CATHARANTHUS ALKALOIDS. XXXV. ISOLATION OF LEUROSIDINE N'$_b$-OXIDE FROM *CATHARANTHUS ROSEUS*[1]

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Clinically and commercially, the most important bisindole alkaloids are the antitumor agents vincaleukoblastine (VLB) and leurocristine (VCR) isolated from the leaves of the Madagascan periwinkle *Catharanthus roseus* (L.) G. Don (2, 3). More alkaloids (over 90) have been isolated from *C. roseus* than any other plant, and it has been the prime focus of biosynthetic studies of the monoterpenoid indole alkaloids (4). Simultaneously, strenuous efforts have been devoted towards the synthesis of the two component "halves" of the bisindole alkaloids.

In the present communication, we would like to report one of several new compounds, leurosidine N'$_b$-oxide (**2**), which we have isolated and characterized from alkaloid fractions of *C. roseus*.

EXPERIMENTAL[2]

SEPARATION OF THE ALKALOID FRACTIONS.—The source, identification and processing of the leaves of *Catharanthus roseus* used in this study have been described previously (5).

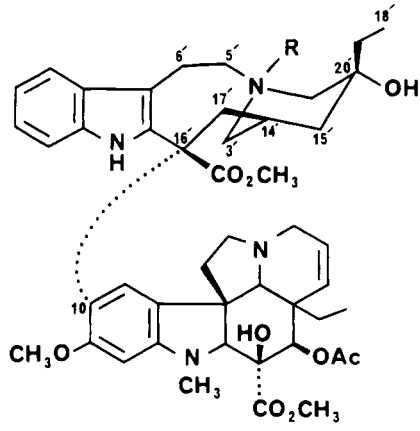
SEPARATION OF THE CRUDE LEUROSINE-CONTAINING FRACTION.—An aliquot (2 gm) of the crude leurosine-containing fraction was applied, in chloroform, to a column of silica gel PF-254 (100 gm) packed in chloroform. Elution with chloroform-methanol (95:5) and crystallization of the residue from the first 100 ml fraction from acetone afforded a white solid (14.4 mg) having the following physical and spectroscopic properties: mp 215–218°, $[\alpha]^{25}D$ +26° (c 0.5, $CHCl_3$); ir, ν max (KBr) 3500 (m, NH and OH), 2980 (m), 1740 (s, ester CO), 1610 (m), 1460 (m), 1280 (m), 1240 (s), 1030 (m), and 750 cm^{-1} (m); uv, λ max (EtOH) (log ϵ) 218 (4.56), 270 (4.14), 288 (4.11), 298 (4.03) and 315 nm (3.83); cd, (MeOH) 225.5 ($\Delta\epsilon$ +34), 214.0 ($\Delta\epsilon$ −67); nmr, δ ($CDCl_3$) 0.76 (t, J=7 Hz, 3H, 18-CH_3), 0.98 (t, J=7 Hz, 3H, 18' -CH_3), 2.10 (s, 3H, 17-$OCOCH_3$), 2.74 (s, 3H, -NCH_3), 3.63 (s, 3H, 16'-CO_2CH_3), 3.78 (s, 3H, 16-CO_2CH_3), 3.79 (s, 3H, 11-OCH_3), 4.44 (broad d, 2H, J=15 Hz, 5'-H and 3'-H), 5.29 (d, J=10 Hz, 1H, 14-H), 5.40 (s, 1H, 17-H), 5.84 (dd, J=10, 4 Hz, 15-H), 6.07 (s, 1H, 12-H), 6.37 (s, 1H, 9-H), 7.44–7.12 (m, 4H, 9', 10', 11', 12'-H), 8.02 (br s, 1H, indole NH); cmr, see table 1; ms, *m/e* 826 (M^-, 12.5), 810 (20.2), 792 (50.2), 779 (25.1), 752 (12.5), 751 (12.0), 733 (5.2), 719 (2.1), 669 (7.5), 649 (6.3), 631 (4.2), 524 (9.9), 510 (15.2), 509 (5.4), 470 (17.9), 469 (35.3), 282 (52.4), 279 (35.3), 206 (52.4), 154 (50.4), 144 (52.2), 136 (51.2), 135 (100), 122 (51.2), 121 (50.2), 107 (25.3); R_f 0.54 (methanol) and 0.18 ethylacetate: absolute ethanol (3:1).

$FeSO_4$-REDUCTION OF LEUROSIDINE N'$_b$-OXIDE (**2**).—The alkaloid (**2**) (1 mg) was heated with reducing agent (1 ml 10% aqueous ferrous sulfate solution) at 100° for 15 min. The reaction mixture was extracted with chloroform (2×2 ml), washed with water, and dried over Na_2SO_4. Analysis of the reaction mixture by tlc eluting with benzene:triethylamine (43:7) indicated complete conversion of the alkaloid (R_f 0.00) to a less polar product (R_f 0.14). The reaction product was identical with leurosidine (co-tlc), but not VLB (R_f 0.45).

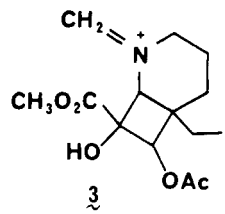
STRUCTURE ELUCIDATION OF LEUROSIDINE N'$_b$-OXIDE (**2**).—The characteristic absorption pattern in the uv spectrum was con-

[1]For Part XXXIV, see Reference 1.

[2]Melting points were determined by means of a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrometer. The ir spectra were determined in Perkin Elmer, model 710 spectrometer. Proton nmr spectra were recorded in $CDCl_3$ on a Varian model T-60A instrument with a Nicolet TT-7 Fourier Transform attachment and carbon nmr spectra were recorded in $CDCl_3$ with a Varian XL-100 instrument and tetramethylsilane was used as an internal standard and chemical shifts are reported in δ-units (ppm). Optical rotations were measured with a Perkin-Elmer, model 241, polarimeter and circular dichroism data were obtained with a JASCO J-40A spectropolarimeter. High resolution mass spectra were obtained with an AEI MS 902 double focussing spectrometer operating at 70 ev. R_f values were obtained by chromatography on silica gel.



1 R = no subst.
2 R = O



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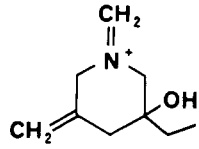

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sistent with an indole-dihydroindole chromophore typical of the vincaleukoblastine group of alkaloids (6, 7), and the ir spectrum indicated the presence of NH, hydroxy and saturated ester functionalities. From the high-field (270 MHz) proton nmr spectrum of the isolate it was evident that the alkaloid was a dimer of the vindoline-cleavamine type displaying an *O*-acetyl, *N*-methyl, a *cis*-substituted olefin, an aromatic methoxy and two carbomethoxy groups. In addition, attachment of the indole portion to the vindoline moiety must be through the C-10 aromatic carbon since only two aromatic protons in a *para* relationship were observed for this unit. The

TABLE 1. Comparison of the ^{13}C-nuclear magnetic resonance spectrum of leurosidine (1) and leurosidine N$^{1}_{b}$-oxide (2).

Carbon	Dihydroindole Unit Chemical Shift[a]		Carbon	Indole Chemical Shift[a]	
	1	2		1	2
2	83.1	83.31	2'	130.2	130.11
3	50.2	50.74	3'	43.9	66.53
5	50.2	50.93	5'	53.9	69.64
6	44.5	44.77	6'	21.4	21.20
7	53.1	53.33	7'	116.6	117.85
8	123.0	123.48	8'	128.9	128.55
9	123.4	124.12	9'	117.9	117.85
10	120.4	120.21	10'	122.0	123.48
11	157.6	158.13	11'	118.6	119.78
12	94.0	94.41	12'	110.2	110.83
13	152.8	153.60	13'	134.5	135.21
14	124.3	124.69	14'	29.8	29.75
15	129.7	131.43	15'	40.4	40.59
16	79.5	79.85	16'	55.4	55.58
17	76.2	76.59	17'	35.1	37.75
18	8.3	8.41	18'	7.1	6.93
19	30.7	30.95	19'	38.5	38.04
20	42.6	43.00	20'	71.8	72.30
21	65.5	66.03	21'	55.1	77.38
*C*OOCH	170.7	170.99	$COOCH_3$	173.9	173.90
$COOCH_3$	51.9	52.16	$COOCH_3$	52.1	52.60
NCH_3	38.2	38.04			
$OCOCH_3$	21.0	21.06			
$OCOCH_3$	171.4	171.75			
$ArOCH_3$	55.7	55.95			

[a]In parts per million from TMS δ ($CDCl_3$) + 76.9.

splitting patterns of the C-18 and C-18' protons indicated that the two ethyl side chains were unsubstituted.

A molecular ion at *m/e* 826 analyzing for $C_{46}H_{58}N_4O_{10}$ indicated the isolate to contain one more oxygen atom than VLB or its 20'-epimer, leurosidine (**1**). The mass spectrum displayed a number of ions typical of a vindoline moiety, particularly the ion at *m/e* 282 (**3**) (8). In addition, the ion fragment at *m/e* 154 (**4**), characteristic of the alicyclic portion of the velbanamine containing dimer (9), indicated the presence of one oxygen atom in the piperidine ring.

The observation of a two-proton doublet with fine splitting at 4.44 ppm arises from non-equivalent aminomethylene protons, and the large coupling constant of 15 Hz reflects *J* gem in a large, cyclic, flexible system. Inspection of a Dreiding model of the velbanamine skeleton revealed that an appropriate modification of the tertiary nitrogen atom could well give rise to this down-field shift of one of the aminomethylene hydrogens. The most plausible group that would produce this shift and would at the same time satisfy other properties (high polarity, molecular formula, and degrees of unsaturation) is an N'$_b$-oxide.

Corroboration of this hypothesis came from the ^{13}C nuclear magnetic resonance spectrum of the isolate on comparison with leurosidine (table 1). Assignment of vindoline as the dihydroindole portion was straightforward and was supported by the chemical shift assignments of all the carbon resonances of this unit on comparison with VLB or leurosidine (**1**); in this way the attachment of the indole unit to C-10 of vindoline was firmly established.

Comparison of the carbon resonances of the indole half with related carbons in leurosidine (or VLB) showed reasonable agreement for most of the carbons, particularly those in the aromatic region. Carbon resonance frequencies of the alicyclic portion corroborated hypotheses for the oxygen substitution, namely, four oxygen functions to be assigned in the indole part. The ester carbonyl group at 173.90 ppm contains two oxygen atoms, and the remaining two oxygens are most probably located in the alicyclic portion. The signal at 72.30 ppm was assigned to C-20', the downfield chemical shift indicating the presence of a hydroxyl function attached to that carbon. This was also in accord with the data for a similar carbon at 71.8 ppm in leurosidine (**1**). The other oxygen function was shown to be a N'$_b$-oxide from the presence of three deshielded methylenes at C-5' (69.64 ppm), C-3' (66.53 ppm) and C-21' (77.38 ppm). Such downfield shifts have been reported previously for the amino-methylene carbons in VLB-N'$_b$-oxide (10, 11). Hence it could be concluded that the alkaloid is an N'$_b$-oxide of either VLB or leurosidine. The CD data indicated that the C-16' chirality is the same as that in **1** (12). Finally, the structure **2** for the alkaloid was established by chemical reduction to leurosidine (**1**). Based on spectroscopic and chemical evidence the structure of the isolate was therefore established as leurosidine N'$_b$-oxide (**2**).

BIOLOGICAL ACTIVITY OF THE ISOLATE.—The isolate was evaluated for anti-cancer activity according to established protocols (13) and was found to be cytotoxic in both the P-388 (ED_{50} 2.7 μg/ml) and KB (ED_{50} 0.26 μg/ml) test systems *in vitro*.

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