Androstane Alkaloids from Musk of *Moschus moschiferus*

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Two androstane alkaloids were isolated from the musk of *Moschus moschiferus*. The structures were elucidated to be 3α-ureido-androst-4-en-17-one (1) and 3α-ureido-androst-4-en-17β-ol (2) by two-dimensional NMR analysis (HMQC, ^{1}H–^{1}H COSY, HMBC, and NOESY).

Key words *Moschus moschiferus*; musk; 3α-ureido-androst-4-en-17-one; 3α-ureido-androst-4-en-17β-ol

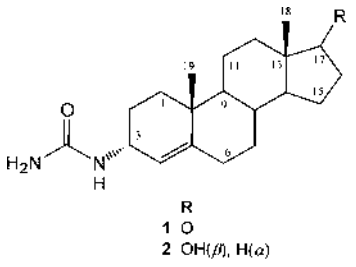
Musk, a dried preputial gland of the male musk deer (*Moschus* sp. Moschidae),[1] is one of the most popular and expensive Chinese drugs and has been used for cardiovascular stimulation, antiinflammation, and potentiation of β-adrenergic activity.[2—4] Because all of this crude drug has been imported from other countries, distinguishing it from imitation musks that usually contain synthetic muscones is important in Korea. In the course of investigations into the characteristic steroids of musk, two androstane alkaloids (**1**, **2**) were newly isolated from a natural source together with eight androstanoids and known cholestanoids, which had already been reported from the musk of *M. moschiferus*.[5,6] Compounds **1** and **2** were synthetically prepared by reaction of androst-4-ene-3,17-dione and testosterone with urea, although the their structural assignments were only given for protons.[7] We report here the identification of **1** and **2**, and assignment of their NMR signals based on two-dimensional NMR analysis (^{1}H–^{1}H-COSY, HMQC, HMBC, and NOESY).

Results and Discussion

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ +250° (c=0.02, MeOH). Its high-resolution fast atom bombardment mass spectrum (HR-FAB-MS) displayed the $[M(C_{20}H_{30}N_2O_2)+Na]^+$ ion peak at m/z 353.2200 (required, 353.2205), indicating seven degrees of unsaturation. The electron-impact mass spectrum (EI-MS, rel/int) showed a molecular ion at m/z 330 (100), and characteristic fragmentations at m/z 287 (23) $[M+H-(NH_2CO)]^+$ and 271 (34) $[M-(NH_2CONH)]^+$. The IR spectrum of **1** contained absorptions at 3480, 1752, 1685, and 1650 cm^{-1}. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, two singlet methyls at δ 0.75 (3H, s) and 0.84 (3H, s), and four methines at δ 4.63 (1H, br s), 5.47 (1H, d, J=4.8 Hz), 6.21 (2H, s), and 6.83 (1H, d, J=8.0 Hz) were detected. The ^{13}C-NMR spectrum indicated 20 carbons, and their chemical shifts and the distortionless enhancement by polarization transfer (DEPT) spectrum indicated the presence of two methyls, eight methylenes, a trisubstituted double bond (δ 120.9, 147.7), four methines, two quarternary carbons (δ 37.4, 47.7), and two carbonyl carbons (δ 159.3, 219.6). The former carbonyl chemical shift (δ 159.3) suggested the presence of an amide group. From these ^{13}C-NMR data, **1** was considered to be a C_{19}-ketosteroid having one substituent composed of one amino-, one imino-, and one carbonyl groups (*i.e.*, an ureido group).[8] This was further confirmed by two proton signals at δ 6.21 and 6.83, which were not correlated with any carbons in the H^1-detected multiple quantum coherence spectrum (HMQC). The entire structure was established by ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H-detected heteronuclear multiple-bond correlation spectrum (HMBC). In COSY, a multiplet methine proton at δ 4.63 showed strong cross peaks with the protons at δ 5.47 and 6.83. In HMBC, an NH proton at δ 6.83 showed strong correlations with carbons at δ 44.5, 120.9, and 159.3, and an olefinic methine proton at δ 5.47 showed strong correlations with carbons at δ 26.6 and 44.5 (Table 1). These results confirmed that a ureido group was present at the C-3 position of the androstane skeleton.[8,9]

Recently, two steroidal alkaloids with the same partial structure of C-2 to C-5 [–CH_2CH(NH)CH=C–] were reported. Salignarine E [(20*S*)-2β-hydroxy-4β-acetoxy-5α,6α-epoxy-20-(dimethylamino)-3β-(tigloylamino)-pregnane], a pregnane-type alkaloid from *Sarcococca saligna* with a 3β-imino configuration, but NOE interactions between NH and H-2α/β and between H-1α and H-3/NH were not described.[10] However, 22*E*,24*R*-3α-ureido-ergosta-4,6,8(14),22-tetraene from the fruit body of *Chlorophyllum molybdites* suggested a 3α-ureido configuration by NOE interaction between NH/H-1α. The stereochemistry of compound **1** at C-3 could be assigned to be the *R* configuration based on nuclear Overhouser effect spectroscopy (NOESY). The NOE interactions of H-1α at δ 1.26 were unambiguously detected with H-9 (δ 0.31) and NH (δ 6.83), whereas H-3 (δ 4.63) showed NOE interactions with H-4 (δ 5.47), H-2 (δ 1.75, 1.95) and NH (δ 6.83), which suggested that the ureido group is α-oriented (Fig. 1). These spectral studies confirmed that the structure of **1** as 3α-ureido-androst-4-en-17-one.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ +115° (c=0.002, MeOH). Its HR-FAB-MS displayed the $[M(C_{20}H_{32}N_2O_2)+H]^+$ ion peak at m/z 333.2540 (required, 333.2542), indicating six degrees of unsaturation. The EI-



R
1 O
2 OH(β), H(α)

Chart 1. Structures of Isolated Compounds

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Table 1. NMR Spectral Data for Compounds **1** and **2** in Pyridine-d_6

Position	**1**[a]		HMBC	**2**[b]	
	δ_C	δ_H		δ_C	δ_H
1	32.9	1.26 m, 1.36 m	3, 19	33.1	1.26 m, 1.42 m
2	26.6	1.75 m, 1.95 m	3, 4	26.7	1.74 m, 1.95 m
3	44.5	4.63 br s	1, 4, NH	44.6	4.65 br s
4	120.9	5.47 (d, 4.8)	6, NH	120.5	5.47 (d, 4.8)
5	147.7		6, 19	148.3	
6	32.3	1.88 m, 2.07 m	7	32.9	1.88 m, 2.11 m
7	31.9	0.62 m, 1.58 m	6	32.5	0.58 m, 1.60 m
8	35.2	1.34 m	7, 9, 14	36.0	1.32 m
9	54.2	0.31 m	8, 12, 19	54.5	0.33 m
10	37.4		1, 19	37.4	
11	20.9	1.12 m, 1.33 m	9, 12	21.4	1.22 m, 1.33 m
12	32.1	1.10 m, 1.79 m	11, 18	37.4	0.93 m, 1.95 m
13	47.7		8, 12, 14, 18	43.5	
14	51.3	0.92 m	8, 12, 15, 18	51.2	0.70 m
15	21.9	1.66 m, 1.69 m	14, 16	23.8	1.26 m, 1.52 m
16	35.9	1.98 m, 2.38 m	15	31.0	1.72 m, 1.97 m
17	219.6		15, 16, 18	81.4	3.84 (dd, 8.8, 8.4)
18	13.7	0.75 s	12, 14	11.8	0.95 s
19	18.3	0.84 s	1	18.4	0.89 s
NH		6.83 (d, 8.0)			6.81 (d, 7.6)
C=O	159.3		NH	159.3	
NH_2		6.21 s			6.19 br s

a) 400 MHz. *b*) 500 MHz.

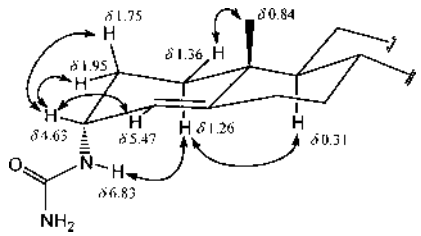

Fig. 1. Selected NOE Interaction of Compound **1**

MS showed a molecular ion at *m/z* 332. The IR spectrum of **2** showed absorptions at 3440, 3375, and 1660 cm^{-1}. The NMR data of **2** were very similar to those of **1**, and the only difference observed is the presence of a hydroxyl group instead of an oxo group at C-17. In the ^{1}H-NMR spectrum, a methine proton at δ 3.84 (1H, dd, J=8.8, 8.4) indicated the vicinal coupling of a methine in the C-17 tetrahedral structure.[11] The ^{13}C-NMR chemical shift (δ 11.8) assigned to C-18 indicated the β-orientation of the C_{17}-OH.[8] Hence the structure of **2** was assigned to be 3α-ureido-androst-4-en-17β-ol.

Of the known steroids in musk extracts, cholesterol and cholest-4-en-3-one were found in free form and as esters with C15—C18 fatty acids, where each of the hydrolysates was confirmed by GC-MS. Androstane steroids (androst-4-ene-3,17-dione, 5α-androstane-3,17-dione, 5β-androstane-3,17-dione, 3α-hydorxy-androst-5-en-17-one, androsterone, *epi*-androsterone, etiocholanolone, and 5β-androstane-3α,17α-diol were identified by TLC, GC-MS, and/or NMR,[8,11,12] and urea was also detected and identified by GC-MS. Among the androstane steroids in musk, compounds **1** and **2** are new characteristic components of *M. moschiferus*, and although the exact mechanism could not be determined, compounds **1** and **2** were deduced to have been produced by stereospecific reductive amination of androst-4-ene-3,17-dione and testosterone with urea.

Experimental

Melting points were measured using an Electrothermal 9100 and are uncorrected. IR was recorded with a Magna 550. FAB-MS, HR-FAB-MS, and EI-MS spectra were recorded using a JEOL JMS-HX 110A, JEOL JMS-HX/HX 110A, and JEOL JMS-HX 110A-Hewlett-Packard 5889A spectrometer, respectively. The ^{1}H- (400 MHz), ^{13}C-NMR (125 MHz), DEPT, HMQC, ^{1}H–^{1}H COSY, HMBC, and NOESY spectra were recorded on a Bruker AMX-400 NMR instrument and the chemical shifts are quoted with TMS as an internal standard. Column chromatography was carried out on Kieselgel 60 (silica gel, Merck, Germany) and on reverse-phase (RP-18) silica gel (YMC ODS-A, YMC Co., Japan). HPLC separation was carried out on a Macherey–Nagel column (ET 250/1″/20 Nucleosil) with an evaporative light-scattering detector (ELSD) (Alltech 500).

Material Musk (40 g) was obtained from the Korea Food and Drug Administration (KFDA). The voucher specimen is deposited in the KFDA.

Extraction and Isolation The content of dried gland (40 g) was extracted three times with MeOH (1 l) to afford a crude extract (11.6 g), which was suspended in water and partitioned with *n*-hexane and $CHCl_3$ to afford an *n*-hexane fraction (7.0 g) and $CHCl_3$ fraction (2.0 g). The $CHCl_3$ extract was chromatographed on a Si gel column and eluted with $CHCl_3$–MeOH of increasing polarity to yield 14 fractions. Both fractions 10 (50.6 mg) and 11 (16.2 mg) were subjected to reverse-phase (RP-18) Si gel column chromatography (65% aqueous MeOH), and preparative HPLC (ODS, 65% MeOH) afforded compounds **1** (13.4 mg) and **2** (0.7 mg).

Compound **1**: an amorphous powder, mp 210—212 °C (>215 °C decomp.), $[\alpha]_D^{25}$ +250° (c=0.02, MeOH). IR (KBr) cm^{-1}: 3480, 1752, 1685, and 1650. Positive HR-FAB-MS *m/z*: 353.2200 $[M(C_{20}H_{30}N_2O_2)+Na]^+$ (required, 353.2205). ^{1}H- and ^{13}C-NMR (C_6D_5N) data are shown in Table 1.

Compound **2**: an amorphous powder, mp. 220—223 °C, $[\alpha]_D^{25}$ +115° (c=0.002, MeOH). IR (KBr) cm^{-1}: 3440, 3375, and 1660. Positive HR-FAB-MS *m/z*: 333.2540 $[M(C_{20}H_{32}N_2O_2)+H]^+$ (required, 333.2542). ^{1}H- and ^{13}C-NMR (C_6D_5N) are shown in Table 1.

Acknowledgments This work was supported by a research grant from the Ministry of Health and Welfare (HMP-99-P-0020), Korea.

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