Withanolides from *Hyoscyamus niger* Seeds

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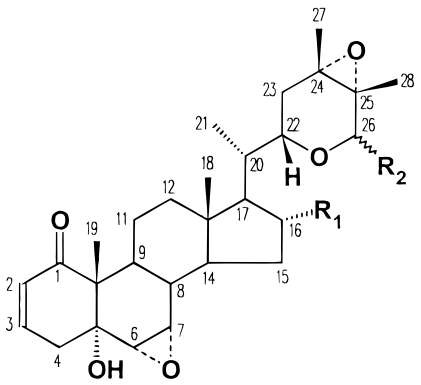
Three withanolide class steroids were isolated from the seeds of *Hyoscyamus niger*. Two of them were identified as daturalactone-4 (**1**) and Nic-3 (which is now named hyoscyamilactol) (**2**). The new compound was elucidated as 16α-acetoxyhyoscyamilactol (**3**) on the basis of spectroscopic properties and X-ray crystallographic analysis.

The genus *Hyoscyamus* (Solanaceae) is well-known for the production of anticholinergic tropane alkaloids such as hyoscyamine and scopolamine (hyoscine). In Chinese medicine, the seeds of *H. niger* (known as *Tian-Xian-Zi*, literally meaning Fairy Lady Seeds) are used as an antispasmodic, sedative and analgesic agent, prescribed for the treatment of stomach cramps, heavy coughs, neuralgia, and manic psychosis.[1] As part of a continuing project to study the chemical composition of toxic Chinese herbal drugs, we have examined the seeds of *H. niger*. Analytical methods have been established for the major alkaloids by using HPLC[2] and capillary electrophoresis (unpublished results).

This paper deals with the isolation and structural determination of three withanolide derivatives. An EtOH extract of the seeds of *H. niger* was concentrated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ fraction afforded three steroidal compounds (**1**−**3**) upon repeated chromatography on silica gel.



(1) R_1=H; R_2= =O
(2) R_1=H; R_2= α-OH, β-H
(3) R_1=OAc; R_2= α-OH, β-H

Daturalactone-4 (**1**) was obtained as colorless needles. The molecular formula $C_{28}H_{38}O_6$ was derived from the DCI-MS ($[M + 1]^+$ at *m*/*z* 471) and ^{13}C NMR data. Its spectral properties were identical in all respects (IR, NMR, MS) with those of daturalactone-4, a withanolide steroid previously isolated from *Datura quercifolia*.[3]

Compound **2** was obtained as colorless needles. The DCI-MS displayed a quasimolecular ion signal at *m*/*z* 473 $[M + 1]^+$ and a base peak at *m*/*z* 455 $[M - OH]^+$. The molecular formula $C_{28}H_{40}O_6$ was derived on the basis of the MS result and ^{13}C NMR data. The IR spectrum exhibited absorption bands of hydroxyl and α,β-unsaturated ketone functional groups, but no absorption was observed for the lactone carbonyl. The ^1H and ^{13}C NMR spectra clearly suggested that **2** was a lactol derivative of **1**. In the literature, a structure identical to that of **2** (coded as Nic-3[4]) was described from the leaves of *Nicandra physaloides*, possessing inhibitory effect on the feeding of insect larvae.[4] More recently the same compound was found in the whole plant of *Exodeconus maritimus*.[5] The X-ray structure of its 26-O-acetate derivative was reported.[4] We now propose a trivial name of hyoscyamilactol for **2**, and provide NMR assignments based on $^1H-^1H$ COSY, HETCOR, and COLOC results.

Compound **3** was obtained as colorless crystals. The DCI-MS contained a quasimolecular ion signal at *m*/*z* 531 $[M + 1]^+$, a base peak at *m*/*z* 513 $[M - OH]^+$, and a prominent fragment peak at *m*/*z* 471 $[M - OAc]^+$. The molecular formula $C_{30}H_{42}O_8$ was derived from the MS and ^{13}C NMR data. Examination of the NMR spectra led to the identification of a lactol ring (δ_C 91.8 and δ_H 4.97) and an acetoxyl group (δ_C 170.9, 21.2 and δ_H 2.08). Compound **3** was therefore an acetoxyl derivative of **2**, and attention was then focused on the location of the acetoxyl group. Examination of the HMBC spectrum revealed that both ^{13}C signals at δ 37.7 (C-20) and 49.1 (C-14) displayed long-range couplings with the methine group (δ_C 78.6, δ_H 5.00) bearing the acetoxyl substituent. The $^1H-^1H$ COSY spectrum also showed couplings between the signal at δ 5.00 and those at δ 1.41 (H-17) and 1.92 (H-15). On the basis of these findings, it was concluded that the acetoxyl substituent was located on C-16 of the steroid structure, and compound **3** was the 16-acetoxy derivative of **2**, i.e., 16-acetoxyhyoscyamilactol.

Finally, a suitable single crystal of **3** was chosen for X-ray crystallographic analysis to confirm the planar structure as well as its relative stereochemistry. The crystal structure is shown in Figure 1, and the bond lengths for **3** are given in Table 1. With the assumption that all other chiral centers conformed to the stereochemistry of daturalactone-4 (**1**), the lactol hydroxyl group in **2** and **3** was α-oriented and thus in a syn relationship with the epoxy ring. The 16-acetoxy functional group in **3** was determined to be in an α orientation; and **3** is therefore 16α-acetoxyhyoscyamilactol. Although withanolides commonly occur in Solanaceae plants[6] and 16-hydroxylated derivatives are

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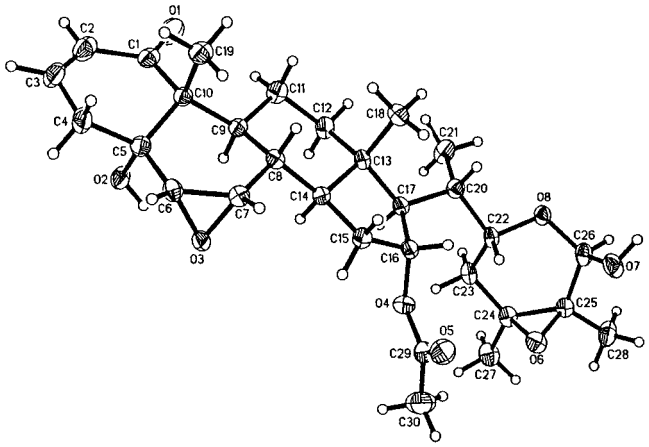

Figure 1. Crystal structure of **3**.

Table 1. Bond Lengths (Å) of **3**

O(1)−C(1)	1.214 (8)	O(2)−C(5)	1.444 (9)
O(3)−C(6)	1.436 (5)	O(3)−C(7)	1.448 (7)
O(4)−C(16)	1.443 (6)	O(4)−C(29)	1.339 (7)
O(5)−C(29)	1.212 (10)	O(6)−C(24)	1.453 (8)
O(6)−C(25)	1.443 (8)	O(7)−C(26)	1.407 (9)
O(8)−C(22)	1.456 (7)	O(8)−C(26)	1.419 (5)
C(1)−C(2)	1.486 (9)	C(1)−C(10)	1.522 (7)
C(2)−C(3)	1.315 (11)	C(3)−C(4)	1.478 (9)
C(4)−C(5)	1.517 (7)	C(5)−C(6)	1.517 (7)
C(5)−C(10)	1.550 (9)	C(6)−C(7)	1.460 (7)
C(7)−C(8)	1.498 (9)	C(8)−C(9)	1.542 (8)
C(8)−C(14)	1.525 (6)	C(9)−C(10)	1.560 (7)
C(9)−C(11)	1.534 (9)	C(10)−C(19)	1.543 (9)
C(11)−C(12)	1.545 (6)	C(12)−C(13)	1.530 (8)
C(13)−C(14)	1.545 (9)	C(13)−C(17)	1.548 (5)
C(13)−C(18)	1.547 (9)	C(14)−C(15)	1.520 (7)
C(15)−C(16)	1.534 (7)	C(16)−C(17)	1.536 (8)
C(17)−C(20)	1.554 (8)	C(20)−C(21)	1.536 (9)
C(20)−C(22)	1.539 (5)	C(22)−C(23)	1.510 (9)
C(23)−C(24)	1.504 (5)	C(24)−C(25)	1.462 (9)
C(24)−C(27)	1.498 (9)	C(25)−C(26)	1.513 (8)
C(25)−C(28)	1.506 (6)	C(29)−C(30)	1.469 (9)

known,[5] 16α-acetoxyhyoscyamilactol is hitherto unreported in the literature.

Experimental Section

General Experimental Procedures. The melting points were measured on a Leica Galen III apparatus and were uncorrected. Optical rotation was measured using a Perkin-Elmer 241 polarimeter. IR and UV spectra were recorded on a Perkin-Elmer 16PC FTIR spectrometer and a Milton Roy Spectronic 3000 array spectrophotometer, respectively. ^{1}H and ^{13}C NMR data were obtained from a JEOL JNM-EX-400 FT-NMR spectrometer at 400 and 98.5 MHz, respectively, with TMS as internal standard. DCI-MS (CH_4) were determined on a Finnigan TSQ7000 triple quadrupole mass spectrometer. The X-ray crystallographic data were obtained using a Siemens P4/RA diffractometer.

Plant Materials. The seeds of *H. niger* were obtained from an herbal drug supplier in Beijing in 1994 and authenticated at the National Institute for the Control of Pharmaceutical and Biological Products, Beijing. A voucher specimen has been deposited in the herbarium of the same institution.

Extraction and Isolation. Air-dried seeds of *H. niger* (5 kg) were ground and defatted with hexane before being extracted with 95% EtOH at room temperature. The EtOH extract was concentrated under reduced pressure and partitioned between hexane and H_2O. The aqueous portion was washed with $CHCl_3$, EtOAc, and *n*-BuOH successively. A residue (2.5 g) was obtained from the $CHCl_3$ fraction after evaporation of excessive solvent under reduced pressure. This residue was chromatographed over silica gel using mixtures of $CHCl_3$−acetone of increasing polarities. The effluents were combined into 10 fractions on the basis of TLC patterns. Fractions 3 and 6 were further purified by silica gel chromatography washed with $CHCl_3$−acetone mixtures to afford **1** (30 mg) and **2** (40 mg), respectively. Compound **3** (13 mg) was purified from fraction 8 by crystallization.

Daturalactone-4 (1): colorless needles, mp 288−290 °C (lit.[3] 282 °C); $[\alpha]_D$ 132.8° (*c* 0.013, MeOH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.2 (s, C-1), 170.0 (s, C-26), 139.6 (d, C-3), 129.0 (d, C-2), 76.3 (d, C-22), 73.2 (s, C-5), 62.2 (s, C-25), 59.3 (s, C-24), 57.2 (d, C-7), 56.3 (d, C-6), 51.9 (d, C-17), 51.3 (d, C-14), 51.0 (s, C-10), 43.5 (s, C-13), 39.8 (t, C-12), 38.7 (d, C-20), 36.7 (t, C-4), 35.7 (d, C-8), 35.5 (d, C-9), 28.7 (t, C-23), 27.2 (t, C-16), 23.5 (t, C-15), 21.8 (t, C-11), 17.9 (q, 27-CH_3), 14.6 (q, 19-CH_3), 13.6 (q, 28- CH_3), 12.9 (q, 21-CH_3), 12.0 (q, 18-CH_3); DCI-MS (CH_4) *m/z* 471 (100%) $[M + 1]^+$.

Hyoscyamilactol (2): colorless needles, mp 241−243 °C (lit.[4] 248 °C); $[\alpha]_D$ 98.4° (*c* 0.012, MeOH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.2 (s, C-1), 139.5 (d, C-3), 129.0 (d, C-2), 91.7 (d, C-26), 73.2 (s, C-5), 65.2 (d, C-22), 65.0 (s, C-25), 63.8 (s, C-24), 57.3 (d, C-7), 56.3 (d, C-6), 52.4 (d, C-17), 51.4 (d, C-14), 51.0 (s, C-10), 43.4 (s, C-13), 39.9 (t, C-12), 39.1 (d, C-20), 36.7 (t, C-4), 35.7 (d, C-8), 35.5 (d, C-9), 29.4 (t, C-23), 27.2 (t, C-16), 23.5 (t, C-15), 21.9 (t, C-11), 18.9 (q, 28-CH_3), 16.5 (q, 27-CH_3), 14.7 (q, 19-CH_3), 12.6 (q, 21-CH_3), 12.1 (q, 18-CH_3); DCI-MS (CH_4) *m/z* 473 $[M + 1]^+$, 455 (100%) $[M - OH]^+$.

16α-Acetoxyhyoscyamilactol (3): colorless plates, mp 262−264 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.60 (1H, ddd, J = 10, 5, 2 Hz, H-3), 5.85 (1H, dd, J = 10, 2.5 Hz, H-2), 5.00 (1H, m, H-16), 4.97 (1H, s, br s, H-26), 3.54 (1H, dt, J = 3.0, 11.5 Hz, H-22), 3.49 (1H, br s, D_2O exhangeable, 26-OH), 3.25 (1H, br d, J = 4 Hz, H-7), 3.14 (1H, s, D_2O exchangeable, 5-OH), 3.05 (1H, d, J = 4 Hz, H-6), 2.68 (1H, br d, J = 19 Hz, H-4a), 2.53 (1H, dd, J = 19, 5 Hz, H-4b), 2.08 (3H, s, CO*CH*$_3$), 1.41 (3H, s, 27-CH_3), 1.32 (3H, s, 28-CH_3), 1.17 (3H, s, 19-CH_3), 0.93 (3H, d, J = 7 Hz, 21-CH_3), 0.79 (3H, s, 18-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.1 (s, C-1), 170.9 (s, *CO*CH_3), 139.6 (d, C-3), 129.0 (d, C-2), 91.8 (d, C-26), 78.6 (d, C-16), 73.2 (s, C-5), 65.0 (d, C-22), 65.0 (s, C-25), 63.8 (s, C-24), 57.9 (d, C-17), 57.0 (d, C-7), 56.2 (d, C-6), 50.9 (s, C-10), 49.1 (d, C-14), 44.1 (s, C-13), 39.8 (t, C-12), 37.7 (d, C-20), 36.7 (t, C-4), 35.5 (d, C-9), 35.2 (d, C-8), 33.6 (t, C-15), 29.5 (t, C-23), 21.5 (t, C-11), 21.2 (q, CO*CH*$_3$), 18.9 (q, 28-CH_3), 16.5 (q, 27-CH_3), 14.7 (q, 19-CH_3), 13.4 (q, 18-CH_3), 12.8 (q, 21-CH_3); DCI-MS (CH_4) *m/z* 531 $[M + 1]^+$, 513 (100%) $[M - OH]^+$, 471 $[M - CH_3COO]^+$.

Crystal Data for 3:[7] $C_{30}H_{42}O_8$, M = 530.6, colorless plates, 2.0 × 0.4 × 0.05 mm, monoclinic, space group C_2, T = 298 K, a = 26.065(5) Å, b = 5.948(2) Å, c = 21.075(5) Å, β = 123.41(2)°, V = 2727.1(12) Å^3, Z = 4, D = 1.292 mg/m^3, F(100) = 1144, μ(Mo Ka) = 0.093 mm^{-1}, a = 0.710 37 Å.

Data Collection and Structure Refinement. Intensities of 3099 reflections were collected on a Siemens P4-RA diffractometer operating at 1 kW using the $\omega/2\theta$ scan mode up to 2θ = 53°. Of these, 2233 were unique and observed $F \geq 4\delta(F)$. The structure was solved by direct methods and refined (343 parameters) by full-matrix least-squares methods using the SHELXTL suite of X-ray programs.[8] All non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms were located in difference Fourier maps and then positioned using geometric constraints. A common isotropic thermal parameter was refined for all hydrogens. The final refinement converged with R = 5.79%, wR = 5.93%, and goodness-of-fit = 1.74. The largest difference peak and largest difference hole in the final difference Fourier map were 0.31 and −0.29 e Å^{-3}, respectively. The absolute configuration was not determined.

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References and Notes

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