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ISOLATION AND STRUCTURE OF HOLARRICINE – A NEW ALKALOID FROM THE SEEDS OF *HOLARRHENA ANTIDYSENTERICA* LINN

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A new alkaloid provisionally named as holarricine has been isolated from the seeds of *Holarrhena antidysenterica* Linn. Its structure as 11-, 16- diketo – holarrhimine as indicated through chemical and spectral data has been further supported through its Clemmensen reduction to holarrhimine.

INTRODUCTION

Chemical studies on *Holarrhena antidysenterica* were undertaken as far back as 1858 when the alkaloid conessine was first isolated by Dr. Haines [1] from the bark of the plant reputed in folklore and traditional medicine as a cure for dysentery [2]. Its molecular formula was, after much controversy, established as $C_{24}H_{40}N_2$ [3]. As a result of isolation studies undertaken since 1858 on the bark and seeds of the plant a large number of alkaloidal bases have been isolated by different groups of workers [4–10]. Most of these studies were , however, based on the bark of the plant apparently because of the non-availability of the seeds of *Holarrhena antidysenterica* which are usually found adulterated with similar looking seeds of *Wrightia tinctoria*. Taking this into account the present work was undertaken on dependably unadulterated seeds of *Holarrhena antidysenterica* supplied through the courtesy of the Hamdard Laboratories and a new alkaloid has been isolated following the procedures described in the experimental and provisionally named as holarricine.

Holarricine m.p. 350 – 1°, $(\alpha)_D^{27}$ = – 31 (EtOH), was isolated from the petroleum ether insoluble non-sulphate forming oxygenous bases. It analysed for the molecular formula $C_{21}H_{32}N_2O_3$ which was confirmed from the mass spectrum which showed the molecular ion at *m/e* 360. In the IR spectrum a broad band was noted between 3380 – 3560 cm^{-1} (– O – H stretching) in addition to other important peaks at 3300, 3240 cm^{-1} (N–H stretchings), 3020, 2950 cm^{-1} (C=C–H and C–C–H stretchings), 1740 and 1710 cm^{-1}, (carbonyl absorptions), 1650 cm^{-1} (C=C absorption), 1620 cm^{-1} (N–H bending) and 1050 cm^{-1} (C–OH stretching). The peak at 1740 cm^{-1} indicated the presence of a carbonyl function in the five-membered ring. In the UV spectrum it showed maxima at 273 nm.

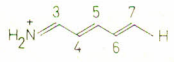
Holarricine formed tetramethyl derivative m.p. 192–3° (M^+, 416), on refluxing with formaldehyde and formic acid, which gave a monoacetyl derivative m.p. 265–6° (M^+, 458) on reaction with acetic anhydride and pyridine. These derivatives supported the presence of two primary amino groups and a hydroxyl function in the molecule which were indicated by the IR spectrum. The remaining oxygen atoms were attributed to the carbonyl functions and this was corroborated through the formation of its 2:4-dinitrophenyl hydrazone, m.p. 120–1°. On reaction with nitrous acid holarricine formed a trihydroxy product m.p. 265–7° (M^+, 362) both the amino groups being converted into hydroxyl functions. The resulting product showed absence of nitrogen atom, and gave positive sterolic tests.

Further, the presence of 1 C=C was supported, as the base absorbed one mole of bromine at 0°. It, however, gave negative Tortelli-Jaffe test.

The molecular formula indicated the presence of 7 double bond equivalence, one of which was accounted for by 1 C=C, 2 for two C=O groups and the remaining 4 for the four rings of the nucleus.

Taking into account the isolation of holarricine from the petroleum ether insoluble fraction of oxygenous bases and its physical and chemical data noted above, it was considered probable that holarricine is genetically related to the holarrhimine series of bases. This fact was further confirmed through the Clemmensen reduction of holar-

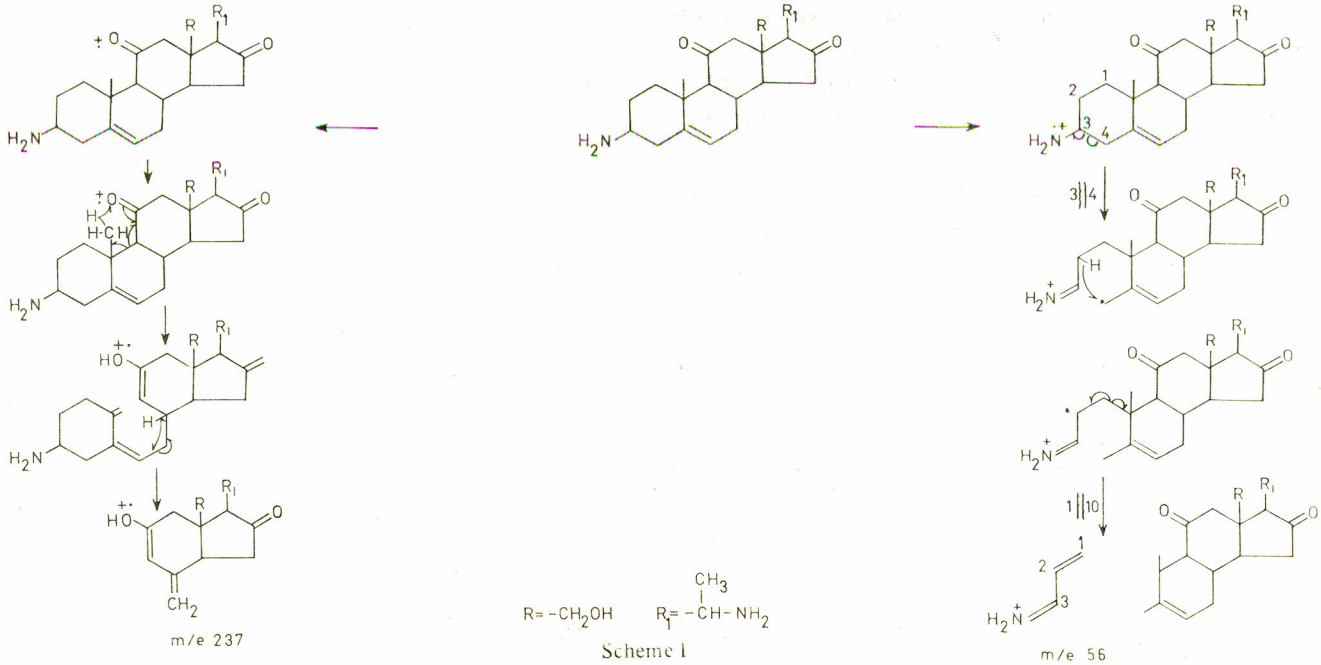
ricine, and the product obtained as a result of the reduction of the carbonyl functions was identified as holarrhimine (M^+, 332) through m.p. 182–3° mixed m.p. and parallel TLC with authentic sample of holarrhimine. Subsequently, the mass spectrum offered almost conclusive evidence to this effect. It gave peaks at *m/e* 360 (M^+), 345 = $(M-CH_3)^+$, 342 = $(M-H_2O)^+$, 316 = (M–side chain)$^+$, 315 = $M^+ - 45$ ($CH_3CH_2NH_2$), 329 = $(M-CH_2OH)^+$ and *m/e* 56 ($CH_2 = CH - CH_2$, $= NH_2$). Ion at *m/e* 56 established the position of one of the two amino groups at C_3 while the second amino group was placed at C_{20} in the side-chain from the fragments at *m/e* 316 and *m/e* 315 [11, 12]. The double bond was assigned the position C_5–C_6 due to the absence of the fragment,



since a conjugated triene can not be generated in such unsaturated amines. [11, 12].

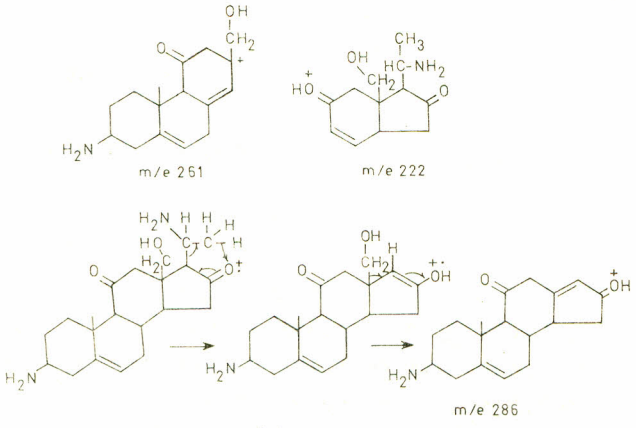
The fragment at *m/e* 329 was taken to indicate the presence of a primary hydroxyl group (–CH_2 OH) and considering the biogenetic relationship of holarricine with holarrhimine the – CH_2OH group was placed at C_{13} which was coroborated by the fragments at *m/e* 237, *m/e* 261 and *m/e* 286, as shown in Scheme I, and by the absence of 18-angular methyl protons in the NMR spectrum. Absence of a conjugated carbonyle function in the IR spectrum and the presence of the fragment at *m/e* 56 indicated that the carbonyl groups are not located in the rings A or B, while a peak at 1740 cm^{-1} in the IR spectrum indicated a carbonyl function in the five-membered ring and thus left C_{11} or C_{12} as the possible positon for the second carbonyl group. Fragments at *m/e* 237 and ***m/e*** 222 (resulting from ring B cleavage) were however, in favour of 11-Keto-structure in accord with the cleavages characteristic of 11-Keto steroids [13, 16]. Taking into account the IR Spectral data and the structural position of the rings A,B and C, as clarified above, the location of the second carbonyl had to be assigned either at C_{15} or C_{16}. Evidence in favour of 16-keto-structure was obtained from the fragments of *m/e* 286 and *m/e* 261, being in agreement with the characteristic fragmentations of the 16-keto steroids [13, 16]. Ion at *m/e* 286 results from the loss of side chain due to McLafferty rearrangement with subsequent loss of C–13 substituent whereas the ion at 261 a.m.u. results from the loss of C–15 to C–17 with an additonal hydrogen (normal ring D cleavage). The structural positon in respect of its various possible fragments is shown in Scheme 1 and 2.

Further support of the structure was provided by the proton–NMR spectrum. It showed a singlet at δ 1.08 (3H, 19-angular methyl protons), a doublet at δ 1.11 (3H, 21-methyl protons), a singlet at δ 3.55 (2H, –CH_2–O–) and a singlet at δ 1.85 (C_3–or C_{20} – NH_2). 12-methylene protons appeared as a relatively broad singlet at δ 2.38 (2H), while C_1 – protons and protons adjacent to 16-ketogroup gave an unresolved multiplet extending from δ 2.8 to δ 3.13 [17]. The C_6 – H appeared at δ 5.38 (1H) while the signals for another – NH_2 – and –OH protons are hidden.



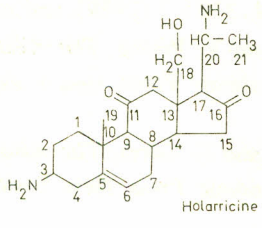
R= –CH_2OH R_1= –$\underset{\underset{1}{|}}{CH}$– NH_2 (CH_3)

Scheme I



Scheme II

In the light of spectral discussion and various chemical reactions, described above, the structure of holarricine may tentatively be represented as,



Holarricine

EXPERIMENTAL

Melting points were recorded in capillary tubes and are uncorrected. IR spectra were measured with SP 200 G spectrophotometer in $CHCl_3$. UV was measured on SP 800 spectrophotometer. Proton NMR spectrum was recorded in deutrated chloroform on JEOL PMX 60 instrument with TMS as internal reference and chemical shifts were measured in ppm (δ). Mass spectra were obtained on V.G. Micromass 12 at 70 electron volt. The purity of the samples was checked on TLC (silica gel).

Seeds of *Holarrhena antidysenterica* Linn (5 kg) were ground to 30-mesh powder, macerated with 10% methanolic sodium hydroxide and repeatedly percolated with methanol at room temperature.

The combined six extracts were neutralized with acetic acid and concentrated *in vocuo* below 50°. The syrupy concentrate was exhausted with petroleum ether to remove the fatty portion and the residue taken up in dilute acetic acid. The acidic solution was made alkaline with 20% ammonia and exhaustively extracted out with ethyl acetate.

The moist ethyl acetate layer was divided into carbonate and non-corbonate forming bases through treatment with vigorous stream of carbon dioxide. Both of these fractions were divided into petroleum ether soluble and insoluble portions. The combined petroleum ether soluble bases ultimately yielded 9 g of conessine directly and after methylation with formaldehyde and formic acid according to the reported isolation procedure [18].

The petroleum ether insoluble bases (mainly from the carbonate forming bases) were dissolved in acetic acid (10 % aqueous) and treated with ammonium sulphate. The resulting colourless crystallizate was identified as holarrhimine sulphate. The sulphate mother liquour was made alkaline with dilute sodium hydroxide and extracted out with ethyl acetate. The ethyl acetate layer was washed, dried, freed of the solvent and dissolved in methanol. The methanolic solution was purified through treatment with ether and petroleum ether, filtered and charcoaled The residue left on removal of the solvent from the filtrate finally yielded clusters of fine needles through repeated crystallizations from dilute methanol (0.85 % on the wt. of total alkaloids). It melted at 350–1° and has been provisionally named as holarricine.

Holarricine is insolube in petroleum ether, ether and benzene, sparingly soluble in ethyl acetate, soluble in chloroform and readily so in methanol and ethanol. It analysed for $C_{21}H_{32}N_2O_3$. (Found after drying over P_2O_5, C, 69.95; H, 9.02; N, 7.68; O, 13.41%; M^+, 360. Calcd. for $C_{21}H_{32}N_2O_3$: C, 70.00; H, 8.88; N, 7.77; O, 13.33 % : and MW, 360).

Bromination – Dibromo Holarricine. Holarricine (50 mg) dissolved in chloroform (1 ml) was titrated with bromine solution in chloroform (1.5 %. w/v) with ice cooling. The reaction mixture showed the presence of free bromine (tested with starch – potassium iodide paper) when 1.5 ml of bromine solution had been added) (0.75 ml ≅ 1 Br). It was freed of the solvent under reduced pressure. The microcrystalline residue thus obtained melted at 219 – 20° (decomp.).

Nitrous Acid Reaction – Holarricinol. A solution of holarricine (50 mg) in 10 % aqueous hydrochloric acid was treated with an aqueous solution of sodium nitrite under ice cooling. The light yellow crystalline powder which separated out immediately was filtered, washed and dried over porous plate. It showed single spot on TLC, melted at 265–7°, and gave molecular ion at *m/e* 362 in the mass spectrum. In the IR spectrum it showed absence of N – H bending or stretching vibration and gave negative test for nitrogen thus supporting the primary nature of both the amino groups. Furthermore it gave positive Liebermann – Buchard and Salkowski tests for sterols.

Methylation – Tetramethyl Holarricine. Holarricine was dissolved in 40 % aqueous formic acid and refluxed with formaldehyde (40 % aqueous solution) with occasional

testing on TLC. It showed nearly complete conversion after three hours when it was cooled, taken in acetic acid and basified with ammonia. The precipitated base was filtered and dried. It crystallised out from methanol in fine needles melting at 192–3° and showed M^+ at *m/e* 416 in the mass spectrum.

Acetylation – Tetramethyl Holarricine Acetate. To a solution of tetramethyl holarricine in acetic anhydride a few drops of pyridine were added and left overnight at room temperature. The reaction mixture was basified with ammonia and the resulting precipitate was filtered, washed well with water and dried over porous plate. It crystallised out from methanol as shining white needles and melted at 265–6°. It showed the molecular ion at *m/e* 458 in the mass spectrum and carbonyl absorption at 1725 cm^{-1} in the infra red spectrum.

Clemmensen Reduction of Holarricine – Holarrhimine. Amalgamated zinc prepared from zinc powder (600 mg) and mercuric chloride (45 mg) following the method of Martin [19] was covered with water (1.5 ml) and concentrated hydrochloric acid (2 ml) and holarricine (25 mg) was immediately added. The reaction mixture was stirred for 3 hr at 60–70° during which a further quantity of concentrated hydrochloric acid (2 ml) was added. It was left overnight at room temperature, filtered and washed with concentrated hydrochloric acid and water. The filtrate and the washings were combined, basified with dilute ammonia and extracted out with ethyl acetate. The ethyl acetate layer on usual working gave white residue which formed very fine neeles from ethyl acetate melting at 182–3°, and showed single spot on TLC. It showed no depression in melting point when mixed with authentic sample of holarrhimine and showed M^+ at *m/e* 332 in the mass spectrum (Holarrhimine MW 332). Furthermore, the IR spectrum showed absence of carbonyl absorption.

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