GLYCOSIDES FROM *NEONAUCLEA SESSILIFOLIA*

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Abstract Seven known compounds were isolated from the n-BuOH fraction of the stems of *Neonauclea sessilifolia* (Roxb.) Merr, and their structures were elucidated by means of spectroscopic method as quinovic acid-3-O-β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester(1), oleanolic acid-(28→1)-β-D-glucopyranosyl ester(2), ursolic acid-(28→1)-β-D-glucopyranosyl ester(3), quinovic acid-3-O-β-D-glucopyranosyl(1→3)-6-deoxy-β-glucopyranoside (4), oleanic acid-3-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside-28-O-β-D-glucopyranosyl ester (5), iridoid loganin(6), 7-methoxy-gentiopicroside(7). All of them were isolated from the genus for the first time.

Key words *Neonauclea sessilifolia*; glycoside; spectroscopic methods

无柄新乌檀中的配糖体

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摘 要 从无柄新乌檀乙醇浸膏的正丁醇部位分离得到7个已知配糖体化合物, 经波谱分析为: 喹诺酸-3-O-β-D-葡萄吡喃糖基(28→1)-β-D-葡萄吡喃糖酯(1), 齐墩果酸-(28→1)-β-D-葡萄吡喃糖酯(2), 熊果酸-(28→1)-β-D-葡萄吡喃糖酯(3), 喹诺酸-3-O-β-D-葡萄吡喃糖基-(1→3)-6-去氧-β-葡萄吡喃糖苷(4), 齐墩果酸-3-O-β-D-吡喃木糖基-(1→2)-β-D-葡萄吡喃糖基-28-O-β-D-葡萄吡喃糖酯(5), 番木鳖甙(6), 7-甲氧基-龙胆苦甙(7)。这些化合物均为首次从该属中分离得到。

关键词 无柄新乌檀; 波谱分析; 配糖体

Introduction

The plant of *N. sessilifolia* is a member of the family Rubiaceae, which distributed abundantly in Xishuangbanna of Yunnan province as timber[1-3]. There were no chemical constituents and biological activity reported for this genus previously. Ethanol extracts from *N. sessilifolia* showed activity on inhibition of P-388 mouse leukaemia and A-549 human lung cancer cell line. In the course of chemical investigation of the stems of *N. sessilifolia*, seven known compounds were isolated as (Fig. 1) quinovic acid-3-O-β-D-glucopyranosyl(28→1)-β-D-glucopyranosyl ester(1)[4], oleanolic acid (28→1)-β-D-glucopyranosyl ester (2)[5], ursolic acid-(28→1)-β-D-glucopyranosyl ester (3)[6], quinovic acid-3-O-β-D-glucopyranosyl (1→3)-6-deoxy-β-glucopyranoside (4)[7], oleanic acid-3-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside-28-O-β-D-glucopyranosyl ester (5)[8], iridoid loganin (6)[9], 7-methoxy-gentiopicroside (7)[9], their structures were elucidated by spectroscopic methods.

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Experimental

General

Melting points were determined using an XRC-1 micro-melting point apparatus, and uncorrected. Optical rotations were determined on a JASCO-20 polarimeter. The ^1H NMR and ^{13}C NMR spectra were obtained on BRUKER AM-400 spectrometer. ^1H- and ^{13}C-NMR chemical shifts were referenced to pyridin-d_5 at δ_H 8.71, 7.55, 7.19 and δ_C 149.9, 135.3, 123.4 respectively. Negative FAB were taken on a VG AUTO.SPCE-3000. Column chromatography was performed either on silica gel (200～300 mesh, Qingdao Marine Chemical Inc, China), silica gel H (60 u; Qingdao Marine Chemical Inc, China). Lichroprep RP_{18} gel (40～63 um, Merck, Darmstadt, Germany), sephadex-LH-20 (25～100 um). Spots of TLC were detected by spraying with 5% H_2SO_4 followed by heating.

Extraction and isolation

The stems of *N. sessilifolia* were collected in Xishuangbanna, Yunnan province, China, in May 2000. The plant was identified by Prof. PENG Hua. A voucher specimen (No.0355188) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, The Chinese Academy of Sciences.

Dried stems of *N. sessilifolia* (5.6 kg) were extracted (three times) with ethanol (95%). After evaporation of ethanol in vacuo, the concentrated extract was suspended in water and extracted successively with EtOAc and n-BuOH. n-BuOH fraction (20 g) was chromatographied on a silica gel column (800 g, 200～300 mesh) using CH_3Cl-MeOH-H_2O (9∶1∶0.1→7∶3∶0.5). Fractions were pooled based on TLC analysis (4 combined fractions). Fraction 1 was further separated on a silica gel H column with CH_3Cl-MeOH-H_2O (9∶1∶0.1) and chromatographied on sephadex-LH-20 (methanol) to get Compound 1(23 mg), 2(48 mg), 3(108 mg). Compound 6 (20 mg), 7 (21 mg) were isolated from fraction 2 using silica gel H and RP-18. Fraction 3 was repeatedly chromatographied over silica gel H with $CHCl_3$-MeOH-H_2O (8∶2∶0.2), and purified by RP-18 silica gel column using MeOH-H_2O (6∶4 to 7∶3) to yield compound 4 (40 mg), 5 (18 mg).

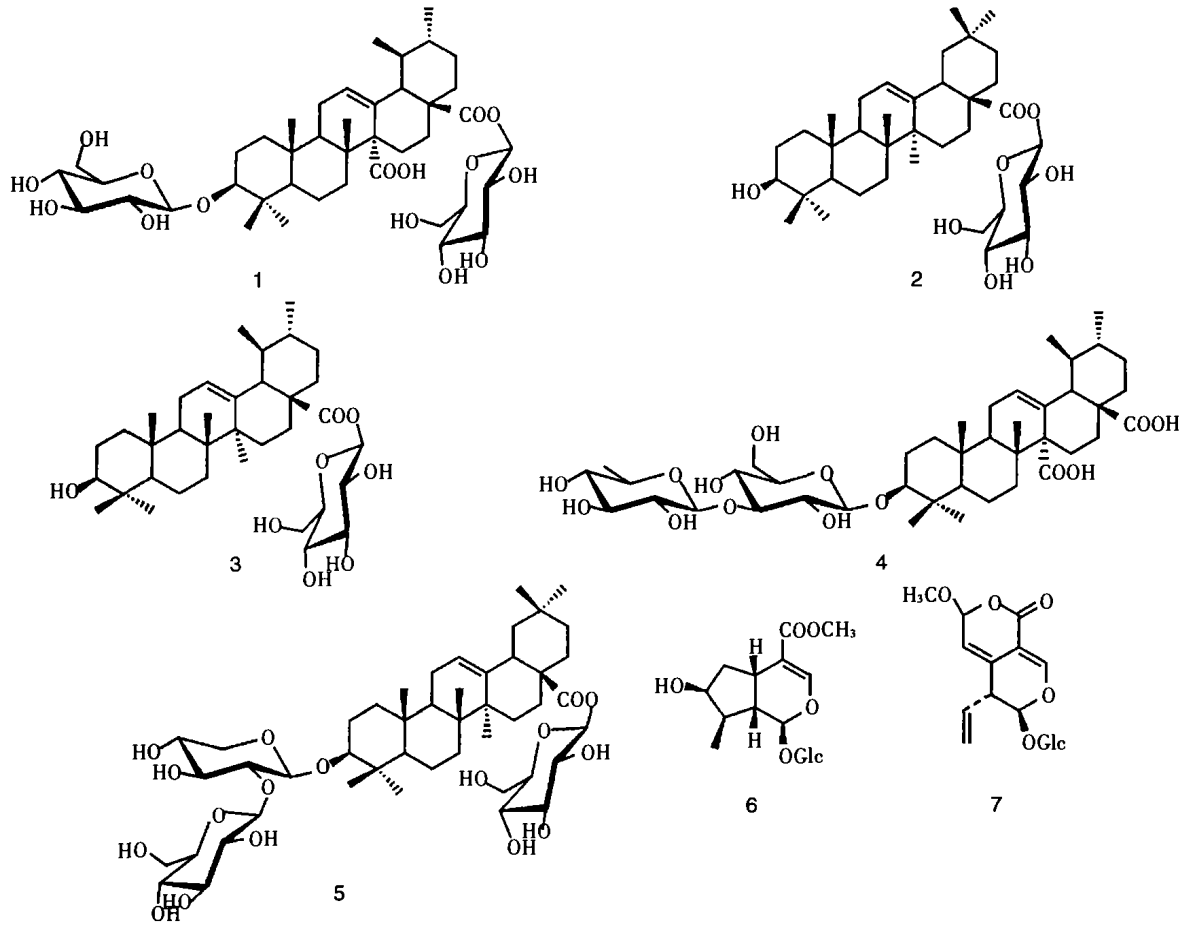

Fig. 1 The structure of 1～7 from *N. sessilifolia*

Results

Compound 1 $C_{42}H_{66}O_{15}$, white powder, mp. 298～300 ℃, ^1H NMR (400 MHz, d_5-pyridine) δ: 3.15 (1H, dd, J = 11.7, 4.3 Hz, H-3), 6.00 (1H, brs, H-12), 2.81 (1H, d, J = 11.4 Hz, H-18), 1.12 (3H, s, H-23), 0.94 (3H, s, H-24), 0.90 (3H, s, H-25), 1.09 (3H, s, H-26), 1.22

(3H,d,J=6.0 Hz,H-29),0.80(3H,d,J=6.3 Hz,H-30),6.35(1H,d,J=8.0 Hz,H-1″),4.05(1H,overlap,H-2″),4.21(1H,overlap,H-3″),4.09(1H,overlap,H-4″),3.89(1H,m,H-5″),4.51,4.38(2H,m,H-6″),4.55(1H,d,J=7.6 Hz,H-1′),4.28(1H,t,J=7.6 Hz,H-2′),4.08(1H,overlap,H-3′),4.05(1H,overlap,H-4′),3.78(1H,m,H-5′),^{13}C NMR (100 MHz) data see Table 1.

Compound 2 $C_{30}H_{47}O_3$, white powder, negative FABMS m/z(%):617(2),603(5),587(15),503(50),483(5),469(5),445(5)。^{1}H NMR(400 MHz, d_5-pyridine) δ:3.55(1H,dd,J=5.4,11.3 Hz,H-3),5.80(1H,brs,H-12),6.37(1H,d,J=8.1 Hz,H-1′),4.41(1H,t,J=8.0 Hz,H-4′),4.26(1H,t,J=8.0 Hz,H-3′,4.18(1H,t,J=8.0 Hz,H-2′),4.09(1H,m,H-5′),4.46,4.43(2H,dd,J=12.1,2.0 Hz,H-6′),^{13}C NMR (100 MHz) data see Table 1.

Compound 3 $C_{36}H_{56}O_{10}$, white crystal, mp. 247～250 ℃, $[\alpha]_D^{20}$ + 62(c 1.0,EtOH)。Negative FAB-MS m/z(%):647(5),587(20),471(556),456(12)。^{1}H NMR (400 MHz, d_5-pyridine) δ:3.20(1H,dd,J=11.0,4.3 Hz,H-3),6.00(1H,brs,H-12),2.63(1H,d,J=11.2 Hz,H-18),0.90(3H,s,H-23),1.12(3H,s,H-24),0.88(3H,s,H-25),1.20(3H,s,H-26),1.16(3H,d,J=6.0 Hz,H-29),0.80(3H,d,J=6.0 Hz,H-30),^{13}C NMR (100 MHz) data see Table 1.

Compound 4 $C_{42}H_{66}O_{14}$, white powder, $[\alpha]_D^{23}$ + 36(c 1.0,MeOH)。Negative FAB-MS m/z(%):794(5),723(26),631(46),587(100),469(6).^{13}C NMR (100 MHz) data see Table 1.

Compound 5 $C_{47}H_{66}O_{18}$, white powder. Negative FAB-MS m/z(%):956(26),794(100),750(15),670(5),646(15),587(56),483(17),423(5)。^{1}H NMR(400 MHz, d_5-pyridine) δ:3.85(1H,dd,J=11.2,4.01 Hz,H-3),5.30(1H,brs,H-12),0.87(3H,s,H-23),0.82(3H,s,H-24),0.94(3H,s,H-25),0.76(3H,s,H-26),1.15(3H,s,H-27),0.91(3H,s,H-29),0.93(3 H,s,H-30),6.36(1H,d,J=8.0 Hz,H-1‴),5.32(1H,d,J=7.7 Hz,H-1″),4.97(1H,d,J=7.6 Hz,H-1′). The data of ^{1}H NMR were same to the reference.

Compound 6 $C_{17}H_{26}O_{10}$, white crystal, mp. 160～162 ℃, $[\alpha]_D^{25}$ + 85(c 0.1,MeOH). Negative FAB-MS m/z(%):389(14),227(100),159(6),127(36)。^{1}H NMR (400 MHz, d_5-pyridine) δ:5.69(1H,d,J=4.4,H-1),7.68(1H,s,H-3),3.50(1H,d,J=7.2 Hz,H-5),2.63,1.72(2H,dd,J=13.8,7.8 Hz,H-6),4.24(1H,m,H-7),1.18(3H,d,J=6.8 Hz,H-10),2.45(1H,ddd,J=4.5,8.9 Hz,H-9),3.56(3 H,s,OCH_3),5.39(1H,d,J=7.8 Hz,H-1′),4.08(1H,t,J=8.0 Hz,H-2′),4.28(1H,t,J=8.1 Hz,H-3′),4.02(2H,overlap,H-4′,H-5′),4.39,4.36(2H,m,H-6′),^{13}C NMR (100 MHz) data see Table 2.

Table 1 The ^{13}C NMR data of compounds 1～4 (100 MHz, in d_5-pyridine)

C	1		2		3		4	
1	39.4	glc	38.6	glc	32.8	glc	39.4	glc
2	26.5	1′ 106.7	28.8	1′ 95.8	23.6	1′ 105.7	26.9	1′ 106.8
3	88.2	2′ 75.9	79.8	2′ 74.1	80.7	2′ 75.1	88.3	2′ 76.0
4	39.6	3′ 78.5	41.1	3′ 78.9	37.6	3′ 78.4	39.5	3′ 85.7
5	55.8	4′ 76.9	56.0	4′ 71.1	55.3	4′ 71.5	39.5	4′ 76.9
6	18.4	5′ 72.1	18.9	5′ 79.4	18.4	5′ 78.6	18.4	5′ 72.3
7	37.5	6′ 62.7	31.8	6′ 62.1	33.0	6′ 62.2	37.8	6′ 62.7
8	40.3	glc	39.1		39.6		40.3	6-deoxy-glc
9	47.3	1″ 95.8	46.4		47.5		47.2	1″ 104.8
10	38.6	2″ 75.3	35.5		37.0		38.6	2″ 75.4
11	23.5	3″ 78.7	45.9		23.3		23.5	3″ 78.8
12	129.3	4″ 71.5	123.0		125.5		129.0	4″ 72.3
13	133.4	5″ 78.9	144.1		138.0		134.2	5″ 78.9
14	56.9	6″ 62.4	41.7		42.0		56.9	6″ 62.3
15	26.4		27.9		28.2		26.4	
16	25.5		24.3		24.0		25.5	
17	48.9		47.9		48.5		48.8	
18	55.1		42.3		52.8		55.1	
19	39.2		45.9		39.0		39.2	
20	37.5		31.4		38.7		37.5	
21	30.5		33.0		30.6		30.5	
22	36.8		31.8		36.8		37.2	
23	28.4		28.1		28.0		28.0	
24	17.8		15.6		15.4		17.8	
25	16.5		13.6		15.7		16.7	
26	18.9		16.7		16.9		18.9	
27	178.1		24.6		23.4		178.2	
28	177.1		177.3		175.8		176.8	
29	19.4		23.6		16.8		19.2	
30	21.3		33.1		21.2		21.4	

Compound 7 $C_{17}H_{22}O_9$, white crystal. Negative FAB-MS m/z(%):385(2),255(7),169(16),127(100)。^{1}H NMR(400 MHz, d_5-pyridine) δ: 5.82(1H, d, J = 4.1 Hz,H-1),7.48(1H,s,H-3),7.27(1H,d,J = 8.3 Hz,H-6),5.86(1H,d,J = 8.3 Hz,H-7),5.35(1H,m,H-10), 5.28(1H,d,J = 7.6 Hz,H-1′),^{13}C NMR (100 MHz) data see Table 2.

Table 2 The ^{13}C NMR data of compounds 6,7 (100 **MHz, in** d_5**-pyridine**)

C	6		7	
1	97.5	glc	97.8	glc
2		1′ 100.9		1′ 100.4
3	151.4	2′ 74.8	153.1	2′ 74.8
4	113.3	3′ 78.5	107.0	3′ 78.4
5	31.8	4′ 71.5	128.2	4′ 71.5
6	43.0	5′ 79.0	118.3	5′ 79.2
7	73.5	6′ 62.7	111.2	6′ 62.7
8	41.7		135.1	
9	45.9		44.8	
10	13.7		119.3	
OCH_3	51.0		51.2	
COO	167.8		167.9	

References

1 Hou KZ. A Dictionary of the Families and Genera of Chinese Seed Plants. Beijing: Science Press, 1982. 328

2 Institute Botanicum Kunmingenese Academiae Sinicae. *Index flora Yunnanensis*. Kunming: The People's Publishing House, 1984. 1247

3 Yunnan Institute of Tropical Botany, Academia Sinica. *List of plants in Xishuangbanna*. Kunming: National Publishing House, 1984. 299

4 Adriana NDG, Cear AN, Jesus GD, *et al*. Sesquiterpen lactones a labdane and other constituents of *Urdepis heatantha* and *Chromolsea arnoltiana*. *Phytochemistry*, 2000,39(4):795-800

5 Cardona L, Garcia B, Peoro JR, *et al*. 6-prenyloxy-7-methoxycoumarin, A coumarin-hemiterpene ether from *carduus tenuiflorus*. *Phytochemistry*, 1992,31(11):3989-3991

6 Tane P, Ayafor JF, Luc BS, *et al*. Chromone glycoside from *schumanniophyton magni-ficum*. *Phytochemistry*, 1990,29(3):1004-1007

7 Kinoshta K, Takizwa T, Koyama K, *et al*. New triterpenes from *Trichocerereus pachanol*. *Joural of Natural Products*, 1995,58(11):1739-1744

8 Ikhlas A, Orro S. New triterpenes from *Trichocerereus pachanol*. *Journal of Natural Products*, 1993, 56: 2663-2665 或 2163-2165

9 Shashi B, Mahato, Ashoke K, *et al*. Pentacyclic triterpenoid sapogenols and their glycoside from *Terminalia Bellerica*. *Tetrahedron*, 1992, 48(12): 2483-2494