# Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don.) XII

## Preparation and Characterization of Trace Alkaloids

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**The processing of this plant (1) for the four new oncolytic agents, vincaleukoblastine, leurosine, leurosidine, and leurocristine, has resulted in obtaining eight new companion dimeric alkaloids, these latter being obtained in yields of not more than $1.5 \times 10^{-4}$ %. To date, a total of 24 new alkaloids have been reported from these laboratories. Procedural details and preliminary characterization of carosidine, carosine, pleurosine, neoleurosidine, vincarodine, catharicine, vindolidine, and neoleurocristine are described.**

As a result of the search for the active antitumor principles contained in various alkaloidal fractions from this plant, 20 crystalline alkaloids, 16 of them being new, have been obtained in these laboratories[1] (2–6). Four of these, leurosine, vincaleukoblastine (VLB),[2] leurosidine, and leurocristine have demonstrated varying degrees of oncolytic activity in experimental tumors, the latter two being capable of producing "indefinite" survivors in DBA/2 mice infected with the P-1534 leukemia, a transplanted acute lymphocytic leukemia (7, 8). Clinically, VLB and leurocristine have elicited responses in human neoplasms, the former being commercially available as the sulfate[3] for the treatment of Hodgkin's disease and choriocarcinoma

The alkaloids obtained from this plant can be categorized into three groups. The first group consists of dimeric indole-indoline alkaloids of approximate composition of $C_{46}H_{52-62}N_4O_{8-10}$. Spectral studies (9) indicate a common structural relationship to the $C_{21}H_{24}N_2O_2$ indole, catharanthine, and the $C_{25}H_{32}N_2O_6$ indoline compound, vindoline, both of which are also obtained from this plant. The second group contains a number of miscellaneous smaller alkaloids ranging in molecular weight of 325–500. This group would include catharanthine and vindoline. A number of symmetrical dimeric alkaloids, primarily dihydroindoles, comprise the third group.

The eight alkaloids reported herein represent minor constituents obtained during the preparation of the four biologically active alkaloids mentioned above, and in no case were they found in greater amounts than $1.5 \times 10^{-4}$ %. Five of these are members of the first group, while the remaining three, carosidine, vincarodine, and vindolidine, belong to the third class.

Carosidine was obtained by rechromatography of the residues remaining in the VLB sulfate mother liquors, while carosine and pleurosine were obtained by rechromatography of certain mixtures of leurosine and isoleurosine. Neoleurosidine resulted from rechromatography of impure leurosidine. The presence of this compound was suspected because of an abnormal pK'a ratio determined by electrometric titration.

Vincarodine was obtained by gradient pH extraction (6) of a fraction resulting from rechromatography of post-VLB column eluate, as were catharicine and vindolidine. Neoleurocristine was found by subjecting leurocristine sulfate mother liquors to the same type of gradient pH technique.

These new alkaloids are listed in Table I, which shows their empirical formulas, m.p.'s, pK'a's, specific rotations, and ultraviolet absorption maxima. Their infrared spectra are reproduced in Fig. 1 as an additional aid to their identification.

### DISCUSSION

**Carosidine.**—Alumina chromatography of the bases obtained from the VLB sulfate mother liquors yielded this alkaloid by elution with chloroform-benzene (3:1). The ultraviolet spectrum is that of a typical substituted dihydroindole. The infrared spectrum (mull) shows the presence of

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[1] For the sake of brevity, experimental techniques repeated from earlier work are not described in detail. A reference is given to the previous paper.

[2] In compliance with the decision of the A.M.A. Council on Drugs, the name vincaleukoblastine should be replaced by vinblastine as the generic name for this alkaloid.

[3] Supplied as Velban, vinblastine sulfate, Lilly.

TABLE I.—NEW TRACE ALKALOIDS FROM *Vinca rosea* LINN.

| Name | Formula[a] | M.P., °C. | $[\alpha]_D^{25}$ ($CHCl_3$) | pK'a in 33% DMF | U.V. $\lambda_{max.}^{EtOH}$, mμ |
|---|---|---|---|---|---|
| Carosidine | ... | 263–278, 283[b] | −89.8° | ... | 212, 254, 303 |
| Carosine | $C_{46}H_{56}N_4O_{10}$ | 214–218 | +6.0° | 4.4, 5.5 | 255, 294 |
| Pleurosine | $C_{46}H_{56}N_4O_{10}$ | 191–194[b] | +61.0° | 4.4, 5.55 | 267, 308 |
| Neoleurosidine | $C_{48}H_{62}N_4O_{11}$ | 219–225[b] | +41.6° | 5.1 | 214, 268 |
| Vincarodine | $C_{44}H_{52}N_4O_{10}$ | 253–256[b] | −197.4° | 5.8 (66%) | 230, 272, 298 |
| Catharicine | $C_{46}H_{52}N_4O_{10}$ | 231–234[b] | +34.8° | 5.3, 6.3 | 214, 268, 293, 315 |
| Vindolidine | $C_{48}H_{64}N_4O_{10}$ | 244–250[b] | −113.2° | 4.7, 5.3 | 261, 311 |
| Neoleurocristine | $C_{46}H_{56}N_4O_{12}$ | 188–196[b] | −57.87° | 4.68 | 220, 257, 298 |

[a] While these molecular formulas agree well with the analytical results for each particular alkaloid, it should be noted that they are to be considered as proximate at this time, in light of our experience with the other dimeric alkaloids (9). [b] With decomposition.

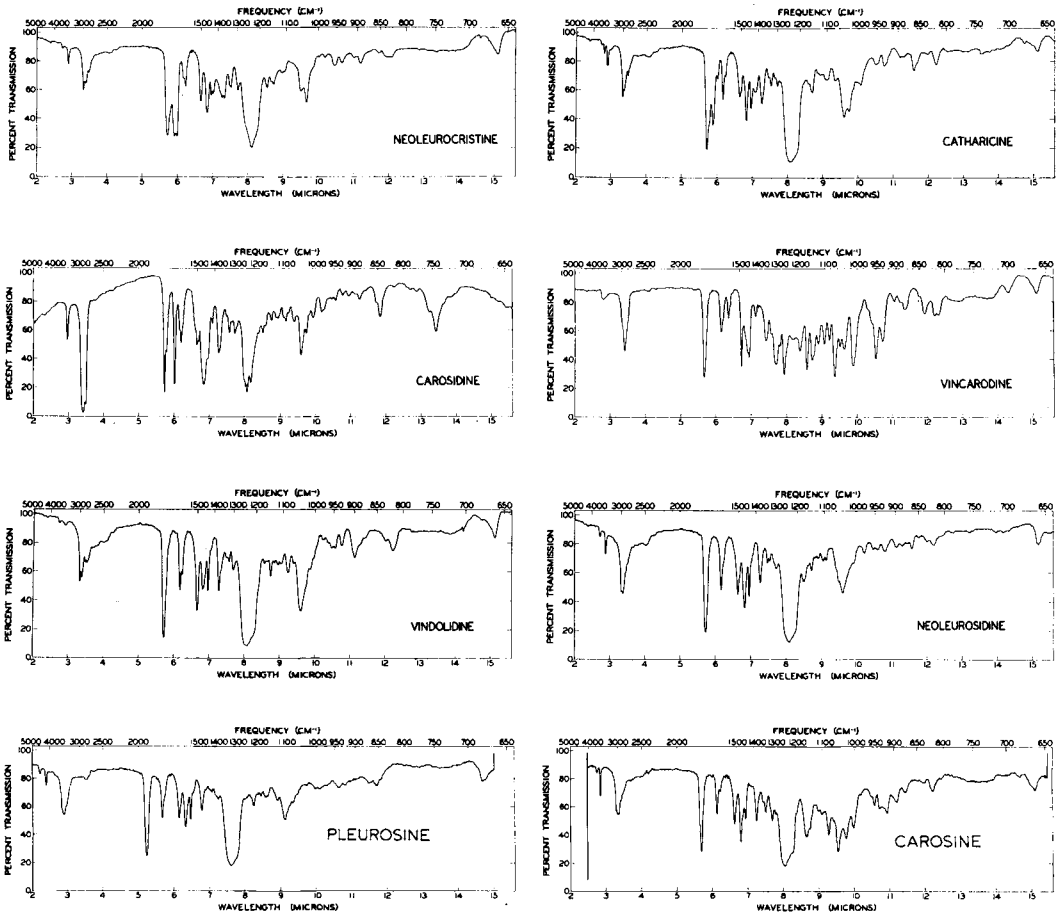


Fig. 1.—Infrared spectra of new alkaloids.

NH at 2.95 μ and carbonyl functions at 5.75 and 6.02 μ.

**Carosine and Pleurosine.**—Rechromatography of crude leurosine-isoleurosine mixtures described previously (5) yielded these two alkaloids, carosine from benzene-chloroform eluates (1:1) and pleurosine from chloroform.

The ultraviolet spectrum of carosine differs from those of the other dimeric alkaloids. Its infrared spectrum shows it to be a dimeric alkaloid containing hydroxyl, indole NH, and ester carbonyls. The NMR spectrum indicates the presence of two methyl esters, an aromatic methoxyl, an N-$CH_3$, and an O-acetyl group.

The ultraviolet spectrum of pleurosine is similar to those of the other dimeric alkaloids except that it does not shift in acid. The infrared spectrum resembles those of the other group 1 dimeric alkaloids.

**Neoleurosidine.**—The presence of this compound was suspected when an abnormal pK'a ratio was found to be associated with a particular crude

crystalline leurosidine sample. Leurosidine exhibits two pK'a groups at approximately 5.0 and 8.8, in equal mole per cent. The presence of neoleurosidine was thus indicated by an increased molar percentage of the lower pK'a. Electrometric titration of this sample, however, in 33% DMF gave the following data: 1.0 mole for pK'a 8.93 and 4.33 moles for pK'a 5.15. Rechromatography gave the new compound from the 1% methanol–99% chloroform eluate.

The ultraviolet spectrum is similar to those of the other dimeric alkaloids except that it does not shift to lower wavelength in acid as do the others. The infrared spectrum is consistent with those of the other related dimeric alkaloids of group 1 and contains the same type of hydroxyl as does leurosidine.

**Vincarodine.**—This alkaloid was obtained by chromatography of the bases from the VLB sulfate mother liquors and subjecting the benzene-chloroform (1:3) eluate to the gradient pH separation. It was found in the pH 4.40, 4.90, and 5.40 extracts.

The ultraviolet spectrum appears to be that of a substituted dihydroindole and closely resembles the alkaloid vincine from *Vinca minor* Linn. (10, 11). The infrared spectrum substantiates the relationship to vincine, showing the presence of a methyl ester and methoxyl substitution on the aromatic portion of the molecule, as well as an N-H.

**Catharicine.**—This new dimeric alkaloid was obtained by rechromatography of the chloroform-soluble post VLB eluate resulting from the commercial production of VLB sulfate (4) and by subjecting an early chloroform cut to gradient pH separation. It was found at pH 2.85 and 3.40.

Its ultraviolet spectrum is similar to those of the dimeric alkaloids which are composed of catharanthine-like and vindoline-like moieties, the slight bathochromic shift in acid, typical of the ultraviolet spectrum of leurosine, VLB, etc., is not found. The infrared spectrum is similar to that of VLB, but shows evidence of additional unsaturation which appears to be in the neighborhood of the basic nitrogen of the indole moiety (which titrates with a pK'a of 7.0 in VLB but does not in catharicine).

**Vindolidine.**—This alkaloid was obtained in the same manner as was catharicine, from the same starting material. The last two benzene-chloroform (1:3) fractions were treated with the gradient pH technique, and vindolidine was found to be present in the pH 3.90 and 4.40 extracts, the bulk of it being associated with the lower level. (Leurosidine was also obtained from these two fractions, but was found at pH levels of 5.40, 5.90, and 6.40.)

The ultraviolet spectrum is that of a typical substituted dihydroindole. The infrared spectrum supports a close structural relationship to vindolicine and vindoline and suggests the possibility that both this alkaloid and vindolicine are dimeric indoline alkaloids.

**Neoleurocristine.**—The presence of this alkaloid in the leurocristine sulfate mother liquors was demonstrated by paper chromatographic examination which indicated a less mobile material than leurocristine. Subjecting these mother liquors to gradient pH separation yielded the new alkaloid from the pH 3.40 fraction. The ultraviolet spectrum is quite similar to that of leurocristine, showing the anomalies to the other dimeric alkaloids that the latter does. The infrared spectrum contains an additional carbonyl band at 6.05$\mu$, which may be due to an amide. As with leurocristine, no N-$CH_3$ is found in the NMR spectrum (1).

## EXPERIMENTAL

**Carosidine.**—A quantity of VLB sulfate crude mother liquors was converted to the base, and 3.6 Kg. of this amorphous alkaloidal residue was chromatographed on 119 Kg. of alumina, deactivated in the usual manner (4). The column results are listed in Table II.

The crude methanol-crystallized carosidine obtained from fractions 32–35 was further purified by warming with 200 ml. of chloroform. The chloroform-insoluble material was filtered off and discarded. Concentration of the clear filtrate *in vacuo* to approximately 40 ml., followed by the addition of 400 ml. of acetone, with subsequent chilling produced 1.54 Gm. of the alkaloid in the form of blades having parallel extinction; gradual decompn. 263–278°, m.p. 283°, $[\alpha]_D^{26}$ −89.8° (c = 1, $CHCl_3$).

*Anal.*—Found: C, 67.75; H, 6.72; N, 8.22; O, 17.46.

Electrometric titration data were not available because of the insolubility of this alkaloid in the systems investigated. The solubility of this compound did not allow for calculation of extinction coefficients; $\lambda_{max.}^{EtOH}$ 212, 254, 303 m$\mu$.

**Carosine and Pleurosine.**—Chromatography of a benzene solution containing 25 Gm. of a crude leurosine-isoleurosine mixture, corresponding to fractions 10 and 11 as described in an earlier paper (5), on 1000 Gm. of alumina, deactivated with 60

TABLE II.—CHROMATOGRAPHY OF BASES FROM VLB SULFATE MOTHER LIQUORS, YIELDING CAROSIDINE

| Fraction | Eluting Solvent | Compound | Wt., Gm. | Crystallizing Solvent |
|---|---|---|---|---|
| 1 (1500 L.) | Benzene | Oil | ... | ... |
| 2–16 (3000 L.) | Benzene-chloroform (3:1) | Leurosine | 3.8 | Methanol |
| 17–31 (3000 L.) | Benzene-chloroform (1:1) | Leurosine | 43.3 | Methanol |
| | | VLB (as sulfate) | 50.4 | Ethanol |
| 32–35 (600 L.) | Benzene-chloroform (1:3) | Carosidine | 2.16 | Methanol |
| 36–41 (1500 L.) | Benzene-chloroform (1:3) | Amorphous residues | ... | ... |
| 42–48 (1500 L.) | Chloroform | Amorphous residues | ... | ... |

TABLE III.—CHROMATOGRAPHY OF CRUDE LEUROSINE-ISOLEUROSINE MIXTURES YIELDING CAROSINE AND PLEUROSINE

| Fraction | Eluting Solvent | Compound | Wt., Gm. | Crystallizing Solvent |
|---|---|---|---|---|
| 1 (1 L.) | Benzene | Oil | 0.95 | ... |
| 2–12 (6 L.) | Benzene | Isoleurosine | 0.60 | Methanol |
| 13–14 (1.2 L.) | Benzene-chloroform (3:1) | Amorphous residues | 0.35 | ... |
| 15–21 (7 L.) | Benzene-chloroform (3:1) | Leurosine | 3.5 | Methanol |
| 22–28 (12 L.) | Benzene-chloroform (3:1) | Amorphous residues | 1.75 | ... |
| 29–41 (20 L.) | Benzene-chloroform (1:1) | Amorphous residues | 3.5 | ... |
| 42–45 (8 L.) | Benzene-chloroform (1:1) | Carosine | 0.300 | Methanol |
| 46–48 (6 L.) | Benzene-chloroform (1:1) | Amorphous residues | 0.12 | ... |
| 49–55 (14 L.) | Chloroform | Pleurosine | 0.225 | Methanol |

TABLE IV.— CHROMATOGRAPHY OF IMPURE LEUROSIDINE, YIELDING NEOLEUROSIDINE

| Fraction | Eluting Solvent | Compound | Wt., Gm. | Crystallizing Solvent |
|---|---|---|---|---|
| 1 (2400 ml.) | Benzene | Oil | 0.01 | ... |
| 2 (2400 ml.) | Benzene-chloroform (3:1) | Oil | 0.01 | ... |
| 3–21 (2600 ml.) | Benzene-chloroform (1:1) | Amorphous residues | 0.91 | ... |
| 22–26 (500 ml.) | Benzene-chloroform (1:3) | Amorphous residues | 0.42 | ... |
| 27–46 (2000 ml.) | Benzene-chloroform (1:3) | Leurosidine | 0.5875 | Methanol |
| 47–73 (3880 ml.) | Chloroform | Leurosidine | 0.5265 | Methanol |
| 74 (500 ml.) | Chloroform-methanol (99:1) | Amorphous residue | 0.04 | ... |
| 75–86 (1410 ml.) | Chloroform-methanol (99:1) | Neoleurosidine | 1.2725 | Methanol |
| 87–90 (360 ml.) | Chloroform-methanol (19:1) | Neoleurosidine | 0.007 | Methanol |
| 91–101 (1280 ml.) | Chloroform-methanol (19:1) | Amorphous residues | 0.40 | ... |
| 102 (1000 ml.) | Methanol | Amorphous residues | 0.33 | ... |

ml. of 5% acetic acid gave the results as listed in Table III.

**Carosine.**—Recrystallization of the crude crystalline base from methylene chloride-ether produced 0.240 Gm. of the base as needles with parallel extinction; m.p. 214–218°; $[\alpha]_D^{26}$ +6.0° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{46}H_{56}N_4O_{10}$: C, 66.97; H, 6.84; N, 6.79; O, 19.39. Found: C, 66.71, 66.71; H, 7.02, 6.88; N, 6.52; O, 19.61.

pK'a 4.4, 5.5, electrometric titration, 33% DMF; mol. wt., 934; $\lambda_{max.}^{EtOH}$ 255 mμ [log $E$(1%, 1 cm.) 2.21], 294 mμ [log $E$(1%, 1 cm.) 2.19].[4]

**Pleurosine.**—Recrystallization of the base from methanol yielded 0.175 Gm. of long blades having parallel extinction; m.p. 191–194° (decompn.); $[\alpha]_D^{26}$ −61.0° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{46}H_{56}N_4O_{10}$: C, 66.97; H, 6.84; N, 6.79; O, 19.39. Found: C, 67.04; H, 7.08; N, 6.49; O, 19.44.

pK'a 4.4, 5.55, electrometric titration, 33% DMF; mol. wt., 908; $\lambda_{max.}^{EtOH}$ 267 mμ [log $E$(1%, 1 cm.) 2.32], 308 mμ [log $E$(1%, 1 cm.) 1.87].

**Neoleurosidine.**—A benzene solution of 8.5 Gm. of impure leurosidine, exhibiting the abnormal pK'a ratio described above, was chromatographed on 343 Gm. of alumina (Alcoa, Grade F-20) deactivated with 11.5 ml. of 10% acetic acid in the usual manner (see Table IV).

The crude methanol-crystallized neoleurosidine from fractions 75–90 were combined and were recrystallized from methanol, yielding 1.017 Gm. of pure alkaloid in the form of rods with inclined extinction; m.p. 219–225° (decompn.); $[\alpha]_D^{26}$ + 41.6° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{48}H_{62}N_4O_{11}$: C, 66.18; H, 7.17; N, 6.43; O, 20.20. Found: C, 66.20; H, 7.26; N, 6.49; O, 20.14; $OCH_3$, 13.4; OAC, 8.65; (C)-$CH_3$, 4.21.

pK'a 5.1, electrometric titration, 33% DMF; mol. wt., 452 × 2 = 904; $\lambda_{max.}^{EtOH}$ 214 mμ [log $E$(1%, 1 cm.) 2.77], 268 mμ [log $E$(1%, 1 cm.) 2.29]; shoulders at 285 mμ [log $E$(1%, 1 cm.) 2.19], 295 mμ [log $E$ (1%, 1 cm.) 2.12], 310 mμ [log $E$(1%, 1 cm.) 1.83].

**Vincarodine.**—The gradient pH technique (6) used to prepare this alkaloid from the benzene-chloroform (1:3) eluate of columned bases from the VLB sulfate mother liquors is as follows: a total of 182 Gm. of amorphous base was dissolved in 7 L. of benzene and filtered. The clear solution was mixed with 9 L. of 0.1 *M* citric acid and the mixture was distilled *in vacuo* to remove the benzene. The aqueous phase was filtered and subsequently extracted with one 9-L. portion of benzene at each of the following pH levels: 3.2, 3.9, 4.4, 4.9, 5.4, 5.9, and 8.0, the pH being raised after each extraction by the addition of 28% ammonia.

The crude cuts from pH 4.4, 4.9, and 5.4 (40.4 Gm.) crystallized from methanol, yielding 3.0 Gm. of crude crystalline vincarodine. Recrystallization from chloroform-methanol yielded 2.55 Gm. of base as blades having parallel extinction; m.p. 253–256° (decompn.); $[\alpha]_D^{26}$ −197.4° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{44}H_{52}N_4O_{10}$: C, 66.31; H, 6.58; N, 7.03; O, 20.08. Found: C, 66.56; H, 6.62; N, 7.20; O, 20.12.

pK'a 5.8, electrometric titration, 66% DMF; mol. wt., 400 if monomeric, 800 if dimeric. The molecular weight by vapor pressure lowering indicated that the dimeric formulation was correct.

[4] Because of the high degree of solvation of these molecules, the samples taken for pK'a determination and ultraviolet spectral measurements at times did not give the same molecular weight as those specially dried for analysis. Therefore, we report the ultraviolet absorption intensities as log $E_{1\%}$ rather than as log $\epsilon_M$.

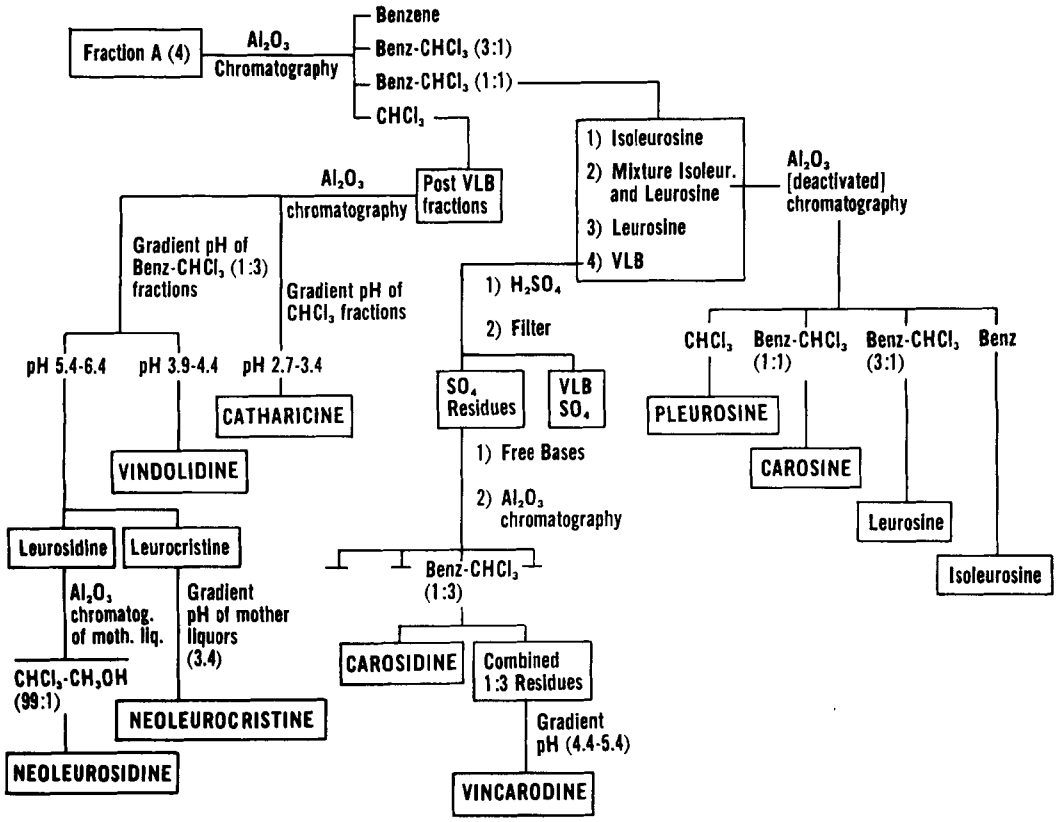


Fig. 2.—Flow diagram.

$\lambda_{max.}^{EtOH}$ 230 mμ [log $E$(1%, 1 cm.) 2.82], 272 mμ [log $E$(1%, 1 cm.) 2.30], 298 mμ [log $E$(1%, 1 cm.) 2.04].

**Catharicine.**—Rechromatography of the chloroform-soluble post-VLB eluate from fraction 1 and subjecting 256.55 Gm. of an early chloroform fraction, corresponding to fraction 47 as described in (6), to the gradient pH technique previously recorded, yielded this dimeric indoline alkaloid from pH cuts 2.70 and 3.40. The amorphous residues from each cut readily crystallized from acetone, yielding 1.1805 and 4.535 Gm. of crude crystalline base, respectively. Recrystallization from acetone, or from any of the commonly-used solvents, was ineffectual, always producing a gel. It was necessary to rechromatograph the base to obtain a pure entity.

The pure base crystallizes from acetone as irregular plates; m.p. 231–234° (decompn.), $[\alpha]_D^{26}$ + 34.8° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{46}H_{52}N_4O_{10}$: C, 66.97; H 6.84; N, 6.79; O, 19.39. Found: C, 66.20; H, 6.70; N, 6.55; O, 20.00.

pK'a 5.3, 6.3, electrometric titration, 33% DMF; mol. wt., 894; $\lambda_{max.}^{EtOH}$ 214 mμ [log $E$(1%, 1 cm.) 2.81], 268 mμ [log $E$(1%, 1 cm.) 2.29], 293 mμ [log $E$(1%, 1 cm.) 2.12], 315 mμ [log $E$(1%, 1 cm.) 1.74]; shoulder at 283 mμ [log $E$(1%, 1 cm.) 2.23].

**Vindolidine.**—Rechromatography of the chloroform-soluble post-VLB eluate from fraction 1 and subjecting 69.25 Gm. of the late chloroform-benzene (3:1) fractions, corresponding to fractions 43 and 44 as described in (6) to gradient pH separation gave 0.642 Gm. of crude crystalline vindolidine from pH cut 3.92 and 0.037 Gm. from pH cut 4.42. Recrystallization from methanol yielded microcrystals; m.p. 244–250° (decompn.); $[\alpha]_D^{26}$ −113.2° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{48}H_{64}N_4O_{10}$: C, 64.84; H, 7.26; N, 6.30; O, 21.60. Found: C, 64.90, H, 7.22; N, 6.56; O, 21.56; $OCH_3$, 17.45; (N)$CH_3$, 3.38; (C)$CH_3$, 4.94; OAC, 4.52.

pK'a 4.7, 5.3, electrometric titration, 33% DMF; mol. wt., 880; $\lambda_{max.}^{EtOH}$ 261 mμ [log $E$(1%, 1 cm.) 2.33], 311 mμ [log $E$(1%, 1 cm.) 2.12].

**Neoleurocristine.**—Subjecting 4.786 Gm. of leurocristine sulfate crude mother liquors to gradient pH separation in the usual manner resulted in obtaining 0.181 Gm. of this new alkaloid from the 0.40 Gm. of crude amorphous residue in the pH 3.40 cut by crystallization from methanol, from which it crystallizes as irregular plates; m.p. 188–196° (decompn.); $[\alpha]_D^{26}$ −57.87° (c = 1, $CHCl_3$).

*Anal.*—Calcd. for $C_{46}H_{56}N_4O_{12}$: C, 64.47; H, 6.59; N, 6.54; O, 22.41. Found: C, 64.62, 64.82; H, 6.55, 6.72; N, 6.57, 6.34; O, 22.05; $OCH_3$, 10.36; (C)$CH_3$, 4.50; OAc, 4.42.

pK'a 4.68, electrometric titration, 33% DMF; mol. wt., 827; $\lambda_{max.}^{EtOH}$ 220 mμ [log $E$(1%, 1 cm.) 2.74], 257 mμ [log $E$(1%, 1 cm.) 2.23], 298 mμ [log $E$(1%, 1 cm.) 2.29]; shoulders at 264 mμ [log $E$(1%, 1 cm.) 2.23], 290 mμ [log $E$(1%, 1 cm.) 2.24].

A flow diagram illustrating the manner in which these alkaloids were obtained is shown in Fig. 2.

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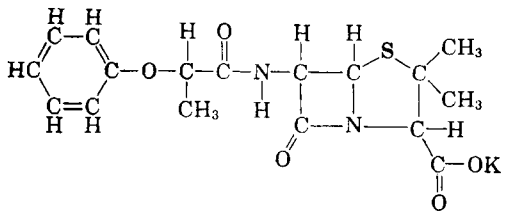
# Stability of Potassium Phenethicillin I

## Kinetics of Degradation in Aqueous Solution

By M. A. SCHWARTZ, A. P. GRANATEK, and F. H. BUCKWALTER

**Kinetic studies of the degradation of potassium phenethicillin in aqueous solution have been carried out and presented with a view toward stability prediction. Phenethicillin, an acid with pKa 2.9, is hydrolyzed by both acid and base with minimum rate of degradation at about pH 6.5 at 35°. In acid solution the ionic form of phenethicillin is hydrolyzed about 13 times as fast as the free acid. The $HPO_4^=$ ion was found to be a general base catalyst. The effect of temperature in the neutral pH region has been determined, and from this data the aqueous solution stability of potassium phenethicillin at 25 and 4° has been predicted.**

Potassium phenethicillin[1] is the first commercially produced semisynthetic penicillin.



Its chemical, microbiological and pharmacological properties have been described (1–3). Clinical studies have also been reported (4, 5). The present work was undertaken to enable the prediction of the optimum stability formulating conditions for potassium phenethicillin in aqueous solution.

## EXPERIMENTAL

One lot of commercially produced potassium phenethicillin containing approximately 60% of the L-form was used throughout this study. All buffer materials were reagent grade.

The kinetic studies were carried out as follows: 90 ml. of buffer (pH range given in Table I) in a 100-ml. volumetric flask was brought to bath temperature and then 1.0 ml. of a freshly prepared 0.1 *M* aqueous solution of potassium phenethicillin was added with mixing. At appropriate intervals, 2.0-ml. samples of the reaction mixture were taken and added to 8.0 ml. of cold 0.5 *M* pH 6 citrate-phosphate buffer to quench the reaction. These solutions were immediately frozen and kept in this state until just before assay. The phenethicillin potency of these samples was determined by the microbiological turbidimetric assay method with *Staph. aureus* strain FDA209-P as the inoculum.

In order to determine the effect of freezing the quenched samples until assay, a solution of 80 mcg./ml. of potassium phenethicillin in the strong pH 6 buffer was divided into 10-ml. samples and frozen. One sample was assayed each day for 13 days. No significant loss of potency was observed despite a day-to-day variability of 14.5%. To avoid this, all samples from a single kinetic run were assayed on the same day. The maximum time these were kept frozen was 6 days.

The ionic strength of all the buffers was adjusted to 0.5 with potassium chloride. The pH was determined with a Beckman Zeromatic pH meter using glass and calomel electrodes.

The pKa of phenethicillin was determined in 0.5 *M* potassium chloride by taking the pH of a half-neutralized 0.01 *M* solution.

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[1] The trade name of Bristol Laboratories, a division of Bristol-Myers Co., for α-phenoxyethyl penicillin is Syncillin. The generic name is phenethicillin or penicillin 152.