

205. *Vinca* Alkaloids XXXIV¹⁾.

Preparation of Des-N_a-methyl-vindoline by Microbiological Conversion of Vindoline, a Major Alkaloid from *Vinca rosea* L.

by Norbert Neuss, D. S. Fukuda, D. R. Brannon and L. L. Huckstep

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206 U.S.A.

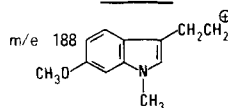
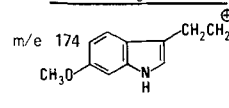
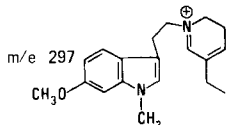
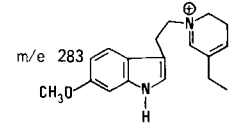
(19. VIII. 74)

Summary. The conversion of vindoline (II) using *S. albogriseolus* led to the isolation of several compounds including des-N_a-methyl vindoline (III) in 8–10% yield.

In an earlier publication [2] from these laboratories we have reported on the isolation of several vindoline ethers (*inter al.* I) formed by the microbiological conversion of vindoline (II) using *Streptomyces* sp. We have now been able to demethylate vindoline using *S. albogriseolus* and obtained des-N_a-methyl-vindoline (III) in 8–10% yield.

The conversion was accomplished as described before [2] using *S. albogriseolus* (A17178, NRLL 5748) and allowed to proceed for five days. After harvesting, an

COMPARISON OF HIGH RESOLUTION MASS SPECTRAL DATA

<p>Vindoline</p>  <p>m/e 188</p> <p>$C_{12}H_{14}NO$</p> <p>Calcd.: 188.1075 Found: 188.1083</p>	<p>Des-N-CH₃-vindoline</p>  <p>m/e 174</p> <p>$C_{11}H_{12}NO$</p> <p>Calcd.: 174.0919 Found: 174.0917</p>
 <p>m/e 297</p> <p>$C_{19}H_{25}N_2O$</p> <p>Calcd.: 297.1967 Found: 297.1959</p>	 <p>m/e 283</p> <p>$C_{18}H_{23}N_2O$</p> <p>Calcd.: 283.1810 Found: 283.1793</p>
<p>m/e 397 M-COOCH₃</p> <p>$C_{23}H_{29}N_2O_4$</p> <p>Calcd.: 397.2127 Found: 397.2111</p>	<p>m/e 383 M-COOCH₃</p> <p>$C_{22}H_{27}N_2O_4$</p> <p>Calcd.: 383.1971 Found: 383.1968</p>
<p>M⁺ 465 C₂₅H₃₂N₂O₆</p> <p>Calcd.: 456.2260 Found: 456.2295</p>	<p>M⁺ 442 C₂₄H₃₀N₂O₆</p> <p>Calcd.: 442.2104 Found: 442.2085</p>

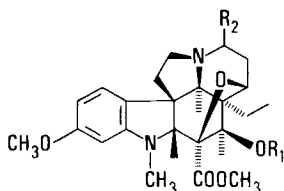
¹⁾ Paper XXXIII in this series see [1].

alkaloidal extract was prepared by conventional procedures. A solution of 2.0 g of this extract in benzene was chromatographed on a column (28 mm wide) using 100 g of silica (*Merck* 7729).

Fractions were collected and examined individually by TLC. using first 1.5 l of benzene, then 2.0 l of benzene/ethyl acetate 95:5, 400 ml of benzene/ethyl acetate 3:1 and finally 1.0 l of benzene/ethyl acetate 1:1 as eluents. Continuing the elution with 1.0 l of the same solvent mixture, eight fractions of approximately 100 ml each were collected and evaporated *in vacuo* (340 mg). After crystallization from ether 210 mg of colorless prisms, m.p. 102–104° were obtained.

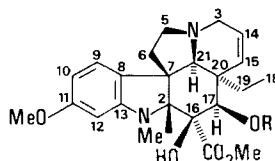
$C_{24}H_{30}N_2O_6$	Calc.	C 65.14	H 6.83	N 6.33%
	Found	64.90	6.53	6.15%

The IR. spectrum of the new derivative shows a typical NH band at 3420 cm^{-1} missing in the spectrum of vindoline.

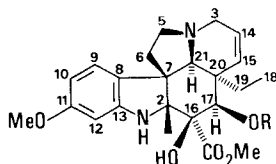


$R_1 = COCH_3$; $R_2 = CH_2COCH_3$; I

3-ACETONYL-DIHYDROVINDOLINE ETHER



$R = COCH_3$; VINDOLINE II



$R_1 = COCH_3$; DES-N- CH_3 -VINDOLINE III

The NMR. spectrum of des-N- CH_3 -vindoline in $CDCl_3$ is characterized by the lack of the N- CH_3 proton signal at $\delta = 2.68$ ppm and appearance of two new peaks: an NH doublet at $\delta = 4.59$ and C(2) proton doublet at 4.15 ppm. Exchange with D_2O resulted in the disappearance of the NH proton (OH at $\delta = 9.48$ has also been washed out) and collapse of the C(2) proton doublet to a singlet at $\delta = 4.13$ ppm. All other protons are quite similar in appearance and chemical shift to those present in the spectrum of vindoline [3]. Additional corroboration of the presence of des-N_a-methyl vindoline was furnished by high resolution mass spectral data and their comparison with those of vindoline (Table). A small sample of the new derivative was hydrogenated

for 7 hours in aqueous formaldehyde and CH_3OH with 5% Pd/C. The reaction mixture was evaporated, crystallized from ether and shown to be vindoline by comparison of TLC. and X-ray powder and mass spectral data with those of an authentic sample of vindoline.

REFERENCES

- [1] *Vinca* Alkaloids XXXIII; N. Neuss, G. E. Mallett, D. R. Brannon, J. A. Mabe & L. L. Huckstep, *Helv.* 57, 1886 (1974).
[2] N. Neuss, D. S. Fukuda, G. E. Mallett, D. R. Brannon & L. L. Huckstep, *Helv.* 56, 2418 (1973).
[3] M. Gorman, N. Neuss & K. Biemann, *J. Amer. chem. Soc.* 84, 1058 (1962).

Erratum

Helv. 57 (1974), Contribution Nr. 155 by F. Gerson *et al.*, pp. 1412 (5. line from the bottom) and 1413 (caption to Fig. 6) read: $E = \alpha - x\beta$ and not $E = \alpha + x\beta$.
