ALKALOIDAL CONSTITUENTS OF THE BARK OF HOLARRHENA ANTIDYSENTERICA

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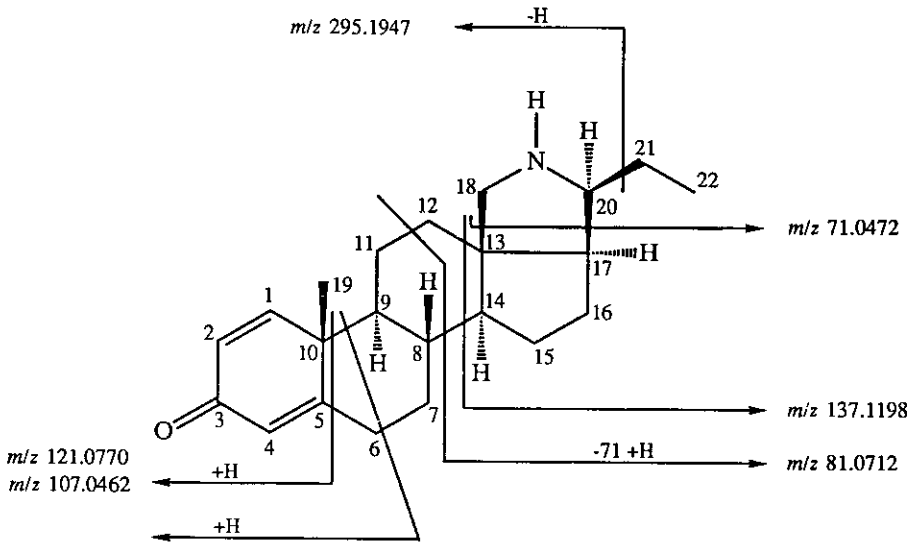
Abstract– Three steroidal alkaloids (**1-3**) of conanine series have been isolated from the bark of H. antidysenterica. **1** and **2** are new natural products while **3** has been reported earlier from this source.

Holarrhena antidysenterica (Apocynaceae) inhibits the drier forest areas of the sub-continent. It has been extensively studied for its alkaloids[1] mainly because the bark commonly known as "Kurchi" is highly reputed in traditional medicine as a remedy for amoebic dysentery and other intestinal ailments.[2] The plant has also been reported to possess anthelmintic, appetiser, astringent and antidiarrhoeal properties.[3] In view of the medicinal properties of the plant, present studies were undertaken on the bark which have led to the isolation of two new alkaloids namely kurchilidine and kurchamide, the structures of which have been elucidated as **1** and **2** employing spectroscopic methods. A known constituent regholarrhenine A[4] has also been identified.

Kurchilidine (**1**) with a molecular formula $C_{22}H_{31}NO$ (hrms, M^+ 325.2379) showed the M^+-15 peak at m/z 310.2172 and the base peak at m/z 71.0742 (C_4H_9N) in the mass spectrum. The peak at m/z 71.0742 is characteristic of *N*-methyl 18,20-epiminoconanine type of steroidal alkaloids.[5] However, in this case the *N*-Me signal ($\delta \sim 2.30$) as well as the secondary methyl group (*C-21* $\delta \sim 1.30$) were not observed in the ^{1}H-nmr spectrum. Instead a triplet of three protons was present at δ 0.85 indicating a $-CH_2-CH_3$ group in the molecule. These observations suggested the 18, 20-epimino ring E with the ethyl group at C-20. It showed strong absorptions in the ir spectrum at 1660, 1620 and 1600 cm^{-1} and uv absorption at 238 nm indicating the presence of a conjugated carbonyl moiety in the molecule. The ^{1}H-nmr spectrum (vide experimental) further showed three deshielded olefinic protons at δ 7.00 (1H, d, $J = 10.1$ Hz), 6.22 (1H, dd, $J = 10.1$, 1.9 Hz) and 6.07 (1H, t, $J = 1.9$ Hz). On the basis of the chemical shifts and coupling constants these signals were assigned to H-1,H-2 and H-4 respectively. These signals and a peak at m/z 121.0770 located a 1,4-dien-3-one moiety[6] in ring A. On the basis of these evidences the structure of kurchilidine has been elucidated as **1**.

Kurchamide (**2**) showed M^+ peak at m/z 341.1995 corresponding to the molecular formula $C_{21}H_{27}NO_3$ showing nine double bond equivalents. Its ir (1660,1620 and 1600 cm^{-1}), uv (238 nm)


m/z 295.1947
-H
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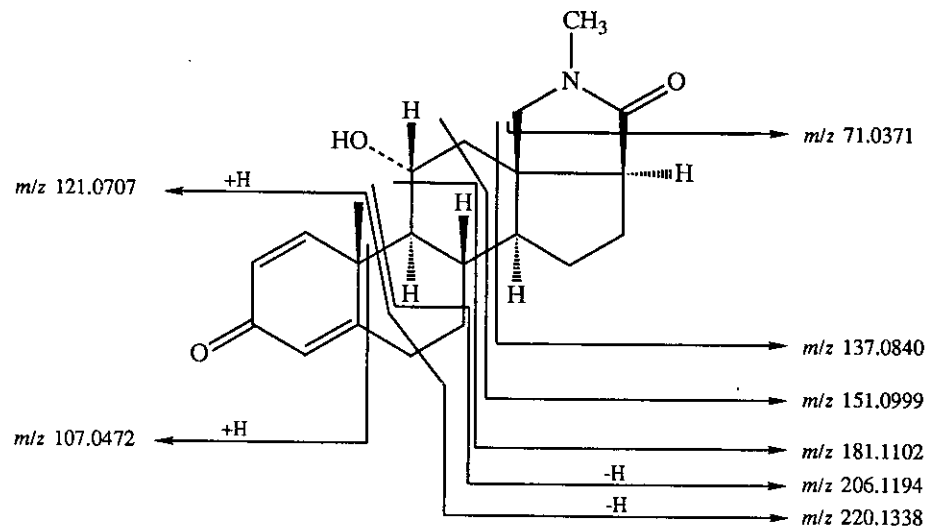
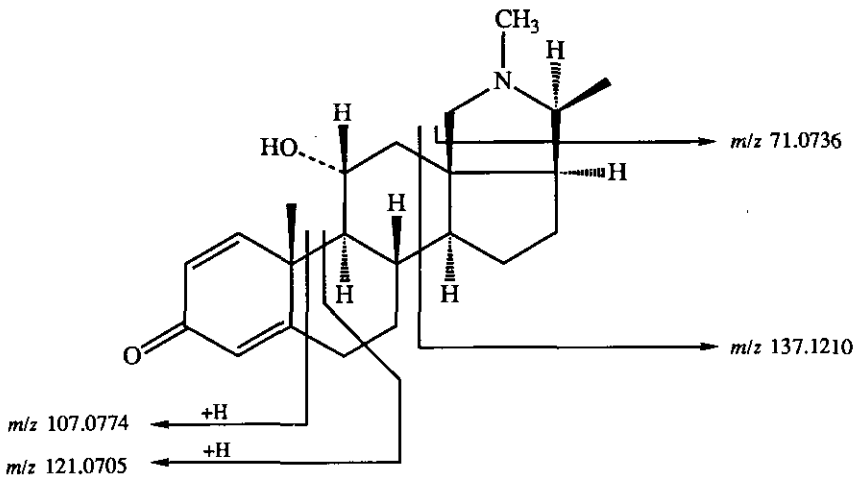
m/z 137.1198
-71 +H
m/z 81.0712
m/z 121.0770
m/z 107.0462
+H
+H

1


m/z 71.0371
m/z 121.0707
+H
m/z 137.0840
m/z 151.0999
m/z 107.0472
+H
m/z 181.1102
-H
m/z 206.1194
-H
m/z 220.1338

2

absorptions and the ^{1}H-nmr signals (Table 1) at δ 7.78 (d, J = 10.3 Hz, H-1), δ 6.16 (dd, J = 10.3, 2.0 Hz, H-2) and δ 6.10 (t, J = 2.0 Hz, H-4) indicated the 1,4-dien-3-one system in ring A which was also supported by a strong peak[6] at m/z 121.0707 (C_8H_9O) in the hrms (vide structure). A signal at δ 3.97 observed as a dt, (J = 10.0, 4.8 Hz) indicated an axial proton geminal to a hydroxyl group . These multiplicities and coupling constants suggested the location of the hydroxyl group at either C-7 or C-11 with α disposition. However, the downfield shift (δ 7.78) of H-1 as compared to H-1 (δ 7.00) of **1** led to its placement at C-11. This assignment was further supported by comparable chemical shifts of H-1 of compound (**2**) with those of other 11-hydroxyl compounds.[4] The compound showed only one tertiary methyl group at δ 1.28 (H-19). The absence of the second angular methyl of steroids and the peak at m/z 71.0371 (C_3H_5NO) indicated the presence of conanine type of skeleton.[6] The remaining oxygen function was placed in ring E as a carbonyl of lactam function due to the downfield shift of *N*-Me (δ 2.84) as compared to its values in other epimino compounds.[5] The lactam moiety in the 21-norconanine skeleton was supported by the presence of ir peak (1710 cm^{-1}) and ^{13}C-nmr shift (δ 176.8; Table 1) and absence of 21-Me signal in the ^{1}H-nmr spectrum. Two AB doublets were present at δ 3.18 and δ 3.06 with a geminal coupling constant of 10.8 Hz assignable to H-18a and H-18b which allowed to locate this amido carbonyl group at C-20. In the case of this carbonyl at C-18 the *H-20 protons would appear as two double doublets. This carbonyl group also caused an upfield shift* of the *N*-Me carbon (δ 29.8) and C-18 (δ 55.4) as against the 20-deoxo analogues (vide supra). On the basis of these observations structure (**2**) has been assigned to kurchamide which was further corroborated by the ^{1}H and ^{13}C shifts (^{13}C-nmr, hmqc) (Table 1) and various fragments in the hrms (vide structure).



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The eims, ir,uv, ^{1}H and ^{13}C-nmr spectral data of **3** were in good agreement with those of regholarrhenine A[4] isolated previously from the same source. It may, however, be noted that exhaustive 2D-nmr (cosy-45, noesy, J-resolved and hmqc) and ^{13}C-nmr DEPT experiments (Table 2) as well as hrms (vide structure and experimental) studies of **3** were undertaken for the first time, which allowed a complete assignment of ^{1}H-nmr and also led to reverse the reported[4] ^{13}C-nmr shifts of C-8 and C-20 with those of C-18 and C-16 respectively (Table 2).

EXPERIMENTAL

Melting points were uncorrected. Mass spectra were recorded on a finnigan MAT 112 and 312 double focussing mass spectrometers connected to a PDP 11/34 computer system; nmr spectra ($CDCl_3$): 400 MHz for ^{1}H and 75 MHz for ^{13}C. The chemical shifts are reported in δ (ppm) and the coupling constants are in Hz. The ^{13}C nmr spectral assignments (Tables 1 and 2) have been made partly through a comparison of the chemical shifts with the published data for similar compounds[7] and partly through the appearance of signals in DEPT and hmqc spectra (Tables 1 and 2). Precoated thin layer cards (DC-karten SiF) were used for tlc. The bark of H. antidysenterica was identified and supplied by the courtesy of Hamdard Foundation Pakistan Ltd. Petroleum ether used was of the boiling range 60-70°C.

Uncrushed bark (10 kg) was macerated with 10% methanolic NaOH (10 l) for 48 h at 28°C and repeatedly percolated with MeOH for 48 h (five times) at the same temperature. Each extract was neutralized with 30% aq. HOAc. The pH of the syrupy concentrate (2 l), obtained on removal of the solvent from the combined extracts under reduced pressure, was stumped down by adding 10% aqueous HOAc at 28 °C and shaken out with EtOAc. The aqueous phase was basified with 20% ammonia and shaken out with EtOAc. The moist EtOAc phase was treated with a vigorous stream of carbon dioxide. The precipitate containing the carbonate bases was filtered and the filtrate was dried over anhydrous Na_2SO_4 and freed of the solvent under reduced pressure. The residue (20 g) was divided into petroleum ether soluble and petroleum ether insoluble fractions.

The petroleum ether soluble fraction yielded conessine (9 g) according to the reported isolation procedure.[8] The petroleum ether insoluble portion (11 g) when dissolved in 10% aqueous AcOH and treated with $(NH_4)_2 SO_4$, furnished colorless precipitate of sulfates which was filtered. The sulfate mother liquor was made alkaline with 10 % aqueous NaOH and extracted out with EtOAc which on usual working afforded a colourless residue (9.5 g). It was subjected to flash column chromatography (Alumina, Merck 90; petroleum ether, petroleum ether-EtOAc, in order of increasing polarity). Nine fractions were ulitmately obtained on combining the eluates on the basis of tlc . A major fraction eluted with 6:4-5:5 petroleum ether-ethyl acetate which was subjected to flash column chromatography (Eyela, 9385, petroleum ether, petroleum ether-ethyl acetate, in order of increasing polarity). The petroleum ether-ethyl acetate (4.5:5.5) eluates furnished crude**1** (12 mg) , **3** (36 mg) and **2** (17 mg) in order of polarity which were purified through thick layer chromatography with solvent system chloroform-methanol in the ratio of 9.2:0.8, 9:1 and 8.3:1.7 respectively.

Table 1. $^{1}H/^{13}C$-nmr data ($CDCl_3$) for kurchamide (**2**).

C	δ C	H	δH	Multiplicity	J Value (Hz)
1	158.4	1	7.78	d	10.3
2	125.5	2	6.16	dd	10.3,2.0
3	186.7	-	-	-	-
4	124.9	4	6.10	t	2.0
5	167.3	-	-	-	
6	32.9	6a	2.06	m	-
		6b	2.40	m	-
7	30.7	7a	1.90	m	-
		7b	1.20	m	-
8	34.4	8	2.04	m	-
9	59.4	9	1.18	m	-
10	43.9	-	-	-	-
11	68.8	11β	3.97	ddd	10.0,10.0,4.8
12	46.9	12a	2.34	m	-
		12b	1.38	m	-
13	46.9	-	-	-	-
14	51.0	14	2.38	m	-
15	26.9	15	1.60	m	-
16	33.6	16a	2.30	m	-
		16b	1.18	m	-
17	53.9	17	2.40	m	-
18	55.4	18a	3.18	d	10.8
		18b	3.06	d	10.8
19	18.6	19	1.28	s	-
20	176.8	-	-	-	-
N-Me	29.8	*N*-Me	2.84	s	-

Table 2. $^{1}H/^{13}C$-nmr data ($CDCl_3$) for regholarehenine A (**3**).

C	δ C	H	δH	Multiplicity	J Value (Hz)
1	159.1	1	7.81	d	10.3
2	125.2	2	6.12	dd	10.3,2.0
3	186.7	-	-	-	-
4	124.6	4	6.06	t	2.0
5	168.2	-	-	-	-
6	33.0	6α	2.01	m	-
		6β	2.35	ddd	13.1,4.2,2.5
7	26.7	7a	1.70	m	-
		7b	1.48	m	-
8	36.2	8	1.52	m	-
9	59.5	9	1.18	m	-
10	44.1	-	-	-	-
11	68.9	11β	3.74	ddd	10.7,10.7, 4.4
12	48.6	12a	2.25	m	-
		12b	1.52	m	-
13	51.2	-	-	-	-
14	52.8	14	2.10	m	-
15	23.8	15a	1.98	m	-
		15b	1.60	m	-
16	33.7	16a	2.42	m	-
		16b	1.12	m	-
17	53.5	17	1.29	m	-
18	62.7	18a	3.29	d	11.3
		18b	2.39	m	-
19	18.6	19	1.28	s	-
20	64.7	20	2.92	m	-
21	13.3	21	1.29	d	6.3
N-Me	40.9	*N*-Me	2.54	s	-

Kurchilidine (**1**): Irregular plates from methanol. mp 118-120°C. Hrms m/z (rel. int.): 325.2379 M^+ (calcd for $C_{22}H_{31}NO$, 325.2405) (10), 310.2172 ($C_{21}H_{28}NO$) (40), 295.1947 ($C_{20}H_{25}NO$) (4), 137.1198 ($C_9H_{15}N$) (20), 121.0770 (C_8H_9O) (5), 107.0462 (C_7H_7O) (35), 81.0712 (C_6H_9) (6) and 71.0742 (C_4H_9N) (100); uv λ_{max} (MeOH) nm: 238; ir ν_{max} ($CHCl_3$) cm^{-1}: 3600-3150 (NH), 1660 (C=O), 1620 and 1600 (C=C); ^{1}H- nmr ($CHCl_3$) δ: 7.00 (1H, d, J=10.1 Hz, H-1), 6.22 (1H, dd, J=10.1, 1.9 Hz, H-2), 6.07 (1H, t, J=1.9 Hz, H-4), 1.17 (3H, s, H-19) and 0.85 (3H, t, J=7.0 Hz, H-22).

Kurchamide (**2**): Irregular plates from methanol. mp 84-86°C. Hrms m/z (rel. int.): 341.1995 M^+ (calcd for $C_{21}H_{27}NO_3$, 341.1990) (32), 220.1338 ($C_{13}H_{18}NO_2$) (100), 206.1194 ($C_{12}H_{16}NO_2$) (24), 181.1102 ($C_{10}H_{15}NO_2$) (4), 151.0999 ($C_9H_{13}NO$) (12), 137.0840 ($C_8H_{11}NO$) (30), 121.0707 (C_8H_9O) (72) 107.0472 (C_7H_7O) (32) and 71.0371 (C_3H_5NO) (42); uv λ_{max} (MeOH) nm: 238; ir ν_{max}($CHCl_3$)cm^{-1}: 3400-3200 (OH), 1710 (C=O amide), 1660 (C=O), 1620, 1600 (C=C) and 1150 (C-O); ^{1}H- and ^{13}C-nmr (Table 1).

Regholarrhenine A (**3**): Irregular plates (36mg) from methanol. mp 198-199°C.Hrms m/z (rel. int): 341.2365 M^+ (calcd for $C_{22}H_{31}NO_2$ 341.2354) (8), 326.2123 ($C_{21}H_{28}NO_2$) (45), 137.1210 ($C_9H_{15}N$) (22), 121.0705 (C_8H_9O) (6), 107.0774 (C_7H_7O) (30) and 71.0736 (C_4H_9N) (100); uv λ_{max}(MeOH) nm: 246; ir ν_{max}($CHCl_3$) cm^{-1}: 3600-3100 (OH), 1665 (C=O), 1620, 1600 (C=C) and 1150 (C-O); ^{1}H- and ^{13}C-nmr (Table 2).

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