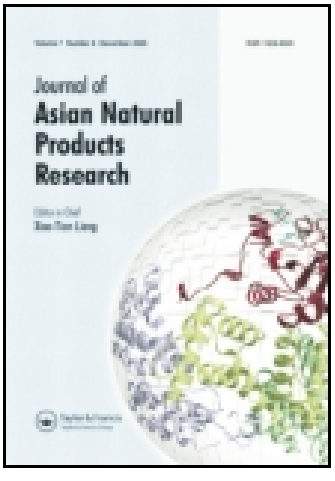


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new sesquiterpenoid glycosides from Nicotiana tabacum

Cai-Yan Yang[ab], Chang-An Geng[b], Yun-Bao Ma[b], Xiao-Yan Huang[b], Xue-Mei Zhang[b], Jun Zhou[b] & Ji-Jun Chen[b]

[a] School of Life Sciences of Yunnan University, Kunming 650091, China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Published online: 09 Jun 2014.

To cite this article: Cai-Yan Yang, Chang-An Geng, Yun-Bao Ma, Xiao-Yan Huang, Xue-Mei Zhang, Jun Zhou & Ji-Jun Chen (2014) Two new sesquiterpenoid glycosides from Nicotiana tabacum, Journal of Asian Natural Products Research, 16:6, 611-616, DOI: 10.1080/10286020.2014.922553

To link to this article: http://dx.doi.org/10.1080/10286020.2014.922553

Journal of Asian Natural Products Research, 2014
Vol. 16, No. 6, 611–616, http://dx.doi.org/10.1080/10286020.2014.922553

Two new sesquiterpenoid glycosides from *Nicotiana tabacum*

Cai-Yan Yang[ab], Chang-An Geng[b], Yun-Bao Ma[b], Xiao-Yan Huang[b], Xue-Mei Zhang[b], Jun Zhou[b] and Ji-Jun Chen[b]*

[a]*School of Life Sciences of Yunnan University, Kunming 650091, China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

(*Received 28 January 2014; final version received 5 May 2014*)

Two new sesquiterpenoid glycosides, nicotabalactonecoside (**1**) and nicotabadiolcoside (**2**), along with four known terpenoids (**3**–**6**) were isolated from the leaves of *Nicotiana tabacum*. The structures of compounds **1** and **2** were determined as dihydrodeacetylphytuberin-2-one 11-*O*-β-D-glucopyranoside and 1,2-dehydro-4-epieremophil-9-ene-11,12-diol 12-*O*-β-D-glucopyranoside by extensive spectroscopic analyses (HR-ESI-MS, UV, IR, 1D, and 2D NMR) and chemical method. Compound **1** is an unusual phytuberin-type sesquiterpenoid with a 6/5/5 tricyclic system.

Keywords: nicotabalactonecoside; nicotabadiolcoside; sesquiterpenoids; *Nicotiana tabacum*

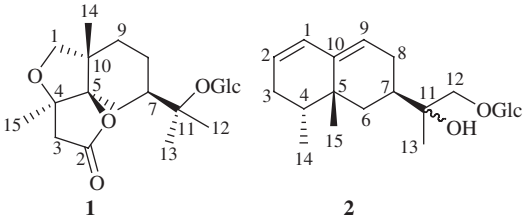
1. Introduction

Phytuberin represents an unusual tricyclic sesquiterpenoid with a 6/5/5 fused system. So far, only three additional cases of this type of sesquiterpenoid, named phytuberol, 2β-methoxydihydrophytuberol, and 2α-methoxydihydrophytuberol, have been reported from *Nicotiana tabacum* since its initial isolation from microbially infected potato [1–5]. The leaves of *N. tabacum* are well-known raw materials in tobacco production, and its aerial parts are also used for sedative, anesthetic, diaphoretic, and emetic purposes in Chinese folk medicines [6]. *Nicotiana* plants are rich in sesquiterpenoids including eudesmane, vatispirane, monocyclofarnesane, and eremophilane types [7,8]. Our chemical investigation on *N. tabacum* afforded two new sesquiterpenoid glycosides, nicotabalactonecoside (**1**) and nicotabadiolcoside (**2**), along with four known terpenoids, 9-*O*-β-D-glucoside of 3-hydroxy-7,8-didehydro-β-ionol (**3**) [9], β-amyrin palmitate (**4**) [10], lupeol palmitate (**5**) [11], and (24*R*)-cycloartane-3β,24,25-triol 3-*O*-palmitate (**6**) [12] (Figure 1). Their structures were determined based on extensive spectroscopic analyses and chemical method. Compound **1** is an important precursor for the understanding of the biogenesis of phytuberin-type sesquiterpenoids, and compounds **4**–**6** were isolated from *N. tabacum* for the first time.

2. Results and discussion

Nicotabalactonecoside (**1**) had a molecular formula of $C_{21}H_{34}O_9$ with five degrees of unsaturation by means of positive HR-ESI-MS which exhibited a quasi-molecular ion peak at *m*/*z* 453.2047 $[M + Na]^+$. Its IR spectrum exhibited hydroxyl (3417 cm^{-1}), ester (1737 cm^{-1}), and C—O—C (1149, 1118, and 1035 cm^{-1}) groups. Acid hydrolysis of compound **1** yielded a D-glucose moiety which was evidenced from its $[\alpha]_D$ value of +51.0 (*c*, 0.06, MeOH). Four methyls [δ_H 1.52,

*Corresponding author. Email: chenjj@mail.kib.ac.cn



1 **2**

Figure 1. The structures of compounds **1** and **2**.

1.36, 1.35, and 1.00 (each 3H, s)] and one anomeric proton (4.99, d, $J = 7.5$ Hz) were observed in its ^{1}H NMR spectrum. Besides a set of signals due to a β-D-glucosyl group, its ^{13}C NMR (DEPT) data (Table 1) exhibited four methyls, five methylenes, one methine, and five quaternary carbons. The NMR data of its aglycone part (Table 1) were similar to those of deacetylphytuberin except that one double bond [δ_H 6.41 and 4.64 (each 1H, d, $J = 2.8$ Hz); δ_C 146.2 and 104.4] in deacetylphytuberin was replaced by one carbonyl group (174.4) and one methylene [δ_H 2.90 and 2.79 (each 1H, d, $J = 18.5$ Hz); δ_C 44.0], and the chemical shift of C-11 was down-fielded from 72.2 in deacetylphytuberin to 78.8 in compound

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (δ in ppm, J in Hz).

	δ_H		δ_C	
No.	**1**	**2**	**1**	**2**
1α	3.53, d, 8.4	5.90, dd, 9.8, 2.2	76.9, t	129.3, d
1β	3.59, d, 8.4			
2		5.33–5.37, m	174.4, s	123.6, d
3α	2.90, d, 18.5	1.67, dd, 18.4, 5.0	44.0, t	33.0, t
3β	2.79, d, 18.5	2.47, d, 18.4		
4		1.48–1.52, m	88.1, s	40.6, d
5			97.2, s	38.0, s
6α	2.26–2.30, m	1.76–1.79, m	29.6, t	39.1, t
6β	1.86–1.89, m	1.28, t, 13.1		
7	1.91–1.94, m	1.72–1.75, m	45.5, d	42.7, d
8α	1.39–1.43, m	1.67–1.69, m 2.10–2.14, m	21.5, t	27.8, t
8β	1.61–1.65, m			
9α	1.29–1.33, m	5.66, d, 7.2	34.3, t	127.1, d
9β	1.78, d, 12.5			
10			45.0, s	141.0, s
11			78.8, s	74.9, s
12	1.36, s	3.89, d, 10.4	23.6, q	76.9, t
		3.30, d, 10.4		
13	1.35, s	1.04, s	24.8, q	20.8, q
14	1.00, s	0.82, d, 6.8	16.6, q	18.0, q
15	1.52, s	1.02, s	25.0, q	31.3, q
1′	4.99, d, 7.5	4.19, d, 7.8	98.7, d	104.9, d
2′	3.94–3.96, m	3.22, dd, 9.1, 7.8	75.4, d	75.2, d
3′	3.94–3.96, m	3.24–3.28, m	78.6, d	78.0, d
4′	4.17–4.21, m	3.20–3.23, m	71.6, d	71.6, d
5′	4.23–4.27, m	3.29–3.33, m	78.2, d	78.0, d
6′	4.35, dd, 12.0, 2.0	3.83, dd, 11.8, 2.2	62.8, t	62.7, t
	4.32, dd, 12.0, 5.1	3.62, dd, 11.8, 5.0		

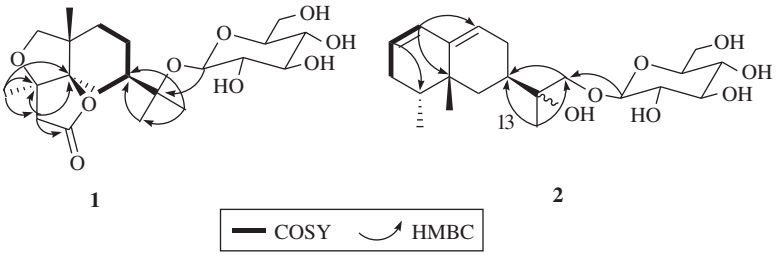


Figure 2. The key $^{1}H-^{1}H$ COSY and HMBC correlations of compounds **1** and **2**.

1 [2]. The above analyses were confirmed by the HMBC correlations of H-15 (δ_H 1.52)/C-3 (δ_C 44.0), C-4 (88.1), and C-5 (97.2), and H-3 (2.90 and 2.79)/C-2 (174.4), C-4 (88.1), and C-5 (97.2). In addition, the HMBC correlation from H-1′ (δ_H 4.99) to C-11 (δ_C 78.8) indicated the β-D-glucosyl group attached at C-11 (Figure 2). The ROESY correlation of H-6β (δ_H 1.86–1.89)/H-15 (1.52) and the undetected ROESY correlations of H-6α (δ_H 2.26–2.30)/H-14 and H-6β (1.86–1.89)/H-14 (1.00) indicated that C-6 and C-15 were situated to the same face of the tetrahydrofuran ring, while C-14 was directed to the opposite face (Figure 3) suggesting the same stereochemistry with deacetylphytuberin (Figure 1). Thus, the structure of compound **1** was characterized as dihydrodeacetylphytuberin-2-one 11-*O*-β-D-glucopyranoside, and named as nicotabalactonecoside.

Nicotabadiolcoside (**2**) showed a molecular formula of $C_{21}H_{34}O_7$ with five degrees of unsaturation by its positive HR-ESI-MS which provided a quasi-molecular ion peak at *m/z* 421.2190 $[M + Na]^+$. Its UV spectrum showed the presence of a conjugated double bond [λ_{max} (log ε): 234 (4.38) nm], and IR spectrum exhibited hydroxyl (3425 cm^{-1}), double bond (1630 cm^{-1}), and C—O—C (1076 and 1032 cm^{-1}) groups. Acid hydrolysis of compound **2** provided a D-glucose moiety which was approved by its $[\alpha]_D$ value of +50.6 (c = 0.09, MeOH). Three methyls (δ_H 1.04, s; 1.02, s; and 0.82, d, J = 6.8 Hz), three olefinic protons (5.90, dd, J = 9.8 and 2.2 Hz; 5.66, d, J = 7.2 Hz; and 5.33–5.37, m) and anomeric proton (4.19, d, J = 7.8 Hz) were revealed by its ^{1}H NMR data (Table 1). Besides one β-D-glucosyl group, three methyls, four methylenes, five methines, and three quaternary carbons were displayed in its ^{13}C NMR (DEPT) spectrum. The ^{1}H and ^{13}C NMR data of its aglycone part were similar with those of 4-epieremophil-9-ene-11,12-diol (**2a**) except that two methylenes (δ_C 31.9,

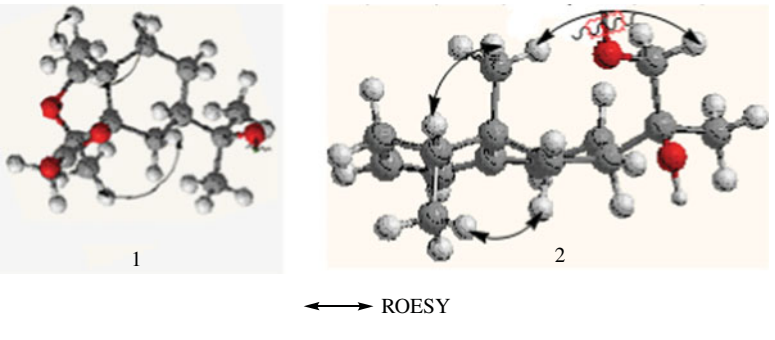


Figure 3. The key ROESY correlations of compounds **1** and **2**.

C-1 and 22.4, C-2) in **2a** were changed to be a pair of olefinic carbons (129.3 and 123.6) in **2**, together with the obvious down-fielded shift of C-12 from 68.5 in **2a** to 76.9 in **2** [13]. The above analyses suggested a double bond between C-1 and C-2 in **2** and the glucosyl group attached to C-12, which was confirmed by COSY correlations of H-1 (δ_H 5.90)/H-2 (5.33–5.37)/H-3α (1.67) and HMBC correlations of H-1′ (δ_H 4.19)/C-12 (δ_C 76.9), H-9 (5.66)/C-1 (129.3), and H-1 (5.90)/C-3 (33.0), C-5 (38.0), and C-10 (141.0). The ROESY correlations of H-4 (δ_H 1.48–1.52)/H-15 (1.02)/H-6β (1.28), H-15 (1.02)/H-12 (3.89 and 3.30), and H-14 (0.82)/H-6α (1.76–1.79) indicated that the configuration of **2** was identical with 4-epieremophil-9-ene-11,12-diol. Therefore, the structure of compound **2** was elucidated as 1,2-dehydro-4-epieremophil-9-ene-11,12-diol 12-*O*-β-D-glucopyranoside, and named as nicotabadiolcoside.

The analog of compound **1**, phytuberin lactone, was an important precursor for the synthesis of phytuberin [2,5]; however, phytuberin lactone and its analogs have not been reported from natural resources. Thus, the first isolation of nicotabalactonecoside (**1**) as natural product was significant for understanding the biogenesis of phytuberin-type sesquiterpenoids.

Compounds **1–6** were assayed on HEK293 cell line *in vitro* for the agitating activities on 5-HT_{2C} receptor using serotonin hydrochloride (5-HT) as the positive control [14] and did not display agitating activities at the concentration of about 0.75 mM.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectrum was recorded using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were carried out on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA, USA). NMR spectra were obtained on an Advance III 600 NMR or Bruker DRX-500 spectrometer (Bruker, Bremerhaven, Germany) using TMS as the internal standard. HR-ESI-MS data were obtained using a Shimadzu LC-MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Semi-preparative HPLC was conducted on a Waters Alliance 2695 with a reversed-phase (RP) C_{18} column (9.4 mm × 250 mm, 5 μm; Agilent, Santa Clara, CA, USA). Silica gel (200–300 mesh; Qingdao Makall group Co., Ltd, Qingdao, China), Sephadex LH-20 (Amersham Bioscience, Uppsala, Sweden), MCI CHP 20P (Mitsubishi Chemical Institute, Tokyo, Japan), and C_{18} (Merck, Darmstadt, Germany) were applied to column chromatography (CC).

3.2 Plant material

The leaves of *N. tabacum* were collected from Luliang County, Yunnan Province, China, and identified as *N. tabacum* Linn. by Prof. Dr Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. The specimen (No. 2011-09-16) was stored in the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried leaves (3.5 kg) were extracted using 90% EtOH (72 h each, 20 l × 3 times) at room temperature and partitioned between EtOAc and H_2O. The EtOAc fraction (615 g) was chromatographed on a silica gel column (3.0 kg, 18.0 cm × 70 cm) using H_2O–MeOH–$CHCl_3$ (0:0:100, 0:5:95, 0:10:90, 2:20:80, v/v/v) as the eluent to give Frs 1–8. Fr 5 (29.5 g) was fractionated by a MCI CHP 20P gel column (310 g, 4.0 cm × 40 cm) eluted with MeOH–H_2O (20:80, 40:60, 60:40, 80:20, 100:0) to

produce Frs 5.1–5.5. Fr 5.3 (3.2 g) was separated on a silica gel CC (100 g, 4.0 cm × 50 cm) eluted with H_2O–MeOH–$CHCl_3$ (0:10:90, 1.0:15:85) to obtain Frs 5.3.1–5.3.6. Fr 5.3.6 (40 mg) was applied to HPLC on an RP C_{18} column eluted with MeCN–H_2O (20:80, $\nu = 4$ ml/min) to generate compounds **1** (3 mg, t_R: 11.3 min) and **3** (2 mg, t_R: 9.6 min). Fr 5.3.3 (500 mg) was applied to HPLC on an RP C_{18} column eluted with MeOH–H_2O (70:30, $\nu = 3$ ml/min) to give **2** (5 mg, t_R: 24 min). Fr 8 (15 g) was fractionated by a MCI CHP 20P gel column (100 g, 2.54 cm × 40 cm) eluted with MeOH–H_2O (20:80, 40:60, 60:40, 80:20, 100:0) to yield Frs 8.1–8.5. Fr 8.1 (5.5 g) was subjected to a silica gel CC (20 g, 1.5 cm × 40 cm) using $CHCl_3$–petroleum ether (PE, 10:90) as the eluent to generate **4** (9 mg) and **5** (44 mg). Fr 3 (40 g) was chromatographed on a MCI CHP 20P gel column (310 g, 4.0 cm × 40 cm) eluted with MeOH–H_2O (60:40, 80:20, 100:0) to provide Frs 3.1–3.4. Fr 3.3 (300 mg) was submitted to a silica gel CC (20 g, 1.5 cm × 40 cm) eluted with EtOAc–PE (10:90) to obtain **6** (22 g).

3.3.1 *Nicotabalactonecoside (**1**)*

White powder, $[\alpha]_D^{16}$ – 11.4 (c = 0.46, MeOH); IR (KBr) ν_{max}: 3417, 1737, 1464, 1384, 1149, 1118, 1035 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data in pyridine-d_5, see Table 1; HR-ESI-MS: *m/z* 453.2047 $[M + Na]^+$ (calcd for $C_{21}H_{34}O_9Na$, 453.2095).

3.3.2 *Nicotabadiolcoside (**2**)*

White powder, $[\alpha]_D^{16}$ +67.8 (c = 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 234 (4.38) nm; IR (KBr) ν_{max}: 3425, 1630, 1377, 1076, 1032 cm^{-1}; for ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data in CD_3OD, see Table 1; HR-ESI-MS: *m/z* 421.2190 $[M + Na]^+$ (calcd for $C_{21}H_{34}O_7Na$, 421.2197).

3.3.3 *Acid hydrolysis and sugar identification*

Compound **1** (1.0 mg) was treated with 1.0 M HCl (MeOH–H_2O, 1:1, 1 ml) on a boiling water bath for 2 h, and neutralized by $NaHCO_3$ and partitioned between CH_2Cl_2 and H_2O. The H_2O part was purified on a silica gel CC (8 g, 1.0 cm × 40 cm) eluted with H_2O–MeOH–$CHCl_3$ (3:30:70) to give glucose (0.2 mg). Acid hydrolysis of compound **2** (1 mg) by the same method of **1** gave glucose (0.3 mg). The glucoses from compounds **1** and **2** were determined to be D-glucoses by their optical rotations $[\alpha]_D^{21}$ + 51.0 (c = 0.06, MeOH) and +50.6 (c = 0.09, MeOH), respectively.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China for Distinguished Young Scholars (No. 81025023) and the Youth Innovation Promotion Association, CAS.

References

[1] D.T. Coxon, R.F. Curtis, K.R. Price, and B. Howard, *Tetrahedron Lett.* **15**, 2363 (1974).
[2] D.T. Coxon, K.R. Price, B. Howard, and R.F. Curtis, *J. Chem. Soc. Perkin Trans. 1* **1**(53) (1977).
[3] Y. Takagi, T. Fujimori, H. Kaneko, and K. Kato, *Agric. Biol. Chem.* **43**, 2395 (1979).
[4] R. Uegaki, T. Fujimori, H. Kaneko, S. Kubo, and K. Kato, *Phytochemistry.* **19**, 1543 (1980).
[5] F. Kido, H. Kitahara, and A. Yoshikoshi, *J. Org. Chem.* **51**, 1478 (1986).
[6] The Editorial Committee of the Administration Bureau of Flora of China, *Flora of China* (Beijing Science and Technology Press, Beijing, 2005), Vol. 67.
[7] R.L. Stedman, *Chem. Rev.* **68**, 153 (1968).
[8] H. Tanaka, R. Uegaki, T. Fujimori, and K. Kato, *Nippon Shokubutsu Byori Gakkaiho.* **49**, 501 (1983).

[9] Y. Yamano, Y. Watanabe, N. Watanabe, and M. Ito, *Chem. Pharm. Bull.* **48**, 2017 (2000).
[10] K.W. Wang, *Chem. Ind. For. Prod.* **28**, 94 (2008).
[11] H.Q. Xue, H.P. Yang, H.Q. Wang, X.L. Xin, and S.X. Wu, *China J. Chin. Mater. Med.* **33**, 272 (2008).
[12] M. Ukiya, T. Akihisa, K. Yasukawa, Y. Kasahara, Y. Kimura, K. Koike, T. Nikaido, and M. Takido, *J. Agric. Food Chem.* **49**, 3187 (2001).
[13] D.G. Watson, D.S. Rycroft, I.M. Freer, and C.J.W. Brooks, *Phytochemistry* **24**, 2195 (1985).
[14] W.J. Liang, C.A. Geng, X.M. Zhang, H. Chen, C.Y. Yang, G.Q. Rong, Y. Zhao, H. B. Xu, H. Wang, N.J. Zhou, Y.B. Ma, X. Y. Huang, and J.J. Chen, *Org. Lett.* **16**, 424 (2014).