CATHARANTHUS ALKALOIDS, XXXVIII. CONFIRMING STRUCTURAL EVIDENCE AND ANTINEOPLASTIC ACTIVITY OF THE BISINDOLE ALKALOIDS LEUROSINE-N′$_b$-OXIDE (PLEUROSINE), ROSEADINE AND VINDOLICINE FROM *CATHARANTHUS ROSEUS*[1,2]

ALY EL-SAYED,[3] GEORGE A. HANDY,[4] and GEOFFREY A. CORDELL*

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612

ABSTRACT.—Additional and confirming chemical and spectroscopic evidence for vindolicine (**4**), roseadine (**5**), and leurosine-N′$_b$-oxide (**6**) is presented. Leurosine-N′$_b$-oxide (**6**) was found to be exceptionally active in the B-16 melanoma test system *in vivo*. Roseadine (**5**), a new isolate of *Catharanthus roseus*, and **6** displayed significant activity in the P-388 lymphocytic leukemia test system *in vivo*. Preliminary spectral studies on the new alkaloid roseamine are also described.

Pleurosine is one of the many bisindole alkaloids originally obtained by Svoboda from the leaf alkaloid fractions of *Catharanthus roseus* (L.) G. Don (Apocynaceae) (2,3). Although a chemical relationship was established with leurosine (**1**), definitive evidence for the location of the N-oxide and the stereochemical integrity of the velbanamine unit was not presented (4).

In the process of isolating additional quantities of leurosine (**1**) for chemical modification, a reasonable quantity of pleurosine was obtained, and it was, therefore, decided to provide additional stereochemical evidence for this alkaloid and to evaluate its anticancer activity.[5]

Continuing our studies (6-12) of several alkaloid fractions of *C. roseus* displaying anticancer activity, chromatography of the highly active fraction (pH 9.0) (9), with concomitant bioassay using the P-388 lymphocytic leukemia and Eagle's carcinoma of the nasopharynx (KB) test systems (5), afforded three bisindole alkaloids. Two of these, roseadine and roseamine, are new natural products.

Most of the bisindole alkaloids of *C. roseus* are composed of an indoline [typically vindoline (**2**)] and an indole [derivative of 16β-carbomethoxy-velbanamine (**3**)] moiety (13,14). But there is one alkaloid, vindolicine, whose structure reflects the joining of two vindoline units through condensation with a single carbon fragment. Although there are other bisindole alkaloids known to be formed by the union of two *Aspidosperma* units (14), vindolicine is the only example linked in the above manner. When these studies were commenced (1977) there was little definitive evidence for the structure (**4**) of vindolicine (15-17). Subsequently, assignments were made for the ^{13}C-nmr spectrum (18). Our work parallels these studies, but indicates that there may be some conformational overlap between the vindoline units.

Also isolated was an alkaloid, hitherto unreported from *C. roseus*, whose structure had been intimated by Wenkert *et al.* (19) in the course of defining the structure of an

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[3]Present Address: Department of Pharmacognosy, Faculty of Pharmacy, University of Cairo, Cairo, Egypt.

[4]Present Address: Department of Chemistry, Northwestern University, Evanston, Illinois.

[5]Fractions and pure compounds were evaluated for antineoplastic activity according to established protocols (ref. 5).

acid rearrangement product of leurosine (**1**). Our more extensive chemical and spectral examination confirms the structural assignment made previously. This alkaloid, to which we have given the name roseadine (**5**), displayed activity in the P-388 lymphocytic leukemia system. Roseamine, a further isolate, also showed activity in this system, and preliminary spectral evidence indicates that it is a structural isomer of vinblastine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined by means of a Kofler hot-plate and are uncorrected. The uv spectra were obtained with a Beckmann, model DB-G, grating spectrophotometer. The ir spectra were determined in $CHCl_3$ solution with a Beckmann model IR 18-A spectrophotometer, and signals are reported as vs (very strong), s (strong), m (medium), and w (weak). Proton nmr spectra were recorded in $CDCl_3$ with a Varian T-60A instrument having a Nicolet TT-7 Fourier Transform attachment, with an IEF spectrometer operating at 240 MHz or with a Brüker spectrometer at 270 MHz. ^{13}C-nmr spectra were recorded in $CDCl_3$ with a Brüker WP-90 instrument operating at 22.68 MHz. Tetramethylsilane was used as an internal standard and chemical shifts and reported in δ units. Low resolution ms were obtained with a Varian MAT 112S instrument operating at 70 eV, and high resolution ms with a Varian MAT 311A double focusing spectrometer operating at 70 eV. Optical rotations were measured with a Carl Zeiss optical polarimeter.

SEPARATION OF THE ALKALOID FRACTIONS.—The origin, identification, and processing of the leaves of *Catharanthus roseus* (L.) G. Don (Apocynaceae) have been discussed previously (8,9).

SEPARATION OF THE CRUDE LEUROSINE-CONTAINING FRACTION.—An aliquot (2 g) of the crude leurosine-containing fraction was applied, in $CHCl_3$, to a column of silica gel PF_{254} (E. Merck, Darmstadt, W. Germany) (100 g). Elution was conducted initially with $CHCl_3$ and then $CHCl_3$-1% MeOH, collecting 100-ml fractions. Column fractions 7 and 8 were crystallized from methanol to afford leurosine N′$_b$-oxide (pleurosine) (**6**, 176 mg) as light orange prisms; mp, 218-219°, $[\alpha]^{25}D$ −57° (c 0.8, MeOH); ir, ν max ($CHCl_3$) 3620 (w), 3010 (s), 1745 (m), 1520 (m), 1480 (w), 1425 (m), 1240 (s), -N↦O), 1100 (m), and 930 cm^{-1} (s, -N↦O); uv, λ max (EtOH) 217 (log ε 4.74), 260 (4.22), and 291 (4.15); ^{1}H-nmr, ($CDCl_3$, 240 MHz) δ 0.69 (3H, t, J=6.9 Hz, 18-CH_3), 1.02 (3H, t, J=6.9 Hz, 18′-CH_3), 2.10 (3H, s, 17-$OCOCH_3$), 2.73 (3H, s, N-CH_3), 3.68 (3H, s, 16′-CO_2CH_3), 3.78 (3H, s, 16-CO_2CH_3), 3.84 (3H, s, 11-OCH_3), 3.99 (1H, d, J=8 Hz, 15′-CH), 4.11 (1H, d, J=14 Hz, 5′α-CH), 4.24 (1H, d, J=12 Hz, 3′β-H), 5.29 (1H, d, J=10 Hz, 12-CH), 5.40 (1H, s, 17-CH), 5.86 (1H, ddd, J=2, 4, 10 Hz, 15-CH), 6.09 (1H, s, 12-CH), 6.65 (1H, s, 9-CH), 7.14 (3H, m, 9′, 10′ and 11′-CH), 7.65 (1H, d, J=8 Hz, 12′-CH), and 8.16 ppm (1H, bs, indole NH, exchanged with D_2O); ^{13}C-nmr, see table 1; ms, *m/z* 838 (5%), 824 (M^+, 20), 822 (8), 808 (45), 792 (22), 764 (35), 750 (37), 690 (12), 612 (45), 610 (45), 590 (20), 469 (42), 353 (52), 339 (70), 282 (80), 221 (40), 213 (40), 201 (3), 187 (45), 157 (20), 153 (30), 143 (17), 125 (72), 121 (100), and 120 (82).

REDUCTION OF LEUROSINE-N′$_b$-OXIDE (**6**).—The isolate (2 mg) was treated with ferrous sulfate solution (1 ml of 10% aqueous solution) at 100° for 15 min. Extraction with $CHCl_3$ (2 ×2 ml), followed by drying (Na_2SO_4) and analysis of the residue by tlc on silica eluting with $CHCl_3$-MeOH (9:1) (Rf 0.25) and $CHCl_3$-MeOH (8:2) (Rf 0.71), indicated a close similarity with leurosine (**1**). Confirmation was obtained by examination of the mass spectrum of the reduction product; ms, *m/z* 808 (M^+), 670, 611, 610, 552, 551, 510, 509, 451, 450, 401, 388, 329, 152 (100%), 144, 135, 122, 121, and 107, in agreement with the published ms data of leurosine (**1**) (8, 20).

The isolate (3 mg) was treated with granulated zinc (1 mg) in 2 N hydrochloric acid (1 ml) and two drops of methanol for 15 min at room temperature. Basification (Na_2CO_3), followed by extraction with $CHCl_3$ (2×2 ml) and drying (Na_2SO_4), afforded a residue that was identified as leurosine (**1**) by tlc and ms analysis.

OXIDATION OF LEUROSINE (**1**).—Leurosine (**1**, 150 mg) was dissolved in CH_2Cl_2 (15 ml) and stirred under nitrogen at 0°. A solution of *m*-chloroperbenzoic acid (33 mg) in CH_2Cl_2 (2 ml) was added dropwise by means of a syringe over 30 min. The progress of the reaction was monitored by tlc [silica, $CHCl_3$-MeOH (95:5)]. A white precipitate of *m*-chlorobenzoic acid appeared after 2 h, and the reaction was terminated after 3 h by the addition of CH_2Cl_2-10% $NaHCO_3$ solution (1:1), 20 ml. After separation, the organic phase was dried (Na_2SO_4), filtered, and evaporated, and the pale yellow residue was recrystallized from absolute ethanol to afford leurosine-N′$_b$-oxide (**6**, 112 mg); mp, 206-208°; ^{13}C-nmr, see table 1.

STRUCTURE CONFIRMATION OF LEUROSINE-N′$_b$-OXIDE (**6**).—The isolate displayed a uv spectrum characteristic of the indole-dihydroindole chromophore of the vinblastine-type alkaloids (21), however, the ir spectrum showed two absorptions characteristic of an N-oxide group (22). The high mass region of the mass spectrum of the isolate was particularly complex, displaying four significant ions above 800 amu

(838, 824, 822, and 808). One explanation for this behavior is that the molecular ion at *m/z* 824 suffers two fates: transmethyleneation (20,23) to afford *m/z* 838, or loss of the N-oxide oxygen to give *m/z* 808 (24). This latter ion can then also undergo transmethyleneation to yield ion *m/z* 822, as observed in the spectrum of leurosine (**1**) itself (8,20). The major fragment ions are derived from *m/z* 808 and parallel those of leurosine (**1**). Thus, cleavage of the vindolinyl ion leads to an intense epoxyvelbanamine ion at *m/z* 353 (20); fragment ions at *m/z* 121, 135, 143, and 282 characterize the vindoline unit (20,25,26).

The ^{1}H-nmr spectrum at 240 MHz also substantiated the presence of a vindoline moiety substituted at the 10-position, displaying characteristic resonances for the methyl group at C-18, the acetate and its associated methine proton, the indoline N-methyl, the aromatic methoxy, the methoxy carbonyl, two *cis*-related olefinic protons and two *para*-related aromatic protons (27-29). Comparison with the ^{13}C-nmr data for the vindoline unit of leurosine (**1**) (8,18,19,30) indicated a close match, and substantiated the inclusion of vindoline, substituted at the 10-position, as an intact unit.

The principal ^{1}H-nmr resonances of the indole moiety were observed at 1.02 (C-18′ CH_3), 3.68 (-CO_2CH_3), 7.14 (C-9′, C-10′ and C-11′H), and 7.65 ppm (C-12′H), with the exchangeable indole NH appearing at 8.16 ppm. From the observation of a triplet at 1.02 ppm, C-19′ is still a methylene group. Three clear signals in the aliphatic portion of the indole unit could be assigned. One of these is a doublet (J=3 Hz) at 2.72 ppm, characteristic of an epoxide methine proton at C-15′, coupling with an equatorial *trans*-related 14′-CH (8). Proton doublets at 4.11 (J=14 Hz) and 4.24 ppm (J=12 Hz) could be attributed to the geminal coupling of different, nonequivalent aminomethylene protons. Although definitive evidence is not available, the proton with the larger coupling constant is tentatively assigned to 5′-CH, being in a large, somewhat flexible, ring (31), and the proton with the smaller coupling constant to 3′-CH, which is in a quite rigid six-membered ring (31).

Another aspect of these two protons is, of course, their downfield chemical shift. One functional group that could account for such a shift is an N-oxide of the aliphatic nitrogen. Such a group would also satisfy the other properties thus far observed. Confirmation of this was obtained by chemical reduction ($FeSO_4$ and Zn/HCl) to afford leurosine, identical with the natural product by tlc on several systems and by

TABLE 1. ^{13}C-nmr data for the isolates and related compounds.[a,b]

Carbon	Leurosine (**1**)	Leurosine N'_b-oxide (**6**)[c]	Leurosine N'_b-oxide (**6**)[d]	Vindoline (**2**)[e]	Vindolicine (**4**)		Vinblastine (**7**)[h]	Roseadine (**5**)
					A	B		
	Dihydroindole Unit							
2	83.1	83.0	82.9	83.2	83.0	83.0	83.0	83.2
3	50.1	50.3	50.2	50.9	50.9[f]	51.0	50.0	50.7
5	50.1	50.2	49.9	51.9	50.8[f]	50.6	50.0	50.7
6	44.4	44.2	44.3	43.9	42.7	41.5	44.3	43.4
7	53.0	53.2	53.1	52.6	52.7	52.8	52.9	52.1
8	122.9	123.4	123.3	124.9	123.4	123.4	122.6	122.8
9	123.4	123.7	123.7	122.4	123.7	124.1[g]	123.1	123.9
10	120.5	119.5	119.6	104.5	124.4[g]	124.4[g]	120.4	119.0
11	157.7	157.6	157.8	161.1	159.0	159.0	157.8	157.7
12	94.1	93.8	93.7	95.6	93.5	93.0	93.9	92.8
13	152.8	153.1	153.2	153.6	152.1	152.1	152.5	153.2
14	124.3	124.5	124.6	123.9	123.9	124.4	124.3	123.9
15	129.8	129.7	129.6	130.2	130.2	130.0	129.7	129.8
16	79.4	79.6	79.5	79.5	79.3	79.5	79.3	79.2
17	76.2	76.4	76.4	76.2	76.0	75.9	76.1	76.1
18	8.2	8.5	8.4	7.5	7.1	7.1	8.1	7.6
19	30.6	30.7	30.7	30.6	30.5	30.3	30.4	30.4
20	42.5	42.6	42.5	42.8	42.5	42.4	42.3	42.3
21	65.5	65.4	65.3	67.0	67.0	67.0	65.2	66.1
CO_2CH_3	170.7	170.9	170.8	170.4	170.6	170.6	170.6	170.5
CO_2CH_3	52.0	52.6	52.6	51.9	52.0	51.8	51.8	51.9
$COCH_3$	171.4	171.5	171.5	171.7	171.6	171.4	171.4	171.6
$COCH_3$	20.9	20.9	20.9	20.8	20.7	20.7	20.7	20.7
NCH_3	38.1	37.9	38.0	38.0	38.0	37.7	38.2	38.1
$ArOCH_3$	55.6	55.1	55.7	55.1	55.1	55.1	55.3	55.4
Ar-CH_2Ar	—	—	—	—	—	34.0	—	—

TABLE 1. Continued.

Carbon	Indole Unit				
	Leurosine (**1**)	Leurosine N'_b-oxide (**6**)[c]	Leurosine N'_b-oxide (**6**)[d]	Vinblastine (**7**)[h]	Roseadine (**5**)
2′	130.7	130.7	130.3	130.9	131.6
3′	42.3	59.0	58.3	47.5	51.5
5′	49.8	65.9	65.7	55.5	57.8
6′	24.8	28.0	28.1	28.7	33.2
7′	116.8	117.6	118.0	115.9	109.5
8′	129.2	130.3	129.6	129.0	128.8
9′	118.1	118.9	118.8	118.1	117.2
10′	122.2	119.4	119.2	122.2	121.0
11′	118.8	117.6	119.0	118.8	118.7
12′	110.3	111.8	111.7	110.2	110.1
13′	134.6	134.5	134.6	134.7	134.3
14′	33.5	33.6	32.0	29.2	34.2
15′	60.3	59.6	59.5	40.0	48.2
16′	55.2	54.7	54.7	55.3	133.2
17′	31.7	32.1	31.8	34.1	142.9
18′	8.4	8.4	8.1	6.7	6.7
19′	28.0	27.6	27.9	34.1	24.3
20′	60.0	59.0	58.7	68.6	74.4
21′	53.9	71.9	71.5	63.1	58.9
CO_2CH_3	174.2	173.8	173.9	174.6	169.2
CO_2CH_3	52.2	52.8	52.6	52.0	51.6

[a]Recorded at 22.68 MHz, in $CDCl_3$, δ (TMS)=δ ($CDCl_3$)+76.9 ppm.
[b]Broad-band and off-resonance decoupled spectra were obtained in all cases.
[c]Natural compound.
[d]Semisynthetic compound prepared from leurosine (**1**).
[e]Data are from Wenkert *et al.* (30).
[f,g]Indicates assignments may be reversed.
[h]Data are from Wenkert *et al.* (19).

mass spectrometry (20). The isolate is, therefore, identical to pleurosine, an alkaloid obtained previously from *C. roseus* (2,32,33), and assigned a structure based on preliminary spectral and chemical evidence (4), or to the 15′,20′-epimer thereof (2).

Final establishment of the structure was derived from the broad band and off-resonance decoupled ^{13}C-nmr spectra of the isolate. The assignment of the resonances of the dihydroindole unit to vindoline has been discussed previously, and examination of the resonances of the indole unit confirmed that the isolate was a member of the vinblastine series of alkaloids (18, 19). However, although the aromatic carbons bore a close resemblance to compounds in this series, particulary those of leurosine (8, 19), a number of the aliphatic carbons displayed quite distinct shifts (table 1).

From the molecular formula, four oxygen atoms remained to be characterized in the indole unit. Two of these were in the methoxy carbonyl group (173.8 and 52.6 ppm), and one more was assigned to an epoxide functionality between C-15′ (59.6 ppm) and C-20′ (59.0 ppm), with a corresponding downfield shift of C-18′ due to a γ-effect of the epoxide oxygen. Such an effect has previously been observed for leurosine (8,19) and 21′-oxoleurosine (8), on comparison with the data for vinblastine and its derivatives (18,19). Because no new methine carbons were observed, the remaining oxygen atom was assigned as an N-oxide. In support of this, three of the methylene resonances C-3′, C-5′ and C-21′ had undergone deshielding on comparison with leurosine (**1**). Similar downfield shifts have been observed for the N'_b-oxides of vinblastine (**7**) (18) and leurosidine (**8**) (10), and are summarized in table 2.

Wenkert *et al.* (34) have recently identified six carbon atoms that display characteristic chemical shifts when the stereochemistry at C-16′ is changed from the natural to the 16′-epi series. Comparison of our data for the isolate with those of Wenkert for C-9, C-10, C-2′, C-7′, C-16′, and C-17′ (table 3) establishes conclusively the C-16′ stereochemistry as being that with the methoxycarbonyl group β.

TABLE 2. Comparison of the effects of the introduction of an N'_b-oxide into the aminomethylene carbons of vinblastine, leurosidine and leurosine.

Compound		Carbon			Reference
		3′	5′	21′	
Vinblastine . .	Base (**7**)	48.0	55.8	64.2	18
	N'_b-oxide	64.0	67.9	77.8	18
	Δδ	+16.0	+12.1	+13.6	
Leurosidine . .	Base (**8**)	43.9	53.9[a]	55.1[a]	19
	N'_b-oxide	66.5	69.6	77.4	10
	Δδ	+22.6	+15.7	+22.3	
Leurosine . . .	Base (**1**)	42.3	49.8	53.9	—
	N'_b-oxide (**6**)	59.0	65.0	71.9	—
	Δδ	+16.7	+16.1	+18.0	

[a]Values may be interchanged.

Based on the data previously available, the structure of pleurosine had been assigned as the N'_b-oxide of leurosine, although those data did not preclude N-oxidation at N_a or N_b in the vindoline moiety, or an inverted stereochemistry at C-15′ or C-20′. Our data have excluded the possibility of the presence of vindoline N-oxide unit in this isolate. However, the question of a possible inversion of the epoxide group was a more serious one, the only evidence being the coupling constant of 15′-CH. Completion of the structure assignment, therefore, required an unambiguous chemical correlation.

Reinvestigation of the N-oxidation of leurosine (**1**) with *m*-chloroperbenzoic acid afforded in high yield a single product identical to natural leurosine-N'_b-oxide as shown by its ^{13}C-nmr spectrum (table 1).

TABLE 3. Comparison of natural and synthetic C-16′ bisindole alkaloids with leurosine N'_b-oxide (**6**).

Carbon	Natural derivative[a]	Synthetic derivative[a]	Leurosine N'_b-oxide (**6**)
C-9	123.6	119.6	123.7
C-10	120.9	126.0	119.5
C-2′	130.7	134.3	130.7
C-7′	117.1	111.1	117.6
C-16′	55.3	53.1	54.7
C-17′	34.2	38.6	32.1

[a]Data are from Wenkert *et al.* (34).

Inasmuch as the previously suggested (19,30) stereochemistry at C-15′, C-20′ in leurosine (**1**) is now firmly established (29,35,37), this reaction and spectral analysis establishes the structure of pleurosine to be **6**, leurosine-N'_b-oxide.

CHROMATOGRAPHIC SEPARATION OF THE GRADIENT pH 9.0 ALKALOID FRACTION.—The pH 9.0 alkaloid fraction (40.01 g) (9) was chromatographed on a column (100×150 cm) of silica gel PF_{254} (2000 g). Elution was initiated with C_6H_6-$CHCl_3$ (1:1), and the eluate was collected in 200-ml portions. Similar fractions were combined based on their tlc patterns [silica gel, $CHCl_3$-MeOH (95:5)] to afford 28 fractions, each of which was bioassayed in the P-388 lymphocytic leukemia system *in vivo* (6 fractions active) or KB carcinoma test system *in vitro* (14 fractions active). The most active fraction *in vitro*, F-084 (2.5 g, ED_{50} 0.43 μg/ml), failed to crystallize and was, therefore, rechromatographed on a column (2.5×10 cm) of silica gel PF_{254} (150 g). Elution was initiated with C_6H_6, and successively with C_6H_6-$CHCl_3$ (1:1), $CHCl_3$, and $CHCl_3$-MeOH, collecting 20-ml fractions. Similar fractions (by tlc) were combined to afford a total of six fractions. Those fractions, eluted with $CHCl_3$-MeOH (98:2) and $CHCl_3$-MeOH (95:5), were processed further.

ISOLATION OF VINDOLICINE (**4**).—The column chromatographic fractions eluted with $CHCl_3$-MeOH (98:2) (198 mg) were evaporated *in vacuo* and the residue dissolved in warm MeOH. On standing at room temperature for several days, a yellowish-brown amorphous powder (160 mg) was deposited. Chromatography indicated this to be a mixture of two components, separated by preparative tlc on silica gel PF_{254} eluting with $CHCl_3$-MeOH (9:1). The major band (Rf 0.57) was removed from the silica with $CHCl_3$-MeOH (1:1) to afford a light yellow powder (47 mg), homogeneous by tlc and having the following physical properties; ir, ν max ($CHCl_3$) 3460 (s), 2980 (s), 1730 (m), 1600 (w), 1585 (w), 1565 (w), 1500

(m), 1415 (m), 1200 (s), 1025 (m), and 910 cm^{-1} (m); uv, λ max (MeOH) 219 (log ε 4.39), 260 (4.37), and 310 nm (4.24); ^{1}H-nmr ($CDCl_3$, 270 MHz) δ 0.44 (3H, t, J=7.3 Hz, 18-CH_3), 0.60 (3H, bd t, 18′-CH_3), 2.07 (3H, s, 17-$OCOCH_3$), 2.10 (3H, s, 17′-$OCOCH_3$), 2.67 (3H, s, N-CH_3), 2.75 (3H, s, N-CH_3), 3.65 (3H, s, 16-CO_2CH_3), 3.82 (6H, s, 11-OCH_3, 16′-CO_2CH_3), 3.84 (3H, s, 11′-OCH_3), 3.97 (2H, bd m, 23-CH_2), 5.30 (2H, bd m, 15-CH and 15′-CH), 5.94 (2H, m, 14-CH and 14′-CH), 6.09 (1H, bd, 12-CH), 6.46 (1H, bd, 12′-CH), 7.03 (1H, bd, 9-CH), and 7.20 (1H, bd, 9′-CH); ^{13}C-nmr, see table 1.

STRUCTURE CONFIRMATION OF VINDOLICINE (**4**).—The uv spectrum of the isolate indicated that it possessed a dihydroindole chromophore (21), and the ir spectrum suggested the presence of NH or OH, a *cis*-disubstituted olefin, and a saturated ester group. No molecular ion was observed in the mass spectrum, but fragment ions were found typical of those in the mass spectrum of vindoline (**2**) (20,25,26). A ^{1}H-nmr spectrum at 270 MHz indicated the dimeric nature of the molecule. Thus, many of the proton resonances of the alkaloid vindoline (38,39) were observed in a repeated or more complex manner (*e.g.*, 18-CH_3, 17-$OCOH_3$, N-CH_3, ArOCH_3, 15-H, 9-H, and 12-H) and indicated a close resemblance to the alkaloid vindolicine (**4**). Previous work (16,17,40) on this alkaloid had suggested that it was a dimer of vindoline joined through C-10 by a methylene group.

The ^{13}C-nmr spectrum of the isolate (table 1) also indicated the presence of two intact vindoline units displaying characteristic (18,19,30,34) resonances on comparison with vindoline (**2**). The methylene group joining the two vindoline units was observed at 34.0 ppm. Previous work (18) had determined that the two vindoline units were identical in the ^{13}C-nmr spectrum of **4**. We do not find this; rather, we observe a conformationally induced interaction between the two units, which causes some carbons to be shifted downfield, while others are shifted upfield. Indeed, only carbons 2, 8, 10, 11, and 18, and the methoxy and methoxycarbonyl groups are overlapping resonances. Under these circumstances, it is not possible to assign resonances definitively to one unit or another; rather, assignments are made to a given carbon, which may be on either vindoline unit (table 1).

The data firmly establish the structure of vindolicine as **4**, in agreement with earlier proposals (17,18) for this alkaloid of *C. roseus* (15,32,33,41,42), *Catharanthus longifolius* (Pich.) Pich. (16,17), and *Catharanthus ovalis* (L.) G. Don (39,43).

ISOLATION OF ROSEADINE (**5**), PERICATHIDINE AND ROSEAMINE.—The fractions from the column eluted with $CHCl_3$-MeOH (95:5) (350 mg) were evaporated *in vacuo* and the residue dissolved in warm MeOH. On standing at room temperature, successive crops of pericathidine (49 mg, 1.2×10^{-6}%), roseadine (87 mg, 2.9×10^{-6}%), and roseamine (13 mg, 6×10^{-7}%) were deposited. Roseadine was subsequently repurified by preparative tlc on silica gel, eluting three times with C_6H_6-$(C_2H_5)_3N$ (5:2) to afford 43 mg of amorphous material possessing the following physical properties: ir, ν max (KBr) 3400 (m), 2830 (s), 1745 (s), 1620 (s), 1505 (w), 1465 (m), 1440 (m), 1370 (m), 1250 (s), 1150 (s), 1040 (w), and 740 cm^{-1} (w); uv, λ max (MeOH) 214 (log ε 4.69), 225 (4.68), 258 (4.08), 286 (4.08), 294 (4.09), and 326 nm (3.93); ^{1}H-nmr ($CDCl_3$, 240 MHz) δ -0.14 (3H, t, J=7.1 Hz, 18′-CH_3), 0.94 (3H, t, J=7.2 Hz, 18-CH_3), 2.06 (3H, s, 17-$OCOCH_3$), 2.66 (3H, s, N-CH_3), 3.78 (9H, s, 11-OCH_3, 16-CO_2CH_3, 16′-CO_2CH_3), 5.07 (1H, d, J=10 Hz, 15-CH), 5.32 (1H, s, 17-CH), 5.74 (1H, d, J=10 Hz, 14′-CH), 5.81 (1H, ddd, J=4, 6, 10 Hz, 14-CH), 5.98 (1H, s, 12-CH), 6.47 (1H, s, 9-CH), 7.07 (3H, m, 9′, 10′, 11′-CH), 7.21 (1H, d, J=8 Hz, 12′-CH), 7.45 (1H, d, J=7 Hz, 17′-CH), and 8.05 (1H, bd, s, indole NH, exchanged with D_2O); ^{13}C-nmr, see table 2; ms, *m/z* 822 (822.4151, $C_{47}H_{58}N_4O_9$, 60.9%), 808 (M^+, 808.4059, $C_{46}H_{56}N_4O_9$, 78.0), 766 (766.3941, $C_{44}H_{54}N_4O_8$, 24.3), 765 (765.3916, $C_{44}H_{53}N_4O_8$, 53.6), 764 (764.3894, $C_{44}H_{52}N_4O_8$, 100), 749 (749.3893, $C_{44}H_{53}N_4O_7$, 36.5), 708 (59.7), 649 (39.0), 648 (30.4), 623 (30.4), 591 (14.6), 528 (39.0), 527 (76.8), 525 (12.1), 469 (19.5), 468 (468.2221, $C_{26}H_{32}N_2O_6$, 17.5), 467 (18.2), 455 (455.2225, $C_{25}H_{31}N_2O_6$, 18.2), 409 (24.3), 296 (18.2), 295 (295.1805, $C_{19}H_{23}N_2O$, 34.1), 282 (282.1345, $C_{14}H_{20}NO_5$, 28.0), 281 (281.1660, $C_{18}H_{21}N_2O$, 16.6), 260 (31.7), 194 (73.1), 185 (76.8), 174 (174.0931, $C_{11}H_{12}NO$, 14.4), 156 (12.1), 154 (25.6), 144 (20.7), 143 (14.6), 135 (82.9), 122 (122.0958, $C_8H_{12}N$, 36.5), and 121 (121.0880, $C_8H_{11}N$, 39.0). With the ceric ammonium sulfate (CAS) reagent, roseadine displays a yellow-brown spot changing, after 5 min, to yellow spot with a red-brown periphery.

The structure elucidation of pericathidine, which showed a red coloration with the CAS reagent, is still underway and will be reported subsequently.

Roseamine exhibited uv, λ max (MeOH) 223 (log ε 4.72), 264 (4.09), 285 (4.08), 294 (4.09), and 303 nm (4.08); ^{1}H-nmr, ($CDCl_3$, 60 MHz) δ 0.61 (3H, t, J=7 Hz, 18-CH_3), 0.99 (3H, m, 18′-CH_3), 212 (3H, s, 17-$OCOCH_3$), 2.78 (3H, s, N-CH_3), 3.56 (3H, s, 16′-CO_2CH_2), 3.70 (3H, s, 16-CO_2CH_3), 3.81 (3H, s, 11-OCH_3), 3.88 (1H, s, 2-CH), 5.20 (1H, d, J=10 Hz, 15-CH), 5.48 (1H, s, 17-CH), 5.87 (1H, m, 14-CH), 6.14 (1H, s, 12-CH), 6.55 (1H, m), 6.88 (1H, m, 9-CH), 7.02-7.26 (3H, m, 9′, 10′, 11′-CH), and 7.45 (1H, m, 12′-CH); ms, *m/z* 810 (M^+, 19.0), 782 (23.8), 766 (38.0), 764 (23.8), 752 (66.6), 751 (76.1), 610 (33.3), 527 (38.0), 469 (66.6), 451 (28.5), 369 (42.8), 355

(95.2), 282 (23.8), 154 (100), 144 (14.2), 135 (71.4), 122 (66.6), 121 (47.6), and 107 (28.5). With the CAS reagent roseamine displays a characteristic blue-grey spot.

PARTIAL SYNTHESIS OF ROSEADINE (**5**).—Leurosine (**1**, 100 mg) was reacted with aqueous sulfuric acid (40%) at room temperature for 30 min. The reaction mixture was poured over ice, rendered basic with concentrated NH_3 solution, and extracted with $CHCl_3$ to afford 96 mg of crude reaction product. Tlc on silica eluting with $CHCl_3$-MeOH (9:1) indicated an extremely complex reaction product in which roseadine could be detected. Chromatography on silica afforded roseadine (**5**, 8% yield) identical (tlc, uv, ms) with the natural isolate.

STRUCTURE ELUCIDATION OF ROSEADINE (**5**).—The uv spectrum of roseadine indicated the presence of both indole and slightly extended dihydroindole (indoline) chromophores (21), and the ir spectrum established the presence of NH and saturated ester functionalities. One-half of the molecule was etablished to be vindoline substituted at the 10-position from consideration of the nmr data (^1H and ^{13}C). Thus, the 17-acetate and its associated proton, the 16-CO_2CH_3, the N-CH_3, the OCH_3, and the 14,15-*cis* double bond were clearly observed in the ^1H-nmr spectrum; their chemical shifts on comparison with the data for leurosine (**1**) indicated this unit to be intact (27,29,44). The indole unit, however, displayed a number of significant differences for compounds in this class. The most outstanding of these was a triplet observed at −0.14 ppm, which is extremely unusual in all alkaloid chemistry (45). This signal was attributed to the 18′-methyl group experiencing particularly strong shielding from the indole nucleus, and clearly must indicate an unusual conformation for those protons. A low field proton resonance at 7.45 ppm (d, J=7.0 Hz) and another signal at 5.74 ppm (d, J=10 Hz) are also not typically observed in the vinblastine series of compounds and indicate significant structural change to have occurred in the alicyclic moiety of the velbanamine unit. There is one compound on which partial data fitting the above criteria have been published. This is a semi-synthetic derivative of leurosine (**1**), produced by the action with sulfuric acid and having the tentative structure (**5**) (19), without stereochemistry.

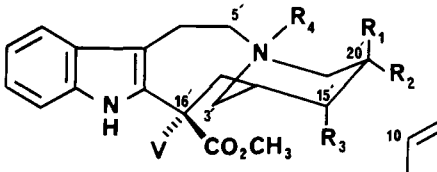
In our hands, the action of sulfuric acid on leurosine at room temperature produced an *extremely* complex mixture, and roseadine, as we named our compound, was detected and isolated in low (8%) yield.

Low and high resolution ms data for roseadine were determined, and, in each case, two potential molecular ions were observed, at *m/z* 822 ($C_{47}H_{58}N_4O_9$) and 808 ($C_{46}H_{56}N_4O_9$), the latter being isomeric with leurosine. The ion at *m/z* 808 was strongly suggested as the true molecular ion because it gave rise to characteristic fragment ions at *m/z* 766, 765 (M^+-$COCH_3$), 749 (M^+-CO_2CH_3), and 648 (M^+-160 amu). The ion at *m/z* 822 is attributed to the addition of CH_2 to the molecular ion; such transmethyleneation processes are well known in this series of compounds (20,23). Typical fragments of the vindoline moiety (20,25,26) were observed at *m/z* 296, 282, 174, 135, 122, and 121 and served to establish the presence of this unit.

Fragment ions at *m/z* 144, 143, and 156 are characteristic of an unsubstituted indole moiety in these compounds (20,23) and, in addition, indicate that C-6 is still a methylene group. Fragment ions at *m/z* 527, 469, and 468 provided some useful information about the alicyclic moiety. From high resolution data, the ion at *m/z* 468 revealed a vindoline moiety plus one carbon atom, and was apparently derived from the ion at *m/z* 527 (**9**) by loss of a methoxycarbonyl group, suggesting that the vindoline unit was linked to the indole moiety through an additional carbon atom bearing a methoxycarbonyl group. This type of cleavage reaction is not typical of the vinblastine series of alkaloids.

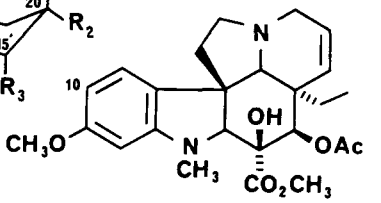
Examination of the broad band and off-resonance decoupled ^{13}C-nmr spectra of roseadine (table 1), through comparison with vindoline (**2**) and leurosine (**1**) permitted assignment of all the carbons of the dihydroindole unit and confirmed that this unit was intact. Subtraction of these carbon shifts provided the carbon resonances of the indole portion. The indole nucleus was assigned by comparison with leurosine (**1**) (8,19) and vinblastine (**7**) (18,19) and revealed the presence of three deshielded carbons, a methine carbon at 142.9 ppm and two quaternary carbons at 133.21 and 169.2 ppm. The latter was assigned to the methoxycarbonyl carbon, shielded somewhat from its characteristic shift of 174±1 ppm in the vinblastine series (18,19), by attachment to the olefinic unit. Assignment of the methine carbon to C-16′ was substantiated by the chemical shift of the attached proton. Assignment of the 18′-methyl group (6.70 ppm) and the methyl of the ester group (51.6 ppm) was trivial.

Five methylene carbons (24.3, 33.2, 51.5, 57.8, and 58.9 ppm), two methine carbons (34.2 and 48.2 ppm) and one quaternary carbon (74.4 ppm) remained to be assigned. Three aminomethylene resonances (51.5, 57.8, and 58.9 ppm) could be attributed to C-3′, C-5′, and C-21′, respectively, in comparison with the shift data of compounds in the vinblastine series (18,19). The two remaining methylene carbons, for C-19′ and C-6′, were assigned to the resonances at 24.3 ppm and 33.2 ppm, respectively; the former by analogy with vinblastine (**7**) (18,19). The signal for C-6′ was substantially deshielded ($\Delta\delta$+8.4 ppm) due to a gauche interaction with C-20′. This carbon was assigned to the quaternary carbon at 74.4 ppm, which is also somewhat deshielded ($\Delta\delta$+14.4 ppm) on comparison with **1** by this interaction. There thus remained two aliphatic methine carbons at 34.2 and 48.2 ppm to be assigned to C-14′ and C-15′, respectively.



V = 10-Vindolinyl

	R_1	R_2	R_3	R_4
1	C_2H_5	—O—		-
6	C_2H_5	—O—		→O
7	OH	C_2H_5	H	-
8	C_2H_5	OH	H	-



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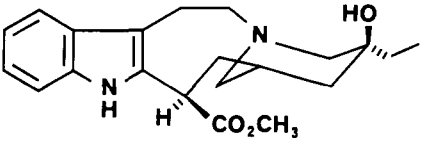

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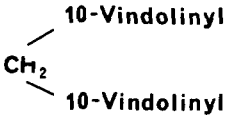

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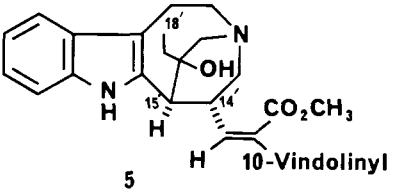
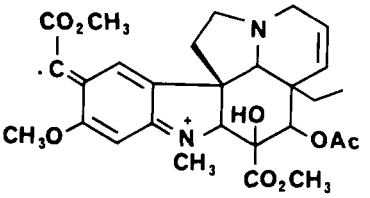
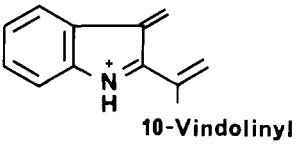


5



9 m/z 527



10 m/z 610

With the complete ^{13}C-nmr spectrum assigned in agreement with the proposed carbon framework, one feature of the ^1H-nmr spectrum deserves mention, namely the sharp doublet ($J = 10$ Hz) at 5.74 ppm. We believe this to be H-14′ coupling with H-15′, although the possibility that this is one of the H-21′ protons cannot be specifically excluded at this time.

The ^{13}C-nmr assignments are in agreement with those made tentatively by Wenkert *et al.* (19), and thus, roseadine is assigned the structure **5**. The stereochemistry of the double bond has not been determined categorically thus far. However, Dreiding models indicate that in the proposed fragmentation reaction (19) leading to roseadine (**5**), the proton lost from C-17′ should come from the α-face resulting in a Z-configuration for the double bond.

PARTIAL STRUCTURAL ANALYSIS OF ROSEAMINE.—Roseamine, from its uv spectrum, is an indole-dihydroindole alkaloid (21) demonstrating from its ^1H-nmr spectrum the presence of several functionalities associated with the vindoline nucleus. Among these are an acetate with its associated methine proton, an N-methyl, a methoxycarbonyl, an aromatic methoxy, and two *cis*-oriented olefinic protons. Signals attributed to the 9-CH and 12-CH were observed at their normal positions (27,29,44) of 6.88 and 6.14 ppm, respectively, thereby indicating attachment of the indole unit to C-10 of vindoline. Four aromatic protons were clearly observed for the unsubstituted indole nucleus, and a second methoxycarbonyl group showed this region of the molecule to be identical with other alkaloids in the series. The ethyl side chain in the indole unit also was intact from the observation of a complex triplet at 0.99 ppm.

Fragment ions at *m/z* 751 and 355 suggested that the ion at *m/z* 810 was the molecular ion, although two transmethyleneation ions at *m/z* 838 and 824 were also observed. Typical fragment ions from the vindoline nucleus were observed (*m/z* 282, 135, 122, 121, and 107) (20,25,26). But, more importantly, the mass spectrum indicated an unsubstituted indole nucleus from an ion at *m/z* 610 (vindoline+155 amu) having the probable structure **10** (20), and a base peak at *m/z* 154. This ion indicates the presence of a single hydroxy group somewhere in the alicyclic moiety. Its precise position remains to be determined, although, because the isolate was not identical with either vinblastine or leurosidine, C-20′ can be eliminated as a possible site for substitution.

ANTICANCER ACTIVITY OF THE ISOLATES.—Leurosine-N′$_b$-oxide (**6**, NSC-304421) was assayed in the following *in vivo* anticancer test systems; L-1210 lymphocytic leukemia, CX-1 colon xenograft, B-16 melanocarcinoma, P-388 lymphocytic leukemia, and the C-8 Colon 38, as well as the Eagles' carcinoma of the nasopharynx test system (5). The results of these tests are presented in table 4.

TABLE 4. Antitumor activity of leurosine-N′$_b$-oxide (**6**, NSC 304421).

Tumor System	Animal (mice)	Vehicle	Dose (mg/kg)	Treatment Schedule (days)	T/C (%)	
					1st test[a]	2nd test[a]
L-1210	CD_2F_1	Water	20.0	1-9	112	117
			10.0		133	117
			5.0		101	110
C-8	Swiss	Water	20.0	1-2	82	
			10.0		108	
			5.0		84	
B1	$B_6C_3F_1$	Water	20.0	1-9	—	—
			10.0		309^5	373^4
			5.0		261^2	246
			2.5		189	185
P-388	CD_2F_1	Water	25.0	1-9	181	
			12.5		163	
			6.25		154	
	CD_2F_1	Saline with Tween 80				
			25.0	3,6,9	181	
			12.5		177	
			6.25		168	
	CD_2F_1	Water	32.0	1	140	171
			16.0		193	134
			8.0		161	122
			4.0		142	130
C-2	NU/NU Swiss athymic	Water	160	4,8,12,16	71	
			80		85	
			40		60	
			20		81	

[a]Superscripts indicate number of survivors.

Leurosine-N′$_b$-oxide (**6**) was quite cytotoxic (ED_{50} 0.019 μg/ml) but, more importantly, activity was also observed in two *in vivo* systems. In the P-388 lymphocytic leukemia system, at doses in the range 4.0-32.0 mg/kg, either on one day only or on days 1-9, typical test/control values were in the range 160-180%. Exceptional, reproducible activity was observed in the B-16 melanocarcinoma system. Although toxicity was observed at 20 mg/kg, good dose responses were observed in the dose range 2.5-10.0 mg/kg. At the highest dose, test/control values in excess of 300 were observed with five cures in one instance and four cures in another. In a parallel test, vinblastine (**7**) showed T/C 309% at 0.5 mg/kg with five survivors. Leurosine N′$_b$-oxide is one of the most active compounds in the B-16 test system thus far isolated.

Roseadine (**5**) was active in the P-388 test system showing T/C 176% at 2 mg/kg, but was not tested further. Roseamine, whose structure was only partially deduced, was also active in this system, displaying T/C 161% at 1.0 mg/kg.

DISCUSSION

A highly active alkaloid fraction of *Catharanthus roseus* (L.) G. Don (Apocynaceae) has been subjected to bioactivity-directed fractionation to afford three bisindole alkaloids, vindolicine (**4**), roseadine (**5**), and roseamine. In the course of isolating additional leurosine (**1**) for the semi-synthesis of roseadine, a quantity of leurosine-N'_b-oxide (**6**) was isolated. The opportunity was taken to determine thoroughly its structure and stereochemistry and to establish the nature of the *m*-chloroperbenzoic acid oxidation product of leurosine.

Roseadine, previously obtained by the action of acid on leurosine, was confirmed to have the structure suggested by Wenkert *et al.* (19), and the structure of vindolicine (**4**) is also confirmed through more complete spectral assignments than have hitherto been available.

Roseamine, an apparently new alkaloid in the vinblastine series was partially characterized from its spectral properties. Roseamine is a structural isomer of vinblastine and displays significant *in vivo* anticancer activity.

Roseadine (**5**) was also very active in the P-388 lymphocytic leukemia system *in vivo*, but the most interesting activity was displayed by leurosine-N'_b-oxide (**6**), which was highly active in the P-388 test system and exceptionally active in the B-16 melanocarcinoma test system, affording some "cures" at a dose of 10 mg/kg.

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LITERATURE CITED

1. S. Mukhopadhyay, A. El-Sayed, G.A. Handy, and G.A. Cordell, *J. Nat. Prod.*, **46**, 409 (1983).
2. G.H. Svoboda, M. Gorman, A.J. Barnes, Jr., and A.T. Oliver, *J. Pharm. Sci.*, **51**, 518 (1962).
3. G.H. Svoboda, in: "Current Topics in Plant Science", J.E. Gunckel, Ed., Academic Press, New York, 1969, p 303.
4. N. Neuss, M. Gorman, N.J. Cone, and L.L. Huckstep, *Tetrahedron Letts.*, 783 (1968).
5. R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972).
6. G.A. Cordell, S.G. Weiss, and N.R. Farnsworth, *J. Org. Chem.*, **39**, 431 (1974).
7. G.H. Aynilian, S.G. Weiss, G.A. Cordell, D.J. Abraham, F.A. Crane, and N.R. Farnsworth, *J. Pharm. Sci.*, **63**, 536 (1974).
8. A. El-Sayed, G.A. Handy, and G.A. Cordell, *J. Nat. Prod.*, **43**, 157 (1980).
9. A. El-Sayed and G.A. Cordell, *J. Nat. Prod.*, **44**, 289 (1981).
10. S. Mukhopadhyay and G.A. Cordell, *J. Nat. Prod.*, **44**, 611 (1981).
11. S. Mukhopadhyay and G.A. Cordell, *J. Nat. Prod.*, **44**, 335 (1981).
12. G.A. Cordell, *Saengyak Hakhoe Chi*, **11**, 173 (1980).
13. A.A. Gorman, M. Hesse, H. Schmid, P.G. Waser, and W.H. Hopff, *Alkaloids (London)*, **1**, 200 (1970).
14. G.A. Cordell and J.E. Saxton, *Alkaloids, NY*, **20**, 1 (1981).
15. G.H. Svoboda, M. Gorman, N. Neuss, and A.J. Barnes, Jr., *J. Pharm. Sci.*, **50**, 409 (1961).
16. P. Rasoanaivo, N. Langlois, and P. Potier, *Phytochemistry*, **11**, 2616 (1972).
17. A. Rabaron, M. Plat, and P. Potier, *Plant, Med. Phytother.*, **7**, 53 (1973).
18. D.E. Dorman and J.W. Paschal, *Org. Magn. Reson.*, **8**, 413 (1976).
19. E. Wenkert, E.W. Hagaman, B. Lal, G.E. Gutowski, A.S. Katner, J.C. Miller, and N. Neuss, *Helv. Chim. Acta.*, **58**, 1560 (1975).

20. D.J. Abraham and N.R. Farnsworth, *J. Pharm. Sci.*, **58**, 694 (1969).
21. M. Hesse, "Indolealkaloide in Tabellen", Springer-Verlag, Berlin, Germany, 1964, and M. Hesse, "Indolealkaloide in Tabellen, Ergansungswerk", Springer-Verlag, Berlin, Germany, 1968.
22. K. Nakaniski and P.H. Solomon, "Infrared Absorption Spectroscopy, 2nd ed.," Holden-Day, Inc., San Francisco, 1977, p 46.
23. P. Bommer, W. McMurray, and K. Biemann, *J. Am. Chem. Soc.*, **86**, 1439 (1964).
24. T.A. Bryce and J.R. Maxwell, *Chem. Commun.*, 206 (1965).
25. B.K. Moza, J. Trojanek, V. Hanus, and L. Dolejs, *Collect. Czech. Chem. Commun.*, **29**, 1913 (1964).
26. M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).
27. N. Neuss, M. Gorman, W. Hargrove, N.J. Cone, K. Biemann, G. Büchi, and R.E. Manning, *J. Am. Chem. Soc.*, **86**, 1440 (1964).
28. S.S. Tafur, J.L. Occolowitz, T.K. Elzey, J.W. Paschal, and D.E. Dorman, *J. Org. Chem.*, **41**, 1001 (1976).
29. A. De Bruyn, L. De Taeye, and R. Simonds, *Bull. Soc. Chim. Belg.*, **90**, 185 (1981).
30. E. Wenkert, D.W. Cochran, E.W. Hagaman, F.M. Schnell, N. Neuss, A.S. Katner, P. Potier, C. Kan, M. Plat, M. Koch, H. Mehri, J. Poisson, N. Kunesch, and Y. Rolland, *J. Am. Chem. Soc.*, **95**, 4990 (1973).
31. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, 1976.
32. N. Neuss, *Bull. Chim. Soc. Fr.*, 1509 (1963).
33. N.J. Cone, R. Miller, and N. Neuss, *J. Pharm. Sci.*, **52**, 688 (1963).
34. E. Wenkert, E.W. Hagaman, H.Y. Wang, G.E. Gutowski, and J.C. Miller, *Heterocycles*, **15**, 255 (1981).
35. Y. Langlois, N. Langlois, P. Mangeney, and P. Potier, *Tetrahedron Letts.*, 3945 (1976).
36. J.P. Kutney and B.R. Worth, *Heterocycles*, **4**, 1777 (1976).
37. J.P. Kutney, A.V. Joshua, P.H. Liao, and B.R. Worth, *Can. J. Chem.*, **55**, 3235 (1977).
38. N. Neuss, D.S. Fukuda, G.E. Mallet, D.R. Brannon, and L.L. Huckstep, *Helv. Chim. Acta.*, **56**, 2418 (1973).
39. N. Langlois, R.Z. Andriamialisoa, and N. Neuss, *Helv. Chim. Acta.*, **63**, 793 (1980).
40. M. Gorman and J. Sweeney, "Abstracts, IUPAC Symposium," Kyoto, Japan, 1964, p 99.
41. L.N. Prista, M.A. Ferreira, and A.S. Roque, *Garcia Orta.*, **12**, 277 (1964); *Chem. Abstr.*, **64**, 3959e (1966).
42. S. Kohlmunzer and H. Tomczyk, *Diss. Pharm. Pharmacol.*, **19**, 213 (1967); *Chem. Abstr.*, **67**, 29850w (1967).
43. N. Langlois and P. Potier, *Phytochemistry*, **11**, 2617 (1972).
44. A. De Bruyn, L. De Taeye, and M.J.O. Anteunis, *Bull. Soc. Chim. Belg.*, **89**, 629 (1980).
45. G.A. Cordell, "Introduction to Alkaloids—A Biogenetic Approach," Wiley-Interscience, New York, 1981.

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