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Microbial Transformations of Glaucine

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Microbial transformation experiments were conducted with the aporphine alkaloid glaucine. Small-scale screening experiments provided a number of micro-organisms which produced three metabolites. In preparative scale studies, *Streptomyces griseus* (UI 1158) produced norglaucine (4) and 2-*O*-demethylglaucine (6) (predicentrine) in 11 and 14% yield, respectively. *Fusarium solani* (ATCC 12823) produced didehydroglaucine (3) and a noraporphinone (10) (an artefact) in 60 and 21% yield, respectively. With racemic glaucine, *F. solani* preferentially dehydrogenated (+)-(*S*)-glaucine, and unchanged, optically enriched (−)-(*R*)-glaucine was recovered from fermentations. *N*- and *O*-dealkylation did not occur in stereoselective fashion.

MICROBIAL transformations of alkaloids and other natural products have been studied.[1-4] Groups of alkaloids investigated to date include morphine derivatives,[5-7] ergolines,[8] and other indole derivatives.[2,9] In general, systematic studies on the types of reaction possible with alkaloids have not been conducted, although these compounds offer unusually rich arrays of functional groups which might be susceptible to chemical transformation by micro-organisms. Reactions reported include *N*- and *O*-dealkylations, hydroxylations, and *N*-oxidations.[10]

Except for a previous report from these laboratories,[11] no microbial transformation studies with aporphines have been described. This paper describes the selective *N*- and *O*-dealkylation of glaucine (1) by a *Streptomyces* species, and its stereoselective dehydrogenation by a *Fusarium* species.

RESULTS AND DISCUSSION

(+)-(*S*)-Glaucine was prepared by methylation of commercially available (+)-(*S*)-boldine in good yield with sodium hydride and 2 equiv. of methyl tosylate,[12] or with diazomethane.[13] Other literature procedures[14-16] were lower yielding. Didehydroglaucine (3) was prepared from (+)-(*S*)-glaucine by treatment with iodine and sodium acetate in dioxan,[17] or in higher yield with palladium–carbon in acetonitrile.[18]

Initial small-scale screening experiments were conducted to discover micro-organisms capable of metabolizing glaucine (1). Micro-organisms were selected on

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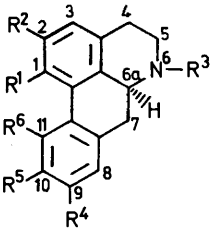
the basis of previous experience,[19,20] and of reports describing cultures capable of *O*- and *N*-dealkylations.[6,7,21,22] Of sixty cultures screened, eight gave sufficiently large amounts of glaucine metabolites to warrant further study (Table 1).

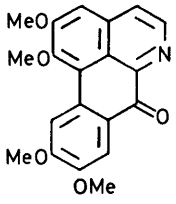
TABLE 1

Cultures yielding metabolites of (+)-(*S*)-glaucine (1)

Micro-organisms	Metabolites (6)	(4)	(3)	Other
Stysanus stemonites (SC 2831)	—	—	+	+
Penicillium claviforme (MR 376)	—	—	+	+
Cunninghamella echinulata (NRRL 3655)	+	—	—	+
Cunninghamella bainieri (ATCC 3065)	+	—	—	—
Streptomyces punipalus (NRRL 3529)	+	—	—	+
Streptomyces griseus (UI 1158)	+	+	—	—
Penicillium brevicompactum (ATCC 10418)	—	+	—	—
Fusasium solani (ATCC 12823)	—	—	+	+

Two metabolites were isolated from preparative scale fermentations with *Streptomyces griseus* (UI 1158). One of these, norglaucine (4) was identified simply on the basis of comparison of n.m.r. and mass spectral data





(10)

(1) $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = Me$, $R^6 = H$

(2) $R^1 = R^5 = OMe$, $R^2 = R^4 = OH$, $R^3 = Me$, $R^6 = H$

(3) 6a, 7-didehydro-(1)

(4) $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = R^6 = H$

(5) $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = Ac$, $R^6 = H$

(6) $R^1 = R^4 = R^5 = OMe$, $R^2 = OH$, $R^3 = Me$, $R^6 = H$

(7) $R^1 = R^2 = R^5 = OMe$, $R^4 = OH$, $R^3 = Me$, $R^6 = H$

(8) $R^1 = R^2 = R^4 = OMe$, $R^5 = OH$, $R^3 = Me$, $R^6 = H$

(9) $R^1 = R^2 = R^4 = H$, $R^5 = R^6 = OMe$, $R^3 = Me$

with published values.[23,24] The resolved *N*-methyl n.m.r. signal of glaucine is absent, and the mass spectrum shows retro-Diels–Alder fragmentation [25,26] ($M - CH_2{=}NH$). The m.p. of the *N*-acetyl derivative (5) was identical with the published value.[23]

The second metabolite was 2-*O*-demethylglaucine (6) (predicentrine;[24] *O*-methylboldine [27]). The mass spectrum indicated that a single methyl ether linkage had been cleaved (*m/e* 341). N.m.r. signals for both the *N*-methyl (δ 2.55) and the 1-methoxy-group (δ 3.58) were evident.[28,29] Methoxy protons at positions 2, 9, and 10 give an unresolved singlet at δ 3.90 in the 60 MHz n.m.r. spectrum of glaucine, and are only marginally separated in a 100 MHz spectrum. The n.m.r. spectrum of the metabolite was comparable with the published spectra of predicentrine (6),[30] *N*-methyllaurotetanine (7),[28,31] and thalicmidine (8) [31-33] (2-, 9-, and 10-*O*-demethylglaucine, respectively). The metabolite was probably not *N*-methyl-laurotetanine (7) since no bathochromic shift occurred in the u.v. spectrum upon the addition of base.[34]

The signals for the aromatic protons at positions 3, 8, and 11 are well separated in the n.m.r. spectrum of glaucine [28] and that of the metabolite. Thus, it was possible to locate the site of *O*-dealkylation by obtaining n.m.r. spectra of the metabolite while titrating the sample with alkali.[31] Under these conditions signals due to aromatic protons *ortho* to a phenolate ion experience a greater upfield shift than those of protons adjacent to methoxy-groups. For comparison, base-titration n.m.r. spectra were obtained for the metabolite, boldine (2), and (later) synthetic 9-*O*-methylboldine (6).[27] The results for the metabolite are shown in Table 2. It

TABLE 2

Relative chemical shifts of protons of the metabolite (6) when titrated with NaOD in $(CD_3)_2SO$–D_2O *

Solvent (equiv. NaOD)	δ H-3	H-8	H-11
$(CD_3)_2SO$	6.52	6.90	7.87
$(CD_3)_2SO$–D_2O (0.28)	6.50	6.87	7.85
$(CD_3)_2SO$–D_2O (0.56)	6.55	6.90	7.85
$(CD_3)_2SO$–D_2O (0.84)	6.50	6.85	7.92
$(CD_3)_2SO$–D_2O (1.10)	6.38	6.90	7.95
Δδ (total)	−0.14	0.00	+0.08

* The experiment was conducted according to the procedure of Pachler;[33] the metabolite (30 mg) was dissolved in $(CD_3)_2SO$ (0.5 ml) and 30% NaOD in D_2O added in 3 μl portions.

was necessary to add base (NaOD) stepwise since it was possible for signals of H-3 and H-8 to cross one another. The H-3 signal was the only one shifted upfield; that of H-11 was shifted downfield, and that of H-8 was unaltered. The magnitude of the shift of H-3 was con-

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sistent with published values for *O*-demethyl derivatives of papaverine.[35]

The structure of the metabolite was confirmed by careful methylation of boldine (2) with diazomethane [27] to yield the two possible isomeric monophenolic trimethoxyaporphines (6) and (7). These were easily separated by t.l.c. and gave different colour reactions with diazotized sulphanilic acid reagent.[27] One of them was identical with authentic *N*-methyl-laurotetanine (7), and exhibited the expected bathochromic u.v. shift upon addition of base.[34] The other (6) behaved chromatographically and spectrally (including the NaOD n.m.r. titration) like the metabolite. The metabolite and (6) prepared from boldine formed identical crystalline hydrobromides.[30]

It is interesting that *O*-dealkylation was restricted to ring A of glaucine, since highly selective *O*-dealkylation of 10,11-dimethoxyaporphine (9) is effected by *Streptomyces* species, and by *Cunninghamella* species.[11] It appears that *O*-dealkylation is restricted to an unhindered methoxy group, as in the *O*-dealkylation of 10,11-dimethoxyaporphine.[11]

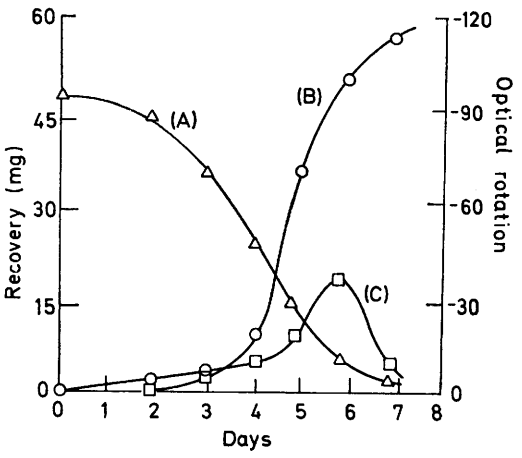
Fusarium solani (ATCC 12823) gave two products, both different from those produced by *S. griseus*. One was identical with synthetic didehydroglaucine (3), showing a broad u.v. absorption maximum from 264 to 278 nm, characteristic of didehydroaporphines.[36,37] The m.p. of the metabolite [38] and the n.m.r. spectrum [37,38] were in agreement with published values.

Lower yields of a noraporphinone (10) were obtained. The product was spectrally identical with a sample described in the literature (i.r.,[39,40] n.m.r.,[41] and u.v. [41]) and possessed the same m.p.[42]

Oxidation of glaucine in air is known to yield a variety of products including didehydroglaucine, and the noraporphinone (10).[43] When didehydroglaucine was incubated under the same conditions as employed for the cultivation of micro-organisms, the noraporphinone was readily detected, in 10% yield as estimated by t.l.c. Glaucine incubated under these conditions produced only traces of didehydroglaucine and the noraporphinone. Thus, the noraporphinone (10) was an artefact produced by aerial oxidation of didehydroglaucine (3).

Stereoselectivity has been demonstrated in a variety of microbial reactions with other substrates.[3,8,44,45] Attempts were made to determine whether *S. griseus* or *F. solani* exhibited substrate enantiospecificity in the reactions described with glaucine. For these studies, (*RS*)-glaucine was prepared by reduction of didehydroglaucine with sodium cyanotrihydridoborate [46,47] at pH 3—4. All attempts to reduce didehydroglaucine with dissolving metals,[48] sodium tetrahydridoborate, and hydrogen over palladium–carbon or platinum oxide [49] were disappointing, giving low yields. Glaucine recovered from fermentations of *S. griseus* was not optically active. On the other hand glaucine recovered from *F. solani* fermentations was 50.8% optically pure (75.4% enantiomerically pure) (—)-(*R*)-glaucine. Thus, an apparent substrate enantiospecificity for natural (+)-(*S*)-glaucine operates during dehydrogenation.

The time course of the conversion of racemic glaucine into didehydroglaucine was studied. Cultures of *F. solani* were grown as usual, and at various times after addition of (*RS*)-glaucine substrate the contents of whole flasks were extracted; unchanged glaucine and didehydroglaucine were separated by preparative t.l.c. and their identities were confirmed by chromatographic and u.v. and mass spectral analyses. Their concentrations were determined spectrophotometrically (u.v. absorptions at 280 and 302, and 260 and 334 nm, respectively). Optical rotations of the recovered glaucine were also measured. The results are shown in the Figure.



Time course of the conversion of racemic glaucine into didehydroglaucine by *F. solani*: (A) glaucine recovery; (B) glaucine optical activity; (C) didehydroglaucine yield

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Optical enrichment in (−)-(*R*)-glaucine was confirmed, and the optical rotation approached the theoretical value (−115°) after 7 days. At the same time, the overall concentration of glaucine diminished. After 7 days, only a few percent of the glaucine originally added remained. Didehydroglaucine yield increased up to 6 days, then decreased. The low recoveries of didehydroglaucine at later times are reproducible. In general, recoveries of both glaucine and dehydroglaucine were low, even at zero time, probably owing to binding of both compounds by the mycelium of *F. solani*. This phenomenon has been observed with glaucine and other aporphines studied in our laboratory. Since little glaucine remains in the fermentation after 7 days, both isomers are probably utilized by the micro-organism.

This is the first report of a microbial dehydrogenation of an aporphine. As with steroids and other compounds studied in microbial transformation systems, this reaction occurred in stereoselective fashion. With the intact micro-organism, a multitude of reactions are possible. The true degree of enantiospecificity of the *F. solani* dehydrogenase enzyme will not be known until the enzyme is purified. It is conceivable that (−)-(*R*)-glaucine is degraded by a completely different metabolic path, or that it is simply dehydrogenated more slowly than the (+)-(*S*)-glaucine isomer. Further studies concerning the metabolic fate of (−)-(*R*)-glaucine are in progress. When purified, the appropriate enzyme may provide a useful tool for the resolution of racemic mixtures of glaucine and other aporphines.

EXPERIMENTAL

Physical data were obtained as follows: n.m.r. spectra, Varian T-60 spectrometer, Me_4Si internal standard; low resolution mass spectra, Finnigan 3200 spectrometer; high resolution mass spectra determined by Battelle Memorial Laboratories, Columbus, Ohio; m.p. (corrected) for samples in open-ended capillaries, obtained with a Thomas–Hoover apparatus; i.r. spectra, Perkin-Elmer 267 spectrophotometer; optical rotations, Perkin-Elmer 141 polarimeter (0.099 8 dm microcell); u.v. spectra, Pye–Unicam SP 1800 spectrophotometer, Didydium external standard.

T.l.c. was performed on 0.25 mm or 1.0 mm thick layers of silica gel GF_{254} (Merck) on glass plates, activated at 120 °C for 30 min prior to use; solvent systems (A) ethyl acetate–ethyl methyl ketone–acetic acid–water (3 : 4 : 1 : 2); (B) benzene–methanol (4 : 1); (C) benzene–methanol (9 : 1); (D) chloroform–diethylamine (9 : 1); (E) benzene–methanol–ammonia (56%) (80 : 20 : 0.1); (F) benzene–methanol (6 : 1). Compounds were located by fluorescence quenching under 254 or 366 n.m. u.v. irradiation, or by spraying with the following reagents: Dragendorff's,[50] cerium(IV) ammonium sulphate (CAS) (1% in 50% v/v H_3PO_4); [51] or diazotized sulphanilic acid–NaOH (5% in 50% ethanol).[50] Column chromatography was performed on silica gel (Baker 3400).

(+)-(S)-*Glaucine* (1) *from* (+)-(S)-*Boldine* (2).[12]—A suspension of boldine (2) (3.27 g, 10 mmol) and sodium hydride (1.0 g, 40 mmol) in dry dimethylformamide (100 ml) was stirred under nitrogen for 0.5 h. A solution of methyl tosylate (4.0 g, 21 mmol) in anhydrous ether (20 ml) was added over 15 min. The reaction was monitored by t.l.c. [system (B); CAS reagent]. After 12 h the mixture was poured over ice (70 g) and exhaustively extracted with ether to yield a residue (3.7 g) after evaporation. The product was purified by column chromatography [silica gel (90 g; 42 × 2.5 cm); benzene–methanol (20 : 1) at 1.5 ml min^{-1}; 15 ml fractions]. Fractions 17—33 gave glaucine (2.45 g) as an oil. Further removal of solvent under high vacuum yielded crystalline glaucine (1) (2.0 g, 56%), m.p. 117—119° (lit.,[52] 120°); $\lambda_{max.}$ (EtOH) 280 (ε 1.5 × 10^4) and 302 nm (1.4 × 10^4); δ($CDCl_3$) 2.55 (3 H, s NMe), 3.67 (3 H, s, 1-OMe), 3.88, 3.90, and 3.92 (9 H, 3× s, 2-, 9-, and 10-OMe), 6.60 (1 H, s, H-3), 6.80 (1 H, s, H-8), and 8.12 (1 H, s, H-11); $[\alpha]_D^{25}$ (*c* 5.04 in EtOH) + 111° (lit.,[52,53] +113)°; *m/e* 355 (90%), 354(100), 341(15), 340(64), 338(15), 330(9), 329(30), 312(18), 308(10), 297(19), 281(30), and 266(9) (Found: C, 71.25; H, 7.3; N, 3.75. Calc. for $C_{21}H_{25}NO_4$: C, 70.95; H, 7.1; N, 3.9%); hydrochloride, m.p. 234° (from methanol–water) (lit.,[52] 234°).

Didehydroglaucine (3).[17]—Didehydroglaucine was prepared either by treating (+)-(*S*)-glaucine with iodine in dioxan [17] or by refluxing (+)-(*S*)-glaucine over palladium–carbon (10%) in acetonitrile.[18] Reactions were monitored by t.l.c. [system (B); R_F (1) 0.5, R_F (3) 0.95]; the product had m.p. 124° (from ethanol) (lit.,[39] 121—122°); $\lambda_{max.}$ (EtOH) 260.5 (ε 3.36 × 10^4) and 334 nm (8.72 × 10^3); [38,39] 3.03 ($CDCl_3$) (3 H, s, NMe), 3.27 (4 H, s, $CH_2{\cdot}CH_2$), 3.90 (3 H, s, 1-OMe), 4.02 (9 H, s, 2-, 9-, and 10-OMe), 6.57 (1 H, s, H-7), 6.95 (1 H, s, H-3), 7.03 (1 H, s, H-8), and 9.20 (1 H, s, H-11); *m/e* 354(31%), 353(100), 388(86), 337(86), 335(46), 307(31), 306(31), 294(31), 280(54), 279(57), and 176(86); all data were consistent with published values.[37,38]

(RS)-*Glaucine* [(RS)-(1)].[46,47]—Sodium cyanotrihydridoborate (0.2 g, 0.45 mmol) was added to a solution of didehydroglaucine (3) (100 mg, 1.3 mmol) in absolute ethanol (30 ml) being purged with nitrogen. Alcoholic hydrogen chloride was added to maintain the pH at *ca.* 3—4. After 18 h, t.l.c. [system (E)] indicated that the reaction was complete. The mixture was evaporated to dryness *in vacuo* and the residue suspended in cold water (30 ml) and exhaustively extracted with ethyl acetate. The extracts were concentrated to an oil. Racemic glaucine, (*RS*)-(1), purified by preparative t.l.c. [system (E)], exhibited no optical rotation; m.p. 136—137° (from methanol) (lit.,[53] 137—139°); δ($CDCl_3$) 2.53 (3 H, s, NMe), 3.65 (3 H, s, 1-OMe), 3.90 (9 H, s, 2-, 9-, and 10-OMe), 6.57 (1 H, s, H-3), 6.77 (1 H, s, H-8), and 8.05 (1 H, s, H-11); *m/e* 355(62%), 354(100), 341(10), and 340(45) (Found: C, 71.1; H, 7.15; N, 3.85%).

Synthesis of 2-O-*Methylboldine* [N-*Methyl-laurotetanine* (7)] *and* 9-O-*Methylboldine* [*Predicentrine* (6)].[27]—Ethereal diazomethane (3 mmol) was added to a solution of boldine (2) (1.0 g, 3 mmol) in methanol (40 ml) cooled in an ice-bath, and the mixture was allowed to warm to room temperature. After 4.5 h more diazomethane (1.5 mmol) was added, and the solution was set aside at room temperature overnight. The reaction was monitored by t.l.c. [system (D); diazotized sulphanilic acid spray showed boldine (2), R_F 0.35, brown; 9-*O*-methylboldine (6), R_F 0.80, orange; glaucine (1), R_F

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0.98, grey; and 2-*O*-methylboldine (7), R_F 0.60, red-brown]. The mixture was evaporated to dryness and the products chromatographed on a silica gel column (150 g, 70 × 2.5 cm) eluted with chloroform–diethylamine (100 : 1) at 2 ml min^{-1} (15 ml fractions). Fractions 68—120 contained 9-*O*-methylboldine (6) (350 mg, 35%), and fractions 133—165 2-*O*-methylboldine (7) (100 mg, 10%), identical chromatographically with *N*-methyl-laurotetanine, kindly supplied by Dr. M. P. Cava. 9-*O*-Methylboldine hydrobromide had m.p. 209—211° (lit.,[30] 209°); [30] the m.p. of a mixture with predicentrine hydrobromide prepared from the metabolite obtained from *S. griseus* showed no depression (Found: C, 56.65; H, 5.75; N, 3.2. Calc. for $C_{20}H_{24}BrNO_4$: C, 56.9; H, 5.75; N, 3.3%).

Fermentation Procedures.—Cultures used in this study are maintained in the University of Iowa College of Pharmacy culture collection, and are stored at 4 °C in a refrigerator. Organisms were grown according to a two-stage fermentation procedure in a medium of the following composition: soybean meal (5 g), glucose (20 g), yeast extract (5 g), NaCl (5 g), K_2HPO_4 (5 g), distilled water to 1 000 ml; pH adjusted to 7.0 with 5N-HCl. Media were sterilized in an autoclave at 121 °C for 15 min.

Fermentations were conducted on rotary shakers, operating at 250 rev. min^{-1} (1 in stroke) at 27 °C in Erlenmeyer flasks holding one-fifth of their volume of medium. The surface growth from slants was suspended in sterile medium (5 ml) and used to inoculate stage I cultures, which were incubated as described for 72 h. The thick 72 h stage I growth was used as inoculum for stage II cultures, the inoculum volume being 10% of the volume of stage II fermentation medium in all cases. Substrates were added to 24 h old stage II cultures, either in dimethylformamide (250 mg in 1 ml), or by solubilizing in a convenient amount of deionized water with hydrochloric acid, followed by adjustment of the solution to pH 7 prior to addition. Unless otherwise specified, the final substrate concentration in stage II cultures was 0.5 mg ml^{-1}. Substrate-containing stage II cultures were sampled at intervals for t.l.c. analyses.

Fermentation Sampling.—Samples (5 ml) were withdrawn at intervals, adjusted to pH 8.5 with saturated aqueous sodium hydrogen carbonate, extracted with ethyl acetate or ether (0.5 ml) and centrifuged if necessary, and spotted on t.l.c. plates.

Screening of Micro-organisms.—Small-scale fermentations were conducted in Erlenmeyer flasks (125 ml) to determine the abilities of sixty selected micro-organisms to metabolize (+)-(*S*)-glaucine (1). Cultures were grown as described above, and a total of 12.5 mg of glaucine (1) was added to each 24 h stage II culture. Substrate-containing flasks were incubated with shaking, and samples (5 ml) were withdrawn 24 and 72 h after substrate addition. Of the sixty organisms screened, fourteen metabolized glaucine. Eight of these cultures gave sufficiently high levels of metabolites to warrant further study.

The experiment was repeated with controls and with only the active cultures. Controls consisted of substrate added to sterile medium and of fermentation blanks containing no substrate. The most active metabolizing cultures were *Streptomyces griseus* (UI 1158) and *Fusarium solani* (ATCC 12823).

Preparative-scale Production of Norglaucine (4) *and* 2-O-*Demethylglaucine* (6) *by* Streptomyces griseus.—*S. griseus* (UI 1158) was grown according to the usual fermentation procedure. Stage II fermentations were conducted in 4.0 l of medium in 500 ml Erlenmeyer flasks. (+)-(*S*)-Glaucine (1.5 g) in dimethylformamide (2 ml) was distributed evenly among forty stage II culture flasks, and the substrate-containing cultures were monitored by t.l.c. [system (B)]. After 90 h the contents of all flasks were combined, adjusted to pH 7.5 with ammonia solution (58%), and exhaustively extracted with ether (liquid–liquid extractor). The extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give a crude extract (5.0 g). The extract was adsorbed on a silica column (300 g; 42 × 5.5 cm) eluted with benzene–methanol (20 : 1) at 2.5 ml min^{-1} (18 ml fractions). Fractions 100—185 contained pure (6) (209 mg, 14%), and fractions 241—331 pure (4) (165 mg, 11%).

Characterization of Norglaucine (4).—The metabolite in fractions 241—331 could not be induced to crystallize. However, a crystalline acetyl derivative was obtained by treating the metabolite with pyridine–acetic anhydride; m.p. 102° (lit.,[23] for *N*-acetylnorglaucine, 102—104°). The n.m.r. spectrum of the metabolite was similar to the published spectrum of norglaucine (D_2O–pyridine),[23] and identical with that of glaucine ($CDCl_3$) except for the absence of an NMe signal at δ 2.55. The metabolite showed *m/e* 341.161 2 (calc. for $C_{20}H_{23}NO_4$: 341.162 7); *m/e* 341(84%), 340(100), 327(17), 326(74), 325(22), 324(22), 312(8), 310(53), 298(38), and 294(36).

Characterization of 2-O-*Demethylglaucine* (6).—The metabolite could not be induced to crystallize, but showed δ($CDCl_3$) 2.58 (3 H, s, NMe), 3.58 (3 H, s, 1-OMe), 3.90 (6 H, s, 9- and 10-OMe), 5.50br (1 H, s, 2-OH, exchanges with D_2O), 6.65 (1 H, s, H-3), 6.82 (1 H, s, H-8), and 7.95 (1 H, s, H-11); $\lambda_{max.}$ (EtOH) 284—312 nm (no bathochromic shift on addition of 5% NaOH); *m/e* 341.161 5 (calc. for $C_{20}H_{23}NO_4$: 341.162 7); *m/e* 341(84%), 340(100), 327(17), 326(74), 325(22), 324(22), 310(53), 298(38), 294(36), and 283(40); the hydrobromide had m.p. 208—211°; the m.p. of a mixture with synthetic material was not depressed (lit.,[30] 209—212°). I.r., u.v., n.m.r., and mass spectral comparisons of the metabolite with synthetic (6) proved the compounds to be identical.

N.m.r.-monitored Titrations of the Metabolite (6) *with Sodium Deuteroxide*.[31]—Base-titration n.m.r. spectra were obtained with [$^2H_6$]dimethyl sulphoxide as solvent and adding sodium deuteroxide (30% in D_2O) in 5 equal portions. Titrations were conducted with boldine (2), synthetic (6), and the metabolite (6). The results are shown in Table 2.

Preparative-scale Production of Didehydroglaucine (3) *and the Noraporphinone* (10) *by* Fusarium solani (ATCC 12823).—*F. solani* (ATCC 12823) was grown according to the usual fermentation procedure. Stage II fermentations were conducted in 2.3 l of medium held in 500 ml Erlenmeyer flasks. (+)-(*S*)-Glaucine (1) (1.15 g) in dimethylformamide (2.3 ml) was distributed evenly among 23 stage II culture flasks. The reaction was monitored by t.l.c. [system (E); Dragendorff reagent spray (all compounds positive) or CAS spray (didehydroglaucine, R_F 0.95, yellow; glaucine, R_F 0.75, blue; aporphinone (10), R_F 0.4, colourless). After 6 days the cultures were adjusted to pH 8.3 with saturated sodium hydrogen carbonate solution, and exhaustively extracted with ether (liquid–liquid extractor). The extract was dried (Na_2SO_4) and dried *in vacuo*. The residue (1.5 g) was subjected to column chromatography on silica gel (350 g; 47 × 5.5 cm) eluted with benzene–methanol–ammonium hydroxide (57%) (200 : 1.0 : 0.1) at 0.5 ml min^{-1} (15 ml fractions). Fractions 225—263 gave

pure didehydroglaucine (700 mg, 61%); fractions 1 600—1 860 gave the noraporphinone (10) (250 mg, 21%).

Characterization of didehydroglaucine (3). The metabolite was similar to synthetic didehydroglaucine (3), giving crystals from 95% ethanol; m.p. 121.5—122° (lit.,[38] 121—122°); $\lambda_{max.}$ 264 and 334 nm;[37] δ ($CDCl_3$)[38] 3.08 (3 H, s, NMe), 3.28 (4 H, s, $CH_2{\cdot}CH_2$), 3.93 (3 H, s, 1-OMe), 4.05 (9 H, s, 2-, 9-, and 10-OMe), 6.67 (1 H, s, D_2O-exchanged, H-7), 6.93 (1 H, s, H-3), 7.05 (1 H, s, H-8), and 9.10 (1 H, s, H-11); *m/e* 353(100%), 352(100), 338(88), 337(76), 335(35), 307(29), 306(29), 294(25), 280(51), 279(51), and 176(63).

Characterization of 4,5,6,6a-*tetradehydro*-1,2 9,10-*tetramethoxynoraporphin*-7-*one* (10). I.r. [$\nu_{max.}$ 1 645 (C=O), 1 735, and 1 590 cm^{-1}][40] and u.v. data [$\lambda_{max.}$ 215, 246, 276, 299sh, 320—395, and 420—445 nm; λ_{min} 233 and 261 nm][41] were identical with those published. The n.m.r. spectrum showed δ($CDCl_3$) 4.05 (s, OCH_3), 4.07 (s, OCH_3), 4.10 (s, 2 OCH_3), 7.20 (1 H, s), 7.8 (1 H, d), 8.1 (1 H, s), and 8.9 (2 H, m); δ ($CF_3{\cdot}CO_2D$) 3.4, 3.47, 3.57, and 3.60 (4 s, 4 OCH_3), 7.21 (1 H, s), 7.75 (1 H, s), 8.33 (2 H, q), and 8.71 (1 H, s). The compound was insoluble in water, turning red on addition of concentrated hydrochloric acid. In chloroform it shows a green fluorescence. It formed reddish crystals (from 95% EtOH), m.p. 225—229° (lit.,[42] 227—229°).

Transformations of (RS)-*Glaucine* [(RS)-(1)].—Stage II cultures of *F. solani* (ATCC 12823) and *S. griseus* were grown in the usual way in 500 ml Erlenmeyer flasks. The substrate, (*RS*)-(1) (50 mg per flask), was then added in dimethylformamide (250 mg ml^{-1}). Sampling was performed in the usual way and the reaction was monitored by t.l.c. as described under ' Preparative-scale Productions.' After 6 days approximately half the glaucine remained. Fermentations were adjusted to pH 8.0 with saturated sodium hydrogen carbonate solution, and exhaustively extracted with ethyl acetate; the extract was dried (Na_2SO_4) and taken to dryness *in vacuo*. Residual glaucine was purified by preparative t.l.c. [system (B)] and identified by mass and u.v. spectra, and co-chromatography with standards. Quantities recovered were: from *S. griseus*, 31 mg, $[\alpha]_D^{25}$ 0.81° (*c* 3.71 in abs. EtOH); from *F. solani*, 15 mg, $[\alpha]_D^{25}$ −56.4° (*c* 2.24 in abs. EtOH) (enantiomeric purity 75.4%).

Transformation of (RS)-*Glaucine*[(RS)-(1)] *by* Fusarium solani.—Further experiments were conducted to evaluate the time course of enrichment in (−)-(*R*) glaucine by *F. solani*, grown according to the usual fermentation procedure. Stage II fermentations were conducted in 1 l Erlenmeyer flasks. (*RS*)-Glaucine (100 mg in 0.4 ml of dimethylformamide) was added to each of six flasks and a sterile media control after 24 h. Whole flasks were harvested at day intervals up to 8 days. The control was also harvested after 8 days. The contents of each flask were adjusted to pH 8.5 with saturated aqueous sodium hydrogen carbonate, and extracted three times with ethyl acetate (50 ml). The extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo*. Preparative t.l.c. was then performed [system (B)]. Bands corresponding to glaucine and didehydroglaucine were isolated and eluted with methanol; the products were dried *in vacuo* and identified by chromatography and u.v. spectra. For optical rotation measurements the glaucine was taken to a convenient volume in absolute ethanol (usually 0.5—1.0 ml, corresponding to a *ca.* 5% solution). For recovery data, u.v. extinctions were measured and compared with the values ε_{280} 1.512×10^4 and ε_{302} 1.445×10^4 for glaucine, and ε_{260} 3.36×10^4 and ε_{334} 8.72×10^3 for didehydroglaucine. U.v. spectra of all samples were run in 95% ethanol. The results of these experiments are shown in the Figure.

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