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Note

Studies on the two new stereo-saponins from *Morchella conica*

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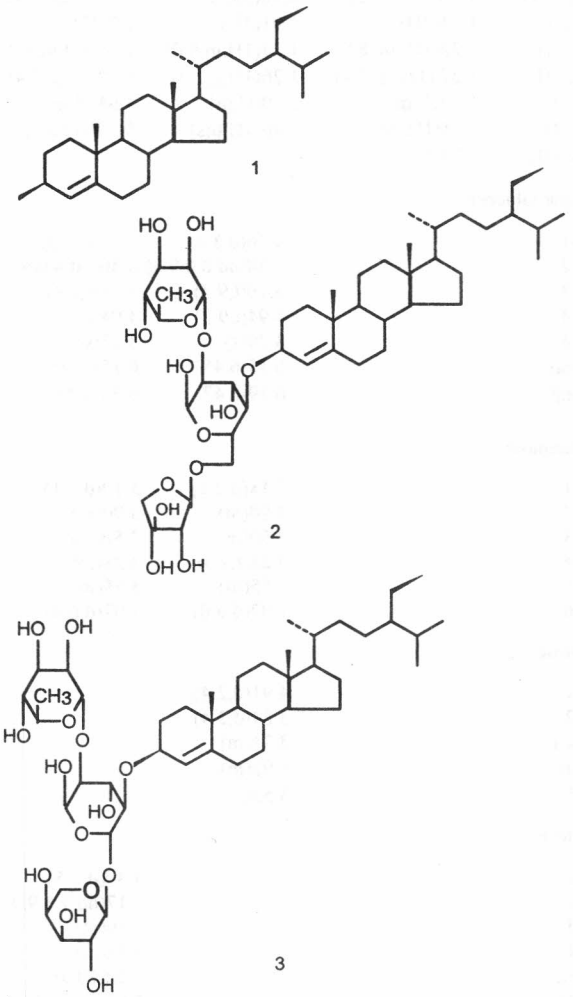
Two new stereo-saponins 3-O-β-[{2-L-rharnnopyranosyl (1→2)-β-D-apiofuranosyl (1→6)}-β-D-glucopyranosyl]rosaterol **2** and 3-O-β-[{2-L-rharnnopyranosyl (1→2)-β-D-xylopyranosyl}-β-D-glucopyranosyl]rosaterol **3** have been isolated from *Morchella conica*. Their structures have been established on the basis of spectroscopic analyses and chemical evidence.

The genus *Morchella* comprises about 12 species in China, of them, many have been used as traditional chinese folk drug for nourishing, anti-cancer and reinforcing immunity[1,2]. However, very little is known about its chemical constituents. We have examined the whole plant of *Morchella conica*, and now report for the first time the isolation and identification of two new rosaterol glycosides **2** and **3**.

Compound **2** was obtained as a white fragmentary crystal, m.p. 250-52°. The FAB-MS of **2** exhibited the quasimolecular ion peaks at m/z 861 [M+Li] and 877 [M+Na], confirming the molecular formula to be $C_{46}H_{78}O_{14}$. The usual colour test indicated **2** to be a steroid saponin.

Acid hydrolysis of **2** resulted in the formation of an aglycone **1**, and monosaccharides identified as L-rhamnose, D-apiose and D-glucose. Purified aglycone **1** showed in its ^{1}H NMR spectrum signals due to six methyl groups at δ 0.65 (3H, 18-H_3), 0.84 (6H, d, *J*=7.0, 26-H_3 & 27-H_3), 0.86 (3H, d, *J*=7.0, 21-H_3), 0.96 (3H, d, *J*=6.0, 29-H_3) and 1.01 (3H, 19-H_3), and four proton signals at δ 1.24 (1H, m, *J*=8.0, 25-H), 1.26 (1H, q, *J*=7.4, 20-H), 3.60 (1H, m, 3-H) and 5.40 (1H, brs, 4H), respectively. The IR spectrum (KBr) showed absorption for hydroxyl groups (3410, 1060 cm^{-1}), double bonds (1634, 950 cm^{-1}) and cyclopentane (1456 cm^{-1}). Based on the comparison of spectral data (Table I) and physical characteristic with those of β-rosaterol[3], aglycone was deduced β-rosaterol **1**.



The FAB-MS of saponins **2** showed signals at m/z 708 (M-146), 722 (M-132) and 413 (M-146-132-163), correspond to the subsequent loss of a rhamnosyl moiety and a apiosyl moiety, thereby indicating the α-L-rhamnose and β-D-apiose were terminal sugars and β-D-glucose was attached to the aglycone. From a detailed comparison of the ^{13}CNMR data of **2** with aglycone (Table II), we found downfield shift of +12.3 ppm for C-3 indicating glycosidic linkage between sugar moiety and hydroxyl at C-3 of aglycone. The sugar carbon signals (Table II) were easily assigned, glycosidation

Table I—^{1}HNMR data of compounds 1-3

Protons	1	2	3
C_{18}–CH_3	0.61(3H)	0.65(3H)	0.68(3H)
$C_{26,27}$–CH_3	0.81(6H,d,7.0)	0.84(6H,d,7.0)	0.82(6H,d,7.0)
C_{21}–CH_3	0.86(3H,d,7.0)	0.86(3H,d,7.0)	0.86(3H,d,7.0)
C_{29}–CH_3	0.93(3H,d,6.2)	0.90(3H,d,6.0)	0.92(3H,d,6.4)
C_{19}–CH_3	1.04(3H)	1.01(3H)	1.01(3H)
C_{25}–H	1.22(1H, m, 8.0)	1.24(1H,m,8.0)	1.24(1H,m,8.0)
C_{20}–H	1.27 (1H, q, 7.4)	1.26(1H,q,7.4)	1.25(1H,q,7.4)
C_3–H	3.5 (H, m)	3.60(H,m)	3.64(H,m)
C_4–H	5.31(1H, brs)	5.40(1H,brs)	5.30(1H,brs)
C_3–OH	2.74(1H)		
Sugar Glucose			
H-1		4.36(d,8.0)	4.36(d,8.0)
H-2		3.38(dd,8.0/9.3)	3.40(dd,8.0/9.3)
H-3		5.70(t,9.3)	5.75(t,9.3)
H-4		4.94(t,9.3)	4.94(t,9.3)
H-5		3.79(s)	3.75(s)
H-6α		6.15-6.45	6.15-6.50
H-6β		6.19-6.47	6.25-6.47
Rhamnose			
H-1		5.18(d,2.1)	5.19(d,2.1)
H-2		3.90(m)	3.90(m)
H-3		3.50(m)	3.50(m)
H-4		3.28(t,9.5)	3.28(t,9.5)
H-5		3.55(m)	3.55(m)
H-6		1.07(d,6.0)	1.07(d,6.0)
Apiose			
H-1		4.91(d,2.4)	
H-2		3.84(d,2.4)	
H-4α		3.74(m)	
H-4β		3.92(m)	
H-5		3.53(s)	
Xylose			
H-1			4.40(d,7.5)
H-2			3.17(dd,7.5,9.0)
H-3			3.30(m)
H-4			3.35(m)
H-5α			3.2(t,10.5)
H-5β			3.85(dd,10.5,3.5)

Table II—^{13}CNMR data of compounds 1-3

Carbon	1	DEPT	2	DEPT	3	DEPT
C-1	37.6	CH_2	37.3	CH_2	37.2	CH_2
2	31.6	CH_2	29.6	CH_2	29.0	CH_2
3	71.8	CH	84.1	CH	84.9	CH
4	121.7	CH_2	118.5	CH_2	119.0	CH_2
5	140.8	C	141.5	C	141.5	C
6	42.3	CH	41.8	CH	41.8	CH
7	31.9	CH_2	31.9	CH_2	31.9	CH_2
8	31.9	CH	32.3	CH	30.0	CH
9	50.6	CH	50.6	CH	51.0	CH
10	36.5	C	37.5	C	37.0	C
11	21.1	CH_2	20.8	CH_2	20.8	CH_2
12	28.3	CH_2	28.1	CH_2	28.3	CH_2
13	42.3	C	41.0	C	42.0	C
14	56.8	CH	57.1	CH	57.2	CH
15	24.3	CH_2	24.3	CH_2	24.0	CH_2
16	39.8	CH_2	39.0	CH_2	39.6	CH_2
17	56.1	CH	55.8	CH	55.9	CH
18	11.9	CH_3	12.3	CH_3	12.0	CH_3
19	19.8	CH_3	21.0	CH_3	21.0	CH_3
20	36.2	CH	36.0	CH	36.2	CH
21	18.8	CH_3	18.7	CH_3	18.8	CH_3
22	34.0	CH_2	33.5	CH_2	34.4	CH_2
23	26.1	CH_2	26.8	CH_2	26.0	CH_2
24	45.9	CH	46.0	CH	45.8	CH
25	29.2	CH	29.3	CH	29.0	CH
26	19.4	CH_3	19.4	CH_3	19.6	CH_3
27	19.1	CH_3	18.7	CH_3	18.8	CH_3
28	23.1	CH_2	24.0	CH_2	22.8	CH
29	12.0	CH_3	12.4	CH_3	12.8	CH_3
Glc						
1			95.2	CH	95.0	CH
2			78.5	CH	77.8	CH
3			74.8	CH	74.8	CH
4			76.3	CH	76.8	CH
5			74.3		74.8	CH
6			63.2	CH_2	63.2	CH_2
Rhamn						
1			102.8	CH	102.9	CH
2			73.0	CH	72.9	CH
3			71.3	CH	71.5	CH
4			74.0	CH	74.3	CH
5			70.2	CH	70.2	CH
6			18.5	CH_3	19.5	CH_3
APT						
1			108.9	CH		
2			77.1	CH		
3			79.8	C		
4			75.8	CH_2		
5			65.8	CH_2		
Xyl						
1					105.0	CH
2					74.9	CH
3					77.3	CH
4					70.5	CH
5					67.0	CH_2

shifts on C-2 (+3.7 ppm), C-6 (+1.7 ppm) and C-4 (+6.0 ppm) of glucose establishing the presence of a nodal rhamnopyranosyl residue glycosylated at C-2 of glucose, a nodal apiosepyranosyl residue glycosylated at C-6 of glucose, and C-4 connected with C-3 of aglycone[4,5]. On the basis of analytical results compound **2** was identified as 3-O-β-[{2-L-rharnnopyranosyl (1→2)}-β-D-apiofuranosyl (1→6)}-β-D-glucopyranosyl]-rosaterol **2**.

Compound **3** was obtained as a white amorphous powder, m.p. 240-42°. The FAB-MS of **3** showed the quasimolecular ion peaks at m/z 861 [M+Li] and 877 [M+Na], confirming the molecular formula to be $C_{46}H_{78}O_{14}$. A detailed comparison of

spectral data of **2** and **3**, showed signals for β-D-xylose and no signals for β-D-apiose, other signals being the same. So, **3** was identified as 3-O-β-[{2-L-rharnnopyranosyl (1→2)β-D-xylopyranosyl}-β-D-glucopyranosyl]-rosaterol **3**.

Experimental Section

Melting points were determined on kofler hot plate and are uncorrected. UV spectra were recorded on a Japan shimadzu UV-300 double beam spectrophotometer; IR spectra on a Perkin-Elmer 986 spectrometer (KBr disks); NMR spectra on a AM-400 BruKer spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C), with TMS as internal standard (chemical shifts in δ ppm); and mass spectra on MAT-44S and ZAB-HS mass spectrometer; silica gel (200-300 mesh, 100-200 mesh).

Collection of plant material. The whole plant of *M. conica*, was collected from Gansu province, P R China, in May 1993, a voucher specimen identified by Prof. Y S Lian and is preserved in the Herbarium of the Botany Department, Northwest Normal University, Lanzhou, 730070, P R China.

Isolation and extraction. Dried plant (3 kg) were pulverized and soaked in petroleum ether-ether-acetone (1:1:1, v/v/v) (three time). The residue obtained by concentration of extract solution under reduced pressure, was subjected to column chromatography over silica gel (100-140 mesh, 0.5 kg), and eluated with petroleum. ether-ethyl acetate and ethyl acetate-methanol in increasing polarity. Rechromatography of selected fractions and purification by preparative TLC yielded a crystalline compound **2** (24 mg), and compound **3** as an amorphous powder (18 mg).

Compound 2: White fragment crystals, m.p. 250-52°, IR(KBr): 3410, 2850, 2960, 1640, 1452, 1382, 1370, 1040 cm^{-1}; MS (m/z): 877, 861, 854, 722, 708, 576, 413, 396, 381, 314, 273, 255, 146, 132; ^{13}CNMR and DEPT data are listed in Table II and sugar proton signals are listed in Table I (Found: C, 64.61; H, 9.51. $C_{46}H_{78}O_{14}$ requires C, 64.46; H, 9.13%).

Compound 3: White amorphous powder, m.p. 240-42°, IR(KBr): 3410, 2854, 2950, 1648, 1450, 1382, 1370, 1040 cm^{-1}; MS (m/z): 877, 861, 854, 722, 708, 576, 413, 396, 381, 314, 273, 255, 146, 132; ^{1}HNMR, ^{13}CNMR and DEPT data are listed in Tables I, II respectively (Found: C, 64.52; H, 9.20. $C_{46}H_{78}O_{14}$ requires C, 64.46; H, 9.13%).

Aglycone 1: white needle crystals, m.p. 123-24°; IR(KBr): 3410, 2822, 2915, 1634, 1456, 1376, 1368, 950 cm^{-1}; MS (m/z): 414, 399, 381, 329, 314, 273, 255, 107; ^{13}CNMR and DEPT data were listed in Table II (Found: C, 84.07; H, 12.16. $C_{29}H_{49}O$ requires C, 84.05; H, 12.02%).

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