Phytochemistry, Vol. 24, No. 11, pp. 2778–2780, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

13a-HYDROXYTYLOPHORINE FROM *TYLOPHORA HIRSUTA**

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(*Received* 2 *January* 1985)

Key Word Index—*Tylophora hirsuta*; Asclepiadaceae; 13a-Hydroxytylophorine; structural determination; conversion to (+)-tylophorine.

Abstract—13a-Hydroxytylophorine isolated from *Tylophora hirsuta* on heating with concentrated hydrochloric acid gave a quaternized salt which was reduced with sodium borohydride to (+)-tylophorine. An amine and a ketoamine having a seco[10-13a] bond were formed after lithium aluminium hydride reduction and chromium trioxide oxidation, respectively.

INTRODUCTION

Recently, we have described the isolation of five new phenanthroindolizidine alkaloids from *Tylophora hirsuta* [2]. Herein, we report the isolation and characterization of 13a-hydroxytylophorine (**1**) which is the principal alkaloid from wild populations of the plant. This alkaloid was not isolated from the cultivated plant investigated by us earlier [2]. However, 13a-hydroxysepticine (**2**) on attempted acetylation was shown to be converted into alkaloid **1** [2].

RESULTS AND DISCUSSION

The total alkaloidal fraction isolated from the aerial parts of the plant collected from wild populations was fractionated as described in our previous work [2]. Instead of 13a-hydroxysepticine (**2**), 13a-hydroxytylophorine (**1**) was obtained from the ethyl acetate insoluble fraction on recrystallization from chloroform–methanol.

13a-Hydroxytylophorine (**1**), mp 274–275°, $[\alpha]_D^{25}$ +45° (*c* 0.5; $CHCl_3$), $[M]^+$ at *m/z* 409 ($C_{24}H_{27}NO_5$) had properties similar to the product obtained from attempted acetylation with acetic anhydride–pyridine of 13a-hydroxysepticine (**2**) reported in our previous publication [2].

13a-Hydroxytylophorine (**1**) on CrO_3 oxidation [3] gave a sticky product (**3**) with a similar $[M]^+$ at *m/z* 409 ($C_{24}H_{27}NO_5$) but with zero optical rotation. Its IR spectrum showed a strong absorption at *ca* 1730 (C=O) and 3400 (*br*, NH str.). The carbonyl of the ketoamine **3** was conveniently reduced with lithium aluminium hydride [4] to the amine **4**, mp 292–293° with no rotation and $[M]^+$ at *m/z* 395 ($C_{24}H_{29}NO_4$). In the ^{1}H NMR spectrum of **4**, the four aromatic protons appeared as *s* integrating for two protons each at δ7.80 and 7.30 were assigned to C-5, C-4 and C-8, C-1 protons, respectively. The four methoxyl groups appeared at δ4.60 (*s*, 6H) and 4.10 (*s*, 6H). Besides this, the methylene multiplets at δ3.30–3.80 centred at 3.55 (*br m*, 4H, C-11 and C-14 CH_2), 2.80–3.06 centred at 2.93 (*m*, 2H, C-13a CH_2), 2.40–2.60 centred at 2.50 (*m*, 2H, C-12 CH_2) and 1.76–2.20 centred at 1.98 (*m*, 2H, C-13 CH_2) and a D_2O exchangeable NH-proton at δ2.30 could only be accounted for in structure **4**. The amine **4** was also obtained from the direct lithium aluminium hydride reduction [4] of **1**.

13a-Hydroxytylophorine (**1**) on heating with conc. HCl afforded a quaternized salt **5** after recrystallization from ethanol. The quaternized salt **5**, mp > 360°, IR 3400 (*br*, $=\overset{+}{N}-$), 1615, 1600, 1460, 1420, 1370, 1240, 1200 and 700 cm^{-1}, had characteristic $[M]^+$ at *m/z* 428 ($C_{24}H_{26}O_4NCl$). The structure **5** was further substantiated by conversion to (+)-tylophorine **6** by $NaBH_4$ reduction. In general a double bond is not reduced with metal hydrides, although exceptions of reduction of carbon–carbon double bond have been noted [5]. However, sodium borohydride has been successfully utilized to reduce the carbon–nitrogen double bond in the quaternized salts during the total synthesis of phenanthroindolizidine alkaloids [6, 7]. The sodium borohydride reduction product (**6**) was identical to an authentic sample of (+)-tylophorine [8].

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR: δ values are given in ppm downfield from TMS. TLC (C_6H_6–EtOAc–Et_2NH, 6:3:1) spots were developed by Dragendroff's spray reagent.

Isolation and separation of 13a-*hydroxytylophorine* (**1**). Aerial parts of *T. hirsuta* (900 g) collected from wild populations during April 1984 were extracted and the EtOAc insoluble alkaloids separated as described in ref. [2]. These on recrystallization from MeOH–$CHCl_3$ gave **1** as a powder, IR ν_{max}^{KBr} cm^{-1}: 3215, 2950, 1610, 1500, 1455, 1410, 1225, 1195, 1180, 1130, 1020 and 995; ^{1}H NMR ($CDCl_3$): δ7.74 (*s*, 2H, H-5 and H-4), 7.24 (*s*, 2H, H-8 and H-1), 4.06 (*s*, 6H, 2 OMe), 4.00 (*s*, 6H, 2 OMe) and 2.02 (*s*, 1H, D_2O exchangeable).

*Part 7 in the series "Investigations of Medicinal Plants". For Part 6 see ref. [1].

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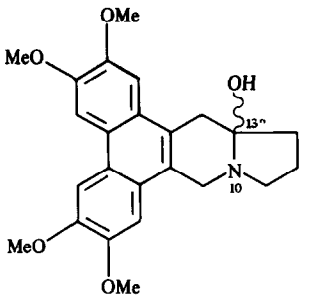

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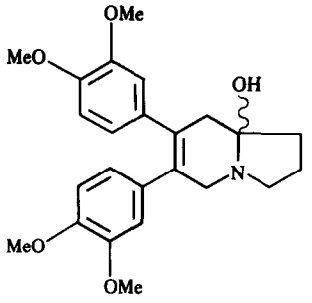

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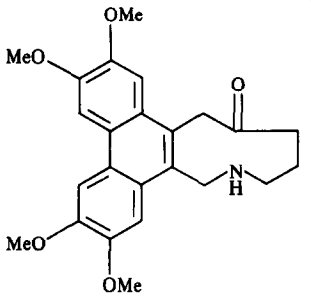

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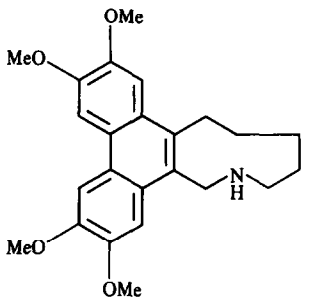

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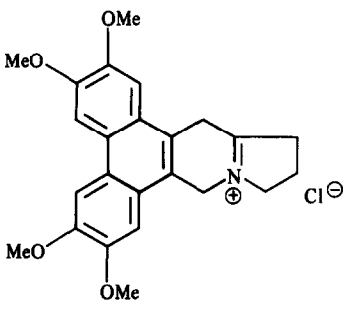

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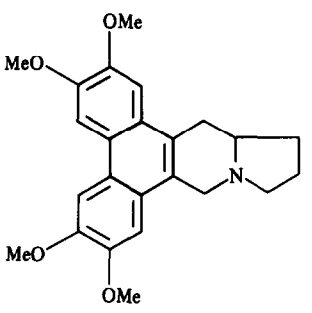

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Reduction of 13a-*hydroxytylophorine* (**1**). 13a-Hydroxytylophorine (**1**, 0.2 g) was dissolved in dry Et_2O (50 ml) and then $LiAlH_4$ (1 g) [4] added portionwise. The reaction mixture was kept at room temp. overnight. After removing solvent, EtOH was added and the soln filtered. The undissolved portion was washed × 3 with hot $CHCl_3$. The combined filtrate and washings were evapd to dryness. The residue was crystallized from $CHCl_3$–MeOH (1:1) to afford the amine **4** (0.19 g, 95% yield), mp 292–293°; IR ν_{max}^{KBr} cm^{-1}: 3300, 2970, 1610, 1500, 1455, 1410, 1230, 1195, 1180, 1135, 1000 and 825.

Oxidation of 13a-*hydroxytylophorine* (**1**). 13a-Hydroxytylophorine (**1**, 100 mg) was dissolved in Me_2CO and then 8 N chromic acid soln (5 ml) [3] was added. The reaction mixture was kept for 10 min at room temp, then diluted to 200 ml and extracted × 3 with $CHCl_3$, dried and distilled to yield **3** as a gummy product (50 mg): IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 2860, 1730, 1610, 1510, 1460, 1425, 1380, 1245, 1205, 1160, 1030, 870 and 740.

Reduction of ketoamine **3**. To the ketoamine **3** (40 mg) dissolved in dry Et_2O (25 ml), $LiAlH_4$ [4] was added and the mixture left at room temp. overnight. Solvent was evapd, EtOH added, filtration carried out and the undissolved mass washed with $CHCl_3$. The filtrate and washings were concd and on cooling gave the product comparable (mp, mmp, TLC, co-TLC) to the amine **4** (25 mg, 60% yield).

Quaternization of 13a-*hydroxytylophorine* (**1**). 13a-Hydroxytylophorine (**1**, 200 mg) was dissolved in conc. HCl (50 ml) and heated at 100° for 4 hr. After evapn to dryness *in vacuo* the salt obtained was recrystallized from EtOH to afford **5** (165 mg, 78% yield), mp > 360°.

Reduction of quaternized salt **5**. To the quaternized salt **5**

(100 mg) in MeOH (50 ml) was added $NaBH_4$ (2 g) [5–7] and the mixture left at room temp. overnight. After *in vacuo* evapn of solvent, the residue was dissolved in H_2O (100 ml) and extracted with $CHCl_3$. This on distillation and recrystallization from $CHCl_3$–MeOH gave (+)-tylophorine **6** (75 mg, 82% yield). The product **6** was comparable (mp, mmp, rotation, TLC, co-TLC) with an authentic sample of (+)-tylophorine [8].

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