CATHARANTHUS ALKALOIDS. XXXIV.[1] CATHARANTHAMINE, A NEW ANTITUMOR BISINDOLE ALKALOID FROM *CATHARANTHUS ROSEUS*

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ABSTRACT.—The isolation and structure elucidation of catharanthamine (**3**), a new bisindole alkaloid with antitumor activity from *Catharanthus roseus*, is described.

Vincaleukoblastine (VLB) and leurocristine (VCR) are the most important bisindole alkaloids for the clinical management of many cancerous states (2, 3), yet there is still a pressing need for new, more active, and less toxic agents. It was with this aim in mind that we began to investigate a number of highly active alkaloid fractions of *Catharanthus roseus* (L.) G. Don (Apocynaceae) which did not contain any of the major antitumor alkaloids from this plant.

TABLE 1. Anticancer activity of the gradient pH alkaloid fractions prepared from the post-VCR column fraction F-010.

Gradient pH fraction	P-388 Lymphocytic Leukemia		9KB Cytotoxic activity
	Dose (mg/kg)	T/C (%)[a]	ED_{50} (μg/ml)[b]
2.7	10	100	25
	5	94	
	2.5	100	
4.0	20	144	3.6
	10	133	
4.5	10	144	3.1
	5	135	
	2.5	148	
5.0	20	250	0.42
	10	216	
	5	160	
5.5	10	241	0.045
	5	194	
	2.5	216	
9.0	10	183	0.15
	5	210	
	2.5	175	

[a]A T/C 125% is considered active (7).
[b]ED_{50} of 20 μg/ml is considered active (7).

A post-VCR alkaloid chromatographic fraction obtained through the courtesy of the Eli Lilly Co. (5) was subjected to pH gradient fractionation (6). Evaluation of the anticancer activity *in vivo* and *in vitro*[3] indicated that activity was concentrated in the pH 5.0, pH 5.5 and pH 9.0 fractions (table 1). Extensive chromato-

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[1]For part XXXIII, see ref. 1.
[3]Fractions and compounds were evaluated for anticancer activity according to established protocols (ref. 7).

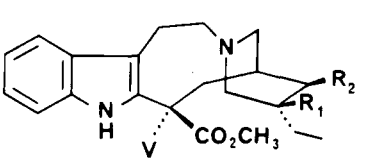
graphic separation has been made of these fractions (8) with concommitant bioassay. One of the biologically active alkaloids isolated during the course of this work, catharanthamine, is the subject of the present disclosure. This is the first bisindole alkaloid having oxygenation at C-17 of the velbanamine unit to be isolated and is, therefore, of considerable biogenetic interest.

EXPERIMENTAL[4]

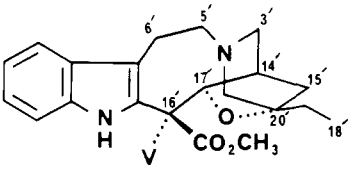
PLANT MATERIAL AND INITIAL FRACTIONATION.—The origin, identification, extraction, and initial separation of the alkaloid fractions have been described previously (5).

pH GRADIENT FRACTIONATION OF THE POST-VCR COLUMN FRACTION F-010.—The alkaloid fraction F-010 (200 gm) was macerated with 2% tartaric acid solution (1 liters) overnight. After filtration, partition with ethyl acetate (3 x 2 liters) was effected to yield a pH 2.7 alkaloid fraction. The aqueous phase was then adjusted to pH 4.0 (pH meter) with ammonia solution (28%) and a further extraction made with ethyl acetate (3 x 2 liters) to afford a pH 4.0 alkaloid fraction. Repetition of this procedure afforded alkaloid fractions corresponding to the pHs 4.5, 5.0, 5.5 and 9.0 (table 1). Samples of these fractions were submitted for biological evaluation, because of the results (table 1), further work was conducted on the pH fractions 5.0, 5.5 and 9.0.

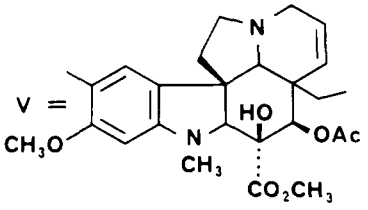
CHROMATOGRAPHIC SEPARATION OF THE PH 5.5 GRADIENT FRACTION OF F-010.—A sample (15.8 gm) of the pH 5.5 alkaloid fraction adsorbed on silica gel PF-254[5] (20 gm) was applied to the top of a prepacked column (5.5 cm. diam.) of silica gel PF-254 (600 gm) packed in benzene-chloroform (1:1) and eluted with this solvent system (1 liter) and chloroform (1 liter). Elution was then conducted with chloroform: 5% methanol (100 ml fractions) and from combined fractions 58–67, a pure, amorphous, alkaloid precipitated slowly on treatment with methanol. With the CAS reagent it exhibited a yellow to greenish-yellow spot with a brown periphery which changed after about two minutes to greyish-white surrounded by a reddish-brown tinge. It displayed the following spectroscopic properties: ir, ν max (KBr) 3400 (s, NH and OH), 2910 (w), 1740 (s, ester CO), 1615 (m, indoline), 1510 (m), 1465 (m), 1240 (s, CO_2CH_3), 1155 (w), 1040 (m) and 745 (m, 1,2-disubstituted benzene) cm^{-1}; uv λ max (MeOH) (log ϵ) 213 (3.76), 223 (3.71), 265 (3.34) and 295 nm (3.04); pmr, δ ($CDCl_3$) 0.68 (t, J=7 Hz, 3H, 18-CH_3), 1.02 (t, J= 7 Hz, 3H, 18'-CH_3), 2.12 (s, 3H, 17-$OCOCH_3$), 2.78 (s, 3H, N-CH_3), 3.51 (s, 3H, 16'-CO_2CH_3)



1 R_1 = OH, R_2 = H
2 R_1, R_2 = —O—



3



[4]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 spectrophotometer, and ir spectra with a Beckman, model IR 18A, spectrophotometer. Proton nmr spectra were recorded in $CDCl_3$ on a Varian T60A instrument with a Nicolet TT-7 Fourier Transform attachment, operating at 60 MHz, and carbon nmr spectra were recorded in $CDCl_3$ with a Varian HA-100 instrument at 25.15 MHz. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ units.

Low resolution mass spectra were obtained at 70 ev on an AEI MS 902 double focusing spectrometer and high resolution spectra on a Varian MAT 311A instrument operating at 70 ev.

[5]E. Merck, Darmstadt, Germany.

3.70 (s, 3H, 16-CO_2CH_3), 3.81 (s, 3H, 11-OCH_3), 3.88 (s, 1H, 2-H), 4.36 (m, 1H, 17'-H), 4.98 (d, J=15 Hz, 1H, 15-H), 5.45 (s, 1H, 17-H), 5.89 (m, 1H, 14-H), 6.14 (s, 1H, 12-H), 7.03 (m, 4H, 9, 9', 10' and 11'-H), 7.67 (m, 1H, 12'-H) and 8.20 (s, 1H, N-H); cmr, see table 2; ms, *m/e* 808 (M^+, 8.9), 792 (14.2), 766 (7.0), 753 (7.1), 752 (14.2), 734 (7.1), 648 (7.1), 527 (64.2), 469 (14.2), 467 (10.7), 451 (7.1), 368 (17.8), 296 (14.1), 289 (42.8), 270 (17.8), 260 (25.0), 256 (42.8), 143 (96.4), 135 (100) and 121 (78.5). Mass measurement, Obsd. 808.4030, Calcd. for $C_{46}H_{56}N_4O_9$ 808.4046.

STRUCTURE ELUCIDATION OF CATHARANTHAMINE (3).—The uv spectrum of the isolate indicated the presence of both indole and dihydroindole moieties (9), and the ir spectrum showed the presence of NH or OH (ν max 3400 cm^{-1}) and saturated ester (1740 cm^{-1}) groups. Importantly, no additional carbonyl absorptions were observed. Typical proton nmr resonances were evident for a substituted vindoline moiety possessing an acetyl methyl (2.12 ppm), *N*-methyl (2.78 ppm), aromatic methoxy (3.81 ppm) and a carbomethoxyl group (3.70 ppm) (10).

Substitution on the vindoline moiety was established to be at the 10-position from the observation of a singlet for the C-12 proton at 6.14 ppm. Unlike other alkaloids in the vinblastine series, however, the C-9 proton was deshielded into the region of the indolic aromatic protons.

It was the ^{13}C nmr spectrum (table 2) which firmly established the 10-vindolinyl moiety

TABLE 2. Comparison of the carbon-13 nuclear magnetic resonance spectrum of catharanthamine (3) and vincaleukoblastine (1).

Dihydroindole unit			Indole unit		
Carbon	Chemical shift[a]		Carbon	Chemical shift	
	3	1[b]		3	1[b]
2	83.48	83.30	2'	131.20	131.4
3	50.50	50.20	3'	48.60	48.0
5	51.20	50.20	5'	55.80	55.8
6	44.71	44.60	6'	26.84	28.2
7	53.20	53.20	7'	117.55	117.0
8	123.12	122.60	8'	129.07	129.5
9	123.67	23.50	9'	117.76	118.4
10	121.43	121.10	10'	123.34	122.1
11	157.80	158.00	11'	119.10	118.7
12	93.19	94.20	12'	110.33	110.4
13	153.55	152.50	13'	134.52	135.0
14	124.10	124.40	14'	38.01	30.1
15	130.20	129.90	15'	43.68	41.4
16	79.50	79.70	16'	58.06	55.8
17	76.35	76.40	17'	65.64	34.4
18	8.30	8.30	18'	6.80	6.9
19	30.76	30.80	19'	33.36	34.4
20	42.74	42.70	20'	69.40	69.4
21	66.64	65.50	21'	65.80	64.2
$COOCH_3$	170.69	170.80	$COOCH_3$	171.00	174.9
$COOCH_3$	52.14	52.10	$COOCH_3$	52.17	52.3
N-CH_3	38.28	38.30			
Ar-OCH_3	55.33	55.80			
$OCOCH_3$	171.82	171.6			
$OCOCH_3$	21.05	21.1			

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

[b]Assignments reported previously for VLB (13).

on comparison with the data obtained for the vindolinyl moiety of vincaleukoblastine (1) (12, 13). From the proton nmr spectrum it was apparent (triplets at 0.68 and 1.02 ppm) that the ethyl side chains in the indole and dihydroindole units had remained intact, and this, too, was confirmed by the carbon-13 spectrum which showed characteristic signals at 8.30 and 6.90 ppm for C-18 and C-18'. Three carbonyl carbons were observed, in agreement with the presence of one acetyl group (171.82 ppm) and two carbomethoxyl groups (170.69 and 171.0

ppm). The latter signal was shielded by 3.9 ppm compared with the corresponding signal in VLB, a point of some importance, as will subsequently be discussed.

With the establishment of all of the carbon atoms of the vindolinyl moiety, attention focused on determining the nature of the substitution in the indole half of the molecule. Bearing in mind the molecular formula, it was apparent that compared with VLB, the molecule contained an additional degree of unsaturation. Potentially this could be in the form of either a double bond, a carbonyl group, or an ether linkage. The first two possibilities were categorically eliminated by examination of the carbon-13 spectrum, for no additional carbon resonances were observed in the regions 95–155 ppm and 165–210 ppm, respectively. The key to the structure was therefore placement of the two terminal points of an ether linkage.

Typical methylene resonances were observed for C-3', C-5', C-6', C-15', C-19' and C-21', the latter being the characteristic most-downfield amino methylene group (12, 13). Substitution of C-20' by oxygen is supported by an identical chemical shift for this carbon atom compared with VLB and a corresponding γ-effect exerted on C-18 which is shielded to 6.8 ppm (12). Elimination of a second quaternary ether linkage could be achieved by examination of the carbon-13 spectrum, leaving possible sites of the ether linkage as C-15' or C-17'. An epoxide function (attachment to C-15') was excluded by the chemical shifts of C-20' and C-18' on comparison with those in leurosine (**2**) (table 3), and the chemical shift of the C-15' resonance (43.6 ppm) which was similar to that in VLB.

A resonance, attributable to C-17', at 65.6 ppm suggested that this carbon was the site of the second carbon-oxygen bond. Typically (table 3), this carbon appears in the region of

TABLE 3. ^{13}C-Chemical shifts of selected carbons in alkaloids related to vincaleukoblastine.

Compound Name	C-15'	C-16'	C-17'	C-18'	C-20'	Ref.
Vincaleukoblastine	41.4	55.8	34.4	6.9	69.4	13
Leurocolumbine	50.5	54.3	40.7	6.9	69.9	13
Desacetoxy-VLB	41.6	55.7	34.5	6.9	69.7	13
Des-*N*-methyl-VLB	41.6	55.9	34.5	6.9	69.8	13
Leurosine	60.3	55.0	30.7	8.1	59.8	12
Vincadioline	75.2	55.8	32.8	6.2	71.3	13
Catharanthamine	43.7	58.1	65.6	6.8	69.4	

30–40 ppm in compounds in the VLB series and its strong deshielding (by about 30 ppm) in the isolate indicated attachment to an oxygen heteroatom. The chemical shifts of some of the neighboring carbons compared with those in VLB can now be rationalized.

Carbon-14' experiences a pronounced (8 ppm) β-effect as a consequence of C-17' substitution and C-16' is also similarly deshielded (to 58.06 ppm). On the other hand the carbomethoxyl carbon experiences a γ-effect (−3.9 ppm), as we have already seen, appearing at 171.0 ppm. The deshielding of C-15' (by 2.28 ppm) is explicable in terms of a gauche intertion of the ether oxygen and this carbon atom.

In summary, the carbon-13 nmr data strongly support an ether linkage between C-17' and C-20'. The multiplet observed in the proton nmr spectrum at 4.36 ppm can now be attributed to the single remaining proton at C-17'. This novel alkaloid, which is tentatively suggested to have the structure **3**, has been assigned the trivial name catharanthamine.

BIOLOGICAL ACTIVITY OF THE ISOLATE.—Catharanthamine (NSC-268275) was cytotoxic in the KB test system *in vitro* and displayed significant (T/C 176% at 1 mg/kg) activity in the P-388 lymphocytic leukemia test system.

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