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COHIRSITININE, A NEW ISOQUINOLINE ALKALOID FROM *COCCULUS HIRSUTUS*

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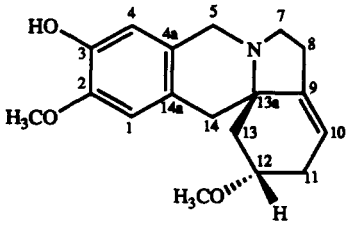
ABSTRACT.—A new isoquinoline alkaloid, cohirsitinine, has been isolated from *Cocculus hirsutus*. Its structure has been assigned as **1** on the basis of spectral studies. Its relative stereochemistry has been determined by homonuclear 2D ^{1}H-nmr (COSY-45, *J*-resolved, NOESY) and nOe difference measurements.

Cocculus hirsutus L. Diels (Menispermaceae), locally known as "jamati-kibel," is a climbing shrub commonly found in Karachi, Sind, and Kutch in Pakistan. Its various parts are reputed for their medicinal properties in folk systems of medicine. The leaves are used in prurigo, eczema, impetigo, and acute gonorrhea, and the roots are used as a substitute for sarsaparilla, antiperiodic in fevers, tonic, alterative, and diuretic, and are given for chronic rheumatism and syphilitic cachexia (1–3).

Previous investigations on various parts of the plant resulted in the isolation of trilobine (4,5), isotrilobine (5), coclaurine (4,5), magnoflorine (5), α-sitosterol, ginnol (6), and a monomethyl ether of inositol (6). As a result of our continuing investigations on *C. hirsutus* (7), we have isolated a new isoquinoline alkaloid, cohirsitinine, to which structure **1** has been assigned on the basis of extensive nmr studies (8–10). The ^{1}H-nmr assignments were made with the help of 2D COSY-45 and *J*-resolved and homonuclear decoupling experiments, and its relative stereochemistry has been determined by a series of nOe difference and NOESY experiments.



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The pure alkaloid, cohirsitinine [**1**], was isolated as a gum as described in the Experimental. Its uv spectrum showed absorptions at 213, 236, and 290 nm, which indicated an isoquinoline skeleton (11). The ir spectrum showed an intense absorption at 3500 and 1230 cm^{-1}, indicating the presence of hydroxyl groups (12). The phenolic nature of the latter was confirmed by a positive reaction with $FeCl_3$ which gave a green color. When the compound was treated with D_2O and the mass spectrum was recorded again, the $[M]^+$ peak was found to be shifted by 1 mass unit to *m/z* 302, suggesting the presence of one exchangeable hydrogen atom in the molecule (13). The hrms indicated the molecular ion peak at *m/z* 301.1663, consistent with the molecular formula $C_{18}H_{23}NO_3$ (calcd 301.1671), indicating eight degrees of unsaturation in the molecule. Other prominent peaks were found at *m/z* 270, 243, 226, 210, 165, 150, and 132. The peak at *m/z* 270.1482 ($C_{17}H_{20}NO_2$, calcd 270.1488) corresponded to the loss of an MeO group from the molecular ion.

There was a prominent peak at *m/z* 243.1177 ($C_{15}H_{17}NO_2$, calcd 243.1254) in the mass spectrum, which showed the loss of 58 mass units (C_3H_6O) from the molecular ion as a result of retro-Diels-Alder fragmentation of ring D. The peak at *m/z* 226.1219 ($C_{15}H_{16}NO$, calcd 226.1227) indicated the loss of an hydroxyl group from *m/z* 243.1177 ($C_{15}H_{17}NO_2$).

The peak at *m/z* 120.0809 ($C_8H_{10}N$, calcd 120.081) indicated the loss of 150 mass units ($C_9H_{10}O_2$) from *m/z* 270.1482 ($C_{17}H_{20}NO_2$, calcd 270.1488), corresponding to the RDA fragmentation process of ring B. The molecular ion was confirmed by fabms (14).

The ^{1}H-nmr spectrum of cohirsitinine [**1**] ($CDCl_3$, 300 MHz) showed the presence of 23 protons in the molecule, each of which was identified by a series of homodecoupling experiments, nOe difference measurements, and COSY-45. H-13β appeared as a triplet at δ 1.68 ($J_{13\beta,12} \sim J_{13\beta,13\alpha}$ = 13.6 Hz), and H-13α appeared as a double doublet at δ 2.26 showing geminal coupling with H-13β ($J_{13\alpha,13\beta}$ = 13.6 Hz) and vicinal coupling ($J_{13\alpha,12}$ = 6.4 Hz) with H-12. The downfield chemical shift observed for H-12 indicated the presence of an MeO function at this carbon (7,15). The multiplets at δ 2.69 and 3.20 were assigned to H-11α and H-11β, respectively. A broad singlet at δ 5.67 was due to the C-10 olefinic proton (16). The H-5α and H-5β appeared as doublets ($J_{5\alpha,5\beta} = J_{5\beta,5\alpha}$ = 13.5 Hz) at δ 3.29 and 2.96, showing only geminal coupling and indicating the presence of a quaternary carbon α to C-4, and the downfield chemical shift of C-5 showed the direct attachment with nitrogen. Similarly, H-14α and H-14β exhibited doublets ($J_{14\alpha,14\beta} = J_{14\beta,14\alpha}$ = 12.4 Hz) at δ 2.42 and 2.40 showing only geminal coupling, thus indicating the presence of two quaternary carbons α to C-14. H-7α appeared at δ 3.53 as a multiplet, while another multiplet at δ 3.07 was assigned to H-7β. H-8α and H-8β resonated at δ 3.54 and 2.68 as multiplets. Two 3H singlets at δ 3.27 and 3.86 were assigned to the 12-OMe and 2-OMe groups, respectively. H-1 resonated at δ 6.66 as a singlet, while another singlet at 7.81 was assigned to H-4.

The nOe NOESY spectrum served to establish the spatial proximities. The signal at δ 6.66 (H-1) showed an nOe interaction with the signal at δ 3.86 (2-OMe). The signal at δ 2.26 (H-13α) showed an nOe interaction with the H-13β proton at δ 1.68 in the NOESY spectrum. In order to confirm the relative stereochemistry of the molecule and to record the subtle nOe effects not visible in the NOESY spectrum, nOe difference measurements were carried out. Irradiation at δ 1.68 (H-13β) resulted in a 14.1% nOe at δ 2.26 (H-13α) and 4.7% nOe at δ 3.74 (H-12). The nOe interaction between H-13β and H-12 could result only if the 12-OMe possessed α stereochemistry. Irradiation at δ 2.26 (H-13α) caused an 11.9% nOe at δ 1.68 (H-13β) and 10.3% nOe at δ 2.40 (H-14β). The nOe interaction between H-13α and H-14β suggested that these protons lie close to each other in the preferred conformation of ring D and established that the C-13a/C-13 bond is β-oriented.

Irradiation at δ 3.53 (H-7α) resulted in 6.7% nOe at δ 7.81 (H-4) and 14.9% nOe at δ 3.29 (H-5α). This established that H-7α lies closer to H-5α and not so close to H-4. It also suggested that the nitrogen lone pair of electrons has a β orientation. Irradiation at δ 7.81 (H-4) and at 3.29 (H-5α) resulted in an 11.6% nOe and 13.7% nOe at δ 3.53 (H-7α), respectively, and established the proximity of H-4 and H-5α in the preferred conformation of ring C. Irradiation at δ 6.66 (H-1) resulted in a 10.8% nOe at δ 3.86 (2-OMe). Irradiation at δ 7.81 (H-4) also resulted in an 11.8% nOe at δ 3.46 (H-8α), establishing that H-8α lies close to H-4; the absence of an nOe effect on the MeO group clearly indicated the presence of an aromatic hydroxy group at C-3 (11).

The ^{13}C-nmr spectrum ($CDCl_3$, 75 MHz) showed the presence of 18 carbon atoms in the molecule. The multiplicity assignments were made by DEPT. C-13a resonated at δ 63.84, its downfield chemical shift suggesting the α nitrogen function. ^{13}C-nmr chemical shifts are listed in Table 1.

TABLE 1. ^{13}C-nmr Chemical Shifts of Cohirsitinine [1].

Carbon	δ	Carbon	δ
C-1	112.75	C-9	129.98
C-2	157.60	C-10	117.99
C-3	155.93	C-11	22.78
C-4	124.40	C-12	73.49
C-4a	134.88	C-13	41.40
C-5	31.86	C-13a	63.84
C-7	51.90	C-14	27.03
C-8	40.45	C-14a	140.16
		2-OMe	51.98
		12-OMe	56.08

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectrum was recorded on a Shimadzu UV-240 spectrophotometer, the ir spectrum was recorded in $CHCl_3$ with a JASCO IRA-1 spectrophotometer, and the hrms was obtained on a Finnigan MAT-312 double focusing mass spectrometer coupled to a PDP 11/34 computer system. Nmr spectra were recorded at 300 MHz in $CDCl_3$ on a Bruker AM-300 NMR spectrometer with TMS as internal reference. Tlc experiments were performed on Si gel (GF-254, 0.2 mm) plates (E. Merck).

For nOe measurements, the sample was frozen under liquid nitrogen and degassed. A lower decoupler power of maximum 0.2 W with 35 attenuations in decibel seconds (dbs) was used. The pre-irradiation time was 11 sec, which is the sum of three delays as used in the nOe difference program of Bruker. The impulse length of 10 μsec was maintained to avoid saturation. The 2D COSY-45 experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2K data points) in ω_2 and 2000 Hz (256, t_1 value zero-filled to 1K) in ω_1. A 2-sec relaxation delay was used and 16 transients were performed for each t_1 value.

PLANT MATERIAL.—The whole plant material was collected from Sindh, Pakistan and was identified by Dr. S.I. Ali, Head of the Department of Botany, University of Karachi. A voucher specimen [no. 14-12-66 (KUH)] has been deposited at the Herbarium of the Department of Botany, University of Karachi.

EXTRACTION AND ISOLATION OF COHIRSITININE [1].—The plant material (40 kg) was chopped into small pieces and extracted exhaustively with EtOH. The EtOH extract was evaporated under reduced pressure. The material thus obtained was extracted with EtOAc. The aqueous layer was basified with NH_3, and the crude alkaloids were extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated, dried with anhydrous Na_2SO_4 (74 g), and subjected to cc. The fraction obtained with hexane-Me_2CO (6:4) was subjected to preparative tlc on Si gel precoated plates with $CHCl_3$-MeOH (9:1). This afforded the pure alkaloid, cohirsitinine [1], R_f 4.5 (6 mg), $[\alpha]^{25}D = 51°$ ($CDCl_3$), which gave a characteristic color reaction with Dragendorff's reagent. Uv (MeOH) λ max 213, 236, 290 nm, min 233, 266 nm; ir ($CHCl_3$) ν max cm^{-1} 3500, 1230 (OH), 2835 (CH); hrms $[M]^+$ 301.1663 (calcd 301.1671) ($C_{18}H_{23}NO_3$, 10%), 270.1482 (calcd 270.1488) ($C_{17}H_{20}NO_2$), 226.1219 (calcd 226.1234) ($C_{15}H_{16}NO$, 20%), 210.0916 (calcd 210.0915) ($C_{14}H_{12}NO$, 13%), 165.1148 (calcd 165.1149) ($C_{10}H_{15}NO$, 63%), 150.0671 (calcd 150.0661) ($C_9H_{10}O$, 33%), 132.0816 (calcd 132.0729) ($C_9H_{10}O$, 29%), 120.0809 (calcd 120.0810) ($C_8H_{10}N$, 16%); ^{1}H nmr ($CDCl_3$, 300 MHz, ppm) 1.68 (1H, t, $J_{13\beta,12} = J_{13\beta,13\alpha} =$ 13.6 Hz, H-13β), 2.68 (1H, m, H-8β), 2.26 (1H, dd, $J_{13\alpha,13\beta} = 13.6$ Hz, $J_{13\alpha,12} = 6.44$ Hz, H-13α), 2.40 (1H, d, $J_{14\beta,14\alpha} = 12.4$ Hz, H-14β), 2.42 (1H, d, $J_{14\alpha,14\beta} = 12.4$ Hz, H-14α), 3.46 (1H, m, H-8α), 2.69 (1H, m, H-11α), 2.96 (1H, d, $J_{5\alpha,5\beta} = 13.5$ Hz, H-5β), 3.07 (1H, m, H-7β), 3.20 (1H, m, H-11β), 3.27 (3H, s, 12-OCH_3), 3.29 (1H, $J_{5\alpha,5\beta} = 13.4$ Hz, H-5α), 3.53 (1H, m, H-7α), 3.74 (1H, m, H-12β), 3.86 (3H, s, 2-OCH_3), 5.67 (1H, br s, H-10), 7.81 (1H, s, H-4), 6.66 (1H, s, H-1); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1.

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