Quinolizidine Alkaloids from *Sophora alopecuroides*

Atta-ur-Rahman,*,† M. Iqbal Choudhary,*,† Khalid Parvez,† Aftab Ahmed,† Farzana Akhtar,† M. Nur-e-Alam,† and Naeem M. Hassan‡

International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan, and Department of Chemistry, University of Baluchistan, Quetta, Pakistan

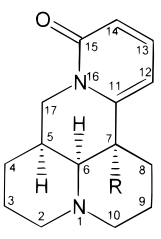
Received July 14, 1999

A new matrine-type alkaloid, 7α-hydroxysophoramine (**1**), was isolated from the aerial parts of *Sophora alopecuroides* together with eight known alkaloids, 12*β*-hydroxysophocarpine (**2**), sophoramine (**3**), 14*β*-hydroxymatrine, matrine, sophoridine, sophocarpine, adenocarpine, and baptifoline. The structures of compounds **1**−**3** were confirmed through single-crystal X-ray diffraction analysis.

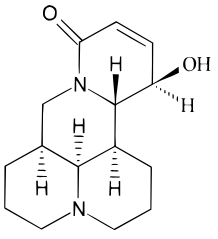
The plant *Sophora alopecuroides* L. (Leguminosae) is widely distributed over a large area of the Asian continent.[1] Biological studies on the constituents found in this plant have been performed in terms of potential sedative, central nervous system depressant, analgesic, hypothermic,[2] antitussive,[3] anticancer,[4,5] nematocidal,[6] antispasmodic,[7] antipyretic,[8] cardiotonic,[9] hypoglycemic,[10] and many other pharmacological activities.[11−14] In this communication, studies on *S. alopecuroides* have led to the isolation of a new alkaloid (**1**) and several known alkaloids. Among these alkaloids 12*β*-hydroxysophocarpine (**2**), 14*β*-hydroxymatrine, and adenocarpine have not been isolated previously from this species.

Results and Discussion

Aerial parts of *S. alopecuroides* were collected from the Baluchistan province of Pakistan and extracted with 80% ethanol. The extract was subjected to solvent−solvent extraction and repeated column chromatography on Si gel to obtain the new alkaloid, 7α-hydroxysophoramine (**1**) together with seven known alkaloids: 12*β*-hydroxysophocarpine[15] (**2**), sophoramine [16,17] (**3**), 14*β*-hydroxymatrine,[18] adenocarpine,[19] matrine,[20] sophoridine,[21,22] sophocarpine[4,23] and baptifoline. The structures of the compounds were determined unambiguously using either X-ray diffraction technique or 1D and 2D ^{1}H and ^{13}C NMR experiments in conjunction with the analysis of mass spectral and other spectroscopic data.



1 R = OH
3 R = H



2

The molecular formula of the new alkaloid **1** was determined by HREIMS to be $C_{15}H_{20}N_2O_2$ (*m*/*z* 260.1511). Its IR spectrum ($CHCl_3$) showed absorption bands of hydroxyl (ν_{max} 3236 cm^{-1}), α,*β*-unsaturated lactam (ν_{max} 1540 cm^{-1} for C═C and ν_{max} 1660 cm^{-1} for C═O), and *trans*-quinolizidine (ν_{max} 2928, 2855, 2793, and 2735 cm^{-1}) functionalities.[24] The EIMS showed a peak at *m*/*z* 243 corresponding to [M − OH]$^+$. The ^{1}H NMR spectrum ($CDC1_3$) was very similar to that of sophoramine (**3**). The downfield protons resonating at *δ* 7.13 (dd, $J_{13,14}$ = 8.9 Hz, $J_{13,12}$ = 7.2 Hz), 6.40 (dd, $J_{12,13}$ = 7.2 Hz, $J_{12,14}$ = 1.2 Hz), and 6.19 (dd, $J_{14,13}$ = 8.9 Hz, $J_{14,12}$ = 1.2 Hz) were assigned to H-13, H-12, and H-14, respectively. Two other downfield signals at *δ* 3.99 (dd, $J_{17\beta,17\alpha}$ = 14.2 Hz, $J_{17\beta,5}$ = 7.0 Hz) and 3.61 (dd, $J_{17\alpha,17\beta}$ = 14.2 Hz, $J_{17\alpha,5}$ = 13.0 Hz) could be assigned to H-17*β* and H-17α, respectively. The lack of any other downfield methine signal indicated that alkaloid **1** might contain a hydroxyl group on a quaternary carbon. The ^{13}C NMR spectra (BB and DEPT) of **1** showed 15 carbon signals with seven methylene, five methine, and three quaternary carbons. The chemical shift of a quaternary carbon (*δ* 69.3) also indicated the presence of a tertiary hydroxyl group. In the HMBC spectrum (Figure 1), the proton resonating at *δ* 6.19 (H-14) showed a long-range heteronuclear connectivity with C-15 (*δ* 163.7), while H-13 (*δ* 7.13) showed HMBC connectivities with C-15 (*δ* 163.7), C-14 (*δ* 118.1), C-12 (*δ* 104.1), and C-11 (*δ* 148.5). H-12, resonating at *δ* 6.40, exhibited HMBC interactions with C-14, C-11, and C-7 (*δ* 69.3), whereas H-17*β* (*δ* 3.99) was coupled with C-15, C-11, C-6 (*δ* 66.5), and C-5 (*δ* 25.6). These results suggested that the new alkaloid **1** is of the matrine-type, in which a hydroxyl group is present at the ring junction (i.e., C-7). The structure of **1** was established unambiguously as 7α-hydroxysophoramine by X-ray diffraction methods. A suitable crystal that formed in the orthorhombic space group, $P2_12_12_1$, was selected for the experiment. Accurate lattice constants were *a* = 7.836(2), *b* = 12.021(2), and *c* = 14.342(8) Å, with four independent molecules in the asymmetric unit. All unique diffraction

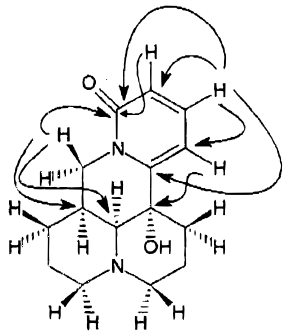


Figure 1. HMBC correlations in compound **1**.

* Authors to whom correspondence should be addressed. Tel.: +92-21-499-0007. Fax: +92-21-496-3373 or 496-3124. E-mail: hejric@digicom.net.pk.
† University of Karachi.
‡ University of Baluchistan.

10.1021/np990351v CCC: $19.00
Published on Web 01/19/2000

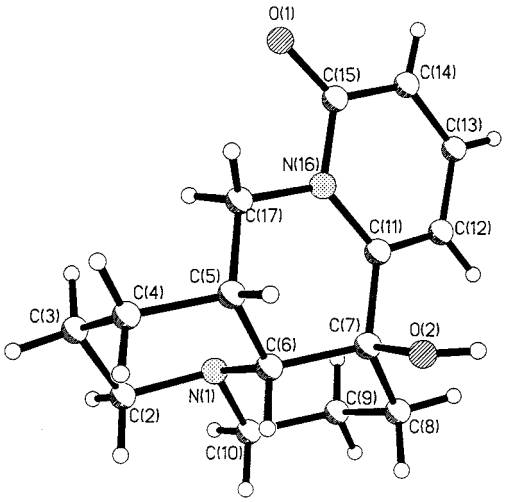


Figure 2. Computer-generated perspective drawing of the final X-ray model of 7α-hydroxysophoramine (**1**).

maxima with $2\theta \leq 135°$ were collected using $\theta-2\theta$ scans and graphite monochromated Cu Kα radiations (1.54178 Å). A total of 3184 unique reflections was collected, and of those 2361 were judged observed [$I > 2\sigma(I)$] and used in subsequent calculations. The structure was phased using direct methods (SHELXTL)[25] and refined using full-matrix least-squares techniques with anisotropic heavy atoms and isotropic riding hydrogens to conventional crystallographic residual of 0.0361 ($R_w = 0.0982$) for the observed data. A computer-generated drawing of the final X-ray model of **1** is given in Figure 2.

12β-Hydroxysophocarpine (**2**) was previously isolated from *Sophora viciifolia*,[15] but this is the first report of its isolation from *S. alopecuroides*. It has not been subjected to X-ray crystallographic structure determination before. Its molecular formula was derived as $C_{15}H_{22}N_2O_2$. The structure was established unambiguously by the single crystal X-ray diffraction technique. Compound **2** was recrystallized from acetone−methanol mixture, and a suitable crystal was selected for the study. The crystal formed in the orthorhombic space group $P2_12_12_1$ was $a = 5.8140(10)$, $b = 14.892(3)$, and $c = 15.189(3)$ Å, with four molecules ($C_{15}H_{22}N_2O_2$) in the asymmetric unit. All unique diffraction maxima with $2\theta < 135°$ were collected using $\theta-2\theta$ scans and graphite monochromated Cu Kα radiations (1.54178 Å). A total of 3047 unique reflections was collected, and of those 2047 were judged observed [$I > 2\sigma(I)$] and used in further calculations. The structure was solved by the direct methods (SHELXTL) and refined by full-matrix least-squares techniques to a final discrepancy index of 0.0359 ($R_w = 0.1001$) for observed data. A computer-generated perspective drawing of the final X-ray model of **2** is given in Figure 3.

Sophoramine (**3**) is an alkaloid previously isolated from *S. alopecuroides*[17] and many other species of *Sophora*. The compound **3** was isolated as large colorless crystals, and X-ray diffraction studies were carried out. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 20 carefully centered strong reflections. This corresponded to a orthorhombic, $P2_12_12_1$, space group with cell constants, $a = 8.0410(10)$, $b = 9.419(2)$, and $c = 16.891(2)$ Å and four independent molecules ($C_{15}H_{20}N_2O$) in the asymmetric unit. A total of 1591 unique reflections was collected using Cu Kα radiations (1.54178 Å) of which 1443 were judged observed [$I > 2\sigma(I)$] and used in further calculations. The structure was solved by direct methods

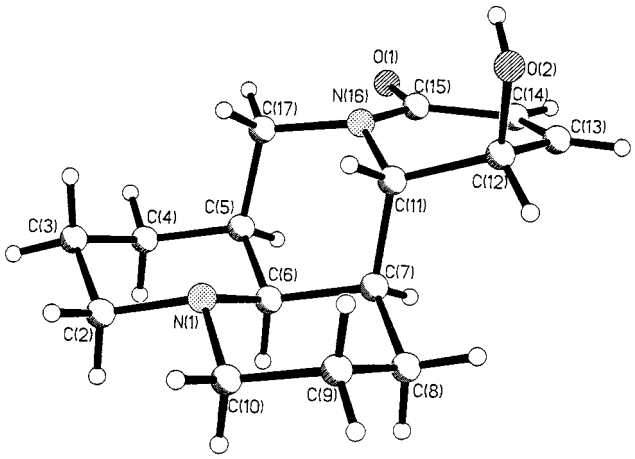


Figure 3. Computer-generated perspective drawing of the final X-ray model of 12β-hydroxysophocarpine (**2**).

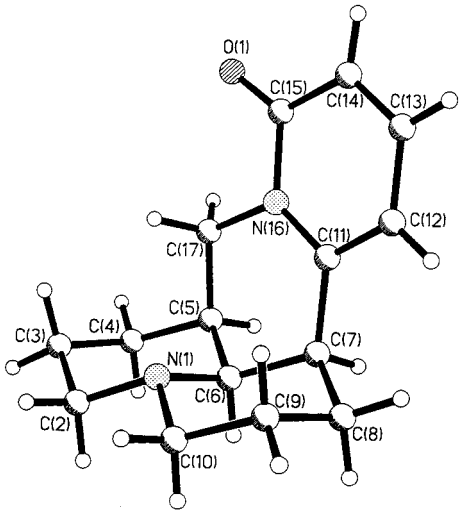


Figure 4. Computer-generated perspective drawing of the final X-ray model of sophoramine (**3**).

(SHELXTL) and refined by full-matrix least-squares techniques to final discrepancy index of 0.0454 ($R_w = 0.1500$) for observed data. A computer-generated perspective drawing of the final X-ray model of **3** is given in Figure 4. This is the first report of its crystal structure.

14β-Hydroxymatrine was previously isolated from *S. tonkinensis*,[18] whereas adenocarpine was isolated initially from *Adenocarpus intermedius* and *A. parvifolius*.[26] This is the first report of the isolation of these compounds from *S. alopecuroides*. Matrine, sophoridine, sophocarpine, and baptifoline have been previously reported from this plant.[27−32] These alkaloids were identified by comparison of their spectral data with the reported values.

Experimental Section

General Experimental Procedures. Melting points were recorded on a Büchi *535* melting point apparatus and are uncorrected. Optical rotations were determined on a polartronic polarimeter. The IR spectra were recorded on JASCO IRA-I IR spectrophotometer. The UV spectra were recorded in CH_3OH on a Shimadzu UV 240 instrument. The ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 500 NMR spectrometer at 500 MHz, while the ^{13}C NMR spectra were recorded on the same instrument at 125 MHz. MS were measured on a JEOL HX-110 mass spectrometer. X-ray diffraction studies (compounds **1**−**3**) were conducted on a Bruker (previously Nicolet) P_4 diffractometer using Cu Kα radiations.

Plant Material. The aerial parts of *S. alopecuroides* were collected from the Hazarganji and Khanuzai areas, located in the Baluchistan Province of Pakistan, in June 1992. The plant was identified by the taxonomist Mr. Saeed-ur-Rahman, Assistant Professor, Department of Botany, University of Baluchistan. A voucher specimen (HS # 35) has been deposited in the Herbarium of the University of Baluchistan, Quetta, Pakistan.

Extraction and Isolation. The air-dried aerial parts of the plant (10 kg) were crushed and extracted three times with 80% ethanol at room temperature. After evaporation of ethanol under vacuum, the concentrate was dissolved in water, acidified to pH 4, and extracted with $CHCl_3$. The aqueous layer was then basified with NH_4OH to pH 8 and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried with Na_2SO_4 and concentrated in vacuo to obtain the crude base (125 g). This was chromatographed on a Si gel column eluted with petroleum ether (40−60°)−acetone mixtures of increasing polarities, which afforded 7α-hydroxysophoramine (**1**) (150 mg), 12β-hydoxysophocarpine[15] (**2**) (50 mg), sophoramine[16,17] (**3**) (90 mg), 14β-hydroxymatrine[18] (15 mg), adenocarpine[19] (7 mg), matrine[20] (535 mg), sophoridine[22] (200 mg), sophocarpine[23] (100 mg), and baptifoline[26] (trace).

7α-Hydroxysophoramine (1): obtained as colorless crystals from acetone−methanol mixture, mp 204 °C; $[\alpha]_D^{25}$ −87 (*c* 0.04, MeOH); IR ($CHCl_3$) ν_{max} 3236 (OH), 2928, 2855, 2793, 2735 (*trans*-quinolizidine), 1660 (lactam C=O), 1540 (C=C) cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 309 (3.84), 233 (3.72), 203 (4.04) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (1H, dd, $J_{13,14}$ = 8.9 Hz, $J_{13,12}$ = 7.2 Hz, H-13), 6.40 (1H, dd, $J_{12,13}$ = 7.2 Hz, $J_{12,14}$ = 1.2 Hz, H-12), 6.19 (1H, dd, $J_{14,13}$ = 8.9 Hz, $J_{14,12}$ = 1.2 Hz, H-14), 3.99 (1H, dd, $J_{17\beta 17\alpha}$ = 14.2 Hz, $J_{17\beta,5}$ = 7.0 Hz, H17β), 3.61 (1H, dd, $J_{17\alpha 17\beta}$ = 14.2 Hz, $J_{17\alpha,5}$ = 13.0 Hz, H-17α), 2.74 (1H, br s, H-5), 2.72 (1H, br s, H-10β), 2.47 (1H, br d, *J* = 13.6 Hz, H-8β), 2.62 (1H, br d, *J* = 10.4 Hz, H-2α), 1.99 (1H, br s, H-6), 1.94 (2H, t, *J* = 11.1 Hz, H-2β and H-10α); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.7 (C-15), 148.5 (C-l1), 138.8 (C-13), 118.1 (C-14), 104.1 (C-12), 69.3 (C-7), 66.5 (C-6), 56.4 (C-2), 56.1 (C-10), 43.7 (C-17), 36.8 (C-8), 26.6 (C-4), 25.6 (C-5), 22.1 (C-9), 20.1 (C-3); EIMS *m/z* 260 $[M]^+$ (100), 259 $[M - H]^+$ (34), 243 $[M - OH]^+$ (65), 152 (40), 134 (58), 124 (81), 96 (96); HREIMS *m/z* 260.1511 ($C_{15}H_{20}N_2O_2$ requires 260.1525).

Crystal Data for 1. $C_{15}H_{20}N_2O_2$, MW = 260.1525, orthorhombic, $P2_12_12_1$, *a* = 7.836(2), *b* = 12.021(2), and *c* = 14.342-(8) Å, *V* = 1351.0(9) Å^3, *Z* = 4, D_x = 1.280 mg/m^3, Cu Kα (λ = 1.54178 Å), *F*(000) = 560, *T* = 293 K, *R* = 0.0361, R_w = 0.0982, for 2362 unique $I > 2\sigma(I)$ (total = 3184), approximate crystal dimension of 0.25 × 0.25 × 0.30 mm^3.

Crystal Data for 2. $C_{15}H_{22}N_2O_2$, MW = 262.1681, orthorhombic, *a* = 5.8140(10), *b* = 14.892(3), and *c* = 15.189(3) Å, *V* = 1315.1(4) Å^3, *Z* = 4, D_x = 1.325 mg/m^3, Cu Kα (λ = 1.54178 Å), *F*(000) = 568, *T* = 293 K, *R* = 0.0359, R_w = 0.1001, for 2047 unique $I > 2\sigma(I)$ (total = 3047), approximate crystal dimension of 0.31 × 0.25 × 0.30 mm^3.

Crystal Data for 3. $C_{15}H_{20}N_2O$, MW = 244.1575, orthorhombic, *a* = 8.0410(10), *b* = 9.419(2), and *c* = 16.891(2) Å, *V* = 1279.29(3) Å^3, *Z* = 4, D_x = 1.269 mg/m^3, Cu Kα (λ = 1.54178 Å), *F*(000) = 528, *T* = 293 °K, *R* = 0.0454, R_w = 0.1500, for 1443 unique $I > 2\sigma(I)$ (total = 1591), approximate crystal dimension of 0.25 × 0.25 × 0.25 mm^3.

All the data were collected in the θ−2θ scan mode on a computer controlled Bruker P_4 (previously Nicolet) diffractometer, maximum 2θ values 3.5 ≤ 135°. The structures were solved by direct methods (SHELXTL, Version 5) and refined by full-matrix least-squares on F^2. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were in riding mode. The crystallographic data of **1**−**3** have been deposited with the Cambridge Crystallographic Data Centre (University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK).

Acknowledgment. We thank Glaxo-Wellcome Pakistan Ltd. for providing financial support to one of us (K.P.).

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NP990351V