the difference in the resistance of the sample and the solvent thermistor beads; ν is the theoretical number of ions per molecule of the solute; ϕ is the molal osmotic coefficient of the solvent; m is the molality of the solute; and α is the calibration constant of the thermoelectric vapor pressure osmometer.

Aqueous solutions of mannitol ranging in concentration from 0.04 to 0.250 molal were used for the calibration of the instrument. Mannitol was chosen because the molal osmotic coefficient of its aqueous solution is very nearly unity up to 1 molal (5). The calibration constant was obtained from the slope of a plot of the measured ΔR *versus* concentration. The plot gave a straight line, the slope of which, as calculated by the method of least squares, was 52.60 ohms/mole.

The accuracy of the method as well as the reliability of the vapor pressure osmometer were determined by calculating the molal osmotic coefficient of aqueous KCl solutions and comparing them with the corresponding reported values (6). The agreement between the two sets of data was excellent.

The amine salts used in this study have pKa's of about 8–9 and the pH's of their aqueous solutions is about 5–6. In this pH range the dissociation of the protonated amines to their corresponding free base is negligible. Thus, in calculation of the molal osmotic coefficients, the samples were treated as simple 1:1 electrolytes. Each ΔR used in either the determination of the calibration constant or the calculation of ϕ is a mean of four separate readings with a standard error of less than $\pm$0.04 ohms.

RESULTS

The osmotic coefficient of aqueous solutions of KCl and the amine salts are summarized in Table I and are plotted *versus* the square root of concentration in Figs. 1 and 2. In these figures are also included the lines representing the Debye-Huckel expression (6) which at 25° is:

$$\phi = 1.0 - 0.392\sqrt{m} \qquad \text{(Eq. 2)}$$

[2] Mechrolab Inc., Mountain View, Calif.
[3] Leeds and Northrup Co., Philadelphia, Pa.

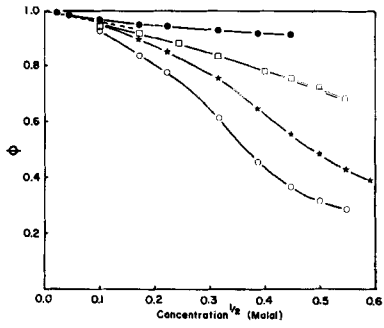

Fig. 1.—Molal osmotic coefficient of aqueous solutions. Key: ●, potassium chloride; □, procaine HCl; ★, tetracaine HCl; ○, dibucaine HCl; ----, Eq. 2.

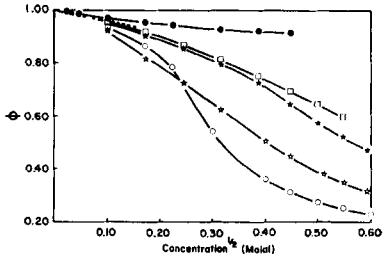

Fig. 2.—Molal osmotic coefficient of aqueous solutions. Key: ●, potassium chloride; □, tripelennamine HCl; ★, diphenhydramine HCl; ○, bromodiphenhydramine HCl; ☆, pyrilamine maleate; ----, Eq. 2.

Equation 2 holds true for extremely dilute aqueous solutions of a simple 1:1 electrolyte. Due to experimental limitations molal osmotic coefficients could not be measured for concentrations less than 0.01 molal. However, Figs. 1 and 2 indicate that at extremely dilute solutions the osmotic coefficient of the solutions should approach that given by the Debye-Huckel expression.

The equivalent conductance of solutions of NaCl and the amine salts used in this investigation are presented in Table II and are plotted *versus* the square root of concentration in Figs. 3 and 4. Each Λ is the average of two separate readings. According to Onsager (7), in extremely dilute solutions and at a temperature of 25° the equivalent conductance of any strong 1:1 salt is given by:

$$\Lambda = \Lambda_0 \left(1.0 - 0.22724 \sqrt{c}\right) - 59.730 \sqrt{c} \quad \text{(Eq. 3)}$$

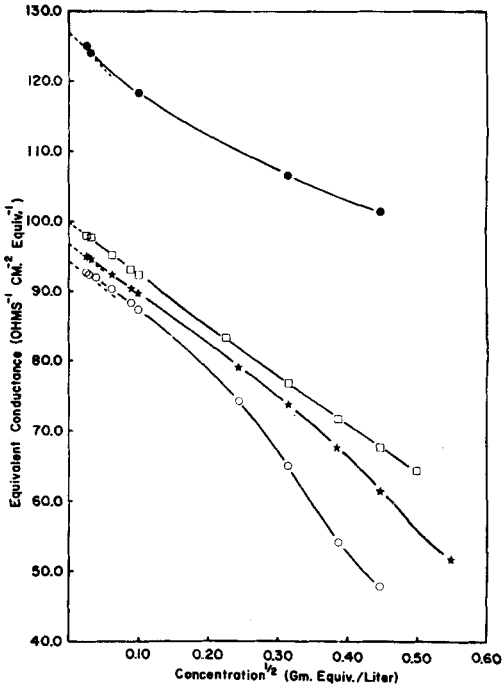

Fig. 3.—Equivalent conductance of aqueous solutions. Key: ●, NaCl; □, procaine HCl; ★, tetracaine HCl; ○, dibucaine HCl; ----, Eq. 3.

Table II.—Equivalent Conductance of Aqueous Solutions of Sodium Chloride and Amine Salts

Concn., *M*	Compd.[a] *1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
0.0007	124.30	98.09	95.03	92.70	97.90		96.00	71.80
0.0008			94.80	92.60	97.80	96.90	95.80	71.30
0.0009		97.99		92.50	97.60	96.60	95.70	70.90
0.0010	123.90	97.70	94.50		97.30	96.50	95.5	70.50
	123.74[b]							
0.0015				92.20		96.00	95.10	69.10
0.0040		95.40	92.40	90.50	95.0	93.90	92.5	65.60
0.0080		93.30	90.50	88.50	92.80	92.10	91.20	62.50
0.0100	118.40	92.50	89.70	87.50	91.70	91.10	90.00	61.40
	118.51[b]							
0.0500		83.40			83.20	82.60	80.50	47.80
0.0600			79.20	74.40				
0.1000	106.85	76.80	73.70	65.00	76.70	76.00	64.10	36.60
	106.74[b]							
0.1500		71.90	67.60	54.20	71.70	70.80	56.10	32.60
0.2000	101.66	67.80	61.46	47.90	67.60	66.20	51.50	28.30
0.2500		64.30				62.00	48.40	25.10
0.3000			51.90			58.42		

[a] The numbers represent: *1*, NaCl; *2*, procaine HCl; *3*, tetracaine HCl; *4*, dibucaine HCl; *5*, tripelennamine HCl; *6*, diphenhydramine HCl; *7*, bromodiphenhydramine HCl; *8*, pyrilamine maleate. [b] Data obtained from literature.

where Λ_0 is the limiting equivalent conductance and c is the concentration of the solute expressed in gram-equivalents per liter of solution. Broken lines in Figs. 3 and 4 represent Eq. 3. The value of Λ_0 for each salt was obtained by extrapolating an expanded plot of Λ *versus* $c^{1/2}$ to zero concentration of the salt solution. In the calculation of the equivalent conductance the salts were treated as 1:1 electrolytes and the contribution by the free bases was neglected.

In Fig. 5 the quantity ϕm, the product of molal osmotic coefficient and the molality of bromodiphenhydramine HCl, is plotted *versus* molality. Figure 6 represents the plot of the specific conductance *versus* concentration for the same salt. Each plot consists of two straight lines with different slopes which intersect each other at the CMC of the salt (8, 9). In Fig. 5 the slope of the straight line segment below the CMC of bromodiphenhydramine HCl coincides with that of an ideal solution of a strong 1:1 electrolyte, *i.e.*, KCl. The CMC's of dibucaine HCl and bromodiphenhydramine HCl as obtained by these two methods were 0.061 and 0.052 M, respectively.

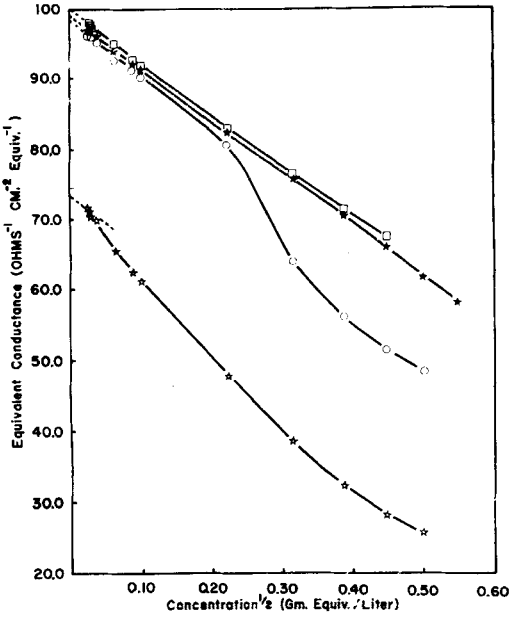

Fig. 4.—Equivalent conductance of aqueous solutions. Key: □, tripelennamine HCl; ★, diphenhydramine HCl; ○, bromodiphenhydramine HCl; ☆, pyrilamine maleate; ----, Eq. 3.

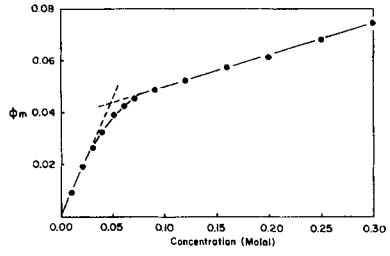

Fig. 5.—Apparent molality of aqueous bromodiphenhydramine HCl solutions.

DISCUSSION

Examination of the plots of Λ *versus* $c^{1/2}$ for the amine salts (Figs. 3 and 4) show that in extremely dilute solutions, where the Onsager expression is accepted to be valid, the experimental points coincide with those obtained from the Onsager equation. This agreement of the experimental and the theoretical data indicates that in these dilute solutions, the salts are totally dissociated into their constituent ions and, therefore, can be considered to be strong electrolytes.

In the concentration range shown in Fig. 1 the values of the molal osmotic coefficients for aqueous solutions of procaine HCl are less than the values predicted by the Debye-Huckel theory. Although this theory does not apply at such high concentrations, all deviations expected and found for a strong 1:1 electrolyte, KCl, are in the opposite direction to that of procaine HCl. These deviations indicate the presence of aggregates in solutions of this amine salt. Since the behavior of the equivalent conductance of procaine HCl solutions (Fig. 3) is similar to that of a strong 1:1 electrolyte, NaCl, the aggregates formed may be of the small ionic type suggested by McBain (10) as an explanation of the similar behavior observed in dilute solutions of colloidal electrolytes. Due to their increased charge, when compared to simple ions, these small aggregates should enhance the electrical conductance of the solutions provided that the decreased mobility of the ions in aggregates is overcome by the increased number of charges carried by the aggregates. The similarity in the behavior of the molal osmotic coefficients and the equivalent conductances of tripelennamine HCl solutions (Figs. 2 and 4) to those of procaine HCl suggests the presence of small ionic aggregates in the moderately concentrated solutions of this salt also.

Assuming that normality approximates molality in the concentration range studied, Figs. 2 and 4 show that up to 0.04 molal the osmotic coefficient as well as the conductance of aqueous bromodiphenhydramine HCl solutions are similar to that of procaine HCl. At concentrations above 0.04

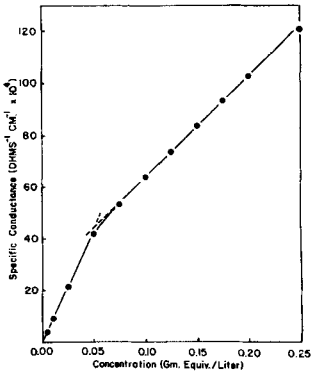

Fig. 6.—Specific conductance of aqueous bromodiphenhydramine HCl solutions.

molal the magnitude of the molal osmotic coefficient and the equivalent conductance decreases sharply over a narrow concentration region. At still higher concentrations the values of these two properties approach asymptotically a constant value. According to theories of micelle formation (2, 11, 12), the behavior of the physicochemical properties of bromodiphenhydramine HCl solutions are in accord with the formation of partially ionized aggregates of approximately constant size which are in equilibrium with the simple ions.

Figures 1 and 3 indicate that the behavior of dibucaine HCl solutions is similar to those of bromodiphenhydramine HCl. The abruptness of change in the physicochemical properties at the CMC is dependent on the micellar size and the ease with which the association equilibrium is established (2). The many polar groups that are present in dibucaine HCl molecules tend to reduce the ease of molecular association. Due to this fact the change in the physicochemical properties of dibucaine HCl, at the CMC, is not as abrupt as that of bromodiphenhydramine HCl.

The behavior of the osmotic coefficient and the equivalent conductance of dilute solutions of tetracaine HCl is similar to those of procaine HCl solutions. At higher concentrations these properties decrease slowly over an extended concentration region as seen in Figs. 1 and 3. These behaviors indicate the weak aggregating tendency of the tetracaine ions which should result in slow multiple equilibria between aggregates of different sizes. Due to the same reason no CMC value could be assigned to this salt. The slight drop in the molal osmotic coefficients together with a nearly linear decrease in the equivalent conductance of diphenhydramine HCl solutions with increasing concentrations also suggests the presence of aggregates of different sizes in moderately concentrated solutions of this salt.

The molal osmotic coefficient and the equivalent conductance of aqueous pyrilamine maleate solutions decreased without any noticeable change of slope at all concentrations studied (Figs. 2 and 4). It has been previously demonstrated by Stokes (13) that maleate ions tend to form ion pairs in aqueous solutions of the sodium salt. It is, therefore, possible that at the pH of the solution pyrilamine maleate ions form ion pairs.

According to Brady and co-workers (14) the amount of gegenions bound per mole of micellar ion is given by:

$$\frac{dm^B}{dm} = 1 - 2\,\frac{d\phi m}{dm} \qquad \text{(Eq. 4)}$$

where m^B is the concentration of the bound gegenions; m is the total concentration of the solute; ϕ is the molal osmotic coefficient of the solution; and $d\phi m/dm$ is the slope of ϕm *versus* m curve at concentrations above the CMC. For dibucaine HCl, this slope is 0.115 while for bromodiphenhydramine HCl (Fig. 5) it is 0.126. Consequently, dm^B/dm for the micelles of these two salts is 0.77 and 0.75, respectively. The excessive counter ion binding indicated by these data is in the expected range. The nonionic polar groups that are present in the molecules of dibucaine HCl and bromodiphenhydramine HCl tend to decrease the aggregation properties of these salts. Consequently, if aggregation is to occur, it is to be expected that the repulsive coulombic forces which come into play when the micelle-forming ions approach each other have to be practically neutralized by the gegenions.

Assuming that micelles are monodispersed and that their formation is governed by the law of mass action, Phillips (15) has shown that the standard free energy change, $\Delta F_m°$, and the standard enthalpy change, $\Delta H_m°$, of this process are given by the following equations:

$$\Delta F_m° = RT\left(\frac{\ln 3 + 2\ln N}{N} + \frac{2N - P - 1}{N}\ln \mathrm{CMC}\right) \qquad \text{(Eq. 5)}$$

and

$$-\frac{\Delta H_m°}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) = \left(2 - \frac{P}{N} - \frac{1}{N}\right)\ln\frac{\mathrm{CMC}_1}{\mathrm{CMC}_2} \qquad \text{(Eq. 6)}$$

where R is the gas constant, N is the aggregation number, P is the charge of the micelle, and CMC_1 and CMC_2 are the critical micelle concentrations at absolute temperature T_1 and T_2. The standard state chosen is mole fraction of unity, and, therefore, the CMC's should be expressed in mole fraction units. The freezing point depression of aqueous solutions of dibucaine HCl and aqueous bromodiphenhydramine HCl have been determined by Hammarlund and co-workers (3, 16). From their data the CMC of these two salts at the freezing points of their solutions were found to be 0.060 and 0.05 M, respectively. These CMC values, when compared with those obtained at 25° indicate that in the temperature range 0–25° the CMC of dibucaine HCl and bromodiphenhydramine HCl are not appreciably affected by the increase in temperature. According to Eq. 6, therefore, the $\Delta H_m°$ of micellization for both salts is practically zero. This finding would imply that $-\Delta F_m°/T = \Delta S_m°$. Since $N > 1$ and $P > 0$ for the micelles formed by dibucaine HCl and bromodiphenhydramine HCl, it follows that their $\Delta F_m° < 0$ and $\Delta S_m° > 0$. It can, therefore, be concluded that the formation of micelles in the solutions of these two salts is practically an entropy effect. A portion of the observed entropy increase should be due to the loss of ordered water that exists around the hydrocarbon moiety of both dibucaine HCl and bromodiphenhydramine HCl ions during micellization process (17, 18). Since the solvated polar groups present in the dissolved molecules of these two salts are practically desolvated during the course of aggregation, it should be expected that this desolvation will also contribute to the total entropy change (19).

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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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