Microbial Transformations of Natural Antitumour Agents. Part 5.† The Structure of a Novel Vindoline Dimer produced by *Streptomyces griseus*

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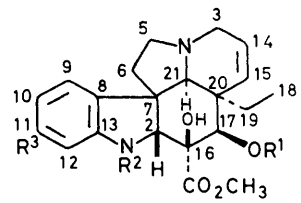
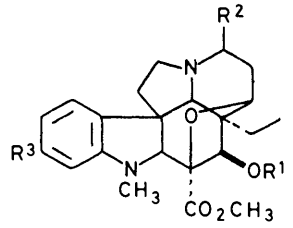
Microbial transformation experiments have been conducted with the alkaloid vindoline. *Streptomyces griseus* (UI 1158) gave good yields of dihydrovindoline ether (2a), and a novel, dimeric vindoline metabolite (4) whose structure was determined by ^{13}C and ^{1}H n.m.r. and high resolution mass spectrometry. It consists of two dihydrovindoline ether (2a) moieties joined by a carbon–carbon bond which is probably formed by means of an enamine coupling reaction.

VINDOLINE (1a) is among the most abundant of the alkaloids of *Vinca rosea*, and its structure is found as one part of the tumour active, dimeric Vinca alkaloids vinblastine and vincristine. Microbial transformation studies have been performed with this compound in order to determine metabolic pathways, and to determine whether physiologically active derivatives of vindoline might be prepared. Earlier reports concerning microbial transformation studies of vindoline described the structures of several microbial metabolites including deacetylvindoline (1b), and deacetyldihydrovindoline ether (2b);[1] dihydrovindoline ether (DHVE)



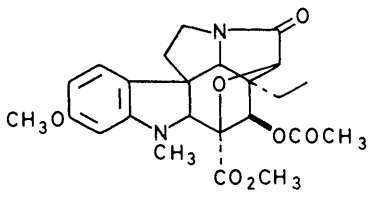
(1)

a; R^1 = $COCH_3$, R^2 = CH_3, R^3 = OCH_3
b; R^1 = H, R^2 = CH_3, R^3 = OCH_3
c; R^1 = $COCH_3$, R^2 = H, R^3 = OCH_3

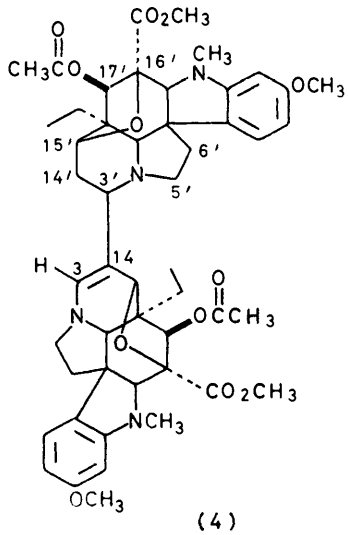


(2)

a; R^1 = $COCH_3$, R^2 = H, R^3 = OCH_3
b; R^1 = R^2 = H, R^3 = OCH_3
c; R^1 = $COCH_3$, R^2 = CH_2COCH_3, R^3 = OCH_3
d; R^1 = $COCH_3$, R^2 = R^3 = H



(3)



(4)

† Part 4, P. J. Davis, D. R. Wiese, and J. P. Rosazza, *Lloydia*, 1977, **40**, 239.

[1] G. E. Mallet, D. Fukuda, and M. Gorman, *Lloydia*, 1964, **27**, 334.

(2a), 3-acetonyldihydrovindoline ether (2c), and a ring contraction product known as 16-dehydroxy-14,15-dihydro-15,16-epoxy-14-oxo-3-norvindoline (3);[2] and de-*N*-methylvindoline (1c).[3] We have also performed microbial transformation studies with vindoline, and have observed several new microbial metabolites.[4] This report describes the structure elucidation of a major microbial transformation product of vindoline by *Streptomyces griseus* (UI 1158). The metabolite (4) is dimeric in nature, consisting of two DHVE moieties joined by a carbon–carbon bond. It is suggested that the metabolite is formed *via* an enamine condensation.

RESULTS AND DISCUSSION

In early screening scale experiments with several micro-organisms, extensive t.l.c. and h.p.l.c. comparisons of fermentation extracts with known vindoline–metabolite standards revealed that *S. griseus* was producing a new vindoline derivative in good yield (30—50% by h.p.l.c.). The new, less polar vindoline metabolite was isolated from a preparative scale fermentation. After purification by column chromatography, the microbial transformation product provided initially confusing structural information.

The low resolution mass spectrum indicated the highest mass at *m/e* 454 (electron impact and chemical ionization) which suggested that the metabolite possessed the vindoline skeleton minus two protons. The 60 MHz ^{1}H n.m.r. spectrum of the metabolite was similar to the spectrum of vindoline. However, signals for the hydroxy-group and for the olefinic protons of vindoline were absent. This indicated that the 16-hydroxy-group was probably involved in ether linkage as with DHVE (2a). Two *N*-methyl (δ 2.71 and 2.78) and two acetyl methyl signals (δ 1.96 and 2.05) of equal intensities were also apparent. The u.v. spectrum of the metabolite was comparable with the spectra of vincoline (1a) and DHVE (2a). When the metabolite was treated with $NaBH_4$ or Pd–C under hydrogen, a product was obtained which gave a presumed molecular ion at *m/e* 456 in the low resolution mass spectrum. This suggested the presence of one double bond in the metabolite relative to vindoline. The purity of the metabolite was verified by h.p.l.c. using the instrument in the recycle mode. The peak for the reduced ($NaBH_4$) metabolite remained symmetrical after more than 14 passes through a microporasil column.[4]

Since the n.m.r. spectral data could possibly be explained on the basis of a dimeric metabolite structure, a Rast molecular weight determination was performed (m.p. depression of camphor, vindoline being used as standard). This indicated that the metabolite had a molecular weight considerably in excess of 454. The high-resolution mass spectrum of the metabolite gave a molecular ion at *m/e* 908 for $C_{50}H_{60}N_4O_{12}$, while the reduced metabolite ($NaBH_4$) gave *m/e* 910. Thus, the metabolite was probably dimeric in nature and possessed one double bond in its structure. Furthermore, the mass spectral fragmentation pattern obtained with the metabolite (Figure 1) was consistent with a dimeric structure. Major ions in the mass spectrum could be assigned to fragmentations of the structure along the lines shown in Figure 1. Thus, by loss of *m/e* 159

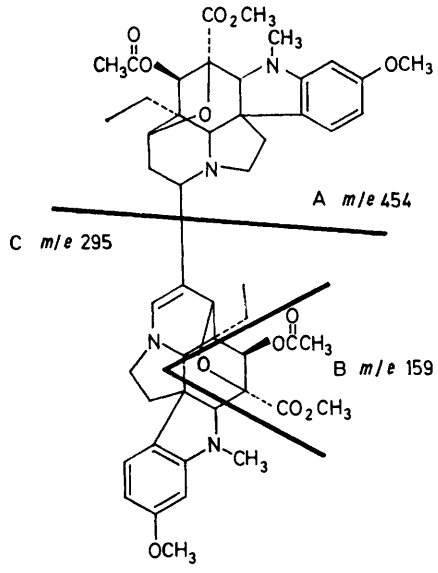


FIGURE 1 Major mass spectral fragments of the dimer (4)

(M^+ — B), the intense *m/e* 749 ion is produced; and by symmetrical cleavage of the molecule into the monomeric pieces, the *m/e* 454 fragment is obtained. Other fragments observed in the mass spectrum could be rationalized according to previously suggested fragmentation pathways for vindoline, dihydrovindoline ether, and aspidospermidine type alkaloids.[2,5]

^{13}C N.m.r. spectral analyses supported the mass spectral data by revealing signals for 49 of the 50 carbon atoms of the metabolite, as against the 25 carbons evident in vindoline itself. Twenty-six of the 49 carbons of the metabolite occurred as 13 distinct pairs of signals, further reflecting its dimeric nature. Tentative assignments for most of the carbon atoms could be made by comparison with the published ^{13}C n.m.r. values for vindoline and dihydrovindoline.[6] Since ^{1}H n.m.r. spectral similarities were apparent between the metabolite and DHVE (2a), ^{13}C n.m.r. spectral comparisons were made between these two compounds. The ^{13}C n.m.r. spectral data for DHVE (2a) and for the metabolite (4) are summarized in the Table.

Chemical shift assignments for the 25 carbons of

[2] N. Neuss, D. Fukuda, G. E. Mallett, D. R. Brannon, and L. L. Huckstep, *Helv. Chim. Acta*, 1973, **56**, 2418.
[3] N. Neuss, G. E. Mallett, D. R. Brannon, and L. L. Huckstep, *Helv. Chim. Acta*, 1974, **57**, 1891.
[4] L. Youel, T. Nabih, and J. P. Rosazza, *Appl. Environ. Microbiol.*, submitted.
[5] B. K. Moza, J. Trojanek, V. Hanus, and L. Dolejs, *Coll. Czech. Chem. Comm.*, 1964, **29**, 1913.
[6] E. Wenkert, D. W. Cochran, E. W. Hagman, F. M. Schell, N. Neuss, A. S. Katner, P. Potier, C. Kan, M. Plat, M. Koch, H. Mehri, J. Poisson, N. Kunesch, and Y. Rolland, *J. Amer. Chem. Soc.*, 1973, **95**, 4990.

DHVE (2a) could be made largely by ^{13}C n.m.r. spectral comparisons with values published for vindoline and dihydrovindoline.[6] All but six of the carbon signals of (2a) could be readily assigned this way. Further comparisons with the ^{13}C n.m.r. spectrum of cathovaline (2d) made it possible to suggest the following assignments for these six signals: C(8) δ 130.28; C(16) 88.11; C(3) 46.62; C(6) 46.30; C(19) 24.76; and C(14) 22.25 p.p.m. (see Table).

^{13}C N.m.r. spectral data for the dimer (4) and dihydrovindoline ether (DHVE) (2a)

	δ (p.p.m.)	
Carbon	DHVE (2a)	Dimer (4)
C(2)	84.89	84.76, 83.69
C(3) [a]	46.62 [b]	46.00
C(3) olefin		136.25
C(5)	50.91 [b]	52.01, 51.07
C(6) [a]	46.30	49.12, 46.00
C(7) [a]	52.34	53.31, 51.46
C(8)	130.28 [b]	130.50, 130.31
C(9)	120.85	121.73, 121.05
C(10)	103.21	103.67, 103.21
C(11)	160.94	161.04, 160.94
C(12)	94.60	95.03, 94.67
C(13)	150.52	151.72, 150.48
C(14) olefin		111.95
C(14)	22.25 [b]	22.32
C(15)	77.58	79.30, 76.77
C(16)	88.11 [b]	88.30, 85.80
C(17)	74.82	74.79, 74.14
C(18)	9.03	9.10, 8.51
C(19)	24.76	32.55, 29.66
C(20) [a]	41.45	48.50, 46.56
C(21)	67.09	67.64, 66.18
Acetyl–C=O	169.78	170.17, 169.91
Ester–C=O	168.94	169.29, 168.94
Aryl–O–CH_3	55.20	55.26, 55.20
Ester–O–CH_3	54.38	52.40, 52.99
NCH_3	36.00	36.91, 36.00
Acetyl–CH_3	20.79	20.92, 20.86

[a] Chemical shift assignments are based on comparisons of ^{13}C n.m.r. spectral data for cathovaline, dihydrovindoline,[6] and craspidospermine,[8] and may be interchangeable. [b] Assignments for these signals in DHVE are based on comparisons with data recorded for cathovaline.

Strong similarities were apparent upon comparing ^{13}C n.m.r. spectral values for (2a) and the metabolite (4). Most of the carbon signals of the monomer and of the metabolite were readily matched except for those involved in the dimer linkage, the olefinic carbons, and seven others. Chemical shift assignments were made for these carbon atoms as follows: the olefinic carbons δ 136.25 and 111.95; C(19) and C(19′) 29.66 and 32.55; C(20) and C(20′) 48.50 and 46.56; C(5) and C(5′) 51.07 and 52.01; and C(16) 85.80 p.p.m. The assignments made for C(19) and C(19′) differ from chemical shift values for the corresponding signal obtained with the monomeric DHVE (2a). This is probably attributable to steric interactions occurring at these positions in the dimer. The chemical shift values of ethyl side-chains attached through quaternary carbons are subject to such steric interactions with other indole alkaloid dimers.[7] In addition, the chemical shift for C(19) of another indole alkaloid, andrangin, has been assigned a value of 35.2 p.p.m.[8] Chemical shift assignments were confirmed by examining the multiplicities of signals through carbon–proton spin–spin coupling experiments. The assignments for most carbons were supported in this way with the exception of C(3), (5)—(7), and (14). Signals for these carbons exist between δ 30 and 60 p.p.m., and it was impossible to discern clearly the multiplicities of all the overlapping signals.

Several features in the 100 MHz ^1H n.m.r. spectrum of the metabolite permit an assignment of the point of linkage of the dimer between positions 3′ and 14. The appearance of the metabolite spectrum was very similar to that reported earlier for DHVE (2a).[2] Signals for protons attached at the two C(15) positions occurred at δ 4.10 (m) and 4.26 (s). In addition, a one-proton olefinic signal (s) was apparent at δ 6.11 (Figure 2). Similar protons in the indole alkaloid craspidospermine resonate at δ 4.22 and 5.95, respectively,[9] and these both correlate well with the position and nature of the signals obtained for the metabolite. Since the 3-H signal was unsplit, one of the points of attachment of the dimer must occur at the adjacent position, C(14). This is confirmed by the finding that the signal assigned to 15-H occurs as a singlet at δ 4.26. The double bond in conjugation with the nitrogen electron pair is consistent with the chemical reactivity of the metabolite with $NaBH_4$, and with a band at 1 653 cm^{-1} in the i.r. spectrum

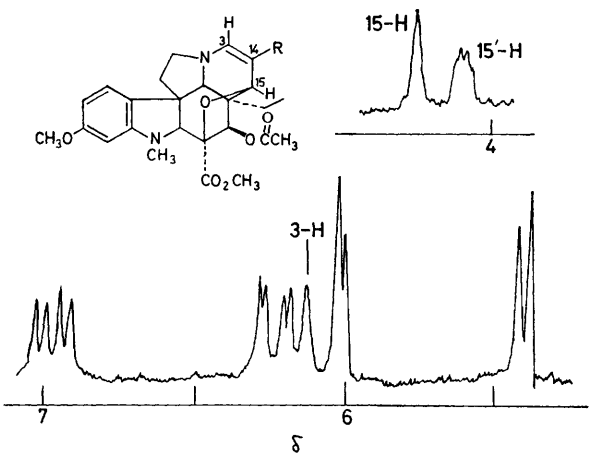


FIGURE 2 Portions of the 100 MHz ^1H n.m.r. spectrum of the dimer (4) where R = the second DHVE moiety attached at the 3′-position

typical for enamines.[10] The presence of a double bond in the metabolite is also consistent with similar aspidospermidine enamines.[9,11] The other point of attachment

[7] J. C. Miller, G. E. Gutowski, G. A. Poore, and G. B. Boder, *J. Medicin. Chem.*, 1977, **20**, 409.
[8] A. Cave, J. Bruneton, A. Ahond, A.-M. Bui, H.-P. Husson, C. Kan, G. Lukacs, and P. Potier, *Tetrahedron Letters*, 1973, 5081.
[9] C. Kan-Fan, H.-P. Husson, and P. Potier, *Bull. Soc. chim. France*, 1976, 1227.
[10] S. F. Dyke, 'The Chemistry of Enamines,' Cambridge University Press, London, 1973, p. 10.
[11] L. Diatta, Y. Langlois, N. Langlois, and P. Potier, *Bull. Soc. chim. France*, 1971, 671; N. Langlois and P. Potier, *Compt. Rend.*, 1971, **C273**, 994.

linking the dimer most likely occurs at the 3′-position of the other DHVE moiety. This may be deduced by examination of the 15′-H signal at δ 4.10 which occurs as a broad signal due to splitting of 15′-H by two non-equivalent protons attached to the 14′-position [2] (Figure 2). This kind of evidence was used in the proof of structure of the 3-substituted DHVE derivative acetonyldihydrovindoline ether (2c) where the multiplicity and breadth of the n.m.r. signal for the proton at C(15) could only be due to the presence of two adjacent C(14) methylene protons.[2]

Although the evidence presented cannot preclude the linkage of the dimer through positions 5′ and 6′ of the other half of the metabolite, several lines of reasoning support our assignment of the point of attachment through the 3′-position. Both ^{13}C and ^{1}H n.m.r. spectral data rule out all other positions as possible sites for this linkage. We attempted to determine the number of protons attached to the carbons at 3′, 5′, and 6′ by using carbon–proton spin–spin coupling. However, all signals occur within the same small region of the ^{13}C spectrum (δ 45.79—56.12 p.p.m.) as complex overlapping multiplets.

Enamine derivatives of compounds like DHVE exist naturally, and they have been prepared. Craspidospermine is a naturally occurring enamine [9] while cathovaline has been converted into an enamine using Polonovski reaction conditions.[11] It is noteworthy that in both these cases, the double bond of the enamine exists within the six-, and not the five-membered ring.

DHVE (2a) was also found as a major metabolite (20—40% yields by h.p.l.c.) of vindoline along with (4) in our cultures of *S. griseus*. The fact that this compound and the dimer (4) are produced in the same fermentation suggests that they may arise *via* a common pathway, and possibly through the same intermediate. In order for either DHVE (2a) or (4) to be formed, the double bond of (1a) must somehow become activated prior to formation of the ether bridge between positions 15 and 16. Such activation may be achieved by formation of reactive iminium intermediates which would undergo initial cyclization to structures like (6).

It is thought that *S. griseus* metabolizes vindoline (1) into enamines with tautomeric structures (6) and (7). The enamines may be produced by microbial *N*-oxidation, a common microbial oxidative transformation [12] followed by conjugation and elimination to form (6) much like the Polonovski reaction. Intermolecular coupling of (6) and (7) by a path similar to that suggested for other heterocyclic alkaloidal enamines [13] allows for the direct formation of the dimer. Bobbitt *et al.* demonstrated how heterocyclic enamines may be linked together to form carbon–carbon dimers with structural features similar to those proposed for the metabolite (4).[13] The enamines proposed in the formation of the dimer may also serve as logical intermediates in the formation of many of the other microbial metabolites of vindoline which are also produced by Streptomyces species. DHVE (2a) would thus be obtained by microbial reduction of (7) while nucleophilic attack of (7) by an acetoacetate moiety generated by the micro-organism leads to the formation of (2c) (Figure 3).

The microbial transformation experiments reported herein indicate that compounds like vindoline are capable of being metabolically converted into chemically reactive species. Similar types of biotransformations may be expected to occur on the vindoline portions of dimeric Vinca alkaloids. Further studies relating to the identification of other vindoline metabolites, and to the chemical synthesis of the proposed dimer are in progress.

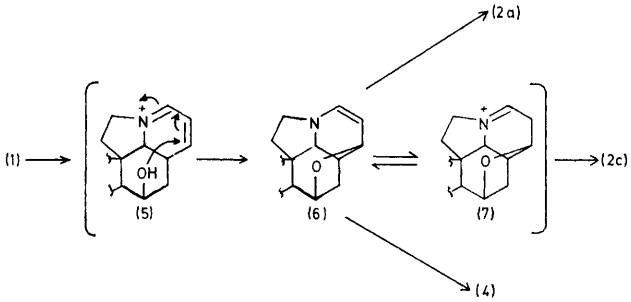


FIGURE 3 Suggested pathway of metabolism of vindoline (1a) through intermediates (5)—(7) to dihydrovindoline ether (2a), 3-acetonyldihydrovindoline ether (2c), and the dimer (4)

EXPERIMENTAL

General.—M.p.s are corrected and were determined in open-ended capillaries with a Thomas–Hoover apparatus. I.r. spectra were obtained with a Beckman IR-5A spectrophotometer, and u.v. spectra were taken with a Philips Pye–Unicam SP 1800 spectrophotometer. ^{1}H N.m.r. spectra were obtained using Varian Associates model T-60 or HA-100 spectrometers with $CDCl_3$ as solvent and Me_4Si as internal standard. Low resolution mass spectra were determined on a Finnigan model 3200 spectrometer (upper mass limit *ca.* 720); high resolution mass spectral data were provided by the Eli Lilly Company, Indianapolis.

^{13}C *N.m.r.*—^{13}C N.m.r. spectra were obtained on a Bruker HX-90E spectrometer at 22.63 MHz incorporating a time-shared internal deuterium lock, a Bruker SXP high power radiofrequency amplifier, a Nicolet BNC-12 computer, and model 293 I/O controller for signal averaging and Fourier transformation of the free induction decay. The 8 μs 30° sampling pulse had a repetition time of 2 s, while broad band proton decoupling was used during data acquisition. The resulting spectra were baseline corrected.[14] Spectra were obtained on 100 mg samples dissolved in deuteriochloroform (0.5 ml) in 10 mm tubes.

Chromatography.—T.l.c. was performed on 0.25 or 1.0 mm thick layers of silica gel GF_{254} (Merck) on glass plates. Prior to use, t.l.c. plates were activated at 120° for 30 min. Solvent systems were: A, ethyl acetate–benzene (2 : 1); B, ethyl acetate–benzene (1 : 1); C, methanol (100%);

[12] K. Kieslich, 'Microbial Trnsformations of Non-steroid Cyclic Compounds,' Wiley, Georg Thieme, Stuttgart, 1976, p. 602.

[13] J. M. Bobbitt, J. M. Kiely, K. L. Khanna, and R. Ebermann, *J. Org. Chem.*, 1965, **30**, 2247.

[14] G. A. Pearson, *J. Magnetic Resonance*, 1977, **27**, 265.

D, ethyl acetate–methanol (6 : 1); E, ethyl acetate–methanol (3 : 1); and F, ethyl acetate (100%). Compounds were detected on developed chromatograms by fluorescence quenching under 254 or 365 nm u.v. light, and were later visualized with Dragendorff reagent,[15] or with cerium(IV) ammonium sulphate (CAS) (1% in 50% v/v H_3PO_4).[16] Column chromatography was performed with silica gel (Baker 3405) activated for 30 min at 120° prior to use. Columns were packed wet by slurrying silica gel in the developing solvent. Fractions were collected with a Fractomette 200 instrument. H.p.l.c. was performed using a Waters ALC 202 instrument equipped with an M6000 solvent delivery system, a U6K universal injector, a 254 nm u.v. detector, and a μ-porasil column. Methanol (100%) was used as the solvent at an operating pressure of 1 100 lb in^{-2}.

Fermentation Procedures.—*Streptomyces griseus* cultures were maintained on Sabouraud–Maltose agar slants in sealed screw-cap tubes at 4° in a refrigerator until required for use.

The fermentation protocol is identical to that previously described [4,17] using a medium of the following composition: glucose (10 g), corn-steep liquor (6 g), $NH_4H_2PO_4$ (3 g), $CaCO_3$ (3.5 g), soybean oil (2.2 g), yeast extract (2.5 g), distilled water (1 000 ml) adjusted to pH 7.0 with HCl. Media were sterilized in an autoclave at 121° at 15 lb in^{-2} for 15 min. Fermentations were conducted in cotton-plugged Erlenmeyer flasks holding 1/5th of their volumes of sterile medium. Cultures were incubated at 26° with shaking on New Brunswick Scientific Co. model G-25 or G-10 gyrotary shakers operating at 250 r.p.m. with a 1 in stroke.

Samples (4.0 ml) of substrate-containing fermentations were withdrawn at various time intervals, adjusted to pH 10 with 58% NH_4OH, extracted with ethyl acetate (1 ml), and the extracts (30 μl) were spotted on t.l.c. plates.

Vindoline.[18]—Vindoline had the following physical values: m.p. 173.5—175.5°; $\lambda_{max.}$(EtOH) 250 (log ε 3.87) and 304 (3.74); δ_H($CDCl_3$) 0.48 (3 H, t, 18-H), 1.35 (2 H, m, 19-H), 2.07 (3 H, s, $COCH_3$), 2.38 (3 H, m, 5- and 6-H), 2.65 (1 H, s, 21-H), 2.68 (3 H, s, NCH_3), 2.9 (1 H, m, 6-H), 3.4 (2 H, m, 3-H), 3.75 (1 H, s, 2-H), 3.80 (6 H, s, OCH_3 and CO_2CH_3), 5.23 (1 H, d, 12-H), 6.20 (1 H, q, 10-H), 6.91 (1 H, d, 9-H), and 9.0 (1 H, s, OH); *m/e* 456 (7%), 397 (9), 381 (17), 308 (10), 296 (31), 281 (40), 188 (89), 173 (90), 161 (100), and 122 (89).

Preparation of Dimer (4) *and DHVE* (2a) *by Microbial Transformation of Vindoline by* S. griseus.—*S. griseus* (UI 1158) was grown according to the usual fermentation procedure. Vindoline (3.0 g) was dissolved in DMF (30 ml) and distributed evenly among 29, second-stage 1-L Erlenmeyer flasks 24 h after inoculation. Metabolite formation was followed by t.l.c. using solvent system F. Both (4) and (2a) were evident in incubation samples within 24 h, and the fermentation was terminated after 72 h. The pooled cultures were adjusted to pH 10 with 58% NH_4OH (68 ml) and exhaustively extracted with diethyl ether (liquid–liquid extractor). The extracts were dried (Na_2SO_4) and concentrated to a viscous brown oil (6.3 g). This concentrate was dissolved in ethyl acetate (500 ml) and partitioned against an equal volume of aqueous solution which was maintained at pH 3.0 by addition of HCl. The acid solution was then partitioned with hexanes (3 × 250 ml) to remove lipid impurities. The remaining aqueous solution was readjusted to pH with 58% NH_4OH and exhaustively extracted with diethyl ether. After combining the ether extracts, drying (Na_2SO_4), and concentration, a clear light yellow oil (2.6 g) was obtained. The oil was dissolved in ethyl acetate (2.5 ml), applied to a silica gel column (250 g; 5 × 60 cm) and eluted with solvent system B at a flow rate of 2 ml min^{-1} while 16 ml fractions were collected. Fractions 80—159 yielded the pure metabolite (4) (300 mg) as an amorphous glass. After collecting 443 fractions, the column was eluted with ethyl acetate (2 l), and then with 95% ethanol from which DHVE (2a) (842 mg) was obtained. Neither metabolite could be induced to crystallize, and physical data were obtained on the chromatographically pure products.

Characterization of the Metabolite (4).—This had m.p. 160—165°; $\lambda_{max.}$(EtOH) 249 (log ε 4.25) and 309 nm (4.05); $\nu_{max.}$(KBr disc) 2 893 and 1 360 (N–CH_3), 1 740 (C=O), and 1 653 cm^{-1} (C=N); δ_H (100 MHz) 0.84 (6 H, t, 18-H), 1.36 (4 H, m, 19-H), 1.58—1.90 (4 H, m), 1.96 (3 H, s, $COCH_3$), 2.05 (3 H, s, $COCH_3$), 2.17—2.28 (1 H, m), 2.71 (3 H, s, NCH_3), 2.78 (3 H, s, NCH_3), 3.20—3.57 (6 H, m), 3.52 (1 H, s, 21-H), 3.62 (1 H, s, 21-H), 3.76 (3 H, s, aryl–OCH_3), 3.77 (3 H, s, aryl–OCH_3), 3.79 (6 H, overlapping s, $2CO_2CH_3$), 4.10 (1 H, m, 15-H), 4.26 (1 H, s, 15-H), 5.37 (1 H, s, 17-H), 5.41 (1 H, s, 17-H), 5.99 (2 H, d, *J* 2 Hz, two overlapping 12-H signals), 6.11 (1 H, s, 3-H), 6.22 (2 H, q, *J* 8, 2 Hz, 10-H), 6.93 (1 H, d, *J* 8 Hz, 9-H), and 6.96 (1 H, d, *J* 8 Hz, 9-H) (Found: *m/e*, 908.419 553. $C_{50}H_{60}N_4O_{12}$ requires *M*, 908.420 788); *m/e* 908 (42%), 879 (17), 849 (33), 749 (100), 661 (12), 579 (13), 502 (14), 454 (52), 402 (32), 395 (31), 295 (100), 188 (100), and 174 (77).

Characterization of DHVE (2a) —Dihydrovindoline ether (2a) could not be induced to crystallize, and it was characterized on the basis of co-chromatographic behaviour when compared to an authentic [2] DHVE standard using solvent systems A, C, and F, and by comparative spectral data, m.p. 95—98°; $\lambda_{max.}$(EtOH) 256 (log ε 3.79) and 308 nm (3.69); δ_H($CDCl_3$) 0.79 (3 H, t, 18-H), 1.36 (2 H, m, 19-H), 1.92 (3 H, s, $OCOCH_3$), 2.73 (3 H, s, NCH_3), 3.51 (1 H, s, 21-H), 3.61 (1 H, s, 2-H), 3.70 (6 H, two overlappings, aryl–OCH_3 and CO_2CH_3), 3.98 (1 H, m, 15-H), 5.27 (1 H, s,1 7-H), 5.87 (1 H, s, 12-H), 6.11 (1 H, d, 10-H), and 6.73 (1 H, d, 9-H); *m/e* 456 (3%), 397 (10), 369 (1), 297 (47), 282 (2), 188 (100), 174 (18), and 122 (10).

Reduction of the Metabolite (4).—(*a*) A mixture of (4) (20 mg, 2.2×10^{-5}M) was suspended in MeOH (20 ml) along with 5% Pd–C (10 mg) and was shaken under hydrogen at 36 lb in^{-2} for 48 h. The catalyst was removed by filtration, and the solvent removed. The resulting oil was purified by preparative t.l.c. using solvent system D to yield the reduced metabolite (10.7 mg, 55%).

(*b*) $NaBH_4$ (100 mg) was added to a chilled solution of (4) (80 mg, 8.8×10^{-5}M) in MeOH (45 ml) with stirring The reaction was complete within 30 min (t.l.c. solvent, system E); it was diluted with four volumes of water and extracted with $CHCl_3$ (4 × 20 ml). The extract was dried (Na_2SO_4) and evaporated to an oil which dried to an amorphous solid (64 mg, 80%). Co-chromatography with

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the product obtained by method (*a*) using solvent systems A and E showed them to be identical.

Physical data were collected on the amorphous, non-crystalline solid, m.p. 178—183°; $\lambda_{max.}$(EtOH) 255 (log ε 4.10) and 307 nm (3.99); $\nu_{max.}$ 3 370, 2 911, 1 732br (C=O), and 1 602 cm^{-1} (indoline); δ_H(60 MHz) 0.44 (3 H, m, 18-H), 0.85 (3 H, m, 18-H), 1.13—1.78 (7 H, m, 19-H), 1.93 and 2.07 (6 H, two s, 2 $COCH_3$), 2.40br (2 H, s), 2.53 (1 H, s), 2.62 and 2.75 (6 H, two s, 2 NCH_3), 2.90 (2 H, m), 3.33 (2 H, m), 3.52 (2 H, s), 3.68 (1 H, s, 2-H), 3.74 (12 H, overlapping s, 2 arylOCH_3 and 2 CO_2CH_3), 4.03 (1 H, m, 15-H), 5.17br (1 H, s), 5.31 and 5.36 (2 H, two s, 17-H), 5.88—6.35 (4 H, m, 10- and 12-H), and 6.68—6.93 (2 H, m, 9-H); *m/e* 910 (39%), 851 (26), 751 (41), 749 (13), 691 (18), 482 (16), 469 (38), 455 (11), 402 (20), 295 (13), 271 (10), 239 (13), 217 (11), 215 (11), 188 (100), and 174 (54).

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