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VINCA ALKALOIDS. XXIX (1). STRUCTURE OF LEUROSIDINE (VINROSIDINE, VRD), AN ONCOLYTIC ALKALOID FROM VINCA ROSEA LINN. (CATHARANTHUS ROSEUS G. DON)

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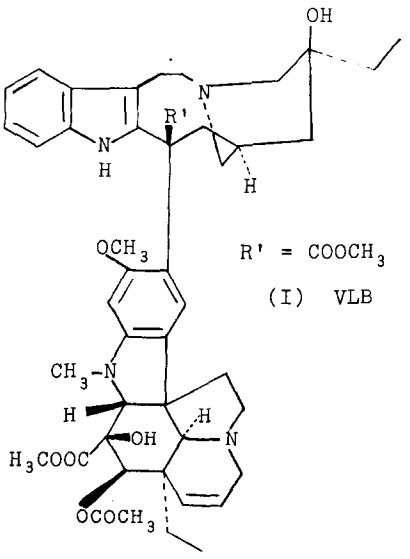
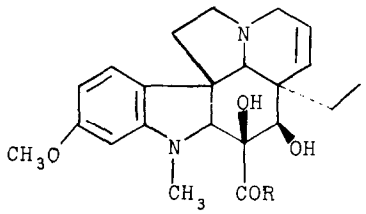
The unique biological properties of leurosine (2), leurosidine (3), leurocristine (3) and vincaleukoblastine (4), dimeric indole-indoline alkaloids from Vinca rosea Linn. (Catharanthus roseus G. Don) have been thoroughly reviewed (5). Two of these compound, leurocristine and vincaleukoblastine* have been introduced as therapeutically useful agents for the treatment of various human neoplasms.

The structures of VLB and VCR have been elucidated (6), and the stereochemistry including the absolute configuration determined by x-ray analysis of vincristine methiodide (7). S nce vincristine has been correlated with vinblastine (6), its stereochemistry is also established (I).



R' = $COOCH_3$

(I) VLB

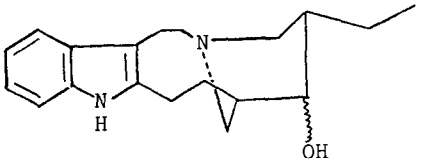


(II) R = OCH_3 Deacetylvindoline

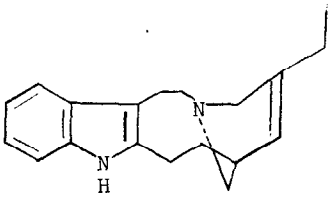
(V) R = $NHNH_2$ Deacetylvindoline-hydrazide

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

The physical properties of leurosidine (VRC) have been reported (3) but no empirical formula was suggested. The spectral data (UV, IR, and NMR), functional group determination, elemental analyses, and mass spectral data of leurosidine and its derivatives are in agreement with formulation of this alkaloid as a $C_{46}H_{58}O_9N_4$ compound, isomeric with vinblastine. The close similarity of leurosidine and vinblastine was corroborated by the identification of the cleavage products of the former using concentrated hydrochloric acid and stannous chloride with tin metal. These were shown to be deacetylvindoline (II) and minute amounts of a new, hydroxyl containing tetracyclic indole derivative, called vinrosamine, $M^+ = 298$, $C_{19}H_{16}ON_2$ (III) as well as cleavamine (IV).



(III) Vinrosamine



(IV) Cleavamine

The isolation of deacetyl vindoline establishes the identity of the dihydroindole moiety of leurosidine and demonstrates that the latter is the same as in VLB. Therefore, the difference between the two alkaloids resides in the indole portion of the molecule.

The higher value (1.4 units) of the second pK'_a of leurosidine (Table I) indicates that the second hydroxyl, located in the indole portion of the molecule, must be in a different environment in relation to N_b than in VLB. Accordingly, the hydroxyl band in the infrared spectrum of VLB is found at 2.80 μ, while the corresponding band in the spectrum of VRD is at 2.72 μ. Leurosidine forms readily triacetate ester with acetic anhydride in pyridine at room temperature. The compound crystallizes from aqueous

methanol and melts at 154-519° (dec.), $[\alpha]^{26}$ = +12.6° ($CHCl_3$). Calcd. for $C_{50}H_{62}O_{11}N_4$: C, 67.09; H, 6.98; N, 6.26. Found: C, 67.01; H, 7.11; N, 6.08. The ease of formation of leurosidine triacetate suggests that the hydroxyl is secondary rather than tertiary as in VLB which requires ketene in benzene for the formation of the corresponding ester in a poor yield. The comparison of chemical shifts of the methyl signals of respective acetyl functions of the ester (Table I) demonstrates clearly the different environment of the acetyl function in the indole moiety of VLB and leurosidine. On the basis of these considerations and physical properties of vinrosamine (vide infra!), the most likely position of the hydroxyl is at C-3'. The isolation of cleavamine (IV) from the mixture of compounds resulting from the cleavage of VRD is also consonant with this assignment.

TABLE I

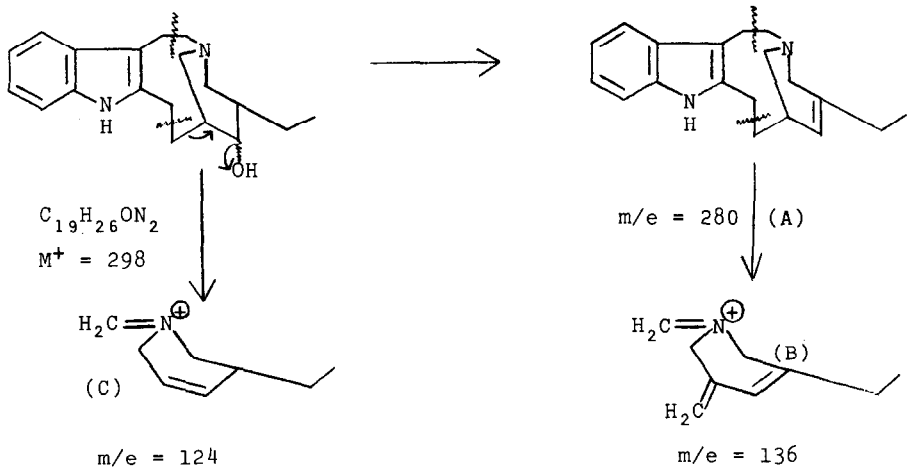
	Leurosidine (VRD) $C_{46}H_{58}O_9N_4$	Vinblastine (VLB) $C_{46}H_{58}O_9N_4$
m.p.	208-211° (dec.	211-216° (dec.) (CH_3OH)
pK'_a (33% DMF)	5.4, 8.8	5.4, 7.4
$[\alpha]_D^{25}$	+55.8° ($CHCl_3$)	+42° ($CHCl_3$)
δ CH_3 (acetyl)	2.10	2.10
	VRD Acetate (tri) $C_{50}H_{62}O_{11}N_4$	VLB Acetate (tri) $C_{50}H_{62}O_{11}N_4$
m.p.	154-159° (dec.)	168-170° (dec.)
$[\alpha]_D^{25}$	+12.6°	-26.4°
δ CH_3 (acetyl)	1.96 (2), 2.03	1.98, 2.08, 2.38
	LSR Acetate (di)	VLB Acetate (di)
δ CH_3 (acetyl)	was not prepared	1.96, 2.08

Vinrosamine is obtained (yield varies from 5-15%) from the cleavage reaction of leurosidinehydrazide (V) which is prepared by reaction of the alkaloid with anhydrous hydrazine in absolute methanol (4 days, reflux). The hydrazide crystallizes from aqueous methanol and melts at 214-219° (dec.), $[\alpha]_D^{25}$ = +20.2° ($CHCl_3$). Calcd. for $C_{41}H_{54}O_5N_6$: C, 69.35; H, 7.76; N, 11.98. Found: C, 69.08; H, 7.68; N, 11.95. M^+, Calcd.: 710.41557. Found: 710.41785. The cleavage of the hydrazide under acidic conditions (HCl, $SnCl_2$, tin metal) affords deacetylvindolinehydrazide, cleavamine, and vinrosamine. The new indole compound crystallizes from methanol and melts at 168-172°. $C_{19}H_{26}ON_2$, M^+ Calcd.: 298.2045. Found: 298.2020. Unlike in velbanamine (6), the mass spectrum of vinrosamine is characterized by two most abundant peaks at m/e 280 ($C_{19}H_{24}N_2$, Calcd.: 280.1939; Found: 280.1944) and m/e 124 ($C_8H_{14}N$, Calcd.: 124.1152; Found: 124.1126).

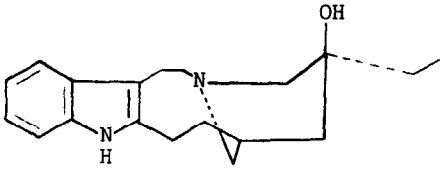
The fragmentation patterns for some of the peaks are shown on Chart 1 and are in agreement with those suggested for related compounds (8).

CHART 1

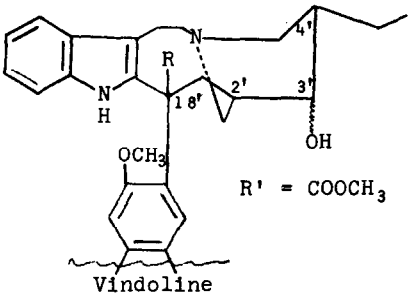
Fragmentation Mechanism of Vinrosamine



The hydroxyl absorption in the infrared spectrum of velbanamine (VI)



(VI) Velbanamine



(VII) Leurosidine

at 0.2 M in chloroform solution is divided between a narrow band, underlying the band for free indole NH at 2.90 μ (intramolecular hydrogen bonding), and a broader band at 3.05 μ (intermolecular hydrogen bonding). In contrast to this spectral change, vinrosamine at 0.2 M concentration in chloroform shows almost entirely free hydroxyl with a band at 2.78 μ and a very weak band at 3.0 μ for the intermolecular form.

Treatment of vinrosamine with acetic anhydride in pyridine affords an acetate, which could not be crystallized. Its NMR spectrum is characterized by acetyl methyl signal at δ = 1.94 with only a very small shift in the NMR spectrum in the region between 3.9-5.5 δ. The corresponding signal in leurosidine is found at δ = 1.96. The mass spectrum of vinrosamine acetate, M^+ = 340, is characterized by fragments of m/e 298, 156, 154, 143, 136. 130, and 122 quite typical for tetracyclic indole derivatives of this type (8). Lastly, it should be mentioned that leurosidine undergoes Moffat oxidation (9) to the corresponding keto derivative (at C-3'). The physical and chemical properties of this compound are also in agreement with the position of hydroxyl at C-3'.

On the basis of these data, the structure of leurosidine is suggested to be as shown (VII). The hydroxyl at C-3' is probably α-oriented since in a Dreiding model of vinrosamine with the same relative stereochemistry of the ethyl group as in velbanamine, α-oriented hydroxyl at C-3 presents much more hindrance to approach for intermolecular hydrogen bonding than in the presence of β-oriented hydroxyl.

Acknowledgement

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