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Atta-ur-Rahman, Azra Pervin, M. Iqbal Choudhary, Naim Hasan, and Bilge Sener

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SOPHAZRINE—A NOVEL QUINOLIZIDINE ALKALOID FROM *SOPHORA GRIFFITHII*

ATTA-UR-RAHMAN,* AZRA PERVIN, M. IQBAL CHOUDHARY,

H.E.J. Research Institue of Chemistry, University of Karachi, Karachi-75270, Pakistan

NAIM HASAN,

Department of Chemistry, University of Baluchistan, Quetta, Pakistan

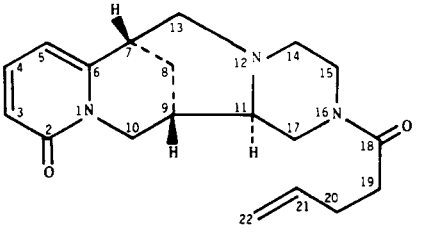
and BILGE SENER

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University Ankara, Turkey

ABSTRACT.—A novel quinolizidine (lupine) alkaloid, sophazrine [**1**], has been isolated from the leafy shoots of *Sophora griffithii*. X-ray crystallography of another related alkaloid, anagyrine [**2**], isolated from the leaves of *Thermopsis turcica*, confirmed its structure.

The aqueous extract of the leafy shoots of *Sophora griffithii* Stocks (Leguminosae), a plant cultivated throughout the warm regions of northern Asia, is occasionally used as a crude drug in folk medicine for its stomachic, diuretic, antipyretic, and analgesic properties and as an insecticide (1,2). Previously a number of quinolizidine alkaloids and flavanoids have been isolated from this plant (3–5). The present study describes the isolation and structure determination of a new type of tetracyclic quinolizidine alkaloid, named sophazrine [**1**], from the leafy shoots. Sophazrine was isolated as an amorphous solid, $[\alpha]_D$ +213° (MeOH, $c = 0.02$). The uv spectrum showed absorptions at λ max 233 (log ϵ 3.81) and 309 (log ϵ 3.87) nm which indicated the presence of a pyridone moiety (7–10). The ir spectrum ($CHCl_3$) showed absorptions at ν max 2865–2729 cm^{-1} (*trans*-quinolizidine), 1660 cm^{-1} (-NC=O), and 1650 cm^{-1} (α,β-unsaturated N-C=O). Sophazrine was assigned the molecular formula $C_{19}H_{25}N_3O_2$ on the basis of accurate mass measurements of a weak molecular ion at *m/z* 327.1951 (calcd 327.1948). The eims of sophazrine showed the major fragment ions at *m/z* 244.1449 (calcd 244.1446 for $C_{14}H_{18}N_3O$), 203.1184 (calcd 203.1183 for $C_{12}H_{15}N_2O$), 160.0740 (calcd 160.0762 for $C_{10}H_{10}NO$), and 146.0593 (calcd 146.0658 for C_9H_8NO). The ms was characteristic of tetracyclic lupine alkaloids having a 3,4,5,6-tetradehydroquinolizidine moiety (6–9).

The ^{1}H-nmr spectrum ($CDCl_3$) showed a doublet for H-10α at δ 4.05 ($J_{10\alpha,10\beta}$ = 14.2 Hz), which was coupled to a double doublet at δ 3.86 for H-10β (J = 14.2 and 6.2 Hz) as established by the COSY-45° experiment. These low field chemical shift values are characteristic for the H-10β/H-10α because of the adjacent nitrogen in the quinolizidine moiety. A 1H doublet of double doublet (Table 1) for H-9β (J = 6.2, 6.0, 4.0 Hz), showed cross peaks in its COSY-45° spectrum with the



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double doublet for H-11α at δ 2.92 (J = 7.4, 6.4 Hz), as well as with the double doublet for H-8β at δ 1.78 (J −13.2, 4.0 Hz) and with the doublet of double doublet for H-8α at δ 1.75 (J = 13.2, 10.3, 6.2 Hz). On irradiating H-10β (δ 3.86) the doublet for H-10α collapsed to a singlet, while H-9β collapsed to a doublet doublet (J = 6.2, 4.0 Hz). A similar effect on H-10α was observed when H-10β was irradiated. Similarly when H-9β (δ 2.40) was irradiated, it resulted in H-10β and H-8β becoming broad doublets, whereas H-8α collapsed to a doublet doublet (J = 13.2, 10.3 Hz). The aromatic region showed three double doublets integrating for one proton each at δ 5.93 (J = 6.8, 1.0 Hz), 6.49 (J = 9.3, 1.0 Hz) and 7.35 (J = 9.3, 6.8 Hz) which were assigned to H-5, H-3, and H-4, respectively. When H-4 (δ 7.35) was irradiated, H-3 (δ 6.49) and H-5 (δ 5.93) collapsed to doublets (J = 1.0 Hz). A downfield 1H multiplet at δ 5.61 was assigned to the H-21 olefinic methine proton, whereas the terminal olefinic methylene protons, H_a-22 and H_b-22, resonated as multiplets at δ 4.87 (J = 16.4, 3.2, 1.5 Hz) and δ 4.82 (J = 11.5, 3.2, 1.5 Hz). Two unresolved multiplets for H-20β and H-20α integrating for one proton each were centered at δ 2.01 and 1.90, respectively. When the H-20β was irradiated, the multiplet at δ 5.61 (H-21) collapsed to a double doublet (J = 16.4, 11.5 Hz), indicating the presence of an H_2C = CH-unit adjacent to the C-20 methylene group. Similarly when H_b-22 (δ 4.82) was irradiated, the multiplet at δ 5.61 (H-21) collapsed to a double doublet (J = 16.4, 8.0 Hz), thereby confirming the assignments to H-21, H-22, and H-20.

The building of the molecular framework of sophazrine was greatly facilitated by 2D nmr techniques. The 2D homonuclear shift correlation spectrum of **1** showed four different sets of mutually coupled spins. The various coupling interactions are presented around Figure 1. The coupling interactions and chemical shift considerations of protons at δ 6.49, 7.35, and 5.93 readily assigned them as H-3, H-4, and H-5, respectively, in ring A of sophazrine [**1**].

In order to gain more structural information and establish the relative stereochemistry at the asymmetric centers, nOe difference experiments were carried out. Irradiation at δ 2.92 (H-11α) resulted in 7.2% nOe at δ 1.75 (H-8α), 6.0% nOe at δ 2.10 (H-14α), 10.7% nOe at δ 2.51 (H-17α), 4.9% nOe at δ 2.34 (H-15α), and

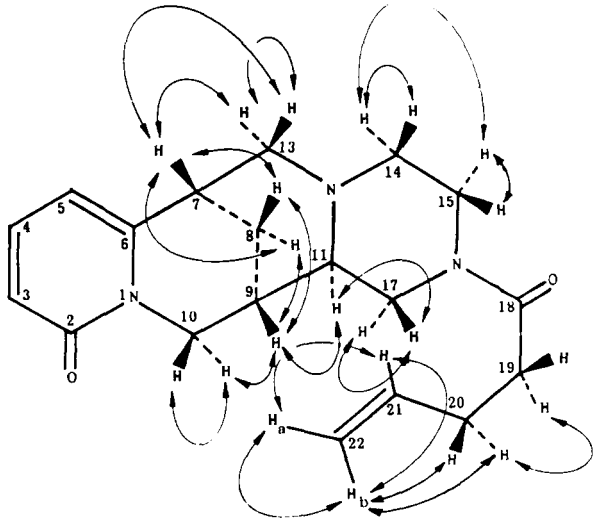


FIGURE 1. ^{1}H-^{1}H connectivities obtained from the analysis of COSY-45° spectrum of **1**.

14.4% nOe at δ 2.40 (H-9β). This established the α configuration for H-11. This was further supported by the irradiation of the signals of H-8α, H-14α, H-17α, and H-15α, which caused nOe at H-11α. Other important nOe interactions are given around Figure 2.

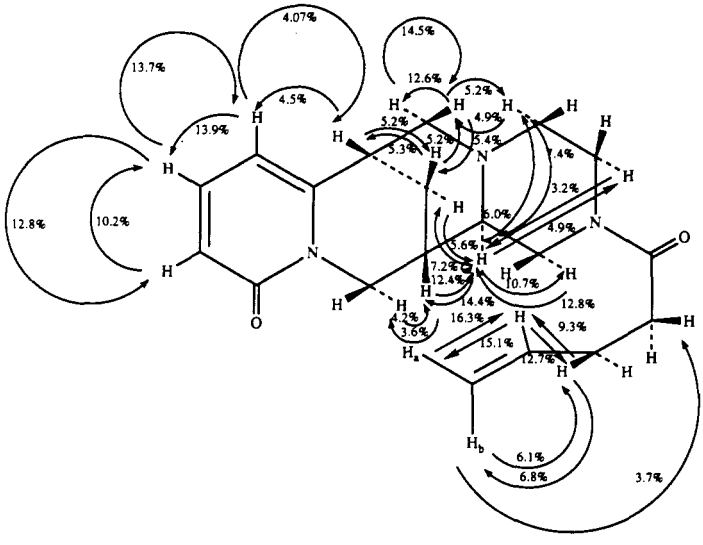


FIGURE 2. NOe measurements of **1**.

The ^{13}C-nmr spectrum of sophazrine [**1**] (in $CDCl_3$) was assigned on the basis of BB, DEPT, and 2D direct CH chemical shift correlation spectra (10,11). The broad band decoupled ^{13}C-nmr spectrum in $CDCl_3$ yielded the 19 carbon signals expected from the molecular formula (Table 1). The DEPT experiment showed the nine methylene and seven methine and hence the presence of three non-protonated carbon signals. The 2D carbon-proton correlation experiment established the proton chemical shift correlations with those of attached carbon atoms. Thus all the carbons and their attached protons were identified (Table 1) as expected from the molecular formula. Substituent effects were then used to assign the oxygen and nitrogen atom connectivities to the individual and carbons of the coupled CH/CH_2 fragments. Thus the five downfield CH_2 groups resonating at δ 50.50, 57.02, 45.23, 60.05, and 60.32 were assigned to C-10, C-13, C-14, C-15, and C-17 carbon signals, respectively, all of which were directly bonded to nitrogens. The nonaromatic region of sophazrine [**1**] showed the three methine signals, two of which resonated at δ 35.63, 28.15 of type C—CH—C and were assigned to C-7 and C-9, respectively, whereas the signal at δ 60.56 was of the C—CH—N type and was assigned to C-11. The upfield chemical shift observed for C-8 at δ 26.08 is characteristic for lupine alkaloids, reflecting the shielding effect due to its spatial proximity to N-12. This is typically encountered in quinolizidines in which H-9 and H-11 are trans-oriented to each other (12,13). In contrast in H-9/H-11 *cis*-quinolizidines, C-8 is known to resonate at values greater than 30 ppm (12,13). This assignment of trans-stereochemistry at C-9/C-11 was also confirmed by nOe experiments. The terminal olefinic methylene carbon appeared at δ 115.40. The olefinic C-21 methine resonated at δ 136.24. The ^{13}C-nmr spectrum showed two carbonyl resonances at δ 163.63 and 166.36, which were assigned to C-2 and C-18, respectively.

Proton-proton through space connectivities obtained by nOe measurements that established the partial structure of the A, B, C, and D rings of sophazrine [**1**] (Figure 3)

TABLE 1. ^{13}C-nmr, HeteroCOSY, and ^{1}H-nmr Spectra of Sophazrine [**1**].[a]

Position	Carbon chemical Shift (BB)	Multiplicities (DEPT)	HeteroCOSY	Multiplicities H^1-H^1, J (in Hz)
2	163.63	—	—	—
3	116.50	CH	6.49 (H-3)	dd, 3, 4 (9.3)
4	138.55	CH	7.35 (H-4)	dd 4.5 (6.8)
5	104.43	CH	5.43 (H-5)	dd 5.3 (1.5)
6	151.60	—	—	—
7	35.63	CH	2.86 (H-7β)	m
8	26.08		1.75 (H-8α)	ddd, 8α, 8β (13.2)
		CH_2	1.78 (H-8β)	dd, 8β, 9β (4.0)
9	28.15	CH	2.40 (H-9β)	ddd, 8α, 9β (6.2)
10	50.50	CH_2	4.05 (H-10α)	d, 10α, 10β (14.3)
			3.86 (H-10β)	dd, 10β, 9β (6.0)
11	60.56	CH	2.92 (H-11α)	brd
13	57.02	CH_2	2.90 (H-13α)	dd, 13α, 13β (14.0)
			2.25 (H-13β)	dd, 13α, 7β (6.0)
				13β, 7β (3.8)
14	45.23	CH_2	2.10 (H-14α)	m
			3.51 (H-14β)	m
15	60.05	CH_2	2.34 (H-15α)	m
			4.31 (H-15β)	m
17	60.32	CH_2	2.51 (H-17α)	d, 17α, 17β (12.8)
			2.97 (H-17β)	dd, 11α, 17β (6.4)
18	166.36	—	—	—
19	31.14		1.95 (H-19α)	brd, 19α, 19β (13.0)
		CH_2	2.28 (H-19β)	dd, 19β, 20α (6.0)
				m
20	30.04	CH_2	1.90 (H-20α)	m
			2.01 (H-20β)	m
21	136.29	CH	5.61 (H-21)	m
22	115.40		4.87 (H-22a)	dq, 22a, 21α (16.4)
		CH_2	4.82 (H-22b)	22a, 22b (3.2)
				22b, 21 (11.5)

[a]Spectra were run in $CDCl_3$ using TMS as internal reference.

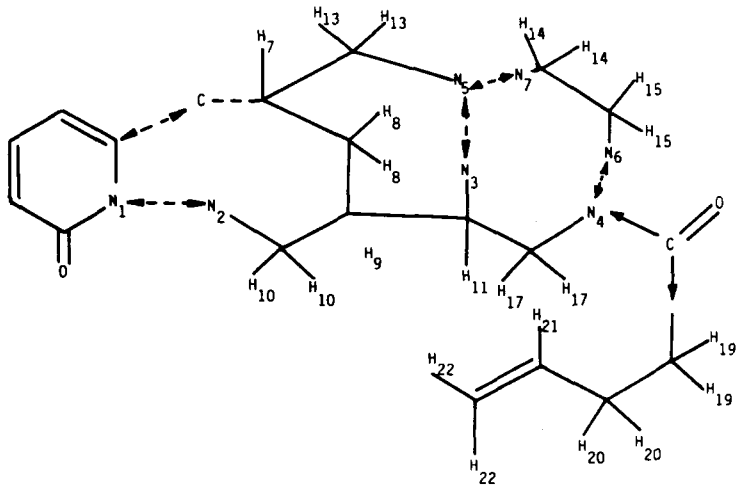
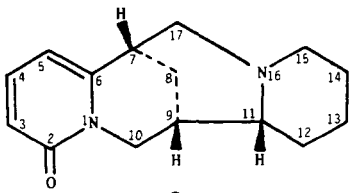


FIGURE 3. Partial structure of **1**.

clearly showed that the methylenes at C-17 and C-15 were connected via C-17—N—C-15 linkage, hence $N_4 = N_6$. Similarly the nOe between the protons at C-13, C-14, and C-11 indicated that $N_3 = N_5 = N_7$, and the tertiary function of nitrogen was C-13—N(C-11)—C-14. Insertion of the carbonyl at δ 166.36 between C-16 and C-19 completed the structure of sophazrine as **1**.

The lupine alkaloid anagyrine [**2**] was first isolated from *Anagyrus foetida* L. (14) and then from other plant sources (15). Alkaloids of the lupine series are renowned for their poisonous effects, and, since teratogenicity is the most important toxic effect of anagyrine [**2**], an X-ray crystallographic study was carried out in order to obtain the coordinates for use in structure-activity studies. We now report the isolation of **2** from the leaves of *Thermopsis turcica* Gandoger (Leguminoseae); it has also been isolated by us from *S. griffithii*. The spectral properties of the compound were identical with those reported earlier (2, 16). Anagyrine [**2**], $C_{15}H_{20}N_2O$, was isolated from *T. turcica* leaves as



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pink-colored crystals. A suitable crystal was selected for single crystal X-ray diffraction analysis. Crystals formed in the tetragonal space group $P4_1$, with $a = 8.260$ (1), $b = 8.260$ (1), $c = 18.689$ (3) Å, V = 1274.887 Å^3, Z = 4. All unique diffraction maxima were collected ($2\theta < 52°$) using a θ:2θ scan with graphic monochromated Mo-Kα radiation (0.71069 Å). Of the 1290 unique reflections, 1203 (93%) had $F_o < 3\sigma F_o$ and were judged observed. The structure was solved by direct methods and refined to a final discrepancy index of 0.05 for the observed data by full-matrix least-squares procedures (16). A computer-generated drawing of the final X-ray model is given (Figure 4).

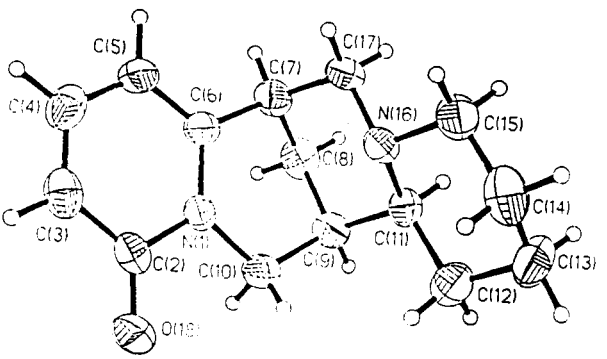

FIGURE 4. Computer-generated X-ray model of anagyrine [**2**].

EXPERIMENTAL

INSTRUMENTATION.—The uv spectra were recorded in MeOH on a Shimadzu UV-240 spectrometer. The ir spectra were recorded in $CHCl_3$ on a JASCO IRA-I spectrometer. The ^1H and ^{13}C-nmr spectra were run in $CDCl_3$ solution on a Bruker AM-400 NMR spectrometer with TMS as internal reference. Mass spectra were obtained on Varian MAT 112 and 312 double focussing mass spectrometers connected to DEC PDP II/34 computer systems. Optical rotations were measured on a Polartronic Universal Australian Standard K-157 spectropolarimeter. The leafy shoots of *S. griffithii* were collected from Quetta, Pakistan

and identified by the plant taxonomist Sauod Omar, Lecturer in the Department of Botany, University of Karachi, where a sample specimen was deposited (Herbarium voucher no. 37247 KUH).

ISOLATION OF SOPHAZRINE [**1**].—Air-dried leafy shoots (5.0 kg) of *S. griffithii* were crushed and extracted with EtOH (20 liters), and the extract was concentrated to a crude gum (150 g). The gum was acidified with 10% HOAc and extracted with $CHCl_3$. The acidic aqueous layer was then basified (pH 9) with NH_3 and again extracted with $CHCl_3$, which afforded the crude alkaloids (80 g). This crude alkaloidal portion was subjected to cc on Al_2O_3 (1.5 kg). Initially the elution was carried out with petroleum ether-$CHCl_3$ (1:1) and then with successively increasing polarities by adding Me_2CO and MeOH. The fractions obtained were: petroleum ether/$CHCl_3$ (1 liter), $CHCl_3$ (2 liters), $CHCl_3$/Me_2CO (1.5 liters), Me_2CO (1.5 liters), Me_2CO/MeOH (1.5 liters), and MeOH (2 liters). The fractions obtained on elution with $CHCl_3$-Me_2CO (85:15–70:30) were combined (16 g) and rechromatographed on a tlc grade Al_2O_3 column, elution being carried out with petroleum ether-$CHCl_3$ (1:2); this afforded a fraction containing sophazrine along with two minor alkaloids. Sophazrine [**1**] was purified on a Chromatotron (circular rotating plate chromatography) using Al_2O_3 as adsorbent; elution with $CHCl_3$ yielded 19.6 mg of sophazrine as a gummy mass, $[\alpha]^{23}D$ +213° (MeOH, $c = 0.02$).

ISOLATION OF ANAGYRINE [**2**].—The leaves of *T. turcica* were collected from Konya, Aksehir area of Turkey, in May 1986. The plant was identified by M. Koyuncu, and a voucher specimen is available in the herbarium of Ankara University, Turkey. The dried and powdered aerial parts (1.0 kg) of the plant were percolated with EtOH at room temperature. The EtOH extract was concentrated in vacuo at 50° to a reddish-brown gum (250 g), dissolved in 5% HCl, and kept in a refrigerator for 48 h. The acidic phase was filtered and basified with NH_4OH (pH 8.0). Repeated cc and preparative tlc of the crude alkaloids (6 g) were carried out to afford pink-colored anagyrine [**2**] (16 mg), which was crystallized from $CHCl_3$, $[\alpha]D = 158°$ (EtOH). It was also isolated from *S. griffithii*. The fraction (6.7 g) obtained on elution with $CHCl_3$ was again chromatographed on an Al_2O_3 column (0.5 kg), and the elution was carried out with petroleum ether-$CHCl_3$ (70:30), (60:40), (50:50), (40:60), (30:70), (20:80), (10:90), and (0:100) and $CHCl_3$-MeOH (90:10), (80:20), (70:30), (60:40), and (0:100), each time collecting 200-ml fractions. The fraction (0.2 g) obtained by elution with petroleum ether-$CHCl_3$ (20:80) contained anagyrine [**2**] as the major alkaloid which was purified on a precoated Al_2O_3 tlc plate using the same solvent system.

CRYSTAL DATA.—$C_{15}H_{20}N_2O$, M = 230.0 monoclinic, $a = 8.260$ (1), $b = 8.260$ (1), $c = 18.689$ (93) Å, $\beta = 91.03$ (3) Å, V = 1274.887 Å^3 (by least squares refinement on diffractometer angles for 15 atomatcially centered reflections, $\lambda = 0.71069$ Å), space group $P2_1$, Z = 4, $D_x = 1.38$ cm^{-3}. Crystal dimensions 0.25 × 0.35 × 0.30 cm.

DATA COLLECTION AND PROCESSING.—Nicolet R_3 M/V diffractometer $\theta/2\theta$ mode with θ scan width = 0.85 + 0.35 tan θ, speed 1.3–6.8 degrees·min^{-1}, graphite-monochromated M_o-K_α radiation; 0.71069 Å, reflection measured ($1.5 \leq Q \leq 25°$, $+h,k,l$), unique [merging R = 0.024 after absorption correction (max., min., transmission factors = 0.37, 0.10)], giving $I > 3\sigma$; linear and approximate isotropic crystal decay, ca. 10% corrected during processing structure analysis and refinement. Direct methods, full-matrix least-squares refinement with all non-hydrogen atoms anisotropic and hydrogen in calculated position. Final R and Rw values are 0.05, 0.06.[1]

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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