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ISOLATION AND STRUCTURE OF HOLACINE A NEW ALKALOID FROM THE BARK OF *HOLARRHENA NTIDYSENTERICA* LINN

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Two new alkaloids provisionally named as holacine and holacimine have been isolated from the bark of *Holarrhena antidysenterica*. The structure of holacine has been elucidated through spectral studies and chemical reactions.

INTRODUCTION

In an earlier communication [1] isolation and structure of a new alkaloid, holarricine, from the seeds of *Holarrhena antidysenterica* has been reported. As a result of extension of this study to the bark of the plant two new alkaloids provisionally named as holacine m.p. 270 – 1° and holacimine m.p. 335–6° have been obtained in yields of 0.8 % and 0.08 % respectively, on the weight of total alkaloids. These have been isolated from the petroleum ether insoluble fraction after its methylation and may therefore be methylated products of the naturally occurring bases.

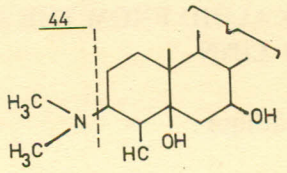
Holacine formed colourless slender needles from a mixture of ethyl acetate and methanol and melted at 270–1° . It showed $(\alpha)_D^{27} = -49$ (EtOH) and analysed for $C_{26}H_{44}N_2O_2$. The molecular formula of holacine was confirmed by mass spectrometery (M^+, 416).

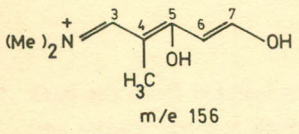
The IR spectrum showed -O-H stretching between 3470-3550 cm^{-1}, C=C at 1660 cm^{-1}, -C-O-Stretching of alcoholic group at 1065 cm^{-1} and in – plane vibration of -O-H- and C-N linkage of tertiary amine at 1350 cm^{-1} and 1110 cm^{-1}. As there is no strong peak between 1670–1800 cm^{-1} showing the absence of carbonyl group (including ketone, aldehyde, ester, lactone and carboxylic function) in the molecule, the two oxygen functions were taken to be present as two hydroxyl groups. On reaction with acetic anhydride and pyridine holacine yielded monoacetate mp. 136–7° (M^+458). The monoacetate showed absorption bands at 1730 cm^{-1} as well as at 3500–3580 cm^{-1} thus suggesting that the second hydroxyl is tertiary in character and was not amenable to acetylation.

The IR spectrum of holacine showed absence of N-H absorption and the two nitrogen fucntions were attributed to tertiary amino groups. Furthermore, the IR spectrum of the acetyl derivative showed a peak at 1730 cm^{-1} which can not be attributed to the absorption of a secondary or tertiary amido-carbonyl groups as they absorb in the range 1630–1680 cm^{-1}. The IR spectrum further showed C=C absorption at 1640 cm^{-1} but due apparent to the fact that holacine did not respond to bromine absorption at O° and absence of olefinic proton in ^{1}H-NMR the double bond appeared to be present between two tertiary carbon atoms.

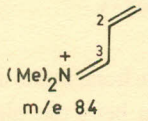
The mass spectrum of holacine showed peaks at *m/e* 416 (M^+), *m/e* 401 $(M\text{-}CH_3)^+$, *m/e* 398 $(M\text{-}H_20)^+$, *m/e* 383 $(M\text{-}H_2O\text{-}CH_3)^+$, *m/e* 380 $(M\text{-}H_2O\text{-}H_2O)^+$, *m/e* 372 $(M\text{-}44)^+$, *m/e* 357$(M\text{-}44\text{-}15)^+$, and *m/e* 336 $(M\text{-}44\text{-}H_2O\text{-}H_2O)^+$. Other prominent fragments were present at *m/e* 156, *m/e* 84 and *m/e* 44 which are in complete agreement with those of kurcholessine of Tschesche *et al.* [2], giving decisive evidence of the structural position of ring A and B. In the light of the data recorded above, and by analogy with Kurcholessine it was concluded that the dimethylamino group is located at C_3, a methyl group at C_4 and the two hydroxyls at C_5 and C_7 respectively. This was further supported bythe dehydration of the mono-acetyl holacine through its treatment with thionyl chloride at O° and subsequent alkaline hydrolysis and oxidation (Jones reagent) of the dehydro-base. The resulting product was characterized as Δ^5-7-one from the absorption bands at 1685 cm^{-1} in the IR and at 238 nm in the UV spectrum (calculated 239 nm for the Δ^5-7-one in absence of α substituent). This observation provided conclusive evidence in favour of the above suggestion. Any alternate location of these groups in Ring A or B would have markedly changed the absorption maxima in the UV spectrum.

A fragment at *m/e* 266, the absence of the well-known fragment at *m/e* 71 resulting from the N-ring cleavage [3,4] and the relatively downfield singlet of 21-methyl protons (δ 1.78) instead of a doublet in the NMR spectrum collec-



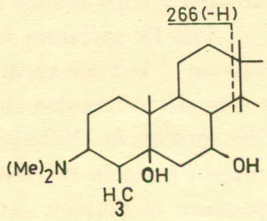


m/e 156

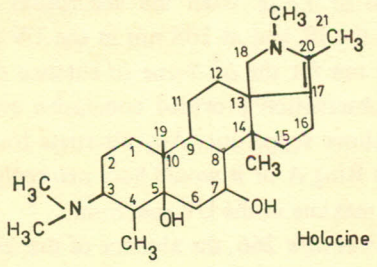


m/e 84

tively pointed to the location of ditertiary double bond between C_{17}–C_{20}.



With the clarification of the structural position of the rings A,B and C, the location of the remaining methyl group had to be at one of the four carbons namely C_{14},C_{15},C_{16} or C_{18}. The proton NMR spectrum however, supported its location at C_{14} as the methyl protons appeared as singlet at δ 0.95 instead of a doublet which would have resulted if it were at C_{15},C_{16} or C_{18}. Fragments at *m/e* 82, 96 and 110 possibly resulting from the cleavage of the N-ring and parts of ring D(C_{17},C_{16} and C_{15} respectively) lend added support to this view. Taking into account the chemical behaviour of the base and the spectral data recorded above the following structure was provisionally ascribed to holacine.



Holacine

Additional confirmation of this structure was obtained from proton NMR spectrum. It showed three singlets at δ 0.83 (3H), δ 0.93 (3H) and δ 1.78 (3H) derived from the protons of the methyl groups located at C_{10} (19-angular methyl), C_{14} and C_{20} respectively. C_4-methyl protons appeared as a doublet at δ 1.28 (3H, J = 7 c.p.s.) while protons of ring N-methyl and side-chain N-methyl groups gave singlets at δ 2.42 (3H) and δ 2.34 (6H) respectively. A broad multiplet at δ 3.55 (1H) was attributed to the proton geminal to the hydroxyl group.

EXPERIMENTAL

Melting points were recorded in capillary tubes and are uncorrected. IR spectrum were measured with SP 200 G spectrophotometer in chloroform. UV was measured on SI 800 spectrophotometer. Proton NMR spectrum was recorded in deutrated chloroform on JEOL PMX 60 instrument with TMS as internal reference. Mass spectra were obtained on. G. Micromass 12 at 70 electron volt.

5 kg 30 mesh powder of *Holarrhena antidysenterica* bark was macerated with 10 % methanolic sodium hydroxide and repeatedly percolated with methanol at room temperature. The combined percolates were neutralized with acetic acid and concantrated *in vacuo* below 50°. The syrupy concentrate was exhaustively extracted out 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 extracted out with ethyl acetate. A sizeable quantity of darkish insoluble material was collected at the interface of ethyl acetate and aqueous layers which was kept aside (Fraction X). The ethyl acetate layer was worked up in the usual manner and the total bases thereby obtained were divided into petroleum ether soluble and insoluble fractions. From the petroleum ether soluble fraction 20g of conessine (yield 0.4%) were obtained before and after methylation with formaldehyde and formic acid following the procedure of Siddiqui *et. al* [5]. The petroleum ether insoluble fraction when similarly methylated finally yielded 2 g tetramethyl holarrhimine (yield0.2%).

The darkish insoluble material (Fraction X referred to above) was digested with 10 % methanolic alkali to break the tannates and the bases from the tannate complex were extracted out with ethyl acetate.

The petroleum ether soluble portion of the residue left on removal of the solvent from the washed and dried ethyl acetate solution gave residual conessine on methylation with formaldehyde and formic acid.

The petroleum ether insoluble portion when similarly methylated gave crystalline base which on repeated crystallisations from a mixture of ethyl acetate and methanol formed colourless slender needles melting at 270–1° and showing $(\alpha)_D^{27}$ = – 49 (EtOH). It has been provisionally named as holacine.

The mother liquor of the holacine was concentrated and kept in cold for a few days when a crystalline mass separated out which on repeated crystallisations from methanol finally yielded prismatic rods melting at 355–6°. It analysed for $C_{22}H_{36}N_2O_3$ (Found after drying over P_2O_5, C, 70.15; H, 9.62; N, 7.37; O, 12.73 % and M^+ 376; calculated for $C_{22}H_{36}N_2O_3$, C, 70.2; H, 9.50; N, 7.4; O, 12.7 % and MW 376. It has been provisionally named as holacimine and awaits further structural studies.

Characterization of Holacine. Holacine is readily soluble in ethanol and methanol sparingly in ethyl acetate and insoluble in ether, benzene and petroleum ether. It analysed for $C_{26}H_{44}N_2O_2$ (Found after drying over P_2O_5: C, 75.13; H, 10.51; N, 6.76; 0, 7.72; and M^+, 416; calculated for $C_{26}H_{44}N_2O_2$; C 75.0, H, 10.57; N, 6.73; 0, 7.69; and MW 416).

Acetylation – Holacine Monoacetate. To a solution of holacine (200 mg) in dry pyridine (1 ml) acetic anhydride (2 ml) was added and the reaction mixture refluxed for two hr. On working up the refluxate in the usual manner the acetyl drivative was obtained, on crystallisation from methanol, as shining rectangular plates m.p. 136–7°. The IR spectrum of the acetate showed absorptions at 1730 cm^{-1} (ester group) and at 3500 –3580 cm^{-1} (-O-H stretching). The molecular ion was present at *m/e* 458 in the mass spectrum corresponding to the monoacetyl derivative.

Dehydration of Monoacetyl Holacine. Holacine monoacetate (150 mg) was dissolved in pyridine (1 ml) and stirred at 0° with thionyl chloride (1 ml) for about 30 min. The reaction mixture was poured over crushed ice, basified and extracted out with ethyl acetate. On working up the ethyl acetate layer in the usual manner the product was obtained, on crystallisation from moist ethyl acetate, as shining needles melting at 143–4°. It showed a band at 1730 cm^{-1} (acetyl group) in the IR spectrum while the O-H absorption was absent. Furthermore, the molecular ion was observed at *m/e* 440 in the mass spectrum corresponding to dehydroholacine acetate.

Hydrolysis of Dehydroholacine Acetate. Dehydroholacine acetate (70 mg) was refluxed with 5 % alcoholic potash (10 ml) on the water bath for 2 hr. The brownish concentrate left on removal of the major portion of the solvent was diluted with water and extracted out with ethyl acetate. The ethyl acetate layer on usual working gave a light yellow semi-crystalline product which formed white needles on crystallization from methanol melting at 280–1°. It showed absorption at 3550 cm^{-1} (-O-H stretching) in the IR spectrum.

Oxidation of Dehydroholacine. To a solutionof dehydro base (40 mg) in acetone (2 ml) Jones reagent [6] (0.5 ml) was added with constant stirring. The stirring was continued for about 1 hr at room temperature when the TLC showed completion of reaction. The reaction mixture was poured in ice cold water and exhaustively extracted out with ether. The residue left on removal of the solvent from washed and dried ethereal solution formed colourless plates on crystallization from methanol melting at 205–6°. The product was found to be a Δ^5-7-one derivative as it showed bands at 1685 cm^{-1} in the IR and at 238 nm in the UV spectrum.

Holacine was also characterized through the formation of the following salts:

Hydrochloride m.p. 295–6°
Picrate m.p. 240–1°
Chloroplatinate m.p. > 320°

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