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OXIDATIVE TRANSFORMATIONS OF 14,15-DIHYDROVINDOLINE BY *STREPTOMYCES GRISEUS*

FRANCES M. ECKENRODE[1] and JOHN P. ROSAZZA*

Division of Medicinal Chemistry and Natural Products, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

ABSTRACT.—Dihydrovindoline was prepared by chemical reduction of vindoline and used as a substrate with *Streptomyces griseus* UI 1158 for the purpose of elaborating pathways by which this organism metabolizes nitrogen heterocyclic compounds. Dihydrovindoline was incubated with growing and resting cells of *S. griseus*, and preparative scale reactions were performed to isolate sufficient quantities of metabolites for structure elucidation. The organism achieves 11-*O*-demethylation of dihydrovindoline, and it performs a variety of oxidation reactions at position 3 of the substrate. Products isolated and characterized included 3-oxo-14,15-dihydrovindoline; $\Delta^{3(14)}$-14-acetyl-14,15-dihydro-17-desacetylvindoline; and the carbinolamine metabolite 3-hydroxy-14,15-dihydrovindoline. The nature of these metabolites suggests the involvement of iminium and enamine intermediates, and a pathway for the oxidation of dihydrovindoline is given.

Microbial transformation studies performed earlier with vindoline (**1a**) have resulted in the production of several interesting metabolites containing an intramolecular ethereal linkage as a common structural feature (1-3). It is believed that vindoline undergoes initial oxidation to an iminium intermediate, which then undergoes intramolecular cyclization to form the various identified metabolites such as dihydrovindoline ether (**2**) (2,4).

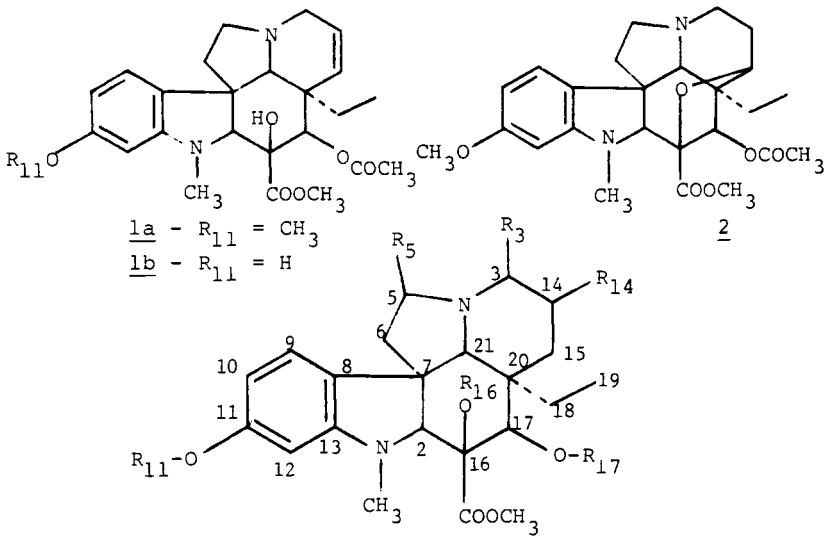
14,15-Dihydrovindoline (**3**) was selected as the substrate of choice in order to shed further light on the pathways by which *Streptomyces griseus* (UI 1158) achieved oxidation of Aspidosperma alkaloids. The simple removal of the 14,15-double bond of vindoline by catalytic reduction would remove a functional group directly involved in the intramolecular cyclization reaction involving the oxygen atom at position-16 and the 15-carbon positions of these alkaloids. By use of dihydrovindoline, it was hoped that less complicated metabolites, and perhaps some putative intermediates in vindoline oxidation processes, might be accumulated in incubation media. One major metabolite of dihydrovindoline transformation by *S. griseus* has already been identified as 11-*O*-desmethyl-14,15-dihydrovindoline (**11**) (5). This report describes the formation, isolation, and characterization of 3-oxo-14,15-dihydrovindoline (**4**); a vinylogous amide, $\Delta^{3(14)}$-14,acetyl-14,15-dihydro-17-desacetylvindoline (**5**); and a carbinolamine, 3-hydroxy-14,15-dihydrovindoline (**6**) as metabolites of 14,15-dihydrovindoline (**3**) by *S. griseus*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points, infrared (ir), ultraviolet (uv), nuclear magnetic resonance (nmr), and low resolution mass spectra were obtained as previously described (5). Fast atom bombardment mass spectra were provided through the courtesy of the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, supported in part by a grant from the National Institute of General Medical Sciences (GM 27029). Vindoline (**1a**) (figure 1) was obtained as a generous gift from Eli Lilly and Co., Indianapolis, Indiana, and has been fully characterized (2). Dihydrovindoline (**3**) (figure 1) was prepared as described in the literature and was fully characterized (ir, uv, nmr, mass spectrometry) before use (5).

CHROMATOGRAPHY.—Thin-layer chromatography (tlc) was performed on 0.25- or 0.5-mm thick layers of silica gel GF_{254} (Merck) prepared on glass plates with a Quickfit Industries Spreader (Quickfit In-

[1]Present address: School of Pharmacy, Oregon State University, Corvallis, Oregon 97331.



Compound	R_3	R_5	R_{11}	R_{14}	R_{16}	R_{17}	Other
3	H	H	CH_3	H	H	$COCH_3$	—
4	=O	H	CH_3	H	H	$COCH_3$	—
5	H	H	CH_3	$COCH_3$	H	H	$\Delta^{3(14)}$
6	OH	H	CH_3	H	H	$COCH_3$	—
7	H	=O	CH_3	H	$COCH_3$	$COCH_3$	—
8	=O	H	CH_3	H	$COCH_3$	$COCH_3$	—
9	H	H	CH_3	H	$COCH_3$	$COCH_3$	—
10	=O	H	CH_3	H	H	H	—
11	H	H	H	H	H	$COCH_3$	—

FIGURE 1. Structures of various derivatives of vindoline (**1**) and dihydrovindoline (**3**).

dustries, London, England). Solvent systems were: A, chloroform-ethanol (30:1); B, ethyl acetate-benzene (4:1); C, ethyl acetate-methanol (3:1); D, chloroform-ethanol (120:1). Chromatograms are visualized by fluorescence quenching under 254 nm uv light and by spraying with ceric ammonium sulfate [1% $Ce(NH_4)_4\cdot(SO_4)_4$ in 50% H_3PO_4] (6). Dihydrovindoline (**3**) possessed Rf 0.70 and 0.24 in tlc solvent systems A and B, respectively.

Column chromatography was performed with silica gel (Baker 3405, 60-200 mesh), which was activated for 60 min at 120° prior to use. Columns were wet packed in the developing solvent, and fractions were collected in a Fractomette 200 instrument.

High performance liquid chromatography (hplc) was performed as previously described (5). Separations were achieved with a μ-Bondapak phenyl column (0.4×30cm, Waters) using methanol and 0.005 M dibasic ammonium phosphate (70:30) at an average flow rate of 1 ml/min and an operating pressure of 1700 psi. Retention volumes were 5-oxo-14,15-dihydro-16-acetoxyvindoline (**7**) 6.9 ml; and 3-oxo-14,15-dihydro-16-acetoxyvindoline (**8**) 6.3 ml. A C-18 μ-Bondapak column (0.4×30cm, Waters) was used to compare 3-oxo-14,15-dihydro-17-desacetylvindoline (**10**) obtained from synthetic and microbial sources. Methanol-water (75:30) at an average flow rate of 2 ml/min and 2600 psi provided a retention volume of 5.0 ml, while methanol-water (50:50) at an average flow rate of 2 ml/min and 3000 psi provided a retention volume of 17.6 ml.

14,15-Dihydro-16-acetoxyvindoline (**9**).—14,15-Dihydrovindoline (**3**) (1.35 g) was dissolved in 65 ml of pyridine, and 85 ml of acetic anhydride was added. The reaction was stirred under a nitrogen atmosphere at 40° for 48 h and was terminated by pouring over 500 g of ice. The resulting solution was adjusted to pH 10 with 58% ammonium hydroxide solution and filtered. The precipitate was washed with water and dried under vacuum to yield 16-acetoxy-14,15-dihydrovindoline (**9**) as a white amorphous solid (1.35 g) (7): uv λ max (EtOH) 211 (log ε 4.59), 247 (3.85), 301 (3.75); ir (KBr) 3380, 2940, 1750, 1600, 1450, 1360 and 1255; ^{1}H-nmr ($CDCl_3$) 0.51 (3H, t, J=7 Hz, H-18), 1.98 (3H, s, $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 2.62 (3H, s, NCH_3), 3.67 (1H, s, H-2), 3.78 (6H, s, OCH_3), 5.90 (1H, s, H-17), 6.05 (1H, d, J=2 Hz, H-12), 6.30 (1H, dxd, J=8 and 2 Hz, H-10), 7.0 (1H, d, J=8 Hz, H-9); high resolu-

tion mass spectrum, *m/e* 500.2501 (9.10%, calcd for $C_{27}H_{36}N_2O_7$ 500.2522), 298.2052 (30.62%, calcd for $C_{19}H_{26}N_2O$ 298.2045), 188.1074 (8.50%, calcd for $C_{12}H_{14}NO$ 188.1075), 174.0918 (3.23%, calcd for $C_{11}H_{12}NO$ 174.0919); 124.1129 (100%, calcd for $C_8H_{14}N$ 124.1126).

Synthesis of 3-oxo- (**8**) *and 5-oxo-14,15-dihydro-16-acetoxyvindoline* (**7**).—The 3-oxo-(**8**) and 5-oxo-(**7**) derivatives were prepared by mercuric acetate oxidation of 14,15-dihydro-16-acetoxyvindoline (**9**) by the method of Kutney *et al.* (7). Mercuric acetate (4.3 g) and **9** (1.35 g) were dissolved in dry dioxane (25 ml) and heated under reflux in an atmosphere of nitrogen for 20 h. The mixture was cooled, diluted with methanol (250 ml), and treated with hydrogen sulfide for 45 min followed by nitrogen for 30 min. The mixture was further diluted with 4 N ammonium hydroxide, filtered, and evaporated. The residue was partitioned between 2 N ammonium hydroxide and dichloromethane. The organic phase was washed with a saturated solution of ammonium chloride, dried over anhydrous sodium sulfate, and concentrated to a red oil (1.03 g). The residue was dissolved in chloroform and applied to a column (*100 g silica gel*, 3×32 cm), which was eluted with chloroform at a flow rate of 2.5 ml/min, while 15 ml fractions were collected. 5-Oxo-14,15-dihydro-16-acetoxyvindoline (**7**) was eluted ion fractions 133 to 235 (163 mg) (7): uv λ max (EtOH) 212 (log ε 4.49), 247 (3.79), 300 (3.66); ^{1}H-nmr ($CDCl_3$), 2.0 (3H, s, $OCOCH_3$), 2.1 (3H, s, $OCOCH_3$), 2.65 (3H, s, NCH_3), 3.5 (1H, s, H-21), 3.75 (1H, s, H-2), 3.8 (6H, s, OCH_3 and CO_2CH_3), 5.75 (1H, s, H-17), 6.1 (1H, d, J=8 Hz, H-9); mass spectrum, *m/e* (rel. int.) 514 (1), 454 (1), 413 (1), 396 (1), 395 (4), 312 (2), 270 (2), 254 (1), 214 (2), 212 (3), 201 (3), 188 (5), 187 (3), 175 (70), 174 (100), 159 (70), 111 (5), 110 (6), 43 (13); tlc-system A, Rf 0.70.

3-Oxo-14,15-dihydro-16-acetoxyvindoline (**8**) (7) was eluted in fractions 270 to 400 (164 mg): uv λ max (EtOH) 213 (log ε 4.42), 252 (3.76), 302 (3.60); ir (KBr) 3400, 2910, 1750, 1650, 1605, 1450, 1362, 1220; ^{1}H-nmr ($CDCl_3$), 2.0 (3H, s, $OCOCH_3$), 2.1 (3H, s, $OCOCH_3$), 2.8 (3H, s, NCH_3), 3.6 (1H, s, H-21), 3.7 (3H, s, OCH_3), 3.8 (3H, s, OCH_3), 4.0 (1H, s, H-2), 5.3 (1H, s, H-17), 6.12 (1H, d, J=2 Hz, H-12), 6.32 (1H, dxd, J=8 and 2 Hz, H-10), 6.85 (1H, d, J=8 Hz, H-9); high resolution mass spectrum, *m/e* 514.2315 (8.26%, calcd for $C_{27}H_{34}N_2O_8$ 514.2315), 312.1851 (2.06%, calcd for $C_{19}H_{24}N_2O_2$ 312.1838), 188.1049 (27.84%, calcd for $C_{12}H_{14}NO$ 188.1075), 187.0997 (100%, calcd for $C_{12}H_{13}NO$ 187.0997), 174.0917 (16.31%, calcd for $C_{11}H_{12}NO$ 174.0919), 138.0938 (71%, calcd for $C_8H_{12}NO$ 138.0919); tlc, solvent system A, 0.60.

Synthesis of 3-oxo-14,15-dihydro-17-desacetylvindoline (**10**).—Synthetic 3-oxo-14,15-dihydro-16-acetoxyvindoline (**8**) (55 mg) was dissolved in 7 ml of 0.5 N sodium methoxide and stirred at room temperature under nitrogen. After 5 h, 100 mg of monobasic sodium phosphate and 30 ml of water were added. The solution was adjusted to pH 0.5 with 58% ammonium hydroxide solution and extracted with 3×50 ml chloroform. The extracts were dried over anhydrous sodium sulfate and evaporated in vacuum to yield 3-oxo-14,15-dihydro-17-desacetylvindoline (**10**) as an amorphous solid (48 mg). The material was further purified by dissolving in 1.0 ml of solvent system D and applying to a column (8 g silica gel, 18×2 cm). The column was eluted with the same solvent system at a flow rate of 4 ml/min, while 8-ml fractions were collected. Fractions 40 to 50 were combined and evaporated under vacuum to yield **10** as a yellow amorphous solid (35 mg); uv λ max (EtOH) 255 nm (log ε 3.68), 308 nm (3.55); ir (KBr) 3420, 2955, 2875, 1725, 1630, 1615, 1595, 1500, 1460, 1250, 1230; ^{1}H-nmr ($CDCl_3$), 2.8 (3H, s, NCH_3), 3.39 (1H, s, H-21), 3.85 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.98 (1H, s, H-2), 6.1 (1H, d, J=2 Hz, H-12), 6.32 (1H, dxd, J=8 and 2 Hz, H-10), 6.9 (1H, d, J=8 Hz, H-9): high resolution mass spectrum, *m/e* 430.2094 (2.17%, calcd for $C_{23}H_{30}N_2O_6$ 430.2103), 312.1830 (4.5%, calcd for $C_{19}H_{24}N_2O_2$ 312.1838), 188.1075 (26.13%, calcd for $C_{12}H_{14}NO$ 188.1075), 187.0992 (100%, calcd for $C_{12}H_{13}NO$ 187.0997), 174.0921 (36.52%, calcd for $C_{11}H_{12}NO$ 174.0919), 138.0923 (1.16%, calcd for $C_8H_{12}NO$ 138.0919), tlc solvent systems A and C, Rf 0.22 and 0.60, respectively.

FERMENTATION PROCEDURE.—Methods used in the cultivation of *S. griseus* (UI 1158, NRRL B8090) and in the use of resting cell preparations with this organisms have been described elsewhere (5,8).

FORMATION OF THE VINYLOGOUS AMIDE (**5**) AND 3-HYDROXY-14,15-DIHYDROVINDOLINE (**6**) BY *S. GRISEUS*.—14,15-Dihydrovindoline (**3**) (780 mg in 7.8 ml DMF) was distributed evenly among 78 Erlenmeyer flasks (125 ml) containing 25 ml of resting cells of *S. griseus*, and the incubation was continued for 96 h. Metabolites were isolated by combining the contents of all flasks, adjusting the pH to 9.7 with 58% ammonium hydroxide solution, and extracting with 3×500 ml ethyl acetate. The extracts were combined, dried over anhydrous sodium sulfate, and evaporated to yield a brown oil (312 mg). The oil was dissolved in a minimum amount of ethyl acetate, applied to five preparative layer plates (0.5 mm thickness), and these were developed with solvent system A.

Isolation and characterization of **5**.—The bands between Rf 0.10 and 0.2 were scraped from the plate and extracted with ethyl acetate. The extracts were evaporated to yield a yellow-brown oil (55 mg). This material was further purified by dissolving in 1 ml of solvent system D and applying to a column (*9 g silica*, 21×1 cm), which was eluted with the same solvent system at a flow rate of 0.5 ml/min, while 3-ml fractions were collected. Fractions 113-150 were combined and evaporated under vacuum to yield the vinylog-

ous amide (**5**) as an amorphous solid (45 mg): tlc, solvent system A, Rf 0.2; uv λ max (EtOH) 214 nm (log ε 4.39), 251 (3.74), 320 (4.31); ir (KBr) 3430, 3320, 2960, 2930, 2870, 1735, 1615, 1600, 1560, 1500, 1270, 1240; ^{13}C-nmr (see table 1); ^{1}H-nmr ($CDCl_3$) 2.10 (3H, s, $COCH_3$), 2.60 (3H, s, NCH_3), 3.80 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.25 (1H, s, H-17), 6.10 (1H, d, J=2 Hz, H-12), 6.25 (1H, dxd, J=8 and 2 Hz, H-10), 6.9 (1H, d, J=8 Hz, H-9), 7.4 (1H, s, H-3); high resolution mass spectrum, *m/e* 456.2265 (21.89%, calcd for $C_{25}H_{32}N_2O_6$ 456.2260), 397.2131 (6.08%, calcd for $C_{23}H_{29}N_2O_4$ 397.2127), 339.2061 (5.83%, calcd for $C_{21}H_{27}N_2O_2$ 339.2072), 338.1997 (7.87%, calcd for $C_{21}H_{26}N_2O_2$ 338.1994), 297.1974 (88.28%. calcd for $C_{19}H_{25}N_2O$ 297.1967), 188.1077 (100%, calcd for $C_{12}H_{14}NO$ 188.1075), 174.0918 (23.33%, calcd for $C_{11}H_{12}NO$ 174.0919), 164.1074 (4.67%, calcd for $C_{10}H_{14}NO$ 164.1075), 122.0972, (7.56%, calcd for $C_8H_{12}N$ 122.0970).

TABLE 1. ^{13}C-NMR chemical shifts for dihydrovindoline (**3**), $\Delta^{3(14)}$-14-acetyl-14,15-dihydro-17-desacetylvindoline (**5**), and 3-hydroxy-14,15-dihydrovindoline (**6**) taken in deuteriochloroform.

	Chemical Shifts (ppm)		
	3[a]	**5**	**6**
C(2)	83.5	80.213 (d)	80.863 (d)
C(3)	52.4	146.943 (d)	89.440 (d)
C(5)	51.4	48.960 (t)	47.400 (t)
C(6)	43.4	42.722 (t)	44.087 (t)
C(7)	52.4	52.728 (s)	54.223 (s)
C(8)	125.2	124.657 (s)	122.058 (s)
C(9)	122.7	122.642 (d)	121.928 (d)
C(10)	104.1	104.514 (d)	104.059 (d)
C(11)	160.6	161.043 (s)	160.848 (s)
C(12)	95.6	96.457 (d)	96.132 (d)
C(13)	154.0	153.896 (s)	154.156 (s)
C(14)	22.4	106.853 (s)	24.204 (t)
C(15)	33.0	35.445 (t)	30.052 (t)
C(16)	78.3	77.809 (s)	82.924 (s)
C(17)	75.6	70.532 (d)	75.340 (d)
C(18)	7.8	7.375 (q)	7.830 (q)
C(19)	29.9	25.504 (t)	30.572 (t)
C(20)	40.0	35.380 (s)	40.253 (s)
C(21)	72.3	68.388 (d)	65.334 (d)
NCH_3	37.7	39.408 (q)	38.694 (q)
OCH_3	55.0	55.198 (q)	55.198 (q)
CO_2CH_3	52.0	53.248 (q)	52.274 (q)
$COCH_3$	20.7	23.684 (q)	20.955 (q)
CO_2CH_3	170.0	175.793 (s)	169.945 (s)
$COCH_3$	172.3	191.972 (s)	172.479 (s)

[a]Data are from reference (10).

s=singlet; d=doublet; t=triplet; q=quartet.

Isolation and characterization of **6**.—The bands between 0.5 and 0.85 from the preparative plates described above were removed and extracted with ethyl acetate. The extracts were evaporated to yield a yellow-brown oil (462 mg). This material was further purified by dissolving in 4 ml of solvent system A and applying to a column (50 g silica, 34×2.5 cm), which was eluted with the same solvent system at a flow rate of 0.6 ml/min, while 5.5-ml fractions were collected. Fractions 105-145 were combined and evaporated to yield unmetabolized 14,15-dihydrovindoline (**3**) (106 mg) as an amorphous solid.

Fractions 336-450 were combined and evaporated to yield 3-hydroxy-14,15-dihydrovindoline (**6**), plus traces of two decomposition products, as an amorphous solid (27 mg). This crude fraction had the following properties: tlc, solvent system A, Rf 0.60; uv λ max (EtOH) 215 nm (log ε 4.30), 254 (3.66), 308 (3.57); ir (KBr) 3310, 2950, 1740, 1615, 1595, 1500, 1230, 1235; ^{13}C-nmr (table 1); ^{1}H-nmr ($CDCl_3$), 2.0 (3H, s, $COCH_3$), 2.7 (3H, s, NCH_3), 3.8 (3H, s, OCH_3), 4.7 (1H, m, H-3), 5.4 (1H, s, H-17), 6.0 (1H, d, J=2 Hz, H-12), 6.2 (1H, dxd, J=8 and 2 Hz, H-10), 6.85 (1H, d, J=8 Hz, H-9); high resolution mass spectrum: *m/e* 456.2254 (13.24%, calcd for $C_{25}H_{32}N_2O_6$ 456.2260); 297.1969 (93.96%,

calcd for $C_{19}H_{25}N_2O$ 297.1967), 188.1073 (100.00%, calcd for $C_{12}H_{14}NO$ 188.1075), 174.0917 (8.59%, calcd for $C_{11}H_{12}NO$ 174.0919), 122.0971 (9.13%, calcd for $C_8H_{12}N$ 122.0969).

Reduction of **6** *to* **3** *with sodium borodeuteride.*—The carbinolamine (**6**) (8 mg) was dissolved in 2 ml of MeOD, and 20 mg $NaBD_4$ was added. The mixture was allowed to react for 20 min, evaporated under a stream of nitrogen until almost dry, redissolved in water, and extracted with ethyl acetate (2×5 ml). The combined organic fractions were dried over anhydrous sodium sulfate and concentrated. The residue was further purified by application to a 0.5-mm preparative plate and developed with solvent system A. The band of Rf 0.70 identical to that of 14,15-dihydrovindoline (**3**) was scraped, eluted with acetone, and evaporated to a pale yellow residue (1 mg): mass spectrum, *m/e* (rel. int.) 460 (13), 459 (32), 458 (8), 401 (8), 400 (22), 399 (12), 300 (40), 299 (100), 298 (26), 188 (23), 174 (18), 125 (100).

FORMATION OF 3-OXO-14,15-DIHYDROVINDOLINE (**4**) FROM 14,15-DIHYDROVINDOLINE (**3**) BY *S. GRISEUS*.—14,15-Dihydrovindoline (**3**) (2.4 g in 24 ml DMF) was distributed evenly among 20 one-liter Delong flasks containing 200 ml of cell suspension and 2 two-liter Microferm fermentors (Model MF102, New Brunswick Scientific Company) containing 1 liter each of cell suspension. The DeLong flasks were incubated according to conditions described above for fermentations. Incubations in Microferm fermentors were conducted at 27° with an agitation rate of 350 rpm, while air was sparged in at a rate of 3.0 liter air/liter medium/min. After 96 h, the incubations were combined and extracted as previously described to yield a viscous red oil (4.72 g). The oil was dissolved in a minimum amount of chloroform and applied to a column (300 g silica gel, dimensions 50×4.5 cm), which was eluted with chloroform (400 ml) followed by chloroform-ethanol (30:1) at a flow rate of 1.5 ml/min, while 12-ml fractions were collected.

Compounds isolated from the column included 11-*O*-desmethyl-14,15-dihydrovindoline (**15**), the vinylogous amide (**5**), and the carbinolamine (**6**) metabolites identified earlier in the first preparative scale incubation, plus crude 3-oxo-14,15-dihydrovindoline (**4**), which was isolated as a yellow-red oil (68 mg, 2.6%) from fractions 450-505. The oil was further purified by dissolving in 1.0 ml of chloroform and applying to preparative plates (silica gel, 0.5 mm thickness). The plates were developed in solvent system A. The band centered at Rf 0.5 was removed, extracted with acetone, and evaporated under vacuum to yield the 3-oxo-14,15-dihydrovindoline (**4**) (tlc, solvent system A, Rf 0.43) as an amorphous solid (22 mg) (13); uv λ max (EtOH) 214 nm (log ε 4.38), 253 (4.05), 305 (3.96); ir (KBr) cm^{-1} 3500, 3400, 2950, 2930, 2870, 1740, 1710, 1655, 1640, 1630, 1614, 1495, 1455, 1365, 1230, 1035; ^{1}H-nmr ($CDCl_3$), 2.0 (3H, s, $COCH_3$), 2.6 (3H, s, N-CH_3), 3.27 (1H, s, H-21), 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.93 (1H, s, H-2), 5.26 (1H, s, H-17), 6.07 (1H, d, J=2 Hz, H-12), 6.34 (1H, dxd, J=8 and 2 Hz, H-10), 6.86 (1H, d, J=8 Hz, H-9); high resolution mass spectrum, *m/e* 472.2187 (2.38%, calcd for $C_{25}H_{32}N_2O_7$ 472.2210), 312.1842 (1.93%, calcd for $C_{19}H_{24}N_2O_2$ 312.1838), 188.1052 (27.92%, calcd for $C_{12}H_{14}NO$ 188.1075), 187.0993 (100%, calcd for $C_{12}H_{13}NO$ 187.0997), 174.0920 (28.10%, calcd for $C_{11}H_{12}NO$ 174.0919), 138.0937 (1.47%, calcd for $C_8H_{12}NO$ 138.0919).

Deacetylation of 3-oxo-14,15-dihydrovindoline (**4**) *to* **10**.—The 3-oxo metabolite (**4**) (15 mg) was dissolved in 3 ml of 0.5 N sodium methoxide and stirred at room temperature under nitrogen. After 5 h, 30 mg of monobasic sodium phosphate and 10 ml of water were added. The solution was adjusted to pH 9.5 with 58% ammonium hydroxide and extracted with 3×15 ml chloroform. The extracts were combined, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 3-oxo-14,15-dihydro-17-desacetylvindoline (**10**) as a yellow amorphous solid (6 mg); mass spectrum *m/e* (rel. int.) 430 (3), 312 (10), 188 (25), 187 (100), 174 (15). This compound was chromatographically identical to synthetic (**10**) by tlc (solvent systems A and C) and by hplc.

RESULTS AND DISCUSSION

Streptomyces griseus (UI 1158) has served as an excellent metabolic system for the preparation of numerous metabolites of the Aspidosperma alkaloid vindoline (2,4). Earlier studies presented strong evidence that the 3-position of these alkaloids was the focal point of metabolic activity, and that quite probably, initial oxidations occurred at that site to result ultimately in a variety of metabolic products. The instability and high reactivity of proposed intermediates in the vindoline metabolic pathways led to a difficulty in the isolation or trapping of such compounds for the purpose of firmly establishing their existence. Thus, approaches were sought to utilize simple analogs of vindoline to simplify profiles of metabolites formed by *S. griseus* and to shed further light on the chemical mechanisms by which *S. griseus* achieves its metabolic interconversions of the alkaloid. 14,15-Dihydrovindoline (**3**) was selected as a simple and logical substrate for further study. Additionally, resting cell suspensions of the metabolizing microor-

ganism were employed in some experiments because the isolation of metabolites from complex fermentation media was sometimes fraught with difficulty. The alkaloid substrate **3** was incubated with both growing cells and resting cells suspensions of *S. griseus*. While growing cultures apparently provided greater yields of metabolites at 24 and 48 h, both these and resting cell suspensions gave essentially the same metabolite profile after 72 h.

Initial incubations with 14,15-dihydrovindoline (**3**) were conducted on a small, screening scale to reveal an apparent pattern of formation of metabolites later isolated and identified. It appeared that the carbinolamine metabolite (**6**) formed first within 24 h of substrate addition, while the lactam (**4**) and the vinylogous amide metabolite **5** were formed at approximately 36 h following substrate addition. Each of the metabolites was isolated from preparative resting cell suspension incubations of *S. griseus*.

IDENTIFICATION OF THE VINYLOGOUS AMIDE **5**.—The first metabolite was isolated in 5.8% yield from preparative scale incubations with 14,15-dihydrovindoline (**3**) and was identified as the novel vinylogous amide (**5**). Comparison of the ^{1}H-nmr spectra of **3** and the metabolite indicated that the dihydroindole aromatic moiety had not been metabolically altered. A singlet at 5.65 ppm (H-17 in 14,15-dihydrovindoline) was absent in the metabolite spectrum and was replaced by a new singlet at 4.25 ppm. Since the H-17 proton signal in 17-desacetylvindoline absorbs at 4.07 ppm (9), these data suggested that the C-17 acetyl group had been eliminated in the structure of the metabolite. A new 3-proton singlet at 2.10 ppm (acetyl-CH_3 protons) and a one-proton singlet at 7.40 ppm suggested the presence of a vinylogous amide functional grouping as in **5**. Typical for α,b-unsaturated ketones, the uv spectrum displayed a bathochromic and hyperchromic shift of the band from 304 nm to 320 nm, and this spectral behavior was absent in the starting material (**3**).

The high resolution mass spectrum of the metabolite indicated a molecular weight of 456.2265 for $C_{25}H_{32}N_2O_6$, in agreement with the proposed structure. Fragment ion peaks at *m/e* 174 and 188 verified that the dihydroindole portion of the metabolite structure was identical to that of **3** (figure 2), while fragments at 338 and 339 indicated that an acetyl functional grouping had been added to the piperidine ring portion of **3**.

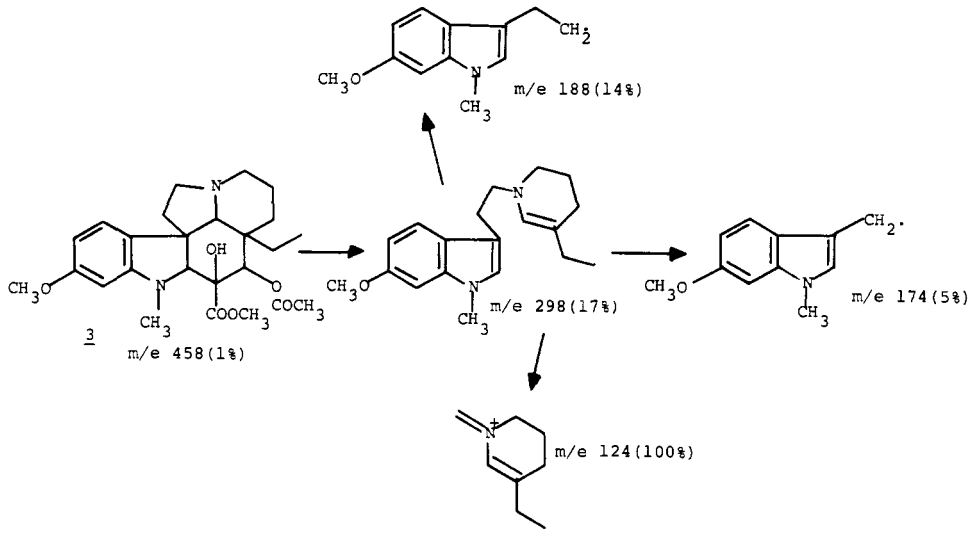


FIGURE 2. Mass spectral fragmentation pattern for 14,15-dihydrovindoline (**3**).

The ^{13}C-nmr spectrum of the metabolite displayed several major differences when compared with the starting material (**3**) (table 1) (10). In the proton coupled ^{13}C-spectrum, signals for two triplets found in 14,15-dihydrovindoline were missing; one of these corresponded to carbon-14 or carbon-19 (signal near 25 ppm), while the other corresponded to either carbon 3 or 5 (51 ppm). Two other absorbances were evident in the spectrum of the metabolite, these being a doublet at 146.943 ppm and a singlet at 106.853 ppm. These signals are attributed to the presence of olefinic carbon atoms in the structure of the metabolite (11), and were assigned to carbons 3 and 14, respectively. The carbonyl absorbance peak at 172 ppm for the 17-acetyl grouping of **3** was absent in the metabolite spectrum, and a new singlet carbonyl resonance at 191.972 replaced it. The chemical shift of this carbon atom is characteristic for α,b-unsaturated ketone moieties, which typically absorb between 185 and 208 ppm (11). These assignments are in excellent agreement with other alkaloidal vinyogous amides (12).

A compound with properties similar to the isolated metabolite **5** was reported as a mercuric acetate oxidation product of 14,15-dihydro-16-acetoxyvindoline (**9**) (7). Attempts to synthesize this compound by published methods were unsuccessful. Most of the spectral data for the metabolite were in complete agreement with that reported for the synthetic vinylogous amide. Based on all of the evidence obtained, the structure of the first metabolite of 14,15-dihydrovindoline was identified as the vinylogous amide (**5**). The origin of the acetyl grouping in the structure of **5** is uncertain, and it could derive either by an intramolecular transfer of the 17-acetyl group of (**3**) to position 14, or from acetate pools found in *S. griseus*. Further experiments designed to identify the origin of the 14-acetyl group of **5** are being conducted.

IDENTIFICATION OF 3-OXO-14,15-DIHYDROVINDOLINE (**4**).—The second and more polar metabolite was isolated from resting cell incubations in 2.6% yield after extensive chromatography. The low resolution mass spectrum of this compound indicated that a single oxygen atom had been incorporated into the dihydrovindoline backbone. The metabolite was assigned the structure of **4** based on the following considerations. The high resolution mass spectrum revealed that the metabolic molecular weight of 472.2225 was consistent with a molecular formula of $C_{25}H_{32}N_2O_7$ and that its structure contained one more oxygen atom than dihydrovindoline itself. Peaks in the mass spectrum at *m/e* 174 and 188 (figure 2) provided evidence that the additional oxygen atom was not in the dihydroindole portion of the molecule, while a peak at *m/e* 138 supported the presence of an oxygenated piperidine ring. These mass spectral properties of the metabolite were nearly identical to those reported for synthetic **4** (13). Attempts to prepare the metabolite directly by chemical methods were unsuccessful; however, the 16-acetoxy derivative had been reported earlier by Kutney *et al.*, (7). Authentic samples of 3-oxo-14,15-dihydro-16-acetoxyvindoline (**8**) and 5-oxo-14,15-dihydro-16-acetoxyvindoline (**7**) were obtained (7), and we also prepared these compounds using 14,15-dihydro-16-acetoxyvindoline (**9**) as starting material for the mercuric acetate oxidation. Spectral and chromatographic characteristics of our synthetic compounds and the authentic standards were identical. Attempts to convert the metabolite **4** to the 16-acetoxy derivative (**8**) were unsuccessful. However, deacetylation of both the metabolite **4** and the synthetic acetyl-3-oxo-derivative (**8**) using sodium methoxide formed a common product, 3-oxo-14,15-dihydro-17-desacetylvindoline (**10**). The deacetylated products obtained from **4** and **8** had identical mass spectra and identical mobilities on two tlc systems and two hplc systems. These results all strongly support the assignment of the structure of the more polar metabolite as 3-oxo-14,15-dihydrovindoline (**4**).

IDENTIFICATION OF THE CARBINOLAMINE **6**.—A third metabolite was isolated

from *S. griseus* incubations. This substance was the first to be observed by tlc in 24-h-old culture extracts. When isolated, the metabolite was subject to rapid decomposition upon exposure to routine chromatography and most organic solvents. Spectral data obtained immediately after isolation procedures were suggestive of the carbinolamine (**6**). An apparent molecular weight of *m/e* 456 was obtained with low and high resolution mass spectrometry. The fragmentation pattern was similar to that of vindoline, which contains a 14,15-double bond (9,14). Key peaks occurred at *m/e* 456, 397, 297, 188, 174 and 122. The major *m/e* 188 peak (99%) includes the dihydroindoline moiety, plus the two carbon atoms representing positions 6 and 5 of **3**, indicating that the metabolic contained a new functionality in the piperidine ring. The carbinolamine (**6**) would possess a molecular ion of *m/e* 474, and the lack of molecular ion could be explained by the simple loss of water from the metabolite. Elimination of water due to thermal decomposition often results in an exaggerated M-18 peak (15). Attempts to obtain a molecular ion through chemical ionization and fast atom bombardment mass spectrometry were similarly unsuccessful.

The ^{1}H-nmr spectra of 14,15-dihydrovindoline (**3**) and the carbinolamine metabolite (**6**) were similar, but no olefinic signals were observed in the spectrum of the metabolite. In addition, chromatographic mobilites of the new metabolite were significantly different from those for vindoline and 14,15-dihydrovindoline.

^{13}C-nmr spectral data for the metabolite (**6**) and 14,15-dihydrovindoline (**3**) were also similar, except for one signal (table 1). A triplet (methylene) signal at 52.3 ppm in **3** was replaced by a doublet (methine) resonance at 89.440 ppm in the spectrum of **6**. This signal was assigned to the carbon atom at position-3 of the metabolite, which is assumed to exist as a carbinolamine. Similar assignments have been made for several alkaloidal carbinolamines (16).

Confirmation of the identity of the metabolite as **6** was obtained by reduction of the compound with $NaBD_4$ in CH_3OD. The reduced metabolite was chromatographically identical to dihydrovindoline (**3**). Key fragments in the mass spectrum of this compound (*m/e* 459, 299, 188, 174, and 125) indicated that a single deuterium atom had been introduced into the piperidine-like ring of 14,15-dihydrovindoline (figure 2). Further attempts to obtain quantities of the carbinolamine (**6**) resulted in its decomposition to two less polar products (tlc, system A, Rf 0.80, system B, Rf 0.30, 0.55). The ^{1}H-nmr spectra of the decomposition products were suggestive of dimeric products, and both high and low resolution mass spectra provided molecular ions in excess of *m/e* 1000.

The use of dihydrovindoline as a substrate has added to our understanding of how *S. griseus* UI 1158 oxidizes Aspidosperma alkaloids. The structures of isolated metabolites clearly confirm earlier work that demonstrated the susceptibility of position-3 of these alkaloids to metabolic oxidation. Evidence in the present work strongly suggests the involvement of an iminium and enamine system in the piperidine-like ring of **3**, and a pathway for the oxidation of **3** is proposed in figure 3.

Metabolic oxidations of aliphatic amines often occur at carbon atoms bonded to nitrogen, with the ultimate formation of carbinolamine intermediates (17). In mammals, it appears that the oxygen atom of the carbinolamine such as **6** derives from molecular oxygen. It is not possible to describe the precise mechanism by which *S. griseus* accomplishes metabolic oxidation of 14,15-dihydrovindoline. However, it is conceivable that either the iminium species **12** or the carbinolamine (**6**) may be the first metabolic products obtained. Because the carbinolamine and the iminium species are essentially interconvertible in aqueous media (18-23), the specific precursors for products **4** and **5** are also difficullt to determine. However, **5** most likely forms by enamine isomerization and acylation, and **4** probably derives from the carbinolamine (**6**), which may be

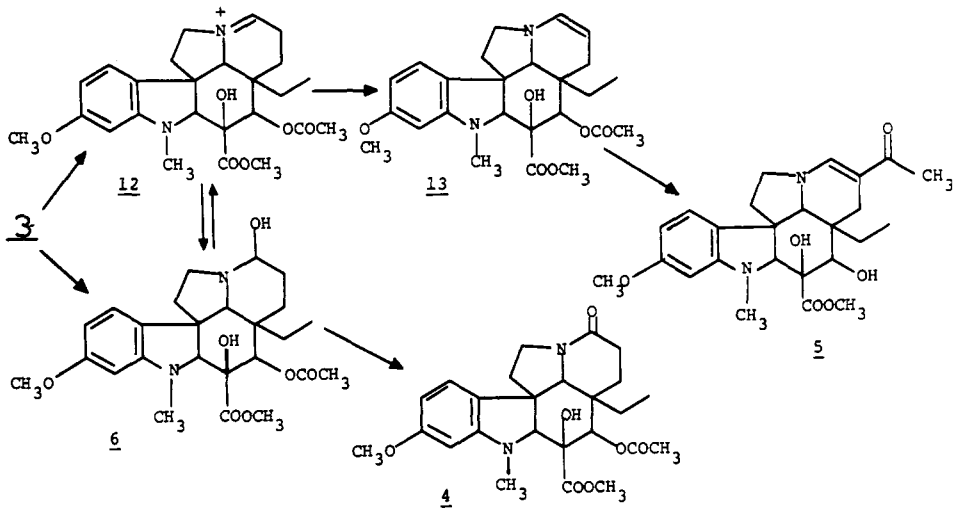


FIGURE 3. Proposed metabolic pathways for the oxidation of 14,15-dihydrovindoline (**3**) by *Streptomyces griseus*.

oxidized directly; or from oxidation of open chain aminoaldehydes that recyclize (21,22).

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