of the collection intervals over which the rates were calculated. Such excretion rate-time plots are much more sensitive and show curvature not nearly as evident on a cumulative amount excreted *versus* time plot.

Figure 3 shows perfect rank-order correlation of per cent of theoretical carboxytolbutamide excreted in the urine in 48 hours with surface area of tolbutamide in the dosage form.

Figure 4 shows a perfect rank-order correlation of average maximum excretion rate of carboxytolbutamide in the urine with surface area of tolbutamide in the dosage form.

These results show that in normal human subjects available surface area of tolbutamide in the dosage form can have a pronounced effect on extent and rate of excretion of the metabolite, carboxytolbutamide, in the urine. Indirectly, they show that available surface area of tolbutamide in the dosage form can influence the rate and extent of absorption of tolbutamide if the surface area is restricted in the range studied.

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

Vinaspine, Vincathicine, Rovidine, Desacetyl VLB, and Vinaphamine

By GORDON H. SVOBODA and ALBERT J. BARNES, JR.

The continued phytochemical investigation of this pantropical plant has resulted in obtaining vinaspine and vinaphamine as free bases and vincathicine, rovidine, and desacetyl VLB as the sulfates. The total number of alkaloids obtained from this plant, utilizing selective extraction, column chromatography, and gradient pH techniques, is now 49.

This investigation was pursued in an effort to elucidate the alkaloid composition of this pantropical plant as completely as possible (1). Occasionally, there is a spillover of leurocristine[1] (and leurosidine) into the B fraction. This investigation centers on the chloroform eluate of so-called post-leurocristine B fractions.

Rechromatography of the benzene-soluble material from the above fractions on deactivated alumina yielded only the known alkaloid perivine directly. Application of the gradient pH technique to each individual fraction yielded the new alkaloid vinaspine only as the base; while vincathicine, rovidine, and desacetyl VLB were obtained only as sulfates. A new alkaloid, vinaphamine, was obtained from crude amorphous leurosidine-containing material from the B fraction.

These new alkaloids are listed in Table I,

Table I.—New Alkaloids from *Vinca rosea* Linn.

Name	M. p., °C.	pK'a in 33% DMF	U.V. $\lambda_{max.}^{EtOH}$ mμ
Vinaspine	235–238	7.85	225, 281, 289
Vincathicine (sulfate)	>320 dec.	5.10, 7.05	231, 264, 300
Rovidine (sulfate)	>320 dec.	4.82, 6.95	214, 265, 286
Desacetyl VLB (sulfate)	>320 dec.	5.40, 6.90	214, 266, 294
Vinaphamine	229–235	5.15, 7.0	214, 262, 292

along with certain pertinent physical data. Their infrared spectra are reproduced separately (Figs. 1–4) as additional aids to their identification.

EXPERIMENTAL AND DISCUSSION[2,3]

Rechromatography of 3.659 Kg. of the chloroform eluate of post-leurocristine material from the B fraction from 90% leaf in benzene on 120 Kg. of deactivated alumina yielded 29.580 Gm. of perivine

Received February 25, 1964, from the Chemical Research and Organic Chemical Development Divisions, Eli Lilly and Co., Indianapolis, Ind.
Accepted for publication March 6, 1964.
The previous paper in this series was presented by Drs. M. Gorman and J. Sweeny to the Third International Meeting on Chemistry of Natural Products, Kyoto, Japan, April 12–18, 1964.
The authors thank the following persons for their aid during this investigation: Drs. M. Gorman, H. E. Boaz, and R. R. Pfeiffer, Messrs. L. G. Howard, P. Landis, D. O. Woolf, Jr., L. A. Spangle, and L. Huckstep, Misses M. L. Hofmann and A. Sheats, and Mrs. N. Cone and Mrs. D. Stephens for physical data; Messrs. W. L. Brown, H. L. Hunter, G. M. Maciak, D. Cline, and A. Brown for microanalysis; and Messrs. A. T. Oliver, D. R. Bedwell, H. Martlage, G. Johnson, R. J. Armstrong, and M. Yager for laboratory assistance.

[1] The A.M.A. Council on Drugs has approved vinblastine, vinleurosine, vincristine, and vinrosidine as generic names for the four oncolytic alkaloids vincaleukoblastine (VLB), leurosine, leurocristine, and leurosidine, respectively. VLB is marketed as Velban (vinblastine sulfate), and leurocristine is marketed as Oncovin (vincristine sulfate) by Eli Lilly and Co., Indianapolis, Ind.

[2] For the sake of brevity, experimental techniques repeated from earlier work (2, 3) are not described.

[3] Melting points were determined on a Kofler microstage. Ultraviolet absorption spectra were obtained using a Cary model 14 spectrophotometer; infrared spectra with a Perkin-Elmer model 21 double beam recording infrared spectrophotometer; NMR spectra with a Varian Associates 60-megacycle spectrometer. A standard Norelco powder camera, 114.6 mm. in diameter, was used in the X-ray examination.

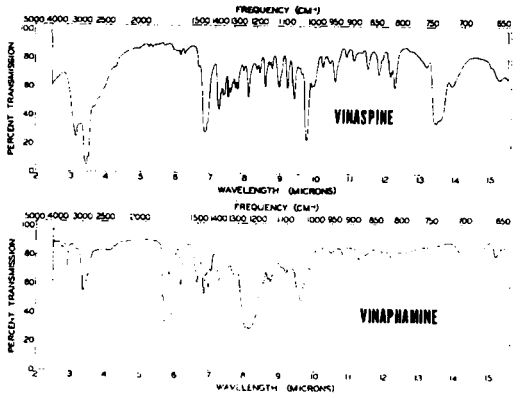

Fig. 1.—Infrared spectra of vinaspine and vinaphamine.

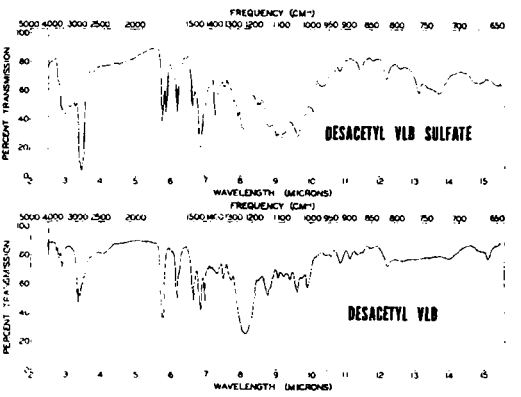

Fig. 3.—Infrared spectra of desacetyl VLB sulfate and desacetyl VLB.

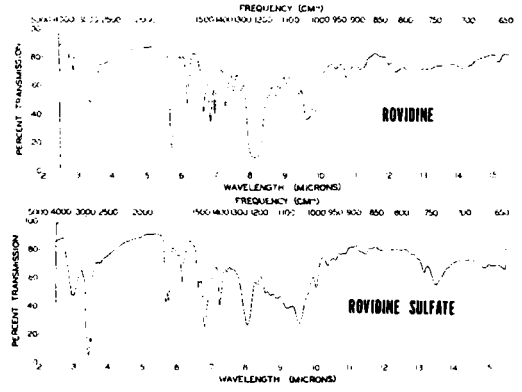

Fig. 2.—Infrared spectra of rovidine and rovidine sulfate.

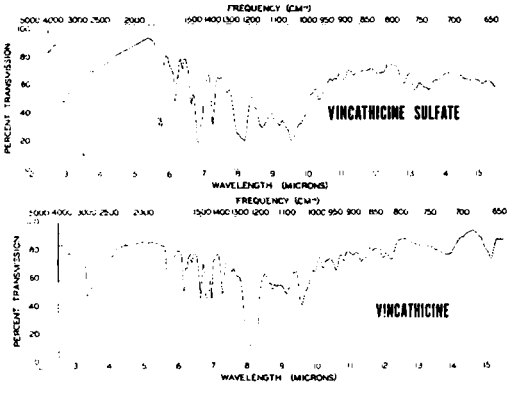

Fig. 4.—Infrared spectra of vincathicine sulfate and vincathicine.

TABLE II.—CHROMATOGRAPHY OF POST-LEUROCRISTINE FRACTIONS

Fraction[a]	Eluting Solvent	Wt., Gm.	Compd.	Wt., Gm.	Crystallizing Solvent
1	Benzene	67.9	...	...	...
2–5	Benzene–chloroform (3:1)	112.5	...	...	...
6	Benzene–chloroform (1:1)	38.8	Perivine	14.505	Methanol, acetone
7	Benzene–chloroform (1:1)	45.4	Perivine	15.075	Methanol, acetone
8–13	Benzene–chloroform (1:1)	370.3	...	...	...
14–21	Benzene–chloroform (1:3)	851.4	...	...	...
22–27	Chloroform	640.0	...	...	...
28–30	Chloroform–methanol (19:1)	387.4	...	...	...

[a] Fraction 1 was 1440 L. in volume; all others were 360 L.

(4). Attempts to crystallize directly other alkaloids from the fractions were unsuccessful. Data are given in Table II.

Utilization of the gradient pH technique with 20-Gm. aliquots of the fractions listed in Table II yielded 0.085 Gm. of vinaspine along with 1.3265 Gm. of leurosine (4), 0.0265 Gm. of mitraphylline (5), 4.267 Gm. of perivine (4), and 1.132 Gm. of lochnerine (4). Residues from the pH levels were subjected to sulfate formation and yielded 7.08 Gm. of vincathicine sulfate in addition to indicating the presence of two new sulfates which were eventually determined as being rovidine and desacetyl VLB sulfates. The following known sulfates were also obtained: 0.373 Gm. of catharanthine (4), 0.420 Gm. of cathindine (6), 0.528 Gm. of perosine (6), 0.261 Gm. of perivine (4), 0.175 Gm. of sitsirikine[4] (4), 0.146 Gm. of VLB (4), and 1.012 Gm. of cavincidine (6). Complete gradient pH data are given in Table III.

Vinaspine.—The base crystallizes from methanol as blades which show parallel extinction under polarized light, m.p. 235–238°. pK'a 7.85, electrometric titration, 33% DMF. The ultraviolet spectrum is that of a simple 2,3-disubstituted indole; $\lambda_{max.}^{EtOH}$ 225 mμ (log $E_{1\ cm.}^{1\%}$ 3.07), 281 mμ (log $E_{1\ cm.}^{1\%}$ 2.37), 289 mμ (log $E_{1\ cm.}^{1\%}$ 2.29).

The infrared spectrum of a Nujol mull confirms the ultraviolet data and in addition shows no carbonyl absorption color.

While thin-layer chromatographic examination on silica in the system ethyl acetate–absolute ethanol

[4] Sitsirikine sulfate is usually obtained as a co-crystallizing mixture of sitsirikine and its dihydro derivative (7).

TABLE III.—WEIGHTS OF GRADIENT pH FRACTIONS AND YIELDS OF ALKALOIDS AND SULFATES

Fraction and Wt., Gm.	Acid Insol.	pH[b] 2.90	3.40	3.90	4.40	4.90	5.40	5.90	6.40	7.50	7.50 (CHCl3)	8.50 (CHCl3)
1 (20.0)	8.21	1.25	0.58	0.59 0.130 C·SO4	0.76	0.56	0.67	0.81	0.71	0.86	0.93	...
2 (20.0)	7.86	1.37	0.68	0.80	0.99 0.243 C·SO4	0.86	0.80	0.86	0.73	1.03	1.12	...
3 (20.0)	6.20	2.54	2.21	1.07	1.02	0.89	1.01	0.97	0.63	0.70	0.89	...
4 (17.98)	6.71	2.07	1.43	1.18	...	1.73	1.48	1.09	0.64	0.53	0.53	...
5 (15.3)	4.08	2.05	1.18	0.85	0.86	0.85	0.96	1.02 0.115P 0.026 PS·SO4	1.07 0.483P	1.71 1.121P	0.94 0.118P 0.055 CI·SO4	...
6 c.m.l.[a] (20.0)	4.69	2.46	0.82	0.95	1.48 0.118L	1.75 0.212L 0.031 VLB·SO4	1.55 0.047 VLB·SO4	1.47	1.28 0.099 P·SO4	2.27 0.830P	1.82 0.244P 0.021 PS·SO4	0.44
7 c.m.l.[a] (20.0)	3.47	2.09	0.66	0.86 0.0385L	1.19 0.235L	1.71 0.416L 0.019 VLB·SO4	1.84 0.084L	1.67 0.049 VLB·SO4	1.39 0.050 PS·SO4	2.48 0.689P 0.0175 S·SO4	2.52 0.431 PS·SO4	0.67 0.085 VA
8 (20.0)	0.83	1.16	0.48	0.47 0.020L	0.79 0.100L	1.09 0.063L	1.01	1.15	1.10	2.19 0.667P	2.36	0.54 0.104LN
9 (20.0)	1.20	1.35	0.45	0.49	0.85 0.040L	1.48 0.011M	1.47 0.0155M	1.98	2.05	3.32	4.28	0.57 0.267LN
10 (20.0)	0.94	1.12	0.45	0.46	0.83	1.34	1.36	2.06	2.52	3.04	4.73 0.761LN	0.65
11 (20.0)	0.83	1.01	0.43	0.60	1.08	...	2.63	2.27 0.162 P·SO4	2.56 0.283 CI·SO4	2.59	3.84	0.43
12 (20.0)	1.17	1.23	0.53	0.69	0.97	1.15	1.61	1.72	3.16	2.65 0.058 CI·SO4	4.47 0.024 CI·SO4	0.39
13 (15.01)	0.83	0.60	0.52	0.50	0.66	0.86	1.37	2.08	1.76	1.72 0.277 CCD·SO4	2.95 0.391 CCD·SO4	0.40 0.037 CCD·SO4
14 (20.0)	0.92	1.08	0.93	0.89	1.15	1.64	2.51	3.41	2.43	1.90 0.307 CCD·SO4	2.78	0.34
15 (20.0)	0.69	1.04	0.78	0.92	1.08	1.59	2.92	3.74	3.08	2.07	1.85	0.18
16 (20.0)	...	...	...	...	...	3.53	2.74	5.32 0.18 VC·SO4	4.66 0.34 VC·SO4	...	...	3.23

continued overleaf

TABLE III.—*Continued*

Fraction and Wt., Gm.	Acid Insol.	pH[b] 2.90	3.40	3.90	4.40	4.90	5.40	5.90	6.40	7.50	7.50 ($CHCl_3$)	8.50 ($CHCl_3$)
17 (20.0)	...	...	...	...	2.23	1.54 0.02 Mixt. of RV·SO_4, DA-VLB·SO_4, and LC·SO_4	3.34 0.05 Mixt. of RV·SO_4, DA-VLB·SO_4, and LC·SO_4 0.06 VC·SO_4	5.07 1.12 VC·SO_4	3.21 0.51 VC·SO_4	...	...	2.32
18 (20.0)	...	...	...	...	...	3.62	3.99 0.25 VC·SO_4	5.85 1.38 VC·SO_4	3.32 0.25 VC·SO_4	...	...	2.45
19 (20.0)	...	...	...	...	...	3.63	4.88 0.10 VC·SO_4	5.23 0.95 VC·SO_4	3.50 0.17 VC·SO_4	...	...	2.26
20 (20.0)	...	...	...	...	...	3.29	3.72	5.69 0.66 VC·SO_4	3.56	...	...	1.74
21 (20.0)	...	...	...	...	...	2.90	5.18	5.44 0.90 VC·SO_4	3.68	...	...	2.41
22 (20.0)	...	...	...	...	...	3.81	4.63	4.92 0.21 VC·SO_4	3.61	...	...	2.69
23 (20.0)	0.39	0.28	0.28	0.30	0.44	1.13	3.90	4.98	2.87	2.21	0.92	...
24 (20.0)	0.31	0.29	0.17	0.25	0.42	1.07	4.19	5.73	2.85	2.09	0.75	...
25 (20.0)	0.51	0.32	0.33	0.39	0.54	1.04	3.05	4.26	3.11	2.31	1.27	...
26 (20.0)	0.53	0.29	0.35	0.44	0.58	1.17	2.83	3.38	3.16	2.83	1.52	...
27 (20.0)	0.65	0.32	0.45	0.35	0.66	1.13	2.58	3.69	3.53	3.15	2.15	...
28 (20.0)	0.87	0.48	0.23	0.26	0.40	0.65	0.94	1.67	2.39	3.63	3.64	0.34
29 (20.0)	1.19	0.34	0.29	0.28	0.38	0.58	0.79	1.64	2.03	3.05	4.00	0.42
30 (20.0)	4.51	...	0.03	0.55	0.28	0.51	1.12	2.07	2.20	2.42	2.86	0.40

[a] c.m.l., crude mother liquor. [b] C·SO_4, catharanthine sulfate; P, perivine; PS·SO_4, perosine sulfate; CI·SO_4, cathindine sulfate; L, leurosine; VLB·SO_4, vincaleukoblastine sulfate; P·SO_4, perivine sulfate; S·SO_4, sitsirikine sulfate; VA, vinaspine; M, mitraphylline; LN, lochnerine; CCD·SO_4, cavincidine sulfate; VC·SO_4, vincathicine sulfate; RV·SO_4, rovidine sulfate; DA-VLB·SO_4, desacetyl VLB sulfate; LC·SO_4, leurocristine sulfate.

(1:1) showed vinaspine to have an R_f identical to that of lochnerine, spraying with ceric ammonium phosphate solution (8) produced a persistent gray-blue *versus* a pinkish-gray color.

No derivatives were prepared.

Vincathicine.—The sulfate crystallizes from ethanol as small blades, m.p. > 320° dec. The base could not be crystallized. pK'a 5.10, 7.05, electrometric titration, 33% DMF. The ultraviolet spectrum suggests similarities to the other dimeric alkaloids. $\lambda_{max.}^{EtOH}$ 213 mμ (log $E_{1\ cm.}^{1\%}$ 2.64), 264 mμ (log $E_{1\ cm.}^{1\%}$ 2.24), 300 mμ (log $E_{1\ cm.}^{1\%}$ 1.85).

The infrared spectrum of the base in chloroform shows it to be a dimeric alkaloid related to the other known dimerics.

The NMR spectrum shows it to be a dimeric alkaloid of the indole-vindoline type. The acetyl, methoxyl, and (*N*) methyl functions are still intact, while the indole is shown to have been oxidized to an oxindole.

Anal.—Found: C, 61.19; H, 6.68: N, 5.94; O, 22.52; S, 3.74.

The base, dissolved in isopropanol, readily forms a crystalline methiodide with excess methyl iodide. Recrystallization from isopropanol yielded a sample of small spheres, m.p. > 320° dec.; its hydrochloride crystallizes readily from ethanol-ether as irregular, submicroscopic crystals, m.p. > 320° dec.

Rovidine.—While the presence of this alkaloid was indicated in certain crude crystalline sulfates (shown in Table III), workable amounts were not obtainable therefrom. This entity subsequently was found to occasionally accompany crude crystalline leurosidine and leurocristine sulfate at pH levels 5.40 and 5.90. Rechromatography and sulfate formation in the usual manner yielded the pure compound. The base could not be crystallized.

The sulfate crystallizes from ethanol as very thin blades, m.p. > 320°. pK'a 4.82, 6.95, electrometric titration, 33% DMF. The ultraviolet spectrum is that of a dimeric alkaloid of the VLB sulfate type; $\lambda_{max.}^{EtOH}$ 214 mμ (log $E_{1\ cm.}^{1\%}$ 2.71), 265 mμ (log $E_{1\ cm.}^{1\%}$ 2.18), 296 mμ (log $E_{1\ cm.}^{1\%}$ 2.05); inflections at 286, 310 mμ.

The infrared spectrum of a chloroform solution of the base also shows it to be closely related to VLB, appearing to contain all of the same functional groups.

The NMR data confirm the I.R. observations.

No derivatives were made.

Anal.—Found: C, 60.76; H, 6.74; N, 5.89; O, 22.06; S, 3.53.

Desacetyl VLB.—As with rovidine, the presence of this compound was indicated in Table III. Rechromatography of leurocristine sulfate crude mother liquors eventually yielded workable amounts of the pure compound.

The sulfate crystallizes from ethanol as blades, m.p. > 320° dec. The free base crystallizes from aqueous ethanol as blades with parallel extinction, m.p. 205–210°. pK'a 5.40, 6.90, electrometric titration, 33% DMF. The ultraviolet spectrum is that of a dimeric indole-indoline alkaloid and is similar to that of VLB sulfate; $\lambda_{max.}^{EtOH}$ 214 mμ (log $E_{1\ cm.}^{1\%}$ 2.76), 266 mμ (log $E_{1\ cm.}^{1\%}$ 2.27), 294 mμ (log $E_{1\ cm.}^{1\%}$ 2.10); inflections at 286, 310 mμ.

The infrared spectrum of a chloroform solution of the base shows it to be closely related to VLB, displaying a less intense carbonyl function. Both the I.R. and NMR spectra were identical to authentic desacetyl VLB (9).

Anal.—Found: C, 59.26; H, 6.68; N, 8.57; O, 21.63; S, 3.71.

Vinaphamine.—While this alkaloid was not encountered during the investigation of the fractions as originally defined, it was associated with crude amorphous leurosidine obtained occasionally from the B fraction at pH levels 5.40, 5.90, and 6.40. Prudent selection of fractions which contained vinaphamine as a major component and leurosidine as a minor component (based on thin-layer monitoring) and crystallization from methanol yielded pure vinaphamine. The base crystallizes from methanol as thin blades with inclined extinction, m.p. 229–235°. pK'a 5.15, 7.0, electrometric titration, 33% DMF. The ultraviolet spectrum suggests a dimeric indole-indoline chromophore; $\lambda_{max.}^{EtOH}$ 214 mμ (log $E_{1\ cm.}^{1\%}$ 2.78), 262 mμ (log $E_{1\ cm.}^{1\%}$ 2.24), 292 mμ (log $E_{1\ cm.}^{1\%}$ 2.08); shoulders at 224, 284, 300 mμ.

The infrared spectrum of a chloroform solution resembles that of a VLB type of dimer, exhibiting an extra carbonyl function at 5.95 μ.

The NMR spectrum resembles that of the other dimeric alkaloids.

No derivatives were made.

Anal.—Found: C, 67.54; H, 6.77; N, 8.70; O, 17.22.

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