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COHIRSINE - A NOVEL ISOQUINOLONE ALKALOID FROM COCCULUS HIRSUTUS

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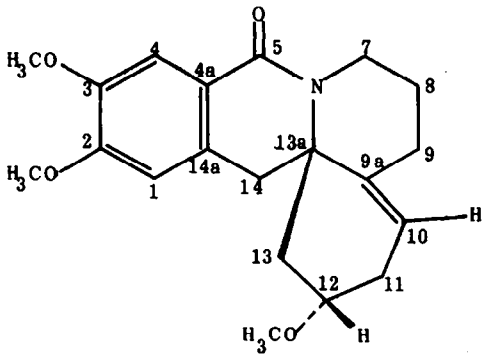
Abstract: A novel alkaloid, cohirsine (1) was isolated from Cocculus hirsutus. Its structure has been investigated by extensive NMR studies including 2D NMR experiments. Its stereochemistry has been determined by 2D NOESY and NOE difference measurements.

Plants of the Indian subcontinent are continuing source of fascinating natural products. Cocculus hirsutus (L.) Diels (Menispermaceae), locally known as "Jamti-ki-bel" is a climbing shrub commonly found in Karachi, Sind and Kutch. Its various parts are highly reputed for their medicinal properties in the indigenous system of medicine[1-4]. As a result of continuing investigations on Cocculus hirsutus, we have isolated a novel isoquinoline alkaloid, to which structure (1) has been assigned on the basis of extensive NMR studies[5-8]. The ^{1}H-NMR assignments were made with help of 2D COSY-45, J-resolved, hetero-COSY and homodecoupling experiments. The ^{13}C-multiplicities were established by carrying out multipulse 1D, DEPT experiments[9,10] while its stereochemistry has been determined by a series of NOE difference and NOESY experiments.

The crude alkaloidal mixture obtained from the EtOH extract of Cocculus hirsutus was basified with ammonia and extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to column chromatography. The fraction obtained with hexane-acetone (8:2) was subjected to preparative TLC on silica-gel plates to afford the alkaloid (1) as a gummy material. Its UV spectrum showed absorptions at 213, 240 and 300 nm, reflecting the isoquinolones-type chromophore.[11] The IR spectrum showed an intense absorptions at 1670 and 1710 cm^{-1}, indicating the presence of α,β-unsaturated 6 membered cyclic amide in the molecule[12]. The HRMS indicated the molecular ion peak at m/z 343.1778 consistent with the molecular formula $C_{20}H_{25}NO_4$, indicating nine double bond equivalents in the molecule. Other prominent peaks were found to occur at m/z 312, 299, 298, 285, 252, 226, 194, 162 and 134. The peak at m/z 312.1576 ($C_{19}H_{22}NO_3$) corresponded to the loss of methoxy from the molecular ion while the peak at m/z 298.1439 ($C_{18}H_{20}NO_3$) suggested the loss of 45 m.u. (C_2H_5O). The prominent peak at m/z 285.1356 ($C_{17}H_{19}NO_3$) corresponded to the loss of 58 m.u.

($C_3H_{16}O$) from the molecular ion suggesting the allylic cleavage of C-11/C-12 and C-13a/C-13 bond. The peak at m/z 134.0962 ($C_9H_{12}N$) indicated the loss of 178 m.u. ($C_{10}H_{10}O_3$) from m/z 312.1576 ($C_{19}H_{22}NO_3$) corresponding to the allylic cleavage of the C-13a/C-14 bond and cleavage along the C-5/nitrogen bond. The molecular ion was confirmed by FAB mass spectrometry[13].



(1)

The ^{1}H-NMR spectrum ($CDCl_3$, 300MHz) showed the presence of 25 protons in the molecule, each of which was identified by a series of homodecoupling experiments, NOE difference measurements, COSY-45 and hetero-COSY experiments. A one-proton singlet at δ6.69 was assigned to the C-1 proton. The C-4 proton appeared at δ 7.74 as a singlet, its downfield chemical shift reflecting the presence of a β-carbonyl function. The presence of two 1H singlets in the aromatic region indicated the substitutions at C-2 and C-3. The C-7α proton appeared at δ 2.62 as a multiplet while another multiplet at δ 3.02 was assigned to the C-7 β proton. The C-8 α and β protons resonated at δ3.52 and δ 3.18 as multiplets, the downfield chemical shifts are due to the β-amidic function[14]. A broad singlet at δ5.62 was assigned to the C-10 olefinic proton. The samll coupling constant indicating that this olefinic proton is gauch to the adjacent methylenic protons[15]. The multiplets at δ 2.67 and δ3.20 was assigned to the C-11α and β protons respectively. The multiplet at δ3.67 was assigned to the C-12 proton, its downfield chemical shift indicating the presence of methoxy function at this carbon. The C-13 α proton appeared as a double doublet at δ 2.26 showing geminal coupling with the C-13β proton ($J_{13\alpha,13\beta}$= 12.3Hz) and vicinal coupling with the C-12 proton ($J_{13\alpha,12}$ = 6.3Hz). The C-13β proton appeared as a triplet at δ1.57 ($J_{13\beta,12}\sim J_{13\beta,13\alpha}$= 12.3Hz). The C-14 α and β H appeared as doublets ($J_{14\alpha,14\beta}$= $J_{14\beta,14\alpha}$= 12.0Hz) at δ 2.44 and δ2.22, showing only geminal coupling, indicating the presence of two quaternary carbons α-to C-14. A two 3H singlets at δ 3.83 and δ 3.85 were assigned to the 2-O$\underline{C}H_3$ and 3-O$\underline{C}H_3$ groups respectively while the third 3H singlet at δ3.25 was assigned to the methoxy group at C-12.

Two dimensional NMR measurements were carried out to verify the ^{1}H-NMR assignments. The coupling interactions were established through correlated spectroscopy (COSY-45) while the multiplicity of the overlapping proton signals was determined from the 2D J-resolved spectrum. The assignment for the C-13 β proton at δ1.57 could thus be confirmed by its COSY-45 spectrum,

which showed strong cross peaks with signals at δ 2.26(C-13α H) and δ 3.67 (C-12H). Similarly the assignments of C-11α H (δ 2.64) and C-11β H (δ 3.20) were confirmed by the COSY-45 spectrum (Fig. 1) since they showed cross peaks with each other and with the proton at δ 3.67 (C-12H). The scalor couplings between the protons indicated that these proton are on vicinal carbons. The C-9α proton at δ 2.71 showed cross peaks with the C-9β H (δ 2.39), C-8αH (δ 3.53) and C-8β proton at δ 3.18. Similarly the C-7α proton atδ 2.61 showed the cross peaks with the C-7β H (δ 3.02), C-8αH (δ 3.53) and C-8β H (δ 3.18). Corresponding cross peaks of C-8 protons with each other and with the C-7 and C-9 protons were also observed in the COSY-45 spectrum. The COSY-45 spetrum is presented in Figure-1 with the important interactions indicated.

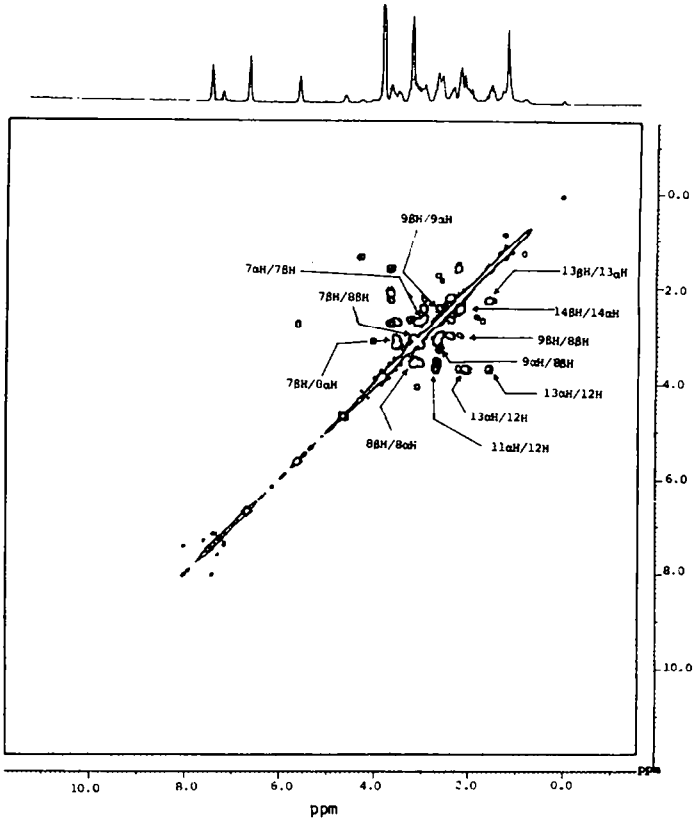


Figure 1: COSY-45 spectrum of cohirsine (1)

The NOESY spectrum served to establish the spatial proximities. The signal at δ 6.69 (C-1H) showed NOE interaction with the signals at δ 3.83 (2-OCH_3). The signal at δ 2.26 (C-13α H) showed NOE interactions with the C-13 β proton at δ 1.57 in the NOESY spectrum. To record the subtle NOE effects which were not observed in the NOESY spectrum NOE differece measurements were carried out (Table-I). Irradiation at δ 1.57 (C-13 β H) resulted in 16.2% NOE at δ 2.26 (C-13α H) and 5.0% NOE at δ3.67 (C-12H). The NOE interaction between C-13βH and C-12H could result only if 12-OCH_3 possessed α-stereochemistry. Irradiation at δ2.26 (C-13α H) caused 14.1% NOE at δ1.57 (C-13βH) and 9.8% NOE at δ2.22 (C-14β H). The NOE interaction between C-13α H and C-14β H suggested that in the preferred conformation of ring "D" these protons lie close to each other. It also established that C-13a/C-13 bond is β-oriented. Irradiation at δ 2.44 (C-14α H) resulted in 8.7% NOE at δ 2.22 (C-14 β H) and 13.0% NOE at δ 2.67 (C-11α H) establishing that in the preferred conformation of ring "D" C-14α proton lie close to the C-11α proton. This again indicated the β-orientation to C-13a/C-13 bond. Irradiation at δ 3.53 (C-8β H) resulted in 18.7% NOE at δ 3.18 (C-8βH) and 2.9% NOE at δ2.62 (C-7αH) and at δ3.02 (C-7βH). This also caused a 7.9% NOE at δ7.74 (C-4H). This established that in the preferred conformation of ring "C" C-8α proton lies close to the C-4 proton. It also suggested the β-orientation of nitrogen lone pair. Irradiation at δ 7.74 (C-4H) resulted in 9.7% NOE at δ3.85 (3-OCH_3) and 12.7% NOE at δ 3.53 (C-8α H), establishing the methoxy substitution at C-3 and again suggesting the proximity of C-4 proton with C-8α proton in the preferred confirmation of ring "C". Irradiation at δ6.69 (C-1H) resulted in 13.9% NOE at δ 3.83 (2-OCH_3). Corresponding NOE interactions were also observed when 2-OCH_3 protons were irradiated (Table-I).

The ^{13}C-NMR spectrum ($CDCl_3$, 75MHz) showed the presence of 20 carbon atoms in the molecule. The multiplicity assignments were made by DEPT pulse sequence with the last polarization pulse angle θ = 45°, 90° and 135°. The C-13a resonated at δ 63.84 its downfield chemical shift suggesting the α-nitrogen function. The C-12 appeared at δ 73.49, while the C-10 olefinic carbon resonated at δ 118.62. The C-8 appeared at δ 40.43, its downfield chemical shift suggesting the β-amidic function. The C-7 resonated at δ47.07 its upfield chemical shift is due to the amidic nature of the nitrogen function[16,17]. The signal at δ 166.22 was assigned to the amidic carbonyl carbon. The C-4 appeared at δ131.05 its downfield chemical shfit reflecting the deshielding influence of β-carbonyl function.The signals at δ 51.97 and δ 55.99 were assigned to the methoxy carbons at C-2 and C-3 respectively while the signal at δ 56.01 was assigned to the 12-OCH_3 carbon. The ^{13}C-chemical shift assignments were confirmed by hetero-COSY spectrum (Fig.2) and are presented in Table-II.

Table-I: NOE difference measurements on cohirsine (1)

Signal irradiated (δ)	Signal enhanced (δ)	% NOE
1.57 (C-13β H)	2.26(C-13α H)	16.2
	3.67 (C-12H)	5.0
2.26 (C-13α H)	1.57 (C-13β H)	14.1
	2.22(C-14β H)	9.8
2.44 (C-14αH)	2.22(C-14β H)	8.7
	2.67 (C-11α H)	13.0
3.18(C-8β H)	3.53 (C-8α H)	16.2
	3.02(C-7β H)	3.6
3.53 (C-8α H)	3.18 (C-8β H)	18.7
	2.62 (C-7α H)	2.9
	3.02 (C-7β H)	2.9
	7.74 (C-4H)	15.4
3.83 (2-$OC\underline{H}_3$)	6.69(C-1H)	7.7
3.85 (3-$OC\underline{H}_3$)	7.74 (C-4H)	9.2
5.62(C-10H)	2.42(C-9β H)	7.3
	2.71(C-9α H)	3.6
6.69(C-1H)	3.83(2-OCH_3)	13.9
7.74(C-4H)	3.53(C-8α H)	12.7
	3.85(3-OCH_3)	9.7

Table-II: ^{13}C-NMR chemical shifts of cohirsine (1)

Carbon No.	(δ)	Carbon No.	(δ)
1	112.75	10	118..62
2	157.53	11	22.78
3	157.68	12	73.49
4	131.05	13	41.40
4a	139.94	13a	63.84
5	166.72	14	27.03
7	47.07	14a	140.16
8	40.43	2-$O\underline{C}H_3$	51.97
9	31.91	3-$O\underline{C}H_3$	55.99
9a	129.97	12-$O\underline{C}H_3$	56.08

Hetero-COSY experiments[5] were carried out to identify the relationship between carbons and their respective protons. The C-11 signal at δ 22.78 showed cross peaks with the protons signals at δ2.67 (C-11αH) and δ 3.20 (C-11βH) thereby indicating the nonequivalence of C-11 protons and also confirming the rather downfield chemical shift of C-11β H (δ 3.20). The C-12 signal at δ 73.49 showed a cross peak with the proton signal at δ 3.67 in the hetero-COSY spectrum (Fig.2). Since these protons are coupled to each other as apparent from the COSY-45 spectrum (Fig.1), the spectral data showed that the methoxy group was located at C-12. Similarly the cross peaks between the carbon at δ 41.40 and the proton signals at δ 1.57 (C-13βH) and δ 2.26 (C-13βH) in the hetero-COSY spectrum and their coupling with the C-12 proton in the COSY-45 spectrum showed that C-13 (methylenic carbon) is adjacent to C-12. Similarly the C-14 signal at δ 27.03 showed cross peaks with the proton signals at δ 2.22 (C-14β H) and δ 2.44 (C-14α H). As these two

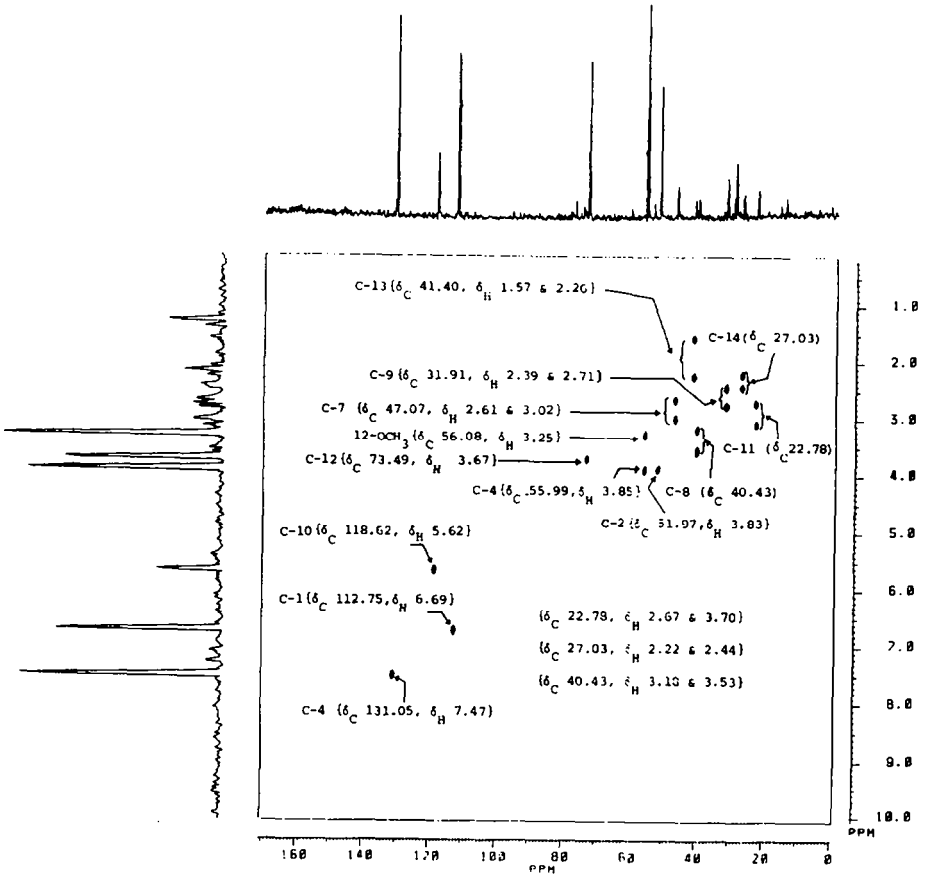


Figure 2: Hetero-COSY spectrum of cohirsine (1)

protons showed only geminal coupling in the COSY-45 spectrum it established the presence of two quaternary carbon atom α- to C-14. The assignments of C-7, C-8 and C-9 protons were also confirmed by the hetero-COSY spectrum (Fig.2), since their carbon signals showed cross peaks with their respective protons in the hetero-COSY spectrum. On the other hand C-8 protons were found to be coupled with C-7 and C-9 protons in the COSY-45 spectrum (Fig. 1) there by indicating that these carbons lie on either side of C-8. The 12-O$\underline{C}H_3$ (δ 56.08) showed cross peak with the proton signal at δ 3.25 similarly the C-10 signal at δ 118.62 showed cross peak with the proton signal at δ5.62 in the hetero-COSY spectrum. The hetero-COSY spectrum thus served to confirm the assignments of carbons and protors and is presented in Figure. 2 with the interactions indicated.

Cohirsine (1) may arise in nature from dehydroprotoberberine system through the oxidative cleavage along 13/14 and 8a/12a bond. Recyclization of C-13 to C-6 followed by intramolecular rearrangement may lead to cohirsine (1)

EXPERIMENTAL

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded on JASCO A-302 spectrophotmeter. HRMS were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. THe ^{1}H-NMR spectra were recorded at 300 MHz on Burker AM-300 NMR spectrometer. The ^{13}C-NMR spectra were recorded at 75 MHz on the same instrument. The optical rotation was recorded on Polartronic Universal Australian Standard K-157 digital polarimeter. TLC experiments were performed on silica gel (GF-254, 0.2 mm) plates (E.Merck).

Isolation of cohrisine (1): The plant material (40 kg) were collected from suburbs of the Karachi city and was identified by the plant Texonomist, Department of Botany, University of Karachi where a voucher specimen is deposited. The plant material was chopped into small pieces and extracted exhausively with EtOH. The ethanolic extract was evaporated under reduced pressure. The material thus obtained was extracted with EtOAc. The aqueous layer was basified with ammonia and extracted with chloroform. The chloroform layer was evaporated, dried with anhydrous Na_2SO_4 (74 gm) and subjected to column chromatography. The fraction obtained with hexane-acetone (8:2) was subjected to preparative TLC on silica gel (GF-254) precoated plates with chloroform-methanol (9.5:0.5) as the solvent system. This afforded a pure alkaloid cohirsine (1) (R_f = 0.4) (20 mg, 5.0 x 10^{-5}% yield) $[\alpha]_D^{26}$ + 147° ($CHCl_3$) gave characteristic cloure reaction with Dragandorff's reagent.

UV (MeOH) λ_{max}: 213, 240 and 300 nm λ_{min} 230 and 266 nm.

IR ($CHCl_3$) ν_{max} cm^{-1}: 2835 (C-H), 1710 (C=O), 1670 (α β -unsaturated amide) 1600 (C=O), and 1090 (C-O).

MS m/z (rel.int.%) : 343 (4), 312 (10), 299 (11), 298 (7), 285 (100), 252 (42), 226 (20), 194 (38), 162 (30) and 134 (14).

^{1}H-NMR ($CDCl_3$, 300MHz) δ : 1.57 (1H, t, $J_{13\beta,13\alpha} \sim J_{13\beta',12}$=12.3Hz, C-13$\beta$H), 2.26 (1H, dd, $J_{13\alpha, 13\beta}$ = 12.3Hz, $J_{13\alpha,12}$ = 6.3Hz C-13αH), 2.44 (1H, d, $J_{14\alpha,14\beta}$ = 12.0Hz C-14αH), 2.22 (1H, d, $J_{14\beta, 14\alpha}$ = 12.0Hz, C-14βH), 3.67 (1H, m, C-12H), 2.64 (1H, m, C-11αH), 3.20 (1H, m, C-11βH), 3.53 (1H, m, C-8αH), 3.18 (1H, m, C-8βH) 2.61 (1H, m, C-7αH), 3.02 (1H, m, C-7βH), 6.69 (1H, s, C-1H), 7.74 (1H, s, C-4H), 3.83 (3H, s, 2-OC$\underline{H_3}$), 3.85, (3H,s, 3-OC$\underline{H_3}$), 3.25 (3H, s, 12-OC$\underline{H_3}$).

^{13}C-NMR ($CDCl_3$, 75MHz) δ : Table-II.

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