Journal of Natural Products
Vol. 49, No. 4, pp. 576-582, Jul-Aug 1986

HIGH FIELD AND 2D-NMR STUDIES WITH THE APORPHINE ALKALOID GLAUCINE

KATHLEEN M. KERR, ALAN M. KOOK,[1] and PATRICK J. DAVIS*

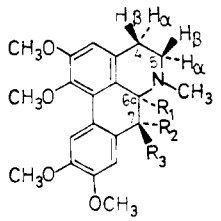
Division of Medicinal Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78705

ABSTRACT.—The aporphine alkaloid glaucine (**1**) was examined by comparison of the high field (600 MHz) ^{1}H-nmr spectra of **1** vs. racemic 6a,7,7-trideutereoglaucine (**4**,**5**), by computer-simulated ^{1}H-nmr spectra at 600 MHz, by using decoupled proton spectra, and two-dimensional COSY and HETCOR experiments with **1** at 500 and 360 MHz, respectively, and using high field (90 MHZ) ^{13}C-nmr of *S*-(+)-glaucine (**1**). Emphasis was placed on the resolution of the chemical shifts and coupling constants for the H-4α, H-4β, H-5α, H-5β, H-6α, H-7α, and H-7β alicyclic protons of the molecule, which were previously unassigned. The complete assignment of the alicyclic protons of **1** by ^{1}H-nmr was required for the structural elucidation of deuterated analogs of glaucine, which will be used in microbial transformation studies to determine the stereochemical course of aporphine dehydrogenation by the fungi *Fusarium solani* (ATCC 12823) and *Aspergillus flavipes* (ATCC 1030).

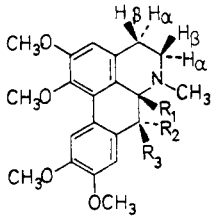
The natural product glaucine (**1**), first isolated from the plant *Glacium flavum* Cranz (Papavaraceae) (1), is an aporphine alkaloid exhibiting antitussive action in humans comparable to that of codeine (2-5). In fact, glaucine, which is nonnarcotic, has been used therapeutically for cough in Bulgaria, Poland, and the USSR. (6). Although the most important pharmacological action of this aporphine is its antitussive action, glaucine and related aporphine alkaloids have additional central nervous system effects (7,8) and are structurally related to alkaloidal antitumor agents (9). Because most aporphines are of the *S*-configuration, pharmacological evaluation of the corresponding *R*-enantiomers has been difficult due to the lack of availability of these compounds. The routine method of resolving racemic mixtures of these alkaloids, fractional crystallization, is a tedious process. However, the resolution of racemic glaucine (**1**,**2**) has been shown to be facile using the microorganisms *Fusarium solani* (ATCC 12823) and *Aspergillus flavipes* (ATCC 1030) (10,11). For example, in the microbial transformation of *R*,*S*-glaucine (**1**,**2**), *F. solani* stereospecifically and quantitatively converts *S*-(+)-glaucine (**1**) to dehydroglaucine (**3**), leaving *R*-(−)-glaucine (**2**) as the residual substrate, while *A. flavipes* stereospecifically and quantitatively converts only *R*-(−)-glaucine (**2**) to **3**, leaving *S*-(+)-glaucine (**1**). This type of destructive resolution may provide a practical method for producing pure enantiomers (either *R* or *S*) of other aporphines from racemic mixtures.

The usefulness of microorganisms in organic synthesis is well documented (12-15), and extending the above reactions to the destructive resolution of other aporphines and related alkaloids could be very useful in organic and natural product chemistry. Our first objective in the study of this microbiological reaction was determining the complete stereochemical course of glaucine dehydrogenation by the fungi *F. solani* and *A. flavipes*. Although the stereochemical requirements at the 6a position of glaucine initially were known, the stereochemistry at the prochiral (diastereotopic) C-7 position was not, and the transformation could be envisioned as proceeding via either an overall *cis*- or *trans*-elimination of hydrogen. Subsequent metabolism studies with C-7 "methyl-blocked" analogs of glaucine, *cis*- (**6**,**7**) and *trans*-7-methylglaucine (**8**,**9**) have shown that this reaction proceeds with an overall *cis*-elimination of hydrogen since only *cis*-7-methylglaucine (**6**,**7**) was converted by the fungi to 7-methyl-dehydroglaucine

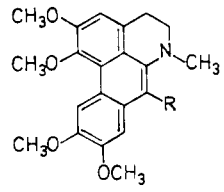
[1]Present Address: Department of Chemistry, Rice University, Houston, Texas 77251.



1 R_1=H,R_2=H(pro *R*,α), R_3=H (pro *S*,β)
4 R_1=R_2=R_3=D
6 R_1=R_2=H,R_3=CH_3
8 R_1=H,R_2=CH_3,R_3=H



2 R_1=H,R_2=H(pro *R*,α), R_3=H (pro *S*,β)
5 R_1=R_2=R_3=D
7 R_1=H,R_2=CH_3,R_3=H
9 R_1=R_2=H,R_3=CH_3



3 R=H
10 R=CH_3

(**10**) (17,18). Theoretically, the presence of a methyl group at the C-7 position of glaucine could effect the formation of the enzyme-substrate complex and the stereochemical course of the microbiological reaction. Consequently, these metabolism studies should be repeated with the corresponding C-7 deuterated analogs of glaucine to confirm the results obtained with 7-methyl analogs of the aporphine. We previously have examined the absolute stereochemistry of residual substrates to elucidate the stereochemical course of aporphine dehydrogenation by *F. solani* and *A. flavipes* (10,11,18). These experiments with specifically deuterated analogs will not employ the same techniques but, instead, will examine the presence of deuterium in the product as an indication of *cis*- or *trans*-elimination. However, experiments with deuterated glaucine analogs will require the unequivocal assignment of the alicyclic protons of glaucine (H-4α, H-4β, H-5α, H-5β, H-6α, H-7α, and H-7β) by ^{1}H nmr in order to confirm the structure of labeled substrates. These data have not previously been reported in studies concerning the ^{1}H nmr of aporphines (19-22). Consequently, this paper describes the complete structural elucidation of glaucine by high field and 2D-^{1}H nmr as a prelude to microbial metabolism studies on the dehydrogenation of deuterated analogs of glaucine by *F. solani* and *A. flavipes*.

RESULTS AND DISCUSSION

The first step in the resolution of the aliphatic protons of glaucine by ^{1}H nmr was obtaining the 600 MHz spectra of **1** and *R*,*S*-6a,7,7-trideutereoglaucine (**4,5**). Inasmuch as the assignment of the aromatic, methoxyl, and *N*-methyl protons of glaucine and other aporphines have previously been determined (20-22), they will not be discussed in this paper. Initially, proton assignments were based upon integration, comparison of spectra of **1** and **4,5**, calculations of relative shielding and deshielding effects of the A and D aromatic rings on each proton, measurements and calculations of coupling constants, and decoupling experiments. Two-dimensional COSY and HETCOR experiments were then conducted, and they supported our assignments.

Figure 1A illustrates the region of the 600 MHz ^{1}H-nmr spectrum (2.4-3.2 ppm) containing the alicyclic and *N*-methyl protons of glaucine. Integration of this region showed that the multiplets at 3.12, 2.67, 2.57, and 2.45 ppm each represent one proton, while the multiplet centered at 3.02 ppm integrates for three protons. The seven alicyclic protons of glaucine are contained in two isolated spin systems, one being the 6a, 7α, and 7β protons, the second being the H-4α, H-β, H-5α, and H-5β protons. These two systems can be identified in the spectrum by the comparison of Figure 1A and the 600 MHz spectral data for *R*,*S*-6a,7,7-trideutereoglaucine (**4,5**) (Figure 1B). Integration of the signals in Figure 1B shows the marked dimunition of the multiplets at 3.02 ppm (1.43 protons) and 2.57 ppm (0.2 proton), suggesting that the 6a, 7α,

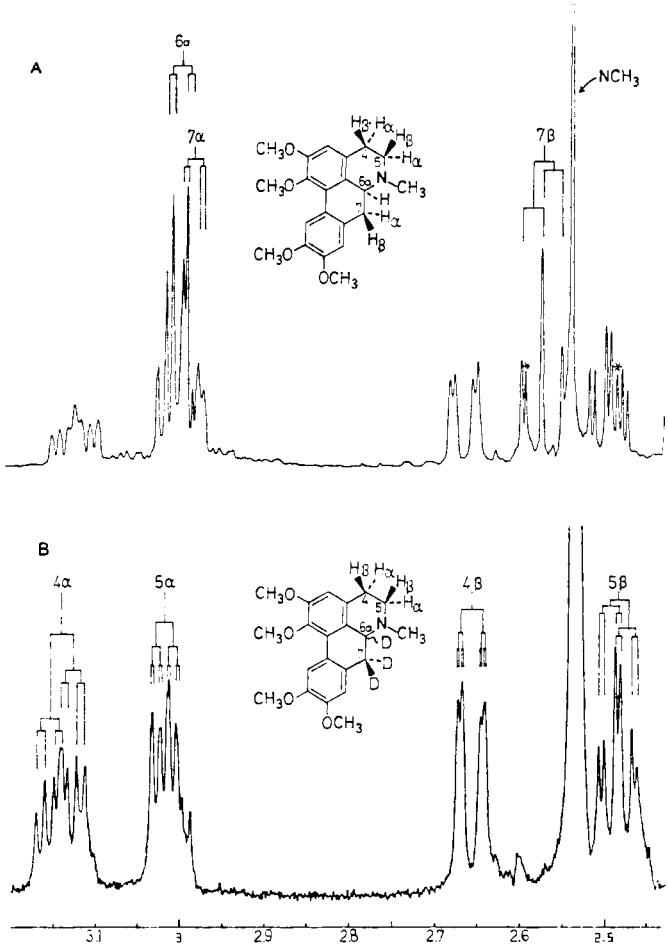


FIGURE 1. The 600 MHz ^{1}H-nmr spectra of glaucine (**1**) and racemic 6a,7,7-trideutereoglaucine (**4, 5**) *sidebands of *N*-Me group due to first order non-spinning shim inhomogeneity.

and 7β protons fall into these two regions while the second spin system (H-4α, H-4β, H-5α, and H-5β) is accounted for by the remaining (unaffected) signals at 3.12, 3.02, 2.67, and 2.45 ppm. This assumption was supported by decoupling experiments at 360 MHz with **1** which showed that irradiation at 3.12 ppm (H-4α) caused the collapse of the signal at 2.67 (H-4β) to a broad singlet and some simplification of the multiplet at 2.45 ppm (H-5β) but no change in the 2.57 ppm (H-7β) multiplet, as expected.

After the two spin systems were isolated, relative anisotropic effects of the A and D aromatic rings on the aliphatic protons of glaucine were calculated, using a formula cited by Abraham and Loftus (23), in order to make initial proton assignments. The formula $\delta=\mu(1-3\cos^2\theta/r^3)$, which was previously employed in making stereochemical assignments for the diastereomers of 7-methylglaucine (17), shows that the relative anisotropic effect an aromatic ring (δ) has on its substituents is related to the angle (θ) the substituent holds from perpendicular to the aromatic ring, its radius (r) in angstroms from the center of the ring, and the dipole ($\mu=27$D) of the induced field (23). Measurements were made from Dreiding models, and Figure 2 illustrates the two conformational extremes of **1** (labeled conformation I and conformation II) that were considered in the calculations. The large vicinal coupling constant (12.6 Hz) observed for the 4α and 5β protons in the 600 MHz spectrum of **1** suggests that these protons are

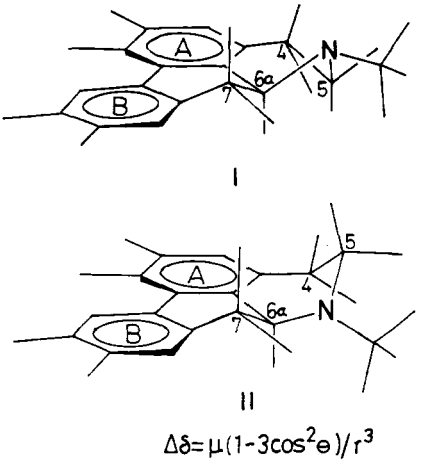


FIGURE 2. Possible conformations of glaucine used in calculations of the relative deshielding effects of the A and D aromatic rings on alicyclic protons.

axial and that conformation I predominates in solution. However, the results from our calculations suggest that, regardless of the conformation of the molecule, the α-protons are deshielded relative to the β-protons, that H-4α is deshielded more than H-5α, and that H-6a and H-7α experience similar deshielding effects. Based upon this and decoupling data, initial proton assignments could be made for the H-4α (3.12 ppm), H-6a and H-7α (3.02 ppm), H-7β (2.57 ppm), and H-5β (2.45 ppm), but the order of the 5α and 4β protons was still unclear.

The 2-D HETCOR experiment for glaucine confirmed assignments of the 5α and 4β protons. Figure 3 diagrams the results of the experiment, displaying the ^{13}C-nmr

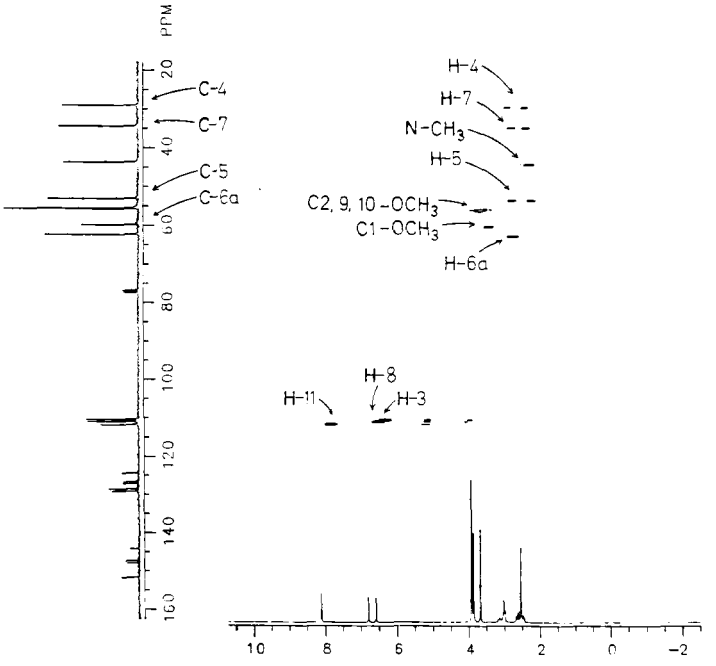


FIGURE 3. Two-dimensional HETCOR experiment for glaucine (**1**).

spectrum on the y-axis and the ^{1}H-nmr on the x-axis, with C-H coupling interactions. The ^{13}C nmr for glaucine has been previously elucidated by Jackman (24), and Jackman's chemical shift assignments for the C-4 and C-5 are confirmed by our 90 MHz C-H coupled ^{13}C-nmr spectra, which show a larger C-H coupling constant for the C-5 (135 Hz) compared to the C-4 (119 Hz) as expected for carbon atoms alpha to a heteroatom (25). In addition, confirmation of the assignment of the two ring systems of glaucine was made by a two-dimensional COSY experiment at 500 MHz. The coupling interactions (cross peaks) between the protons of the 6a, 7α, and 7β spin system and coupling within the second spin system (4α, 4β, 5α, and 5β protons) is depicted in Figure 4. Table 1 lists the final chemical shift assignments for the aliphatic protons of glaucine.

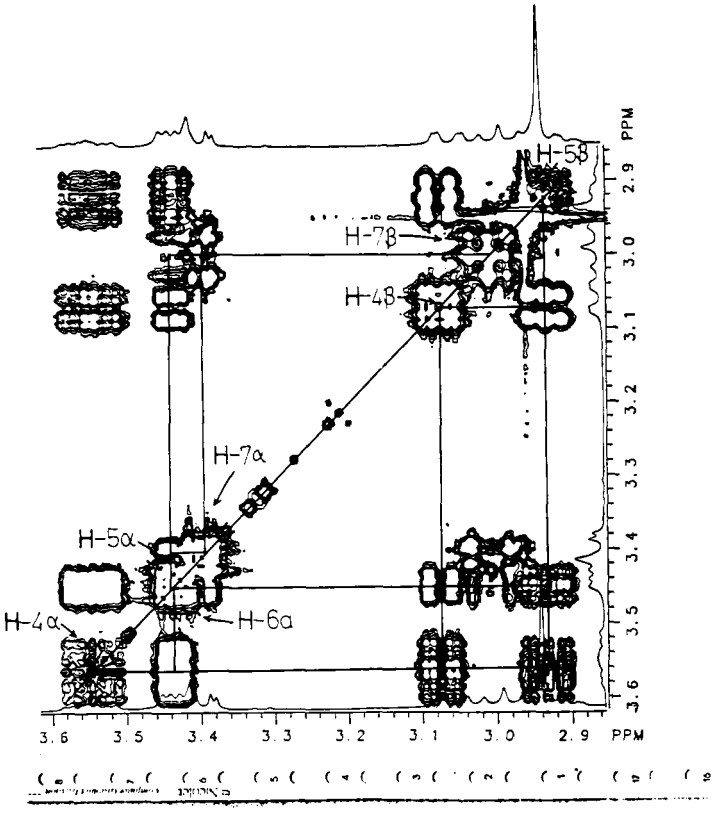


FIGURE 4. Two-dimensional COSY spectrum of glaucine (**1**).

The last step in the elucidation of the high field ^{1}H nmr of glaucine was the measurement of coupling constants. Coupling constants were measured directly from the 600 MHz ^{1}H-nmr spectra of **1** and **4,5** (see Figure 1). These data were used to simulate a spectrum which was consistent with the original. Table 1 lists the final chemical shift and coupling constant data for the alicyclic protons of glaucine.

Full elucidation of the ^{1}H-nmr spectrum of glaucine has made it possible to continue with metabolism experiments examining the stereochemical course of aporphine dehydrogenation by the fungi *F. solani* and *A. flavipes*. These data will allow for the structural elucidation of the microbiological substrates deuterated at the 6a, 7α, and/or 7β positions. Our assignments of chemical shift and coupling constants for the aliphatic protons of this molecule are supported by high field ^{1}H nmr and ^{13}C nmr, comparison of the ^{1}H-nmr spectra for deuterated and nondeuterated compounds, decoupling experiments, calculation of deshielding effects, 2-D HETCOR and COSY experi-

TABLE 1. ^{1}H-nmr Chemical Shift and Coupling Constant Assignments for the Aliphatic Protons of Glaucine at 600 MHz

Proton	Chemical Shift (ppm)	Observed Coupling Constant (Hz)	
4α	3.12	J_{gem}	16.4
		$J_{4\alpha-5\alpha}$	6.3
		$J_{4\alpha-5\alpha}$	12.6
4β	2.67	J_{gem}	0.63
		$J_{4\beta-5\beta}$	3.2
5α	3.02	J_{gem}	12.6
5β	2.45		—
6a	3.03	$J_{6a-7\alpha}$	3.8
		$J_{6a-7\beta}$	13.9
7α	3.01	J_{gem}	13.9
7β	2.57		—

ments, and C-H and H-H coupling constants. In addition, these data should prove useful in the structural elucidation of other aporphines and related alkaloids.

EXPERIMENTAL

REAGENTS AND CHEMICALS.—All solvents were analytical grade or better. The compounds *S*-(+)-glaucine (**1**) and dehydroglaucine (**3**) were prepared as described previously (10).

ANALYTICAL PROCEDURES.—All nmr spectra were taken in $CDCl_3$ with TMS as the internal standard. The ^{1}H-nmr and ^{13}C-nmr spectra were generated on either Varian EM-390 (90 MHz), Varian FT-80 (80 MHz), Nicolet Model 600 (600 MHz), Nicolet Model NT-360 (360 MHz), or General Electric QN-500 (500 MHz) spectrometers. All 2-D spectra and decoupling experiments were run at 360 MHz. Simulated 600 MHz ^{1}H-nmr spectra were generated on an IBM-XT computer using the RACCOON spectrum generation program (Version 2.0) developed by P.F. Schatz, University of Wisconsin, Madison (available from NSF Project SERAPHIM, J.W. Moore, Director, Department of Chemistry, Eastern Michigan University, Ypsilanti, Michigan). Mass spectra were taken on a Dupont Model 21491 unit by direct probe insertion, and deuterium isotope content was determined from ms data (M-15 fragment with its corresponding isotope peaks of labeled and unlabeled material).

Tlc was conducted on plastic-backed 0.25 mm silica gel GF-254 plates (Polygram, Brinkman, Houston, Texas) eluted with EtOAc-MeOH-diethylamine (90:10:1). Plates were visualized by fluorescence quenching under 254 nm light and by spraying with ceric ammonium sulfate (1% in phosphoric acid).

SYNTHESIS OF (*R,S*)-6a,7,7-TRIDEUTEREOGLAUCINE (**4,5**).—Dehydroglaucine (**3**) (700 mg) was dissolved in 2 ml of 2.5 N DCl (Aldrich Chemical Company, Milwaukee, Wisconsin in a two-necked flask (100 ml) fitted with a condenser. For the reduction, Zn(Hg) was formed by first shaking 5 g zinc dust (Aldrich) and 300 mg of $HgCl_2$ (Fisher Scientific Company, Fair Lawn, NJ) with 10 ml of D_2O (Aldrich) and, secondly, by adding 75 ml of 2.5 N DCl/D_2O and shaking vigorously to yield fine globules of Zn(Hg). The aqueous layer was decanted and the Zn(Hg) was added to the flask, which was heated to 80°, stirred, and refluxed under N_2. After bubbling subsided (1 h), reduction was initiated by the slow addition of 2 ml of 6N DCl with an addition funnel, and the reaction was allowed to proceed overnight (27). The progress of reduction was monitered by tlc (17), and when complete, the mixture was basified to pH 9 by the addition of concentrated NH_4OH (500 ml). The product was extracted into EtOAc, and after backwashing with H_2O, the EtOAc layers were combined and dried under vacuum before analysis by ^{1}H nmr, ^{13}C nmr, and ms. The yield of product (**4,5**) was 77.8% (545 mg). The product *R,S*-6a,7,7-trideutereoglaucine (**4, 5**) gave the following analytical data for ^{1}H nmr on a Varian EM-390 spectrometer: [ppm (multiplicity, integration, assignment)]: 8.05 (s, 1, H-11); 6.75 (s, 1, H-8); 6.55 (s, 1, H-3); 3.90-3.99 (3s, 9, C2,8,10-OMe); 3.65 (s, 3, C1-OMe); 3.25-2.5 (m, 4, C-4 and C-5 aliphatic protons); 2.55 (s, 3, N-Me). The ^{13}C-nmr data (normal spectrum, C-H decoupled, Varian FT-80) was as follows (ppm, assignment): 151.7 (C-2); 147.8 (C-9); 147.3 (C-10); 144.1 (C-1); 129.2 (C-7a); 128.7 (C-3a); 126.9 and 126.8 (C-1b and C-1a); 124.5 (C-11a); 111.9 (C-11); 110.9 (C-8); 110.2 (C-3); 60.3 (C1-OMe); 56.4 (C2,9,10-OMe); 53.5 (C-5); 44.1 (N-Me); 29.4 (C-4). Carbons with deuterium labeling (C-6a and 7) "drop out" of the spectrum due to to C-D coupling, which greatly lowers the signal intensity, due also to the increased spin-lattice relaxation time of the deuterated carbon and to a decreased nOe. Mass spectra gave the following results (*m/z*,

% rel. int.): 360 (35), 359 (63), 358 (100), 357 (59), 356 (57), 347 (2), 346 (8), 345 (19), 344 (35), 343 (58). The overall percent deuterium labeling in **4,5** was calculated as 77%

ACKNOWLEDGMENTS

This research was supported by Grant F-780 from the Robert A. Welch Foundation and Grant GP-41570 from the National Institutes of Health. K. Kerr was supported on the 1984-1985 AFPE-Robert Wood Johnson Memorial Fellowship. All 600 MHz spectra were run at the Carnegie-Melon Institute and supported by PHS/NIH Grant RR-00292, courtesy of R.E. Betts. This research was conducted by K. Kerr in partial fulfillment of requirements for the Ph.D. degree at the University of Texas at Austin.

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Received 18 September 1985