Microbial Transformations in Organic Synthesis. 3. Synthesis, Characterization, and Fungal Metabolism of *cis*- and *trans*-7-Methylglaucine

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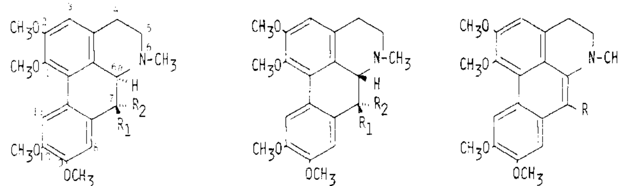
The synthesis of racemic *cis*-7-methylglaucine (**4**/**5**) and *trans*-7-methylglaucine (**6**/**7**) is described, involving the condensation of glaucine (**1**) with formaldehyde to yield 7-methyldehydroglaucine (**8**), followed by dissolving metal reduction. The relative stereochemistry of the two diastereomeric sets of enantiomers was assigned on the basis of chemical reactivity as well as ^{1}H NMR and ^{13}C NMR spectroscopy. These compounds were synthesized as "methyl-blocked" substrates for determining the stereochemical course during the oxidation of aporphines by fungal cultures. In both *Fusarium solani* (ATCC 12823) and *Aspergillus flavipes* (ATCC 1030), only racemic *cis*-7-methylglaucine (**4**/**5**) was oxidized to the corresponding dehydro-7-methylaporphine, to the expected extent of 50%. Thus, an overall cis elimination is operative with both organisms in aporphine oxidations.

The microbiological oxidation of aporphine alkaloids to the corresponding dehydroaporphines represents a potentially useful synthetic reaction on the basis of excellent chemical yield and a high degree of substrate stereoselectivity.[1-3] In particular, the oxidation of (*S*)-(+)-glaucine (**1**, Chart I) to dehydroglaucine (**2**) by *Fusarium solani* proceeds quantitatively, and with complete substrate stereoselectivity since (*R*)-(−)-glaucine (**3**) is not metabolized.[2] Conversely, the unnatural aporphine **3** is quantitatively and stereospecifically oxidized to **2** by *Aspergillus flavipes*.[3] In order to elucidate the mechanism of these reactions and to extend the method to other aporphines and related substrates, it is necessary to determine not only the substrate stereoselectivity but also the stereochemical course of the reaction involving the 6a-proton and a proton at the prochiral 7-position, i.e., an overall "cis or trans elimination" of hydrogen. One approach is to examine "methyl-blocked" derivatives allowing for elimination to proceed only in a cis or trans fashion. Such an approach was successfully used with methyl-steroid analogues to demonstrate that microbial 1,2-dehydrogenation follows a trans-1,2-diaxial course.[4,5] The use of *cis*-7-methylglaucine (6a*S*,7*S* and 6a*R*,7*R*, **4** and **5**, respectively) and *trans*-7-methylglaucine (6a*S*,7*R* and 6a*R*,7*S*, **6** and **7**, respectively) represents an analagous set of substrates since each would restrict the stereochemical course of oxidation to 7-methyldehydroglaucine (**8**). This report describes the synthesis, characterization, and examination of **4**–**7** as substrates for determining the stereochemical course of aporphine oxidations by *F. solani* and *A. flavipes*.

Experimental Section

Reagents and Chemicals. All solvents were analytical grade or better in quality. Solvents for high-performance liquid chromatography (HPLC) were of HPLC grade (Omnisolv, MCB Manufacturing Chemists, Cincinatti, OH). (*S*)-Glaucine (**1**) and dehydroglaucine (**2**) were prepared as previously described.[2] Pd/C (10%) was purchased from Sargent-Welch Scientific Co., Skokie, IL. Papaverine (HPLC internal standard) was purchased from Sigma Chemical Co., St. Louis, MO. Formaldehyde (37% aqueous) was purchased from Spectrum Chemical Manufacturing Company, Redondo Beach, CA.

Chart I



1, R_1 = H (pro-*S*); R_2 = H (pro-*R*)
3, R_1 = H (pro-*S*); R_2 = H (pro-*R*)
2, R = H
8, R = CH_3
4, R_1 = CH_3; R_2 = H
5, R_1 = H; R_2 = CH_3
6, R_1 = H; R_2 = CH_3
7, R_1 = CH_3; R_2 = H

Analytical Procedures. All NMR spectra were taken in $CDCl_3$ with tetramethylsilane as the internal standard. ^{1}H NMR spectra were generated on a Varian EM-390 (90 MHz) spectrometer, while decoupled proton experiments were conducted on a Nicolet Model 200 (200 MHz) unit. Normal (totally decoupled) and off-resonance continuous wave decoupled (ORCWD) ^{13}C NMR spectra were generated on a Bruker Model NH-90 spectrometer. Mass spectra were taken on a Du Pont Model 21491 unit by direct probe insertion. Melting points were taken by using a Fisher Model 355 digital melting point apparatus and are corrected.

Thin-layer chromatography (TLC) was conducted on plastic-backed 0.25-mm silica gel GF-254 plates (Polygram, Brinkman, Houston, TX), eluted with one of the following solvents systems: system A = 4:1 benzene:methanol [R_f **1**, 0.43; **4**/**5** 0.50; **6**/**7**, 0.37; **8**, 0.77]; system B = 9:1 diethyl ether:methanol [R_f **1**, 0.20; **4**/**5**, 0.50; **6**/**7**, 0.23; **8**, 0.87]; system C = 9:1 chloroform:methanol [R_f **1**, 0.63; **4**/**5**, 0.66; **6**/**7**, 0.50; **8**, 0.77]; system D = 9:1 ethyl acetate:methanol [R_f **1**, 0.20; **4**/**5**, 0.38; **6**/**7**, 0.20; **8**, 0.83]; system E = 90:10:1 ethyl acetate:methanol:diethylamine [R_f **4**/**5**, 0.77; **6**/**7**, 0.44]. Plates were visualized by fluorescence quenching under 254-nm light and fluorescence under 280-nm light and by spraying with ceric ammonium sulfate (1% in 50% phosphoric acid; **1**, **4**/**5**, and **6**/**7** blue, fading to yellow; **2** and **8**, brown-yellow).

HPLC was conducted as described previously[6] by using a μ-Phenyl Bondapak column (Waters Associates, Milford, MA), 250 × 3.9 mm, eluted with 0.05 M KH_2PO_4:methanol:acetonitrile (5:4:1) at a flow rate of 2.0 mL/min [Tr **4**/**5** and **6**/**7** = 3.74 min; Tr **8** = 10.0 min]. Low-pressure liquid chromatography (LPLC) was conducted by using an FMI Model RP-SY low-pressure pump (Fluid Metering Inc., Oyster Bay, NY) and a 2.5 × 25 cm column (Ranin Instruments, Woburn, MA) packed with 66 g of Merck Kieselgel 60, 0.040–0.063 mm (230-400 mesh ASTM) from MCB Manufacturing Chemists, Cincinatti, OH. The eluting solvent was ethyl acetate:methanol:ammonium hydroxide (100:5:1) at a

(1) Davis, P. J.; Rosazza, J. P. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1.
(2) Davis, P. J.; Rosazza, J. P. *Bioorg. Chem.* **1981**, *10*, 97.
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(6) Davis, P. J. *J. Chromatogr.* **1980**, *193*, 170.

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Table I. ^{1}H NMR (90 MHz) Assignments (ppm) for Glaucine Derivatives

position	1	4	6	2	8
C7-CH_3		0.89^a	1.50^a		2.64
N-CH_3	2.52	2.46	2.32	3.07	2.74
C4-CH_2					3.16
C5-CH_2	2.3-3.2	2.3-3.2	2.3-3.2	2.31	3.48
C1-OCH_3	3.64	3.61	3.64	3.91	3.84
C2,C9,C10-OCH_3	3.86	3.86	3.87	4.02	3.97
	3.89	3.90	3.91	4.04	4.02
	3.91				
C7-H				6.58	
C3-H	6.57	6.50	6.56	6.90	7.02
C8-H	6.77	6.70	6.93	7.07	7.30
C11-H	8.08	8.04	8.07	9.10	9.27

^{a} d, J = 6.5 Hz.

flow rate of 1.0 mL/min. Fractions were collected by using an LKB Model 2070 Ultrorac fraction collector (LKB Instruments, Gaithersburg, MD).

Synthesis of 7-Methyldehydroglaucine (8). The synthesis of 8 was conducted according to the method of Mollov and Philipov.[7] (*S*)-Glaucine (1, 2.0 g) and 37% aqueous formaldehyde (15 mL) were sealed in a tube and heated to 105 °C for 72 h. The tube was cooled, and the large amorphous mass and green crystals (1.28 g, 68% yield) were filtered off and washed with water. The product in both forms (amorphous and crystalline) was shown to be homogeneous by TLC. The product was crystallized from absolute ethanol (150 mL) to yield 750 mg (36% yield) of 8: mp 148–150C (lit.[7] mp not reported); mass spectrum (% relative abundance), *m/e* 367 (100), 352 (56), 337 (6), 322 (56), 309 (10), 294 (12), 278 (12), 183 (25), 119 (33); ^{1}H NMR ($CDCl_3$), see Table I.

Synthesis of *cis*-7-Methylglaucine (4/5) and *trans*-7-Methylglaucine (6/7). Zinc amalgam (Zn[Hg]) was prepared as follows:[8] zinc dust (15 g), mercuric chloride (1 g), and 30 mL of water were combined and shaken vigorously. A total of 200 mL of 2.5 N HCl was added and the suspension shaken vigorously to yield fine globules of Zn[Hg]. The aqueous supernatant was decanted, and the amalgam was washed twice with methanol and then resuspended in 300 mL of methanol in a three-neck flask. 7-Methyldehydroglaucine (8) (2.0 g) was added, and the vessel was taken to 60 °C. To initiate reduction, 40 mL of 12 N HCl was added slowly by an addition funnel (1 drop/5 s) with rapid stirring (vigorous hydrogen evolution). Initially 8·HCl separated from the reaction mixture and was redissolved by the addition of 20 mL of water. After 24 h, an additional 30 mL of 12 N HCl was added by slow addition. The yellow solution turned water-clear when reduction was complete. The progress of the reaction was monitored by taking an aliquot of the reaction solution to dryness under a nitrogen stream, partitioning between 1 mL of ethyl acetate and 1 N sodium hydroxide, and examining the organic layer by TLC on solvent system E or by HPLC. Twenty-four hours after the HCl addition, the entire reaction was taken to dryness in vacuo and partitioned between 25% aqueous ammonium hydroxide and ethyl acetate. The organic layer was washed once with water, dried over anhydrous sodium sulfate, and taken to dryness in vacuo to yield a gold oil, 2.85 g.

The diastereomeric sets of enantiomers *cis*-7-methylglaucine (4/5) and *trans*-7-methylglaucine (6/7) were separated by using the LPLC system described above to yield 830 mg of 4/5 (impure) and 750 mg of pure 6/7. A second passage through the column for the impure *cis*-7-methylglaucine yielded 750 mg of pure 4/5. Crystallization from petroleum ether (bp 60–68 °C) yielded light yellow crystals, mp 4/5 137–141 °C, mp 6/7 125–127 °C.

Racemic *cis*-7-methylglaucine (4/5) gave the following analytical data: ^{1}H NMR ($CDCl_3$), see Table I; in decoupled spectra (Nicolet 200), irradiation at 3.05 ppm (on C7-H multiplet), the signal at 0.92 ppm (d, J = 6 Hz) collapsed to a singlet; irradiation at 2.65 ppm on the C6a-proton caused the C7-proton multiplet to collapse to a quartet, with no effect on the C7-methyl; irradiation at 0.9

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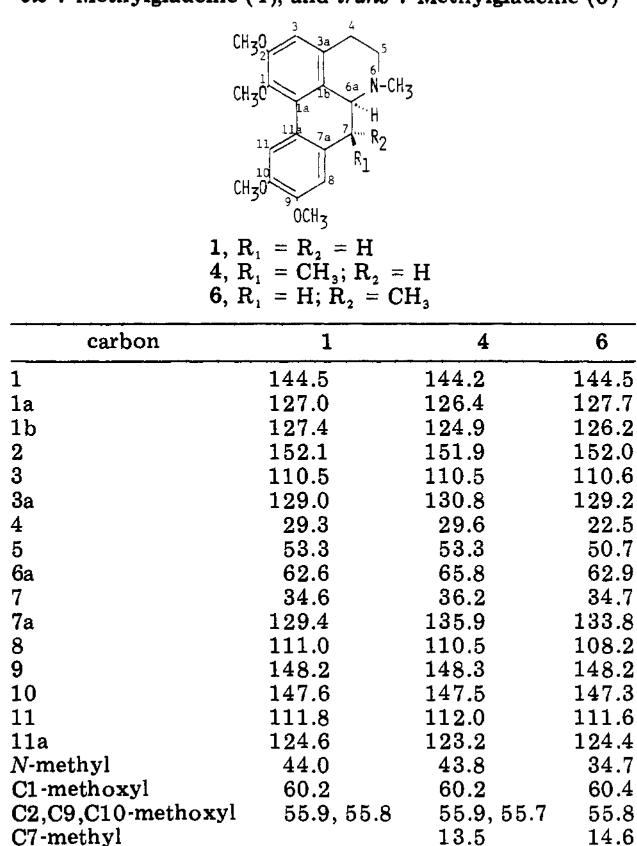
(8) Venkateswarlu, A; Cava, M. P. *Tetrahedron* **1976**, *32*, 2079.

Table II. ^{13}C NMR Assignments for Glaucine (1), *cis*-7-Methylglaucine (4), and *trans*-7-Methylglaucine (6)



1, R_1 = R_2 = H
4, R_1 = CH_3; R_2 = H
6, R_1 = H; R_2 = CH_3

carbon	1	4	6
1	144.5	144.2	144.5
1a	127.0	126.4	127.7
1b	127.4	124.9	126.2
2	152.1	151.9	152.0
3	110.5	110.5	110.6
3a	129.0	130.8	129.2
4	29.3	29.6	22.5
5	53.3	53.3	50.7
6a	62.6	65.8	62.9
7	34.6	36.2	34.7
7a	129.4	135.9	133.8
8	111.0	110.5	108.2
9	148.2	148.3	148.2
10	147.6	147.5	147.3
11	111.8	112.0	111.6
11a	124.6	123.2	124.4
N-methyl	44.0	43.8	34.7
C1-methoxyl	60.2	60.2	60.4
C2,C9,C10-methoxyl	55.9, 55.8	55.9, 55.7	55.8
C7-methyl		13.5	14.6

ppm (on C7-methyl) resulted in no change for C6a-H, and C7-H collapsed to a doublet, J = 5 Hz; ^{13}C NMR ($CDCl_3$), see Table II; mass spectrum (% relative abundance), *m/e* 369 (92), 368 (83), 354 (56), 340 (32), 339 (25), 338 (100), 326 (35), 322 (20), 311 (23), 295 (15).

trans-7-Methylglaucine (6/7) gave the following analytical data: ^{1}H NMR ($CDCl_3$), see Table I; in decoupling experiments (Nicolet 200), irradiation at 1.67 ppm (on C7-methyl; d, J = 6 Hz) caused the signal at 2.90 ppm (C7-H) to collapse to a doublet (J = 12 Hz); ^{13}C NMR ($CDCl_3$), see Table II; mass spectrum (% relative abundance), *m/e* 369 (100), 368 (94), 354 (70), 340 (50), 339 (42), 338 (97), 326 (50), 322 (25), 311 (40), 295 (20).

Catalytic Dehydrogenation of *cis*- and *trans*-7-Methylglaucine. Portions of 2 mg each of 4/5 and 6/7 were dissolved in 1.0 mL of acetonitrile in a screw-cap tube, and 2.0 mg of Pd/C (10%) was added.[9] The tubes were sealed and heated to 90 °C. Samples of 20 μL were removed for analysis at 0, 45, 90, and 120 min, dissolved in 200 μL of methanol, pressure filtered (Whatman, GF/F glass fiber filters), and analyzed by HPLC as described above. Only 4/5 was cleanly dehydrogenated to 8.

Catalytic Reduction of 7-Methyldehydroglaucine (8) to *cis*-7-Methylglaucine. A total of 686 mg of 8 and Pd/C (10%, 75 mg, Sargent-Welch) in 200 mL of absolute ethanol was subjected to hydrogenation at 40 psi and 27 °C in a Parr hydrogenation apparatus. Samples were removed periodically and examined directly by TLC using solvent system E. Results indicated production of only 4/5, with no production of 6/7. The reaction was terminated after 4 days with a resultant conversion of approximately 50%.

Microbial Transformation Studies. *Fusarium solani* (ATCC 12823) was grown according to the two-stage fermentation procedure as described previously.[2] A total of 600 mL of 72-h second-stage culture was centrifuged at 27 °C and 1650*g* (IEC Centra 7R centrifuge) for 12 min, and resuspended in 0.05 M phosphate buffer (pH 8.0). This process was then repeated. The resultant cell pellet was resuspended in 225 mL of 0.05 M phosphate buffer (pH 8.0). Aliquots of 24 mL were placed in each

(9) Cava, M. P.; Edie, D. L.; Saa, J. J. *J. Org. Chem.* **1975**, *40*, 301.

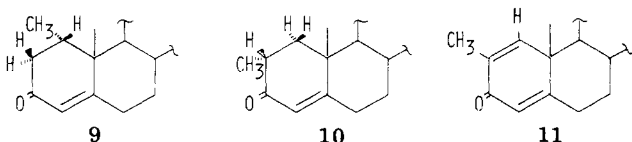
of seven 125-mL Bellco-DeLong flasks and equilibrated at 27 °C and 250 rpm[2] for 1 h. The substrates (*S*)-(+)-glaucine (single flask, control), *cis*-7-methylglaucine (**4**/**5**, triplicate flasks), and *trans*-7-methylglaucine (**6**/**7**, triplicate flasks) were added at a level of 3.5 mg dissolved in 1 mL of 0.01 N HCl/25 μL of DMF per flasks, and the incubation was allowed to continue. Samples of 1 mL were harvested at 1, 2, 4, 6, 24, 72, 96, 120, and 168 h after substrate addition. Samples were extracted and analyzed by HPLC as described previously.[2]

Aspergillus flavipes (ATCC 1030) was grown according to the two-stage fermentation procedure as described previously.[3] The substrates (*R*)-(−)-glaucine (metabolism control) and *cis*- and *trans*-7-methylglaucine were added to 24-h second-stage flasks as described above (3.5 mg/flask). Samples were harvested and analyzed in a manner identical with that for *F. solani*.

Results and Discussion

The use of microbial cultures and their isolated enzymes to catalyze reactions of organic synthetic interest is well documented[10–16] and shows certain advantages over classical reagents on the basis of mild conditions, high-yielding reactions, and, in particular, the high degree of stereoselectivity often observed in such reactions.[17] In previous studies, it was observed that the aporphine alkaloid (*S*)-(+)-glaucine (**1**) was quantitatively and stereospecifically oxidized to dehydroglaucine (**2**) by using growing cultures of the fungus *Fusarium solani*.[1,2] In a subsequent study, the fungus *Aspergillus flavipes* was shown to catalyze this reaction with the reverse sterochemistry in quantitative yield since **3** is oxidized to **2**, with no activity in oxidizing **1**.[3]

In order to extend these reactions to other aporphines and related structures, the mechanism of oxidation must be determined. In particular, the stereochemistry of the overall oxidation (direct dehydrogenation or hydroxylation/dehydration) involves not only the proton at position 6a but also the prochiral center at position 7. For example, the oxidation of (*S*)-(+)-glaucine (**1**) to **2** by *F. solani* could involve an overall cis elimination by loss of the 6a*S*- and 7-*pro-R*-hydrogen atoms or an overall trans elimination by loss of the 6a*S*- and 7-*pro-S*-hydrogen atoms. Our approach to resolving this question is similar to that used by Hayano et al.[4,5] to examine the 1,2-dehydrogenation of steroids by *Bacillus sphaericus*. Steroids of the type illustrated by **9** containing a 1α-methyl substituent yielded



no dehydrogenated product with this organism, while 2α-methylsteroids such as **10** were smoothly oxidized to the corresponding 1,2-dehydro-2-methylsteroids (**11**). The same results were obtained with 1α- or 2α-hydroxysteroids. These results suggested an overall trans-diaxial elimination of hydrogen with loss of the 1α- and 2β-hydrogens. These results were later verified by using the appropriate isotopically labeled steroids.[5]

Thus, the preparation of 7-methylglaucine stereoisomers would yield substrates that would allow only (depending on the stereochemistry) 6a,7-cis or trans elimination by the fungi to yield **8**. For example, *Fusarium solani*, which requires the 6a*S* chirality for oxidation,[1,2] would metabolize (6a*S*,7*S*)-methylglaucine (**4**) if a cis elimination were operative, or (6a*S*,7*R*)-methylglaucine (**6**) if a trans elimination were operative.

Few natural, synthetic, or semisynthetic aporphines contain substituents as the C7-position, and all are 7-hydroxyaporphines[18,19] or 7,7-dimethylaporphines.[20] Aporphines containing a 6a-methyl substituent have also recently been reported.[21] An entree to 7-alkylaporphines was recently reported by Mollov and Philipov,[7] and the condensation of **1** with formaldehyde yielded **8** in good yield as described by these investigators. The product gave the expected mass and ^1H NMR spectra data (see Table I), including the expected deshielded C11 aromatic proton and *N*-methyl signal characteristic of dehydroaporphines[18] relative to the corresponding aporphine, as well as two triplets for the C4- and C5-methylene groups. Dissolving metal reduction using Zn[Hg] in methanol/concentrated hydrochloric acid allowed for the slow but quantitative reduction of **8**. As expected, the reduction yielded two diastereomeric sets of enantiomers representing *cis*-7-methylglaucine [6a*S*,7*S*/6a*R*,7*R* (**4**/**5**)], and *trans*-7-methylglaucine [6a*S*,7*R*/6a*R*,7*S* (**6**/**7**)]. The diastereomeric sets were easily separated by TLC as indicated by excellent resolution on systems A–E, as well as by preparative low-pressure liquid chromatography, with the cis set always showing the higher mobility.

Proof of the relative stereochemistry of **4**/**5** vs. **6**/**7** (i.e., cis vs. trans) was based on chemical and spectral data. Since catalytic hydrogenation involves a cis addition of hydrogen, it would be anticipated that reduction of **8** using Pd/C according to the method of Cava[22] would yield only the cis isomers (**4**/**5**), and this was found to be the case. Further, only the cis isomers were smoothly dehydrogenated to **8**.[9] The mass spectral fragmentation of both *cis*- and *trans*-7-methylglaucine was entirely consistent with the proposed structures, as well as with glaucine, including the expected M^+, M − 1 (6,6a-dehydro), M − 15 ($M - CH_3$), and M − 43 (retro-Diels–Alder) ions.[23,24] A major fragment at M − 31 can be rationalized by the $M - CH_3$ ion losing the 6aH and 7-CH_3 to yield the stable dehydroaporphine.

Major emphasis was placed on NMR spectroscopy for proving the relative stereochemistry of *cis*- and *trans*-7-methylglaucine. Since the enantiomeric sets have not been resolved and to facilitate the following discussion, we will refer to only (6a*S*,7*S*)-methylglaucine (**4**) to represent *cis*-7-methylglaucine and (6a*S*,7*R*)-methylglaucine (**6**) to represent *trans*-7-methylglaucine.

The ^1H NMR spectra of **4** and **6** correlate well with glaucine (**1**, see Table I), but with important differences from one another indicating different stereochemical en-

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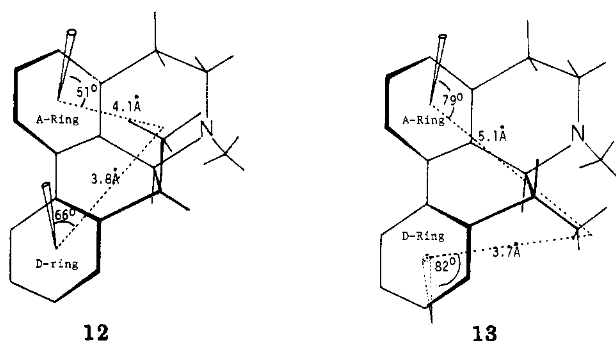
vironments at position 7. In particular, the 7-methyl substituent and aromatic proton at position 8 are shielded in the cis-isomer 4 in comparison to the trans-isomer 6. The relative shifts of the C7-methyl groups can be rationalized on the basis of their spatial relationships to the A and D rings and the resultant anisotropic influence. In the cis isomer (see 12) the methyl group occupies a position

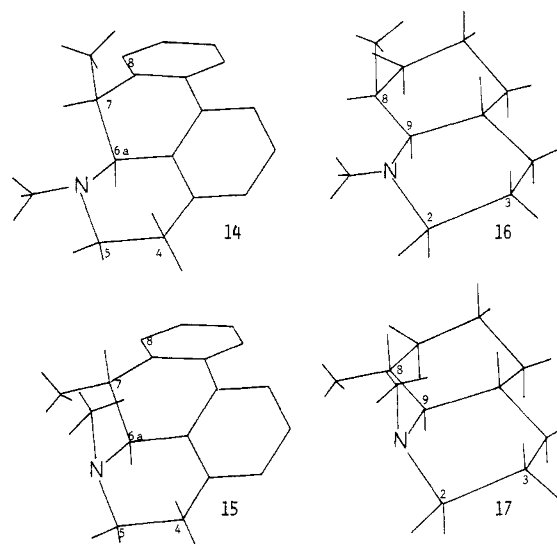


above both the A and D rings, while in the trans isomer (see 13) the methyl group lies nearly in the plane of the A ring and slightly below the D ring. The relative shifts may be calculated as described by Abraham and Loftus[25] on the basis of the equation $\Delta\delta = \mu(1 - 3\cos^2\theta)/r^3$, where $\Delta\delta$ equals the chemical shift change due to the anisotropic effect, θ equals the angle from perpendicular to the aromatic ring to the substituent, r equals the radius from the substituent to the center of the ring, and μ is the equivalent dipole of the anisotropic field (μ = 27.0 D). The appropriate values are given in 12 and 13 (from Dreiding model measurements). In the cis isomer (12) the D-ring effect of +0.24 ppm and the A-ring effect of −0.07 ppm yields a net deshielding effects of +0.17 ppm. In the trans isomer (13) the D-ring effect of +0.52 ppm and the A-ring effect of +0.18 ppm yields a net deshielding effect of +0.70 ppm. Thus, in comparison, the methyl group of the trans isomer should appear downfield from that of the cis isomer, and the difference should be approximately 0.53 ppm (observed 0.61 ppm). Essentially the same results were obtained from the isoshielding line graphs of Johnson and Bovey.[26]

The difference in resonance frequency for the C8-aromatic proton in 4 vs. 6 may be rationalized on the basis of a "steric compression" shift resulting from increased steric interaction with the C7-methyl group (in the D-ring plane) in the trans isomer resulting in a deshielding of the C8 proton, as opposed to little interaction in the cis isomer (see 12 and 13). Such an observation is consistent with the observed shift for the C8-aromatic proton in dehydroglaucine (2) relative to 7-methyldehydroglaucine (8, see Table I), where the only structural difference is the 7-methyl group in the plane of the D ring in 8, with a resultant deshielding of the C8-proton of 0.27 ppm. The difference in shift for this proton in 4 vs. 6 is 0.23 ppm.

Assignments of stereochemistry were further substantiated by decoupled ^{1}H NMR spectra. In 4, irradiation of the C7-proton multiplet caused the C7-methyl doublet to collapse to a singlet. Irradiation of the C6a-proton signal caused the C7-proton multiplet to collapse to a quartet, with no effect on the C7-methyl signal. Finally, irradiation of the C7-methyl signal resulted in no change for the C6a-proton, while the C7-proton yielded a doublet, $J_{6a,7}$ = 5 Hz, indicating a cis-proton relationship. In 6, irradiation of the C7-methyl group yielded a doublet for the

Chart II



C7-proton, $J_{6a,7}$ = 12 Hz, indicative of a trans-proton relationship. Interpretation of other decoupling experiments with 6 was difficult due to the complexity of the spectrum. Such interpretations are considerably more difficult than in the case of 7-hydroxyaporphines, where the C7-proton doublet ($J_{6a,7}$) is resolved from the rest of the spectrum by the deshielding influence of the hydroxy group.[21]

The characterization of 4 and 8 was also based on comparative ^{13}C NMR spectroscopy. An examination of chemical shifts and multiplicities for these compounds in relation to glaucine, as well as an analysis of the observed chemical shift differences between 4 and 6 attributable to steric factors, allowed for further confirmation of the relative stereochemistry. The ^{13}C NMR data for 1, 4, and 6 are listed in Table II. The ^{13}C NMR spectrum for glaucine was virtually identical with that previously reported by Jackman et al.[27] As expected, a majority of the aromatic carbons of 4 and 6 (C1, C1a, C2, C3, C3a, C9, C10, and C11) displayed minimal differences in chemical shift when compared to 1. The meta coupling displayed by C1, C1b, C7a, C9, C10, and C11a in the ORCWD spectra enabled their positive assignment. The methyl-substituent at C7 caused a downfield shift of the 6a-, 7-, and 7a-carbons and a shielding effect at C1b and C11a in both 4 and 6, consistent with related aporphines.[20,21] All assignments were supported by their multiplicities.

The relative stereochemical assignments were based on a comparative analysis of the shifts for carbon 4, 5, 6a, and 8, as well as the *N*-methyl and C7-methyl substituents of 4 vs. 6. Several previous ^{13}C NMR studies[28,29] were used as models in the interpretation of these effects, most of which were attributable to steric compression shifts. A previous examination of cyclohexane derivatives[28] resulted in the observation that steric hindrance associated with gauche interactions of methyl groups caused a shielding of the carbon nuclei involved, and conversely, deshielding resulted from relief of these interactions. An inverse relationship was observed for protons in these systems. In addition, a ^{13}C NMR conformation analysis of *trans*-decahydroisoquinoline derivatives enable Vierhapper and Eliel[29] to determine the axial or equatorial orientation of ring substituents on the basis of chemical shift data. This

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Table III. Comparison of ^{13}C NMR Chemical Shifts due to Steric Interactions for 7-Methylglaucine Derivatives 4 and 6 and 8-Methyl-*trans*-decahydroisoquinoline Derivatives 16 and 17

carbon	4	6	carbon	16	17
4	29.6	22.5	3	25.8	19.4
5	53.3	50.7	2	58.2	55.0
6a	65.8	62.9	9	71.9	70.7
N-methyl	43.8	34.7	*N*-methyl	42.2	33.2
7-methyl	13.5	14.6	8-methyl	12.1	18.9
8	110.5	108.2			

second study was especially helpful in the analysis of 4 and 6. The preferred conformations of 4 and 6 were assigned on the basis of steric interactions as depicted with Dreiding models and are illustrated in 14 and 15 (Chart II). These two diastereomers were examined in comparison with the 8-methyl-*trans*-decahydroquinoline isomers 16 and 17,[29] and the sterically induced shifts were interpreted as follows (see Table III). In the *trans*-7-methylglaucine isomer (15), 1,3-diaxial interactions between the *N*-methyl group and C4-hydrogen cause a shielding at the C4 carbon (−7.1 ppm) and the *N*-methyl carbon (−9.1 ppm). Shielding was also observed in this stereoisomer at C5 (−2.6 ppm) and C6a (−2.9 ppm). Comparable shifts were observed in 8α-methyl-*trans*-decahydroquinoline (17) for the *N*-methyl (−9.0 ppm), C2 (−3.2 ppm), C3 (−6.4 ppm), and C9 (−1.2 ppm) positions. The C7-methyl moiety was shielded (−1.1 ppm) in the cis isomer (14) due to its axial orientation as also occurs in the 8β-methyl-*trans*-decahydroquinoline (16). Conversely, the equatorial orientation of the C7-methyl group in the trans-isomer 15 caused an upfield shift (−2.3 ppm) of the C8-carbon due to C7-methyl/C8-H interactions.

The racemic mixtures represented by *cis*-7-methylglaucine (4/5) and *trans*-7-methylglaucine (6/7) were utilized as substrates with *Fusarium solani* and *Aspergillus flavipes*. In cell suspensions of *F. solani*, only *cis*-7-methylglaucine was metabolized, and to the expected extent of 50%, presumably due to stereoselective oxidation of the 6a*S*,7*S* stereoisomer, 4 (assuming the organism retains its 6a*S* stereochemical preference as observed with (*S*)-(+)-glaucine[2]). Cell-suspension cultures were used since only sluggish metabolism was observed in growing cultures. *A. flavipes* also metabolized only *cis*-7-methylglaucine, again to the expected extent of 50%, presumably due to stereoselective oxidation of the 6a*R*,7*R* stereoisomer, 5 (assuming the organism retains its 6a*R* stereochemical preference as observed with (*R*)-(−)-glaucine[3]). Thus, in both organisms, an overall cis elimination appears to be operative. We recognize, as was suggested earlier for methyl-blocked steroid substrates,[4,5] that the presence of an alkyl substituent in the 7-position may hinder approach of the substrate to the requisite enzyme(s) (reactions were observed to be slower than with glaucine) and that stereochemical preference may be altered. Thus, such studies will require confirmation with the appropriate isotopically labeled substrates.[5]

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Registry No. (*S*)-(+)-1, 475-81-0; 2, 22212-26-6; (*R*)-(−)-3, 38325-02-9; (±)-cis-4/5, 84681-50-5; (±)-*trans*-6/7, 84681-51-6; 8, 72498-26-1; 16, 52008-64-7; 17, 55970-12-2; formaldehyde, 50-00-0.

Fluoride Ion Catalyzed Aldol Reaction between Enol Silyl Ethers and Carbonyl Compounds

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A new aldol reaction effected by the reaction of enol trimethylsilyl ethers and a quaternary ammonium fluoride is reported. Under the influence of a catalytic amount of tetrabutylammonium fluoride at low temperatures, enol silyl ethers react with various aldehydes to give the corresponding aldol trimethylsilyl ethers in fair to good yields. The silyl group of these products can be smoothly removed under mild conditions. Ketones, epoxides, and esters do not serve as electrophiles in this reaction. The reaction proceeds in a regiospecific manner with respect to the enol silyl ethers; the reactions of two regioisomers of 2-methylcyclohexanone with benzaldehyde cleanly give the respective regioisomeric aldol products. The reaction of 4-*tert*-butyl-1-methyl-2-(trimethylsiloxy)cyclohexene proceeds exclusively by the axial attack of the electrophile, making a strong contrast with the related cases reported on this cyclohexanone system. The aldol addition of a ketone and an aldehyde can be performed without the isolation of the enol silyl ether of the ketone by effecting both the silylation of the ketone and the aldol reaction with the aid of a fluoride anion. The characteristic behavior of the enolate species in this reaction can be rationalized by considering a mobile equilibrium in which the combination of fluorotrimethylsilane and a quaternary ammonium enolate functions as the key controlling factor.

The aldol reaction is one of the standard tools for creating new carbon–carbon bonds,[1] but its utility in organic synthesis has been severely limited because of the difficulties in controlling the course of the reaction. Attempts to effect the heterocoupling of carbonyl compounds have been fraught with troubles. Since the aldol reaction is controlled by a series of equilibria centered around the aldol anion 2 shown in Scheme I, which do not necessarily favor the adduct formation, undesired self- or polycon-

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