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ISOLATION AND STRUCTURE OF HOLARRIFINE, A NEW ALKALOID FROM THE BARK OF *HOLARRHENA ANTIDYSENTERICA* LINN.

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A new alkaloid, provisionally named as holarrifine has been isolated from the fresh, undried bark of *Holarrhena antidysenterica* and its structure elucidated through chemical and structural studies.

Key words: Isolation, Holarrifine, New alkaloid, *Holarrhena antidysenterica.* Linn.

INTRODUCTION

Chemical studies on *Holarrhena antidysenterica* go back to 1858 when the alkaloid conessine was first isolated by Haines [1] from the bark of the plant reputed in folklore and traditional medicine as a cure for dysentery [2]. Since 1858, a large number of alkaloids have been isolated from the bark and seeds of the plant by different groups of workers [3-9]. The present paper deals with the isolation and sturcture elucidation of a new oxygenated base from the bark.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. IR spectrum was measured on Perkin Elmer Spectrophotometer. Proton NMR spectrum was recorded in deutrated chloroform on 60 MHz Varian instrument with TMS as internal reference. Mass spectrum was obtained on V.G. Micromass 12 at 70 electron volt. The purity of the sample was checked on TLC (silica gel).

18 kg fresh undried bark, was cut into 2-3 inches long pieces, and extracted four times with ethanol. The extracts were freed of the solvent in *vacuo* below 50° to a semi solid (A). The barks were then dried at room temperature, powdered to 30 mesh and macerated with 5% alcoholic sodium hydroxide and then repeatedly macerated with 7% alcoholic sodium hydroxide to liberate the alkaloids from their tannate complex. The combined percolates were neutralized with 50% acetic acid and freed of the solvent in *vacuo* to a dark brown syrupy mass (B).

The extracts (A) and (B) were combined and exhaustively extracted with petroleum ether to remove the fatty constituents. The residue was taken up in 10% acetic acid with the help of 15% hydrochloric acid and the acid solution was made alkaline with 10% sodium hydroxide to pH 7.5. The liberated weak bases were extracted with ethyl acetate, which was washed with water, dried over anhydrous sodium sulphate and freed of the solvent at reduced pressure to yield a reddish brown syrupy mass (C). Comparatively stronger bases were liberated form the aqueous layer by adding 20% alkali upto pH 12.0, extracted with ethyl acetate and worked up as above to get reddish brown residue (D).

Fraction (D) containing stronger bases was divided into petroleum ether soluble (E) and insoluble (F) fractions and conessine was separated from fraction (E) following the procedure of Siddiqui *et al.* [5]. The carbonate forming petroleum ether soluble fraction was added to the fraction (F), which were then taken in 10% acetic acid, saturated with ammonium sulphate and kept overnight for complete precipitation of the insoluble sulphates, which were filtered off. From the filtrate, containing soluble sulphates, bases were liberated, extracted with ethyl acetate and worked up in the usual manner to give a light brown powder which on recrystallization from methanol-benzene (4:1) formed white needles m.p. 218-219° (0.1% on the weight of total alkaloids). It has been provisionally named as holarrifine.

Characterization of holarrifine. Holarrifine R_f = 0.76 (MeOH-NH_3-H_2O; 15:2:3), is sparingly soluble in ethyl acetate, methanol and chloroform, readily soluble in the mixture of methanol-benzene and insoluble in ether and petroleum ether. It analysed for $C_{24}H_{38}N_2O_2$ found after drying over P_2O_5: C, 74.85; H, 9.39; N, 7.29; O, 8.47; calculated for $C_{24}H_{38}N_2O_2$; C, 74.61 H, 9.84; N, 7.25; O, 8.30%; and MW 386.

IR ν max; 3450 (NH), 2950 (C-H stretching), 1630, (C=C) 1670 (C=O) and 870 (substituted double bond) cm^{-1}. H-NMR δ: 7.73 (2H, m, 2xNH), 7.57 (1H, s, CHO), 5.42 (1H, m, H-5), 4.08 (1H, m, H-20), 2.83 (1H, m, H-3) 1.92 (3H, s, $COCH_3$), 1.40 (3H, d, J = 7.0 Hz, CH_3-20) and 1.07 (6H, s, CH_3-10, 13). MS m/z (rel. int. %): 327 (3), 325 M–$COCH_3$-H_2O, (3), 310 (50), 309 (100), 308 (10), 295 (15), 292 (10), 282 (10), 277 (7), 267 (12), 255 (2), 250 (4), 249 (6), 236 (6), 234 (3), 206 (5), 189 (68), 173 (4), 162 (12), 161 (6), 160 (3), 148 (15), 146 (9), 130 (8), 120

(11), 119 (10), 113 (7), 107 (27), 106 (3), 95 (13), 83 (20), 72 (10), 67 (25), 57 (10), 45 (26) and 44 (10).

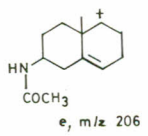
Acetylation - holarrifine monoacetate. To holarrifine (20 mg), dissolved in dry pyridine (1 ml), acetic anhydride (2 ml) was added and the reaction mixture kept over night at room temperature. On working up the reaction mixture, in the usual manner, a light yellow crystallizate was ultimately obtained, which was taken in ethyl acetate, concentrated and kept in cold, when clusters of short rods of the acetate came out mp 248-249°, R_f = 0.88 (MeOH-NH_3-H_2O; 15:2:3). It analysed for $C_{26}H_{40}N_2O_3$, (found after drying over P_2O_5 C, 72.18; H, 10.21; N, 6.24; O, 11.37%; calculated for $C_{26}H_{40}N_2O_3$; C, 72.89; H, 9.35; N, 6.54; 0, 11.22%; and MW 428.

RESULTS AND DISCUSSION

Holarrifine isolated from the more soluble sulphate forming basic fraction, as described in the experimental, formed white needles, m.p. 218-219° on recrystallization from methanol - benzene (4:1), and analysed for $C_{24}H_{38}N_2O_2$. The mass spectrum did not show the molecular ion peak, but a peak at m/z 325 possibly resulting from the loss of $-COCH_3$ and H_2O molecules. IR spectrum showed absorption bands at 3450 (NH), 2950 (CH stretching), 1630 (C=C), 1670 (C=O) and 870 cm^{-1} (trisubstituted double bond). It gave positive test for the secondary nitrogen, while the test for tertiary nitrogen was negative.

The proton NMR spectrum showed a three - proton singlet for the acetyl function at δ 1.92, a one - proton multiplet at δ 7.57 attributable to aldehydic proton and a two-proton multiplet at δ 7.73, exchangeable wtih D_2O, due to two –NH functions. Further, a six - proton singlet at δ 1.07 and a three - proton doublet at δ 1.40 (J=7.0Hz) have been assigned to two angular methyl groups at C-10 and C-13 and a methyl group at C-20 respectively. H-20 and H-3 are represented by two broad multiplets at δ 4.08 and δ 2.83 respectively, while a multiplet at δ 5.42 is due to the olefinic proton H-5.

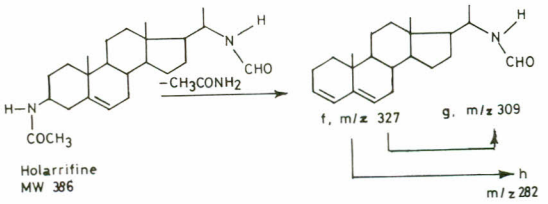
The data recorded so far suggested that the molecule contains four rings of the carbocyclic nucleus, an N-formyl and an N-acetyl function and one C=C which has been placed at C-5 on biogenetic considerations. The substitution of the N-formyl and N-acetyl functions at C-20 and C-3 respectively could be done through mass spectral data. The location of N-formyl function at C-20 is supported by the cleavage pattern, a, b [10,11], c [12,13] and d [14,15] resulting to yield fragments at m/z 44; m/z 72; m/z 113 and m/z 234 respectively as shown in the structure I. N-acetyl function at C-3 is corroborated by the fragment e, m/z 206, retaining $-NHCOCH_3$ group in the ring A [16].



e, m/z 206

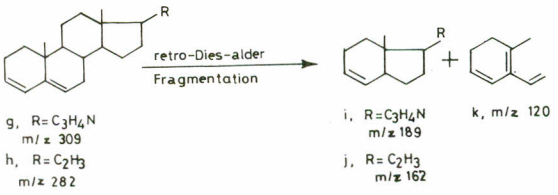
Further, the base peak at m/z 309 (fragment g) possibly resulting from the elimination of acetamide from C-3 followed by the loss of a water molecule (Scheme I) also supports the location of the acetamide function at C-3. Moreover 1,2-elimination of $HNCONH_2$ from the fragment f, (m/z 327) produced the fragment h, m/z 282.

Scheme 1

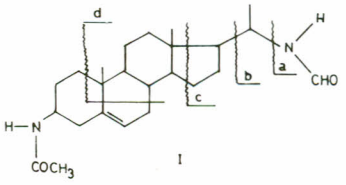


Major fragments g and h, undergo retro-Diels-Alder fragmentation [17] yielding i, m/z 189 and j, m/z 162 respectively along with the common fragment k, m/z 120 (Scheme 2). The fragment for its analogue are present in the mass spectra of Δ^5- steroids and are the most characteristic fragments for such an olefin [17]. Thus the position of the double bond at C-5 and the locations of $NHCOCH_3$ at C-3 holarrifine (I) are substantiated by these fragments.

Scheme 2



In the light of the above discussion, structure I has been assigned to holarrifine.



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