conversion of eq. 9–12 (see 4.3) in terms of absorbance is: $A_0-A_t/(A_t-A_\infty) = makt$ where $rom = 1$, 2, 4 for eq. 9 and 11, respectively; $1n[(b/a)(A_0-A_\infty) - m(A_0-A_t)/(b/a)(A_t-A_\infty)] = (b\text{-}ma)kt$ where $m = 1$ for eq. 10 and 2 for eq. 12.

5.4. *Stopped-flow measurements.* A *Durrum-Gibson* model D-110 stopped-flow spectrophotometer equipped with a thermostated valve block, drive syringes, and mixing chamber was used for the stopped flow measurements. Data acquisition and computation of rate constants has been described previously [16]. Pseudo-first-order rate constants, k_{ob}, were obtained with 1–2% standard deviations in almost all cases. Reproducibility of independent experiments was generally within $\pm 5\%$.

We would like to express our thanks to the *Swiss National Foundation for Scientific Research* for the grant allowed.

REFERENCES

[1] *F. Basolo & R. G. Pearson*, "Mechanisms of Inorganic Reactions", ch. 5, Ed. John Wiley & Sons 1967.
[2] *A. D. Goolsby & D. T. Sawyer*, Anal. Chemistry *40*, 1978 (1968).
[3] *R. L. Rich & H. Taube*, J. physic. Chemistry *58*, 1, 6 (1954).
[4] *D. De Filippo, F. Devillanova & C. Preti*, Inorg. Chim. Acta *5*, 103 (1971).
[5] *F. H. Brain, C. S. Gibson, J. A. J. Jarvis, R. F. Phillips, H. M. Powell & A. Tyabji*, J. chem. Soc. *1952*, 3686.
[6] see for example *P. Templet, J. R. McDonald, S. P. McGlynn, C. H. Kendrow, J. L. Roebber & K. Weiss*, J. chem. Physic *56*, 5746 (1972).
[7] *C. K. Jørgensen*, "Absorption Spectra and Chemical Bonding in Complexes", Addison-Wesley Publishing Co., Reading Mass. 1962.
[8] *E. L. Muetterties & C. W. Alegranti*, J. Amer. chem. Soc. *92*, 4114 (1970).
[9] *J. M. Meyer & A. L. Allred*, J. inorg. nucl. Chemistry *30*, 1328 (1968); A. D. Westland, Can. J. Chem. *47*, 4135 (1969).
[10] *D. D. Perrin, W. L. F. Armarego & D. R. Perrin*, "Purification of Laboratory Chemicals", Pergamon Press 1966.
[11] *Von G. Bergerhoff*, Z. anorg. allg. Chem. *327*, 139 (1964).
[12] *W. R. Mason & H. B. Gray*, Inorg. Chemistry *7*, 55 (1968).
[13] *A. D. Beveridge, G. S. Harris & F. Inglis*, J. chem. Soc. (A). *1966*, 526.
[14] *O. Bravo & R. T. Iwamoto*, J. electroanal. Chemistry *23*, Interfacial 419 (1969).
[15] *O. Bravo & R. T. Iwamoto*, Inorg. Chim. Acta *3*, 663 (1969).
[16] *W. R. Mason*, Inorg. Chemistry *9*, 2688 (1970).

---

249. Vinca Alkaloids XXXII[1]). Microbiological Conversions of Vindoline, a Major Alkaloid from *Vinca rosea* L[2])

by **N. Neuss, D. S. Fukuda, G. E. Mallett, D. R. Brannon** and **L. L. Huckstep**
The Lilly Research Laboratories Eli Lilly and Company
Indianapolis, Indiana USA

(30. VIII. 73)

Summary. Vindoline, a major alkaloid from *Vinca rosea* L was subjected to microbiological conversion using *Streptomyces* cultures.

Several new metabolites were isolated and their structures elucidated.

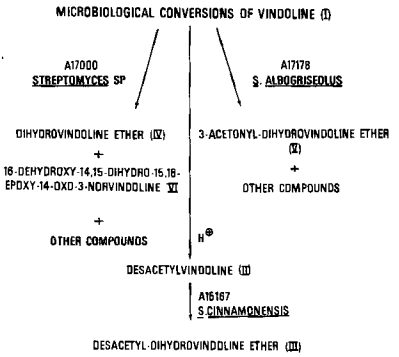
---

1) Paper XXXI in this series, see [1].

2) Presented in part at the joint meeting of the American Academy of Pharmacognosy and Natural Products Section, Academy of Pharmaceutical Sciences Symposium on Biotransformations and Fermentations, Jekyll Island, Georgia, USA, July 15–20, 1973.

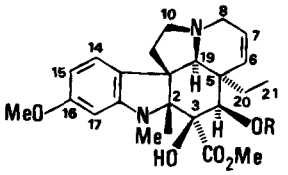
The clinical use of dimeric *Vinca* alkaloids vinblastine (VLB) and vincristine (VCR) prompted extensive chemical investigations on other alkaloids [2] obtained from the pantropical shrub, *Vinca rosea* L (*Catharanthus roseus* G. DON). In search for new anti-tumor agents, biological transformations of major alkaloids from this plant have been initiated using different microorganisms. In an earlier report [3] from these laboratories it was mentioned that about 400 cultures from the families, *Actinomycetaceae* and *Streptomycetaceae*, were screened for the ability to convert one of the major *Vinca* alkaloids, vindoline (**I**). Approximately 20% of these cultures produced a variety of products, the most common being desacetylvindoline (**II**). Incubation of vindoline with *S. albogriseolus* or *Streptomyces sp.* A17000, or desacetylvindoline with *Streptomyces cinnamonensis* resulted in the formation of several new metabolites (Table 1). Physical properties and tentative partial structures of some of these products have been reported [4]. We wish to present new data which permit structural assignments for these new microbiological conversion products.

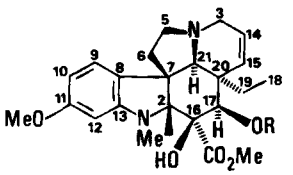
Table 1. *Microbiological Conversions of Vindoline*



Incubation of vindoline with *Streptomyces sp.* A17000 afforded several compounds. Extraction of the alkaloidal material after five days of fermentation followed by chromatography on silica gel (7729 *Merck*) using benzene/ethyl acetate 1:1, afforded several hundred fractions which were combined according to TLC. (silica, EtOAc/MeOH 7:3, ceric ammonium sulfate spraying reagent). Due to difficulties encountered in purification, only two of the several compounds could be characterized and identified. The major component was shown to be dihydrovindoline ether **IV**, reported [4] earlier to contain an ether linkage between C(16) and another locus in the alicyclic portion of the molecule. Comparison of the NMR. spectral data of **IV** and the previously described desacetyl derivative **III** (both amorphous but chromatographically homogeneous materials), and those of more recently isolated alkaloid cathanneine [5][3] (**VII**) (Table 2), clearly indicated that the ether linkage was between C(16) and C(15). In fact, irradiation at $\delta = 2.02$ in **IV** caused collapse of the multiplet ($d \times d$) at 4.05 ppm to a singlet; corresponding experiment in **III** at $\delta = 1.93$ resulted in coalescense of the multiplet to a singlet at $\delta = 4.22$ ppm. (Fig. 1 and 2

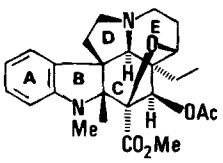
[3] Cathanneine is apparently identical with cathovaline[6], an alkaloid isolated from *Catharanthus lanceus*. The structures of the alkaloid have been proposed by both groups independently. The structure and absolute configuration of cathanneine was established unequivocally by chemical correlations with alkaloid vindovosine [7].



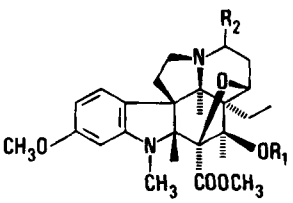


R = $COCH_3$: VINDOLINE I

R = H: DESACETYLVINDOLINE II



CATHANNEINE (CATHOVALINE) VII



R_1 = H; R_2 = H: III

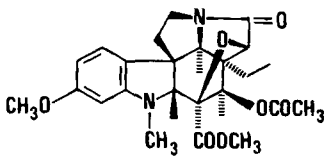
DESACETYL-DIHYDROVINDOLINE ETHER

R_1 = $COCH_3$; R_2 = H: IV

DIHYDROVINDOLINE ETHER

R_1 = $COCH_3$; R_2 = CH_2COCH_3: V

3-ACETONYL-DIHYDROVINDOLINE ETHER



16-DEHYDROXY-14,15-DIHYDRO-15,16-EPOXY-14-OXO-3-NORVINDOLINE VI

Table 2. *Chemical Shifts of Transformation Products and Model Compounds*

CHEMICAL SHIFTS OF TRANSFORMATION PRODUCTS AND MODEL COMPOUNDS IN PPM (δ)

Cpd.	C–2	C–17(4)*	C–15(6)*	C–14(7)*	C–21(19)*	N–CH_3	$COOCH_3$	$OCOCH_3$	Arom. OCH_3
III	3.63	3.97	4.22 m	1.93 m	3.55	2.78	3.88	none	3.75
IV	3.68	5.39	4.05 m	2.03 m	3.59	2.78	3.80	1.95	3.75
V	3.73	5.43	4.06 m	2.0 m	3.64	2.78	3.78	1.92	3.76
VI	4.02	5.55	4.22 s	none	3.57	2.79	3.78	1.98	3.77
	All spectra are 100 MHz in $CDCl_3$ solution								
Vindoline I	3.75	5.43			2.65	2.68	3.80	2.07	3.80
Cathanneine VII	3.81	5.32	4.08 m	2.12–2.67	3.58	2.80	3.68	1.94	none
Cathovaline VII		5.28	4.05 m	1.8–2.0		2.78	3.76	1.93	none

*Old numbering system

respectively). Additional corroboration was obtained from the mass spectra (Fig. 3). Thus, metabolite **III** and **IV** must have structures as shown. Their analytical data and spectral properties (see Exper. Part) were also in agreement with these formulations.

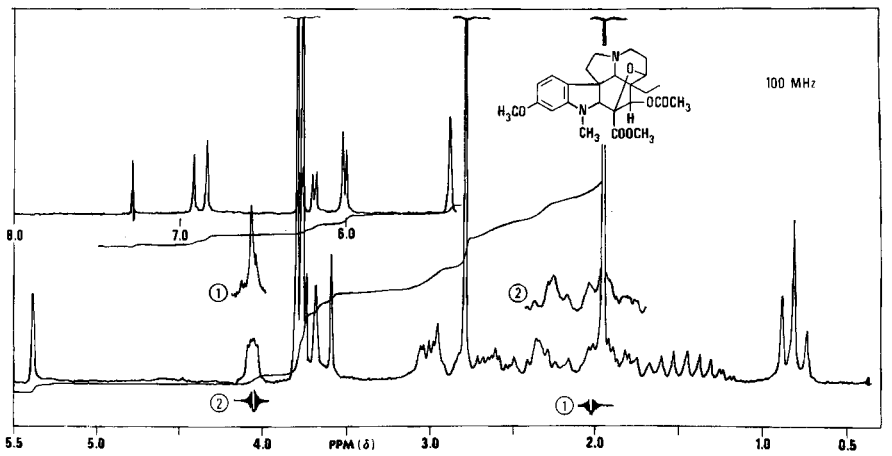


Fig. 1. *NMR. Spectrum* (100 MHz) *of* **IV**

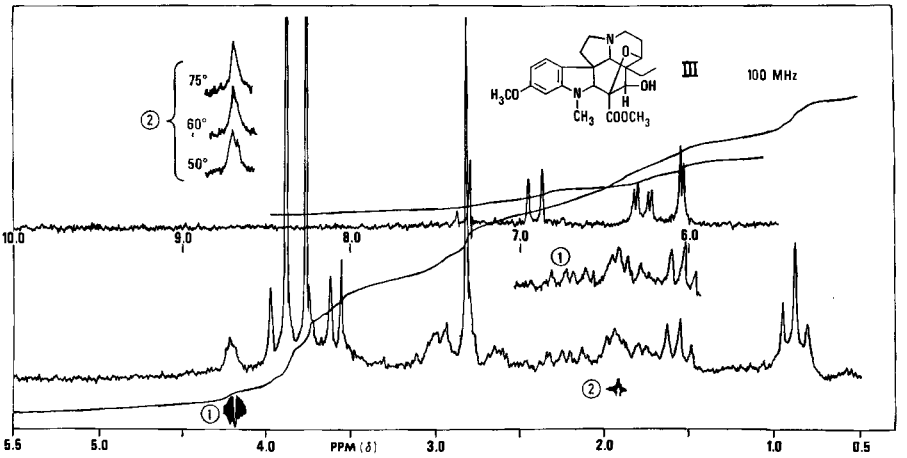


Fig. 2. *NMR. Spectrum* (100 MHz) *of* **III**

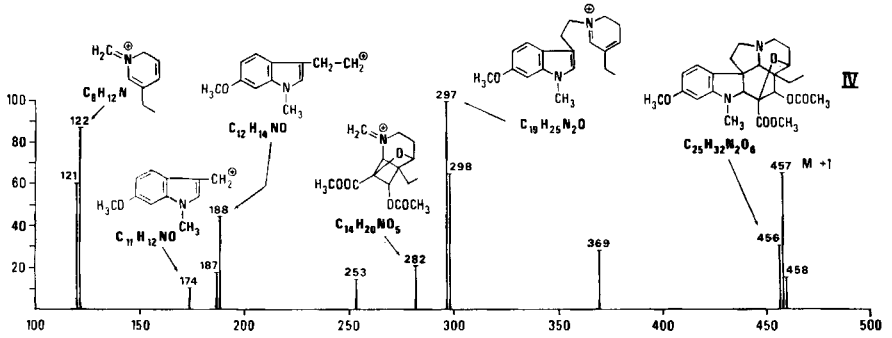
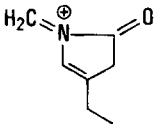
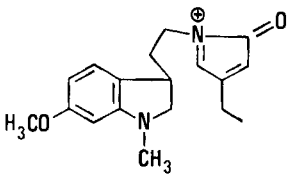


Fig. 3. *Mass Spectrum of* **IV**

The minor metabolite resulting from this microbiological conversion represented a *novel type of transformation involving contraction of ring E to a five-membered lactam* (**VI**). This compound, $C_{24}H_{28}N_2O_7$, crystallized from methanol, m.p. 225–226° (dec.) and did not contain any titratable group. Its UV. spectrum was quite similar to that of vindoline [8] and in the IR. spectrum, there were now two carbonyl bands, 1710 cm^{-1} corresponding to methyl ester and acetate and 1750 cm^{-1} arising from the five-membered lactam carbonyl fused to other rings [9][4]). Small amounts of this metabolite did not permit extensive chemical transformations but NMR. and mass spectral data were in good agreement with the proposed structure. The most striking

Table 3. *High Resolution Mass Spectral Data of* **VI**

m/e 124		$C_7H_{10}NO$	CALCD.: 124.07623 FOUND: 124.07657
m/e 297		$C_{18}H_{21}N_2O_2$	CALCD.: 297.16030 FOUND: 297.15981
m/e 397	M−$COOCH_3$	$C_{22}H_{25}N_2O_5$	CALCD.: 397.17635 FOUND: 397.17956
M^+		$C_{24}H_{28}N_2O_7$	CALCD.: 456.18965 FOUND: 456.18576

feature in the NMR. spectrum of **VI** was a sharp singlet at 4.22 ppm (C(15)) corresponding to the multiplet at 4.05 ppm (C(15) in **IV**) indicative of the lack of the methylene group at C(14) (Fig. 4). This was substantiated further by the presence of only two methylene multiplets at C(5) and C(6) (the third methylene from the ethyl group was present as a quartet) which were completely resolved in the 220 MHz spectrum. Characteristic geminal couplings of 13.7 and 12.0 Hz as well as four vicinal couplings of 4.3, 10.6, 9.3 and 7.2 Hz were also in agreement with this formulation. The mass spectrum also corroborated this structure by the presence of a few unique and characteristic fragments absent from the spectra of related compounds; *e.g.*, vindoline, as shown by high resolution data (Table 3).

The ions resulting from fragmentation in the aromatic portion of the molecule were identical to those arising from fragmentation of vindoline itself [1].

Incubation of vindoline with *S. albogriseolus* (A17178) had been reported [4] to yield a number of unidentified metabolites one of which seemed to contain 56 mass units more than dihydrovindoline ether (**IV**). The new compound (**V**) was re-isolated

[4]) The frequency assigned to this type of absorption should be 1750–1700 cm^{-1} but fusion to other rings would tend to shift this value to a higher wavenumber.

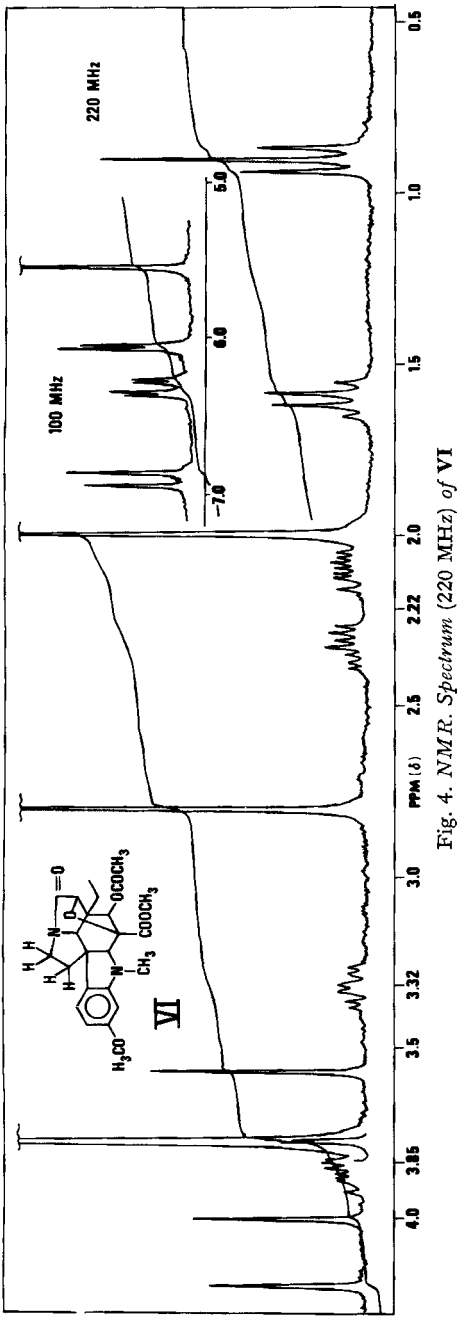


Fig. 4. *NMR. Spectrum* (220 MHz) *of* **VI**

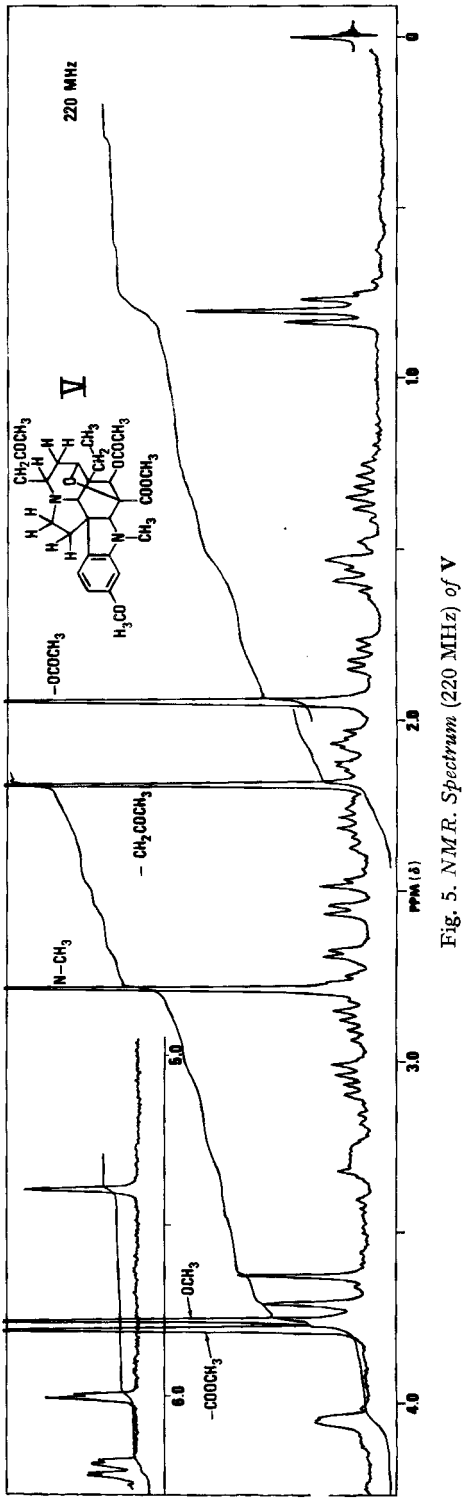
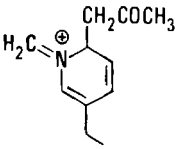
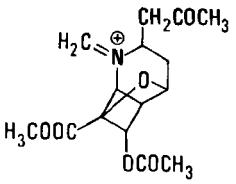
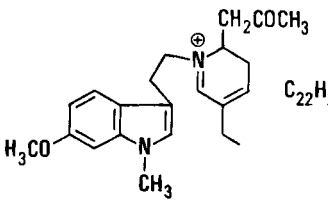


Fig. 5. *NMR. Spectrum* (220 MHz) *of* **V**

by silica column chromatography with benzene/ethyl acetate 3:1, followed by gradient pH extraction at pH 2.8 and finally preparative TLC. The material appeared to be homogeneous and although amorphous, gave consistently good micro-

Table 4. *High Resolution Mass Spectral Data of* **V**
3-Acetonyl-dihydrovindoline Ether

m/e 178		$C_{11}H_{16}NO$	CALCD.: 178.12319 FOUND: 178.12241
m/e 338		$C_{17}H_{24}NO_6$	CALCD.: 338.16036 FOUND: 338.16051
m/e 353		$C_{22}H_{29}N_2O_2$	CALCD.: 353.22290 FOUND: 353.22245
m/e 453	$M-COOCH_3$	$C_{26}H_{33}N_2O_5$	CALCD.: 453.23895 FOUND: 453.23746
m/e 469	$M-COCH_3$	$C_{26}H_{33}N_2O_6$	CALCD.: 469.23386 FOUND: 469.23000
M^+		$C_{28}H_{36}N_2O_7$	CALCD.: 512.25225 FOUND: 512.24867

analytical and physical data. This new metabolite, $C_{28}H_{36}N_2O_7$, displayed a one proton multiplet (C(15)) (Fig. 5) at 4.06 ppm similar in shape and chemical shift to that of the dihydrovindoline ether (**IV**) at 4.05 ppm. It was coupled to the methylene multiplet at C(14) as could be demonstrated by irradiation at 2.0 ppm resulting in collapsing of the multiplet at C(15). In addition to the $OCOCH_3$ signal (*s*) at 1.98 ppm, the new metabolite displayed an equally strong methyl signal (*s*) at 2.17 ppm arising from the new function containing 56 additional mass units (*vide supra*). Since decoupling experiments clearly indicated the presence of the methylene protons at C(14), the only available location for this function is C(3). The 56 mass units correspond to an acetonyl moiety, $-CH_2COCH_3$, and this was demonstrated as follows.

The UV. spectrum of the metabolite was again almost identical with that of vindoline [8] and in the IR. spectrum there were two carbonyl bands; 1750 cm^{-1} corresponding to methyl ester and acetate and 1710 cm^{-1} arising from aliphatic ketone

carbonyl[5]). Ester carbonyl in vindoline [8] is at 1740 cm^{-1}. Additional corroboration of the presence of the acetonyl moiety in the ring E was furnished by high resolution mass spectrum characterized by a few unique fragments. Of particular interest were those present in the spectrum of **IV** with the corresponding increase of molecular weight arising from the presence of the acetonyl moiety in the ring E. The ions containing the aromatic portion of the molecule were the same as in vindoline ether (**IV**) (Table 4).

The work on the identification and structure elucidation of other, as yet incompletely characterized metabolites, is continuing.

The authors thank the following members of the *Lilly* Research Laboratories for their help in this investigation: Mr. *J. B. Deeter* and associates, UV. and IR. spectra; Mr. *G. M. Maciak* and associates, microanalyses and electrometric titrations; Mr. *J. P. Hettle*, high and low resolution mass spectra; Mr. *T. K. Elzey*, recording of the NMR. spectra and decoupling experiments, and Dr. *H. E. Boaz* for the interpretation of some of the spectral data.

Experimental Part

^{1}H-NMR. Spectra. The spectra in $CDCl_3$ were measured using Me_4Si as an internal standard and were recorded on *Varian* HA-100 MHz and HR 220 MHz instruments.

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

Numbering System. The two numbering systems are shown in order to facilitate the comparison of the new system with the old one still in use by Chemical Abstracts.

Microbiological Conversions. An appropriately prepared[6]) culture of A 17178, A 17000 or A 15167 was incubated on a rotary shaker (250 rpm) at 30° and vindoline or desacetylvindoline was added at a concentration of 50 mg in 2 ml of acetone/water mixture 1:1 per 500 ml flask after 24 h (48 h for A 17000). The conversion was allowed to proceed for 5 days. After that time the culture was harvested and extracted after adjusting to pH 9.5 with methylene dichloride.

Dihydrovindoline ether from A 17000 conversion (**IV**). A crude alkaloidal extract (3.1 g) was chromatographed using 200 g of silica (7729 *Merck*) and benzene, and elution continued by benzene/ethyl acetate. Fractions of 25 ml were collected and combined according to TLC. Trace amounts of unidentifiable compounds were isolated from fractions resulting by eluting with 500 ml of benzene, then 4.6 l of benzene/ethyl acetate 1:1 and 16.0 l of benzene/ethyl acetate 1:3. Following the elution with the same solvent, fractions corresponding to a volume of additional 6 l gave 160 mg of still slightly impure ether. This material was further purified by preparative TLC. (silica, 2 mm thick, *Merck*) and ethyl acetate/methanol 7:3 as a solvent. The usual work up gave 55 mg of homogeneous material. All physical data were obtained using this material. UV. spectrum was practically identical to that of vindoline. IR. indicated the absence of the hydrogen bonded OH [8].

$C_{25}H_{32}N_2O_6$	Calc.	C 65.77	H 7.07	N 6.14%
(456.54)	Found	,, 66.04	,, 7.39	,, 6.02%

16-Dehydroxy-14,15-dihydro-15,16-epoxy-4-oxo-3-norvindoline from A 17000 conversion (**VI**). 3 g of the crude alkaloidal extract were chromatographed on 150 g of silica (3.5 × 40 cm column). After washing with 400 ml of benzene, elution proceeded with 1.6 l of benzene/ethyl acetate 3:1. Solvents were then changed to benzene/ethyl acetate 1:1 and the first 100 ml were discarded. The next fractions resulting from elution using additional 160 ml of the same solvent mixture

---

[5]) See [a], p. 243. Ketone carbonyls between saturated hydrocarbon group absorb strongly at 1725–1705 cm^{-1}.

[6]) Details pertaining to conditions of conversion (preparation of inoculum, media, etc.) will be described elsewhere.

afforded 60 mg of crude compound. This crystallized from 5 ml of methanol to give 42 mg of colorless crystalline material, m.p. 225–226° (dec.).

$C_{24}H_{28}N_2O_7$	Calc.	C 63.15	H 6.18	N 6.14	O 24.53%
(456.19)	Found	,, 62.76	,, 6.26	,, 5.96	,, 24.40%

3-Acetonyldihydrovindoline ether from A 17178 conversion (**V**). 2 g of an alkaloidal extract were chromatographed on silica (100 g) and eluted first with 1.5 l of benzene, 2.0 l of benzene/ethyl acetate 95:5, 400 ml 3:1, 4.1 l 1:1 and finally 2.2 l of the same mixture yielding 1.0 g of crude ether (A). Another batch containing 3.6 g of alkaloidal extract was chromatographed on 175 g of silica and washed successively with 9.7 l 3:1, 1.6 l 1:1 and 1 l 1:3 of benzene/ethyl acetate. Following elution with 3.2 l of 1:1 mixture, 1.5 g of crude ether were obtained (B). Combined 'A' + 'B' were dissolved in 100 ml of 0.1M citric acid, (pH 2.8) and extracted with 50 ml of CH_2Cl_2, dried over sodium sulfate and evaporated to give 1.5 g of purified material (C). 200 mg of 'C' was chromatographed on TLC. (2 mm silica *Merck* in EtOAc/MeOH 7:3). After the usual work-up 130 mg of homogeneous ether were obtained.

$C_{28}H_{36}N_2O_7$	Calc.	C 65.61	H 7.08	N 5.47	O 21.85%
(512.25)	Found	,, 65.39	,, 7.04	,, 5.63	,, 21.60%

Desacetyldihydrovindoline ether from A 15167 conversion of desacetylvindoline (**III**). A solution of 1.54 g of alkaloidal extract in benzene was chromatographed on 100 g of *Camag* Alumina (Neutral, Activity III) and washed with benzene. Elution using 4.5 l of this solvent afforded several fractions of similar Rf on TLC. and gave a total of 200 mg. Preparative TLC. on this slightly inhomogeneous material on G-plates (2 mm thick), using benzene as a solvent, afforded 125 mg of homogeneous metabolite.

$C_{23}H_{30}N_2O_5$	Calc.	C 66.64	H 7.30	N 6.76	O 19.30%
(414.50)	Found	,, 66.24	,, 7.87	,, 6.38	,, 19.16%

REFERENCES

[1] *N. Neuss, H. E. Boaz, J. Occolowitz, E. Wenkert, F. M. Schell, P. Pottier, C. Kau & M. Plat*, in press.

[2] *G. H. Svoboda*, p. 9 in Antitumoral Effects of *Vinca Rosea* Alkaloids, Proceedings First Symposium, European Cancer Chemotherapy Group, Paris, June 1965, Editors S. Garatini, Milan, and E. M. Sproston, Basingstoke. Excerpta Medica Foundation, Amsterdam, New York, London, Milan, Tokyo, Buenos Aires, 1966.

[3] *G. E. Mallett, D. S. Fukuda & M. Gorman*, Lloydia, *27*, 334 (1964).

[4] *G. E. Mallett, M. Gorman, W. W. Hargrove & D. S. Fukuda*, Abstracts – 35 of the 151st Meeting of the Amer. chem. Soc. Fall, 1967, Pittsburgh, Pa.

[5] *G. H. Aynilian, M. Tiu-Wa, N. R. Farnsworth & M. Gorman*, Tetrahedron Letters, *1972*, 89.

[6] *N. Langlois & P. Pottier*, C. r. hebd. Séances Acad. Sci., *273*, 994 (1971), and *275*, 219 (1972).

[7] *G. H. Aynilian, B. Robinson, N. R. Farnsworth & M. Gorman*, Tetrahedron Letters, *1972*, 391.

[8] *N. Neuss*, Collection of Physical Data of Indole and Dihydroindole Alkaloids, Eli Lilly and Company, Indianapolis, Indiana, USA, 1968.

[9] *N. B. Colthup, L. H. Daly, S. E. Wiberley*, Introduction to Infrared and Raman Spectroscopy, Academic Press, New York and London, 1964, p. 265.