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VINCA ALKALOIDS. XXX (1). CHEMISTRY OF THE DEOXYVINBLASTINES (DEOXY-VLB), LEUROSINE (VLR), AND PLEUROSINE, DIMERIC ALKALOIDS FROM VINCA ROSEA LINN. (CATHARANTHUS ROSEUS G. DON)

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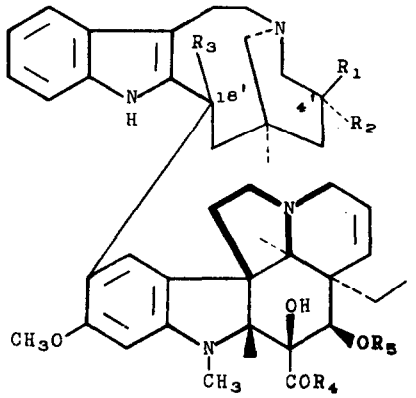
In the course of our investigations of the plant Vinca rosea Linn., several dimeric alkaloids have been isolated (2). Structures of oncolytic dimeric indole-indoline alkaloids vincaleukoblastine (VLB),* leurocristine (VCR)* and leurosidine (VRD)* (3,4,5,1) have been established. During the process of preparing oncolytic alkaloids, it was found that one of these, leurosine (VLR),* is always accompanied by substantial amounts of another alkaloid, called isoleurosine, believed to be isomeric with leurosine (6). However, on the basis of new data, it was shown that leurosine is a $C_{46}H_{58}O_9N_4$ (I) and isoleurosine, now designated as deoxyVLB "A", a $C_{46}H_{58}O_8N_4$ compound (II). The elemental composition of this alkaloid was substantiated by high resolution mass spectrum of the corresponding 18'-decarbomethoxy-4-deacetyl monohydrazide (III) which does not undergo internal methylation when introduced into the ion source (4). The acid cleavage of III (conc. HCl, $SnCl_2$ and Sn, reflux) afforded a good yield of deacetyl vindoline hydrazide (IV) (4) and 4α-dihydrocleavamine (V) (7). Since the signals of aromatic protons in the NMR spectrum of deoxyVLB are found in the typical 1,4 pattern at δ = 6.16 and 6.70 as in VLB, VCR

*A.M.A. approved generic names are vinblastine (VLB), vincristine (VCR), vinrosidine (VRD), and vinleurosine (VLR), respectively. VCR is supplied as ONCOVIN (vincristine sulfate, Lilly) and VLB as VELBAN (vinblastine sulfate, Lilly).

and VRD (3), the mode of attachment in the vindoline moiety is the same as in these alkaloids, and the structure of deoxyVLB "A" is as shown in II.

Treatment of leurosine with prehydrogenated Raney Nickel (W1) in refluxing absolute alcohol affords a new compound, called deoxyVLB "B" (VI), accompanied by varying amounts of II. These are separated by gradient pH extraction, followed by column chromatography. By acidic cleavage of deoxyVLB "B" or its deacetyldecarbomethoxy hydrazide (VII), there were obtained the appropriate vindoline derivatives and 4-β-dihydrocleavamine (VIII) (7). Therefore, deoxyVLB "B" (VI) differs from deoxyVLB "A" (II) in the configuration at C-4'. The partial synthesis of VII was attempted by reacting 4-β-dihydrocleavamine-chloroindolenine (IX) (8,9) with IV in 1.5% HCl, in analogy to the preparation of dihydrovoacamine (10). The



II Deoxy VLB "A"
R_1=H, R_2=C_2H_5, R_3=$COOCH_3$, R_4=OCH_3, R_5=$COCH_3$

III Decarbomethoxy Deacetyl Deoxy VLB "A" Hydrazide
R_1=R_3=R_5=H, R_2=C_2H_5, R_4=$NHNH_2$

VI Deoxy VLB "B"
R_1=C_2H_5, R_2=H, R_3=$COOCH_3$, R_4=OCH_3, R_5=$COCH_3$

VII Decarbomethoxy Deacetyl Deoxy VLB "B" Hydrazide
R_1=C_2H_5, R_2=R_3=R_5=H, R_4=$NHNH_2$

resulting dimer was purified by gradient pH extraction followed by chromatography on alumina and was shown to be a $C_{41}H_{54}O_4N_6$ compound isomeric with III and VII. M^+, Calcd.: 694.42468. Found: 694.42333 (high resolution mass spectrum). In the NMR spectrum of this compound, designated deacetyldecarbomethoxy deoxyVLB "C" hydrazide (X), there is a doublet centered at δ = 4.5, J = 10 cps, attributable to the C-18' proton found upfield in other hydrazides. All other signals are very similar to those found in the NMR spectra of III and VII. Therefore, deoxyVLB "C" differs from deoxyVLB "B" in the stereochemistry at C-18'. While attempts to isomerize C-18' in the deoxyVLB hydrazide

series failed, it was possible to convert decarbomethoxy deacetyl VLB hydrazide (XI) (4) to its C-18' epimer in refluxing 1.5% HCl. This new $C_{41}H_{54}O_5N_6$ compound displayed the same NMR spectral shift as X (doublet centered at δ = 4.6, J = 10 cps) attributable to the C-18' proton and is, therefore, designated 18'-isodeacetyl decarbomethoxy VLB hydrazide (XII). Some of the physical properties of the above-mentioned compounds have been summarized in Table I.

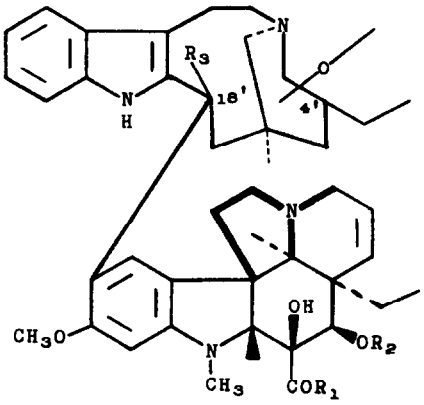
TABLE I

COMPARISON OF HYDRAZIDES OF DIMERIC ALKALOIDS

	Compound	$[\alpha]_D^{26}$	pK'a	Empirical Formula
III	DeoxyVLB A hydrazide*	+15.5°	5.5 and 6.8	$C_{41}H_{54}O_4N_6$
VII	DeoxyVLB B hydrazide*	-10.9°	5.8 and 8.2	$C_{41}H_{54}O_4N_6$
X	DeoxyVLB C hydrazide*	-47.2°	6.3 and 8.8	$C_{41}H_{54}O_4N_6$
XI	VLB hydrazide*	+40.8°	5.6 and 6.8	$C_{41}H_{54}O_5N_6$
XII	Iso-VLB hydrazide*	-92.8°	5.7 and 8.4	$C_{41}H_{54}O_5N_6$
XIII	Leurosine hydrazone*	+2.4°	4.8 and 6.2	$C_{44}H_{56}O_5N_6$
XIV	Deacetyl leurosine hydrazide	+117.9°	5.2 and 6.95	$C_{43}H_{54}O_7N_6$

*Unless mentioned otherwise, all hydrazides and the hydrazone are decarbomethoxydeacetyl compounds. All compounds melt with decomposition over a wide range of temperatures depending upon the rate of heating. Rotations were recorded in a 1% chloroform solution. Microanalyses and high resolution mass spectra gave satisfactory values for these formulae.

The $C_{46}H_{56}O_8N_4$ formula of leurosine was confirmed by analyses of several derivatives and salts as well as high resolution mass spectral studies on decarbomethoxy deacetyl leurosine isopropylidene hydrazone (XIII) (obtained by recrystallization of the hydrazide from aqueous acetone), deacetyl-leurosine hydrazide (XIV), and leurosine itself. Acid cleavage of leurosine or preferably its hydrazide afforded cleavamine (XV) together with deacetylvindoline (XVI) and its hydrazide (IV), respectively (3).



I Leurosine
R_1=OCH_3, R_2=$COCH_3$, R_3=$COOCH_3$

XII Decarbomethoxydeacetyl Leurosine Isopropylidene Hydrazone
R_1=$NHN{=}C(CH_3)_2$, R_2=R_3=H

XIII Deacetyl Leurosine Hydrazide
R_1=$NHNH_2$, R_2=H, R_3=$COOCH_3$

In the NMR spectrum of leurosine, aromatic protons are distributed again in a 1,4 ratio (vide supra) indicative of a similar mode of attachment in the vindoline moiety. Isolation of cleavamine does not account for the additional oxygen which must be present in the indole moiety of the parent alkaloid as an oxide [the presence of only one hydroxyl in the molecule (vindoline moiety) was substantiated by the preparation of an o-acetate (Ac_2O in Py, or ketene)]. The strongest mass spectral peak in the fragmentation pattern of dihydrocleavamine (11,12) or its moiety in III, VII and X is found at m/e 138 and corresponds to $C_9H_{16}N^+$. Calcd.: 138.12827. Found: 138.12844. The corresponding fragment from leurosine or its hydrazide is found at m/e 152, $C_9H_{14}ON^+$. Calcd.: 152.10833. Found: 152.10812 and 152.10754, respectively. This ion obviously includes the oxide function. Therefore, the partial structure of leurosine must be as shown in I.

Another dimeric alkaloid pleurosine (2), $C_{46}H_{56}O_{10}N_4$, is characterized by an I.R. spectrum virtually identical with that of leurosine, however, the pK'a value of the most basic N_b is two units lower (from 7.5 to 5.5). Treatment of pleurosine with Zn and acetic acid at room temperature afforded a quantitative yield of leurosine. Therefore, pleurosine is the N_b-oxide of leurosine.

It is interesting to note that while leurosine and its quaternary salts (13) have shown significant activities in a variety of experimental

tumors, the clinical response to these agents has been rather limited (2). Other related alkaloids mentioned above have not shown any reproducible activity in experimental tumors.

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