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HOLACETINE, A NEW STEROID ALKALOID FROM *HOLARRHENA ANTIDYSENTERICA*

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Key Word Index—*Holarrhena antidysenterica*; Apocynaceae; conessine; holacetine.

Abstract—The occurrence of a new steroid alkaloid in the root-bark of *Holarrhena antidysenterica* is reported. It is shown to be 20S-acetamido-5α-pregnan-3β-ol from spectroscopic investigations, chemical reactions, correlation with compounds of known structure and stereochemistry and subsequently by the stereo-specific synthesis from 3β-hydroxybisnorcholenic acid.

INTRODUCTION

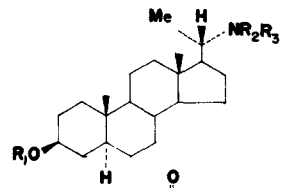
The seeds and leaves of the plant *Holarrhena antidysenterica* are reported to contain a number of steroid alkaloids, such as alkamines [1], pyrrolidine bases [1], amino-gluco steroids [2–4] and amino-gluco cardenolides [3, 4]. However the root-bark, though used extensively in folk medicine for curing dysentery, has not been analysed. We have now isolated a new alkamide, holacetine, in addition to conessine (yield: 0.04%) from the basic fraction of the ethanolic extract of the petrol-defatted root-bark of this plant. The structure, stereochemistry and the stereospecific synthesis of holacetine are described in the present communication.

RESULTS AND DISCUSSION

Holacetine (**1**), $C_{23}H_{39}NO_2$ (M^+ 361), mp 258°, $[\alpha]_D^{25}$ + 6.9° was separated from the congeners by the following procedure. The alkaloid-mixture was digested with petrol and the petrol-insoluble base acetylated and chromatographed over alumina to yield holacetine-*O*-acetate (**2**), $C_{25}H_{41}NO_3$ (M^+403), mp 244–45°. On mild acid hydrolysis it yielded holacetine found to be identical by TLC with the major alkaloid present in the petrol-insoluble alkaloids of the root-bark. A small amount of holacetine (0.002 g) was prepared by PLC and this was used as an authentic sample for comparison with holacetine purified by the procedure described above. This confirmed that holacetine was not an artefact but did occur in the plant cells.

Strong absorptions at 3280 cm^{-1} (broad) (–OH) and 1635 cm^{-1}(–NH–CO–Me) were discernible in the IR spectrum. This was consistent with IR spectral data of holacetine-*O*-acetate $[\nu]_{max}^{KBr}$: 3294 and 1635 cm^{-1} (–NH·CO·Me), 1722, 1237 and 1027 cm^{-1} (–O·CO·Me). The NMR spectrum of the acetate in conjunction with its MS suggested a 20-acetamido-pregnane skeleton [5] for the alkaloid. The resonance signals in the NMR spectrum appeared at δ0.73 (3H, *s*, C-18), 1.02 (3H, *s*, C-19), 1.15 (3H, *d*, J = 6.5 Hz, C-21), 1.93 (3H, *s*, –NH·CO·Me) and *ca* 5.40 (2H, *m*, one of the two protons disappeared on deuteration, C-20 and –NH·CO·Me). The sharp singlet at 2.02 (3H, –O·CO·Me) in the NMR spectrum of *O*-acetylholacetine was absent in the spectrum of **1**. The NMR spectrum of holacetine in d_6-DMSO showed a one-proton signal at 1.52 due to a hydroxyl group. In other respects the NMR spectra of **1** and of its *O*-acetate were practically the same, both lacking any chemical shifts for olefinic protons.

O-Acetylholacetine on acid hydrolysis yielded 20S-amino-5α-pregnan-3β-ol (**3**) [6], $C_{21}H_{37}NO$ (M^+ 319), mp 169–71°. This pregnane derivative was synthesised from (20S)-3β-hydroxy-5-pregnen-20-carboxylic acid (3β-hydroxybisnorcholenic acid) (**4**) by Curtius degradation followed by catalytic hydrogenation [7]. Product **3** was subsequently methylated by Eschweiler–Clarke's method yielding 3-deamino-3β-hydroxychonemorphine (**5**) [8], $C_{23}H_{41}NO$ (M^+ 347), mp 170–71°. The latter on oxidation furnished funtumafrine-C (**6**) [9], $C_{23}H_{39}NO$ (M^+ 345), mp 168–70°. Both 3-deamino-3β-hydroxychonemorphine and funtumafrine-C were found to be identical with the corresponding authentic samples by mmp, co-TLC and superimposable IR spectra. 20S-Amino-5α-pregnan-3β-ol on acetylation yielded holacetine-*O*-acetate. The latter on mild acid hydrolysis afforded holacetine (**1**) identical with the natural product.

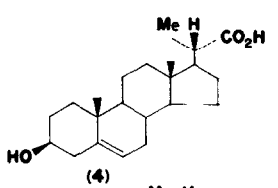


(1) R_1 = R_2 = H; R_3 = –C(=O)–Me

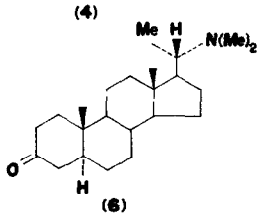
(2) R_1 = R_3 = –C(=O)–Me; R_2 = H

(3) R_1 = R_2 = R_3 = H

(5) R_1 = H, R_2 = R_3 = Me



(4)



(6)

The MS ion fragments of the natural product and its *O*-acetate were typical of 20S-acetamido-5α-pregnan-3β-ol, the diagnostic peaks appearing at *m/e* 361 (M^+), 346 (M − 15), 302 (M − NH = C(OH)Me), 86 (base peak) and 43 and at *m/e* 403 (M^+), 388 (M − 15), 343 (M − $MeCO_2H$), 86 (base peak) and 43 respectively. Based on all these results the structure and stereochemistry of holacetine are firmly established as shown in **1**.

EXPERIMENTAL

All mps were recorded in open capillaries in a Kofler block and are uncorr. UV spectra were recorded using 95% aldehyde-free EtOH, IR spectra were taken as a Nujol mull and in KBr disc. The NMR spectra were run with TMS as the internal standard. Alumina was used for column chromatography and Si gel G for TLC. The spots on the chromatoplate were detected with Dragendorff reagent. The analytical samples were routinely dried in *vacuo* at 80° over P_2O_5 for 24 hr unless otherwise stated.

Isolation of O-*acetylholacetine* (**2**) *and conessine.* The finely powdered root-bark of *Holarrhena antidysenterica* (Roxb.) Wall (1.8 kg) was exhaustively extracted (24 hr) with petrol (bp 60–80°) in a Soxhlet. The petrol-defatted marc was extracted with EtOH for 3 weeks at room temp. The alcoholic concentrate was mixed with 5% aq. citric acid soln (1 l.). The acid-soluble portion on basification with aq. NH_3 (pH 10) liberated the base. The $CHCl_3$ extract of the base was freed from the solvent, digested with petrol (3 × 100 ml) and the petrol-soluble portion afforded conessine, $C_{24}H_{40}N_2$ (M^+ 356), mp 122–24° when chromatographed over alumina. The petrol-insoluble crude base was acetylated with Ac_2O (20 ml) and C_5H_5N (10 ml) and allowed to stand overnight at room temp. The acetylated crude base was diluted with H_2O and the weakly basic fraction taken up in Et_2O (3 × 100 ml). The ethereal extract was washed with ammoniacal H_2O (3 × 30 ml), and H_2O and finally dried. The ethereal concentrate was chromatographed over alumina and the column was eluted with solvents of increasing polarity, using petrol, petrol–C_6H_6 mixtures, C_6H_6, C_6H_6–$CHCl_3$ mixtures of different proportions and $CHCl_3$. The C_6H_6 eluates furnished *O*-acetylholacetine (**2**), $C_{25}H_{41}NO_3$ (M^+ 403) which could be crystallized as white needles, mp 244–45°, from a mixture of petrol–C_6H_6 (3:1) (yield: 0.024%), R_f = 0.51 (developing system: EtOAc), $[\alpha]_D^{25}$ −53.6° ($CHCl_3$) (Found: C, 74.40; H, 10.15; N, 3.50; O, 11.95. $C_{25}H_{41}NO_3$ requires: C, 74.44; H, 10.17; N, 3.47; O, 11.92%); IR ν_{max}^{KBr} cm^{-1}: 3294, 1722, 1237, 1027, 1635; NMR ($CDCl_3$): δ 0.73 (3H, *s*, C-18), 1.02 (3H, *s*, C-19), 1.15 (3H, *d*, *J* = 6.5 Hz, C-21), 1.93 (3H, *s*, –NH·CO·CH₃), 2.02 (3H, *s*, –O·CO·Me), *ca* 4.60(1H, *m*,C-3), *ca* 5.40 (2H, *m*, one of them disappeared on deuteration, C-20 and –NH·CO·Me); MS *m/e* (rel. int.): 403 (M^+ 0.5), 388 (M − 15, 0.5), 343 (M–CH_3CO_2H, 71), 86 (100) and 43 (55).

Mild acid hydrolysis of O-*acetylholacetine* (**2**) *to holacetine* (**1**). *O*-Acetylholacetine (0.1 g) dissolved in MeOH (5 ml) was refluxed at 110–15° in the presence of 5N H_2SO_4 (8 ml) for 3 hr. MeOH was removed and the reaction mixture was basified with NaOH. The liberated base was taken up in Et_2O (3 × 50 ml). The ethereal extract was then washed with H_2O and dried. The solvent was removed and the solid residue (0.07 g) was crystallised from a mixture of C_6H_6–$CHCl_3$ (1:1) as white needles, $C_{23}H_{39}NO_2$ (M^+ 361), mp 258°, $[\alpha]_D^{25}$ 6.9° (EtOH), R_f = 0.37 (developing system: EtOAc) (Found: C, 76.40; H, 10.85; N, 3.90; O, 8.85, $C_{23}H_{39}NO_2$ requires: C, 76.45; H, 10.80; N, 3.88; O, 8.87%); IR ν_{max}^{KBr} cm^{-1}; 3280 (broad) and 1635; NMR (d_6-DMSO): δ 0.61 (3H, *s*, C-19), 0.72 (3H, *s*, C-18), 0.99 (3H, *d*, *J* = 6.5 Hz, C-21), 1.52 (1H, *br. s*, –OH), 1.71 (3H, *s*, –NH·CO·CH₃), 7.54 (1H, *m*, –NH–); MS *m/e* (rel. int.): 361 (M^+, 5) 346 (M − 15, 14), 302 (33), 86 (100) and 43 (82).

Isolation of holacetine from the petrol-insoluble crude base. The petrol-insoluble crude base (0.5 g) was subjected to PLC on Si gel G using multiple development with EtOAc. The band containing the major alkaloid was separated and eluted with EtOAc (200 ml). The solvent was removed and the solid residue was crystallised from a mixture of C_6H_6–$CHCl_3$ (1:1) as white needles (2 mg), mp 258°, R_f = 0.37 (developing system: EtOAc). The basic constituent thus obtained was found to be identical with holacetine (**1**) by the usual procedures.

Acid hydrolysis of O-*acetylholacetine* (**2**) *to* 20S-*amino-5α-pregnan-3β-ol* (**3**). *O*-Acetylholacetine (0.2 g) dissolved in MeOH (3 ml) was heated in an evacuated sealed tube at 115–20° in the presence of 10 N H_2SO_4 for 20 hr. The reaction mixture was basified with 2 N NaOH and the organic portion was extracted with Et_2O, washed and dried. Removal of the solvent afforded a gummy residue which on trituration with petrol (bp 60–80°) gave an amorphous solid. The latter crystallised from Me_2CO as colourless needles, $C_{21}H_{37}NO$ (M^+ 319), mp 169–71°, R_f = 0.64 (developing system: Me_2CO–diethylamine, 16:1), $[\alpha]_D^{25}$ +12° ($CHCl_3$) (Found: C, 79.10; H, 11.55; N, 4.45; O, 4.90. $C_{21}H_{37}NO$ requires: C, 79.00; H, 11.60; N, 4.39; O, 5.01%).

Synthesis of 20S-*amino-5α-pregnan-3β-ol* (**3**). 20S-Amino-5-pregnen-3β-ol (10 g) was prepared by the Curtius degradation of (20S)-3β-hydroxy-5-pregnen-20-carboxylic acid (3β-hydroxybisnorcholenic acid) following the procedure of Chatterjee and Das [10]. The compound was hydrogenated at a pressure slightly greater than one atmosphere in the presence of platinum dioxide (0.2 g) in glacial HOAc (20 ml). After filtering off the catalyst, the soln was diluted with H_2O, cooled in ice and basified with aq. NH_3. The liberated base was extracted with Et_2O, washed and dried. Removal of the solvent afforded a crystalline residue which crystallized from Me_2CO in colourless needles (0.5 g), $C_{21}H_{37}NO$ (M^+ 319), mp 169–70°, R_f = 0.64 (developing system: Me_2CO–diethylamine, 16:1), $[\alpha]_D^{25}$ +12° ($CHCl_3$) (Found: C, 79.05; H, 11.65; N, 4.35; O, 4.95. $C_{21}H_{37}NO$ requires: C, 79.00; H, 11.60; N, 4.39; O, 5.01%).

Conversion of N-*deacetylholacetine to* 3-*deamino-3β-hydroxychonemorphine* (**5**) [8]. N-Deacetylholacetine (0.1 g) was heated on a steam bath under reflux in the presence of 85% formic acid (2 ml) and 40% formaldehyde (1.0 ml) for 4 hr. The reaction mixture was diluted with H_2O and extracted with Et_2O to remove neutral material and then treated with 10 ml HCl (2 N). The aq. acid extract on basification with 5% NaOH yielded a base. It was dissolved in Et_2O from which 3-deamino-3β-hydroxychonemorphine (**5**) [8] was obtained. It crystallized from MeOH in white needles, $C_{23}H_{41}NO$ (M^+ 347), mp 170–71°, R_f = 0.55 (developing system: Me_2CO-diethylamine, 16:1) and $[\alpha]_D^{25}$ +23° ($CHCl_3$) (Found: C, 79.40; H, 12.00; N, 4.07; O, 4.53. $C_{23}H_{41}NO$ requires: C, 79.54; H, 11.81; N, 4.04; O, 4.61%).

Chromic acid oxidation of 3-*deamino-3β-hydroxychonemorphine* (**5**) *to funtumafrine-C* (**6**). To a soln of 3-deamino-3β-hydroxychonemorphine (0.04 g) in HOAc (5 ml), chromic acid (0.06 g) dissolved in a mixture of HOAc (2 ml) and H_2O (1 ml) was added dropwise. After 24 hr the excess chromium trioxide was destroyed by the addition of MeOH. The reaction mixture was diluted with H_2O and basified with aq. NH_3. The base liberated was extracted with Et_2O (3 × 40 ml), washed with H_2O and dried over anhydrous Na_2SO_4. Removal of the solvent furnished funtumafrine-C (**6**) [9] (0.03 g) crystallizing from Me_2O as colourless needles, $C_{23}H_{39}NO$ (M^+ 345), mp 168–70°, $[\alpha]_D^{25}$ +33° ($CHCl_3$) (Found: C, 79.90; 11.30; N, 4.10; O, 4.70; $C_{23}H_{39}NO$ requires: C, 80.00; H, 11.30; H, 4.06; O, 4.64%).

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