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204. *Vinca* Alkaloids XXXIII [1]. Microbiological Conversions of Vincaleukoblastine (VLB, Vinblastine), an Antitumor Alkaloid from *Vinca rosea*. LINN.

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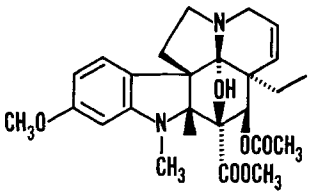
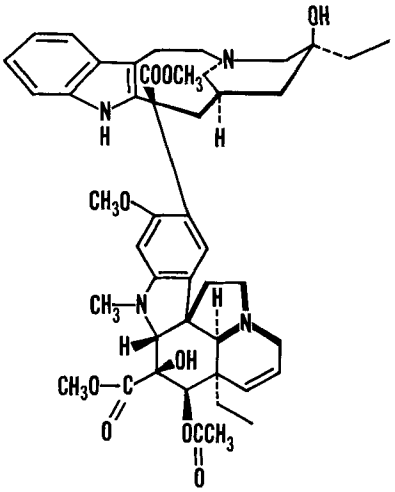
Summary. The conversion of VLB using different *Streptomycetes* led to the isolation of VLB-ether (**3**) and Hydroxy VLB (**6**). The structural assignments have been made by NMR. and high resolution mass spectral data.

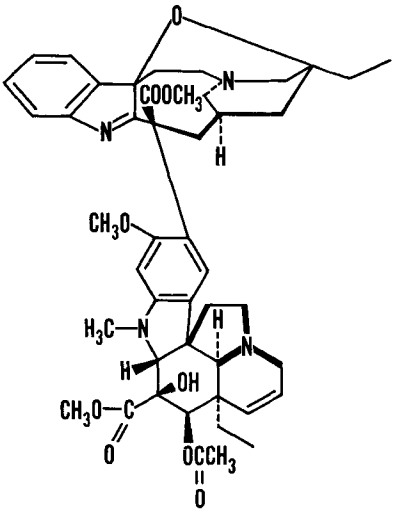
In a recent communication [1] from these laboratories we have reported some interesting transformations of one of the major alkaloid from *Vinca roesa* L., vindoline (**1**) using *S. albogriseolus*, A17178. Since Vincaleukoblastine [2] (Vinblastine, VLB) is an indole-indoline alkaloid with vindoline as the indoline moiety, we have subjected VLB (**2**) to microbiological conversions using the same organism. Examination of the alkaloidal extract using TLC., after six days of fermentation, indicated the presence of several new components in addition to unchanged VLB. One of the new metabolites was an indolenine-indoline compound (**3**). This new metabolite was shown to contain an ether linkage between C(7′) and C(20′) with rearrangement of the indole-portion to indolenine with the intact vindoline (indoline) moiety. These structural assignments could be made on the basis of IR., NMR. and MS. data. The absence of the NH band in the IR.-spectrum [3] and of the corresponding signal in the NMR.-spectrum was obvious. In addition, unlike in the NMR.-spectrum of VLB, the two methyl signals from the two ethyl groups were well separated and centered at $\delta = 0.90$ (*t*, 3H, vindoline portion) and at $\delta = 0.39$ ppm (*t*, 3H, indolenine moiety). The corresponding two methyl signals in the NMR.-spectrum of VLB are grouped together and are centered at $\delta = 0.90$ ppm (*t*, 6H). The presence of the unchanged



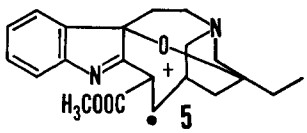
1 Vindoline



2 VLB



3 VLB-Ether



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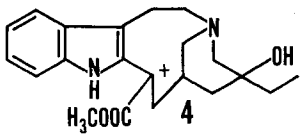

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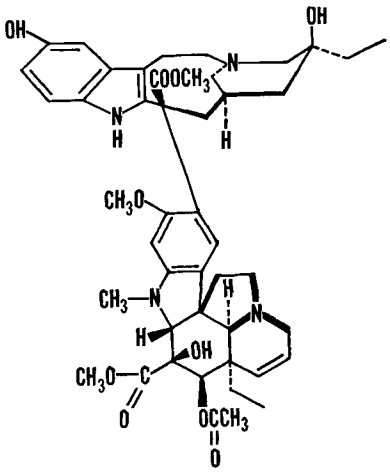


6 Hydroxy-VLB

hydroxyl in the vindoline moiety was indicated by an extremely broad signal at δ = 9–10 ppm and strongly hydrogen bonded, broad band in the IR.-spectrum at 2700–2800 cm^{-1}. Of greatest significance for the structure assignment **3** was the high resolution mass spectrum of the new metabolite with the molecular ion M^+ at

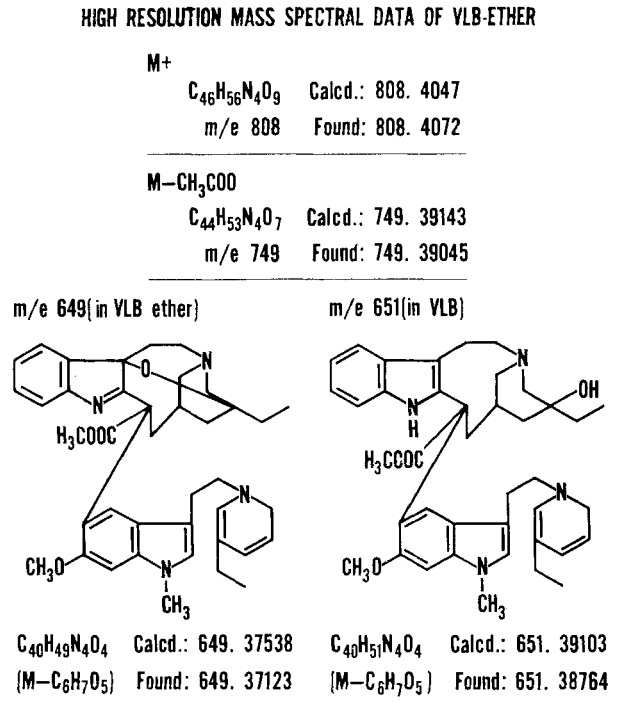


Fig. 1

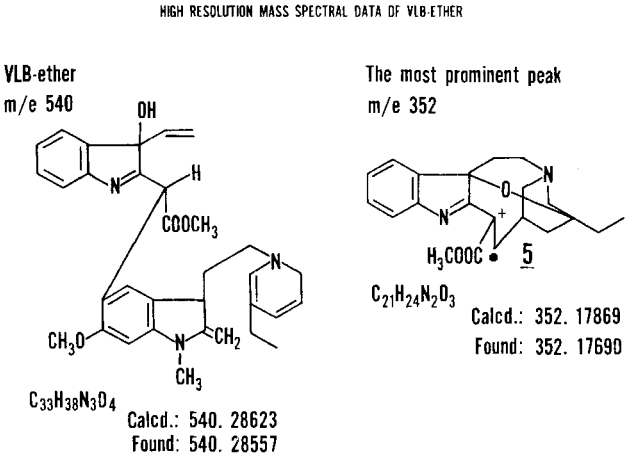
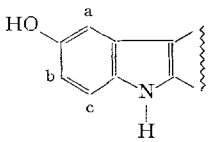


Fig. 2

808.4072. Calculated for $C_{46}H_{56}N_4O_8$. 808.4047. The absence of the *m/e* 355 ion (**4**) typical in the fragmentation pattern of VLB [2], and the appearance of the new ion at *m/e* 352 (**5**) in the spectrum of **3** was also of importance. These and other important fragments are illustrated in Fig. 1 and 2.

When VLB was subjected to the conversion using *S. panipalus* A36120, hydroxylation at C(10′) in the indole moiety of the alkaloid took place without any other changes in the molecule. This type of transformation **6** has been observed with several indole alkaloids using other organisms [4]. As in previously mentioned conversions (see Experimental Part) purification of the material was rather tedious, and although the metabolite was shown to be homogenous by TLC., it could not be induced to crystallize as a free base. Due to the scarcity of the material and its instability toward acids, formation of a crystalline salt has not been attempted.

The structure of the new compound was quite apparent from the NMR. and MS. data. In addition to the two signals from the two protons in the aromatic portion of vindoline moiety at δ = 6.62 and 6.12 ppm, there were also three signals H_a at δ = 6.91, H_b at 6.71 and H_c at 6.95 ppm with the J_{ab} of 8 Hz and J_{bc}, 2 Hz.



The mass spectrum of **6** showed, in addition to M^+ of 826, the transmethylation ion at *m/e* 840. The MS. of the metabolite esterified with diazomethane was characterized by M^+ of 840 and transmethylation products at *m/e* 854 and 868. Comparison of pertinent high resolution mass spectral fragments of VLB and 10-hydroxyderiva-

HIGH RESOLUTION MASS SPECTRAL DATA OF INDOLE FRAGMENTATION PRODUCTS OF VLB, HYDROXY-VLB AND METHOXY-VLB

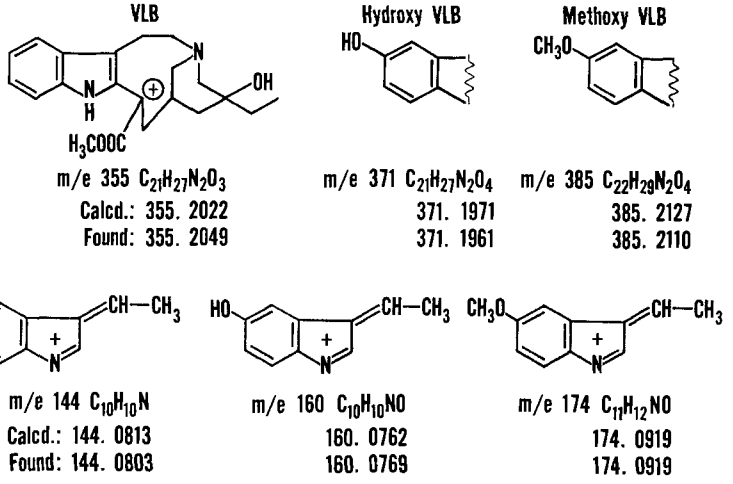


Fig. 3

tive was of particular significance as shown in Fig. 3. These data are consistent with structural assignments as portrayed in **6**.

Experimental Part

Proton Magnetic Resonance Spectra. The spectra in $CDCl_3$ were measured using Me_4Si as an internal standard and were recorded on *Varian* HA-100 MHz instrument.

High Resolution Mass Spectra. The spectra were recorded using a *CEC* high resolution model 21-110 instrument.

Microbiological Conversion. An appropriately prepared[1]) culture of A17178 and A36120 was incubated on a rotary shaker (250 rpm) at 30° and VLB was added at a concentration of 50 mg in 2 ml of acetone/water 1:1 per 500 ml flask after 24 h. The conversion was allowed to proceed for six days, except in the case of A36120 where it was terminated after five days. After that time the culture was harvested, filtered after adjusting the pH to 3.4, and made basic (pH 9–10), and unless otherwise mentioned, extracted with CH_2Cl_2.

VLB Ether (A17178). A total of 2.75 g of VLB sulfate, in ethanol, was added to fifty-five 1-l-flasks, each containing 100 ml of a 48 h culture of *Streptomyces sp.* A17178, grown in a complex medium containing the following constituents per l: cerelose 25, soluble starch 10, *Wilson*'s peptone 159 40, NZ Amine A 4, $MgSO_4 \cdot 7\ H_2O$ and Backstrap molasses 5. The medium was adjusted to pH 7.6 with KOH prior to autoclaving.

After an additional 120 h, the contents of the flasks were pooled and the pH of the whole broth adjusted to 3.0 with citric acid. After stirring for 2 h, the cells were removed by filtration and the pH of the filtrate adjusted to 9.5 by addition of ammonium hydroxide. The resulting solution was extracted with two portions of ethyl acetate. The ethyl acetate solutions were pooled, washed with water until neutral, and concentrated to give 1.8 g of non-crystalline material. TLC. (silica gel G, methanol/ethyl acetate 3:7, vanillin spray) showed a major component of Rf 0.57, compared to an Rf value of 0.27 for VLB in the same system. This material was chromatographed on a column of 100 g of *Merck* silica gel packed in ethyl acetate. Elution of the column with ethyl acetate gave 910 mg of material which precipitated upon addition of hexane. This material corresponded to the major component of Rf 0.57.

500 mg of the material was now chromatographed on 75 g of silica (*Merck* 7729) using first ethyl acetate as a solvent. After discarding 450 ml of the eluate, fractions of 25 ml were collected.

Fraction 4 appeared to be homogenous on TLC. and contained 60 mg. It could not be induced to crystallize and therefore was used for recording of physical and spectral data.

Hydroxy VLB (A36120). A solution of 4.7 g of the crude alkaloid extract in ethyl acetate was chromatographed on 200 g of silica (*Merck*, 7729; column 3.5 × 50 cm). After collecting 3.8 l of ethyl acetate, the elution was followed by 1.4 l of ethyl acetate/absolute alcohol 9:1.

Fractions resulting from elution using additional 2.8 l of the same solvent mixture were collected (Fractions 14–19) and gave 550 mg of crude metabolite. This material was dissolved in 50 ml of 0.1 M citric acid (pH 2.7) and extracted two times with (20 ml) of methylene dichloride after changing pH to 3.5, 4.0, 4.5, 5.0, 5.5, 6.5 with dilute ammonia. Fractions at pH 4.0, 4.5 and 5.0 yielded 103, 230 and 85 mg respectively and appeared homogenous on TLC. (silica, ethylacetate/methanol 1:1). This material was submitted for the recording of physical data and pharmacological testing.

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