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Two novel innovanoside dimers from *Daphne aurantiaca* and a concise total synthesis of diinnovanoside A†

Shuang Liang,‡[ab] Shan-Xiang Liu,‡[c] Hui-Zi Jin,[c] Lei Shan,[a] Shi-Chong Yu,[d] Yun-Heng Shen,[a] Hui-Liang Li,[a] Qiu-Ye Wu,[d] Qing-Yan Sun*[d] and Wei-Dong Zhang*[a]

Chemical examination of the methanolic extract from the stem bark of *Daphne aurantiaca* led to the isolation of two innovanoside dimers (1 and 2) with an unusual four-membered cyclobutane ring, together with the isoinnovanoside 3. Their chemical structures and configurations were elucidated by extensive spectral analysis and synthesis.

Daphne aurantiaca Diels is a common evergreen shrub native to the Yunnan and Sichuan provinces of China. Its stem bark is used as a traditional folk medicine for the treatment of impact injuries sustained from falls such as bruises.[1] As part of our ongoing research on thymelaeaceous plants, we recently developed an interest in studying the chemical constituents of *D. aurantiaca* and have studied this plant species over the last 3 years.[2–4] During the course of this study, two interesting new innovanoside dimers, diinnovanosides A and B (**1** and **2**), were isolated from the titled plant and were found to contain an unusual four-membered cyclobutane ring. Confirmation of the structural configurations of compounds **1** and **2** proved to be particularly challenging only by extensive spectral analyses. Based on these difficulties, we developed and implemented a synthetic strategy for the construction of diinnovanoside A (**1**) to provide further confirmation of its absolute configuration and the relative configuration of **2** using single-crystal X-ray diffraction as well as other chemical methods. Herein, we have described the structural elucidation, synthetic methods and inhibitory activities against LPS-induced NO production in macrophages of compounds **1**, **2** and **3**.

The EtOAc-soluble fraction of the methanolic extract from the stem bark of *D. aurantiaca* was sequentially subjected to repeated column chromatographies over silica gel, RP-18, and Sephadex LH-20 columns eluting with a variety of different solvent systems to afford the two new innovanoside dimers, diinnovanosides A and B (**1** and **2**), as well as the isoinnovanoside (**3**). By comparing the physical and spectroscopic data of these compounds with other data reported in the literature,[5] compound **3** was identified to be an isoinnovanoside.

Compound **1**§ was obtained as a colorless oil. The molecular formula of the material was determined to be $C_{42}H_{44}O_{20}$ using HRESIMS, which provided a mass ion of $[M - H]^-$ at *m*/*z* 867.2357. The ^{1}H, ^{13}C and DEPT NMR spectra of **1** (Table 1) revealed the presence of resonance signals typical of several moieties, including (1) two maltol groups[5] [δ_H 6.43 (1H, d, J = 5.6 Hz), 7.90 (1H, d, J = 5.6 Hz), 2.32 (3H, s); 6.43 (1H, d, J = 5.6 Hz), 7.96 (1H, d, J = 5.6 Hz), 2.30 (3H, s); δ_C 164.7, 143.4, 177.1, 117.4, 157.2, 15.9; 164.6, 143.2, 177.0, 117.4, 157.3, 15.9]; (2) two 4-hydroxyphenyl rings [δ_H 7.06 (2H, d, J = 8.4 Hz), 6.72 (2H, d, J = 8.4 Hz); 7.04 (2H, d, J = 8.4 Hz), 6.69 (2H, d, J = 8.4 Hz); δ_C 130.9, 129.8, 116.3, 157.7, 116.3, 129.8; 130.9, 129.8, 116.3, 157.7, 116.3, 129.8]; (3) two β-glucose moieties[5] [δ_H 4.65 (1H, d, J = 5.6 Hz), 4.78 (1H, d, J = 7.6 Hz); δ_C 105.0, 75.2, 77.7, 71.4, 76.1, 64.5; 104.7, 75.2, 77.7, 71.4, 76.0, 64.4]; and (4) two C=O groups (δ_C 177.1, 173.4) (Table 1). The structure was further confirmed by comparison with a very similar NMR spectrum observed for the corresponding region of isoinnovanoside (**3**). The ^{1}H–^{1}H correlations of H-7″ (δ_H 4.25) with H-8″ (δ_H 3.81) and H-8‴ (δ_H 3.78), and of H-7‴ (δ_H 4.20) with H-8″ and H-8‴ allowed for the assignment of the cyclobutane ring. The structure of **1** was consequently established and named diinnovanoside A on the basis of the HMBC spectroscopic analysis (Fig. 1).

To identify the stereochemistry of compound **1**, compounds **1** and **11** were synthesized for the first time according to the

[a] *Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, People's Republic of China. E-mail: wdzhangy@hotmail.com; Fax: +86-21-81871244; Tel: +86-21-81871244*

[b] *Shanghai University of Traditional Chinese Medicine, Engineering Research Center of Modern Preparation Technology of TCM, Ministry of Education, Shanghai 201203, People's Republic of China*

[c] *School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China*

[d] *Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, People's Republic of China. E-mail: sqy_2000@163.com; Fax: +86-21-81871244; Tel: +86-21-81871244*

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‡ These authors have contributed equally to this work.

Table 1 ^{13}C and ^{1}H NMR spectroscopic data[a] for compounds **1** and **2**

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
2	164.7		164.6	
3	143.4		143.3	
4	177.1		177.1	
5	117.4	6.43 (d, 5.6)	117.5	6.52 (d, 5.6)
6	157.2	7.90 (d, 5.6)	157.5	8.07 (d, 5.6)
7	15.9	2.32 (s)	16.1	2.40 (s)
1′	105.0	4.65 (d, 5.6)	105.0	4.77 (d, 5.6)
2′	75.2	3.32 (m)	75.3	3.29 (m)
3′	77.7	3.32 (m)	77.8	3.41 (m)
4′	71.4	3.13 (m)	71.2	2.93 (dd, 8.8, 10.0)
5′	76.1	2.99 (m)	76.2	2.60 (m)
6′	64.5	4.34 (dd, 12.0, 1.6)	64.4	4.53 (dd, 1.6, 12.0)
		3.70 (dd, 12.0, 7.6)		4.32 (dd, 5.2, 12.0)
1″	130.9		131.2	
2″	129.8	7.06 (d, 8.4)	128.9	6.98 (d, 8.4)
3″	116.3	6.72 (d, 8.4)	116.2	6.66 (d, 8.4)
4″	157.7		157.3	
5″	116.3	6.72 (d, 8.4)	116.2	6.66 (d, 8.4)
6″	129.8	7.06 (d, 8.4)	128.9	6.98 (d, 8.4)
7″	42.1	4.25 (dd, 10.4, 7.2)	42.4	3.97 (dd, 8.4, 10.4)
8″	48.8	3.81 (dd, 10.4, 7.2)	50.3	3.83 (t, 8.4)
9″	173.4		174.8	
2‴	164.6		164.6	
3‴	143.2		143.1	
4‴	177.0		177.0	
5‴	117.4	6.43 (d, 5.6)	117.2	6.37 (d, 5.6)
6‴	157.3	7.96 (d, 5.6)	157.3	7.89 (d, 5.6)
7‴	15.9	2.30 (s)	15.7	2.16 (s)
1⁗	104.7	4.78 (d, 7.6)	104.6	4.63 (d, 7.6)
2⁗	75.2	3.33 (m)	75.2	3.29 (m)
3⁗	77.7	3.33 (m)	77.7	3.23 (m)
4⁗	71.4	3.13 (m)	71.1	3.34 (m)
5⁗	76.0	3.13 (m)	76.1	3.45 (m)
6⁗	64.4	4.14 (dd, 12.0, 1.6)	64.2	4.23 (dd, 1.6, 12.0)
		3.96 (dd, 12.0, 7.6)		3.36 (m)
1′′′′′	130.9		130.9	
2′′′′′	129.8	7.04 (d, 8.4)	128.5	6.93 (d, 8.4)
3′′′′′	116.3	6.69 (d, 8.4)	116.2	6.64 (d, 8.4)
4′′′′′	157.7		157.2	
5′′′′′	116.3	6.69 (d, 8.4)	116.2	6.64 (d, 8.4)
6′′′′′	129.8	7.04 (d, 8.4)	128.5	6.93 (d, 8.4)
7′′′′′	42.4	4.20 (dd, 10.4, 7.2)	42.0	3.95 (dd, 8.4, 10.4)
8′′′′′	48.4	3.78 (dd, 10.4, 7.2)	47.0	4.10 (t, 10.4)
9′′′′′	173.1		172.7	

[a] Data were recorded in CD_3OD at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR.

route shown in Scheme 1. The single-crystal X-ray structure of **11** was obtained as shown in Fig. 2. Compound **4** was prepared according to the reported method.[6] The material was then reacted with TBDMS triflate, and subsequently selectively deprotected to remove the 6-*O*-TBDMS protecting group to afford compound **5**.[7] In a separate sequence, *p*-coumaric acid was coupled with TBDMS triflate, and subsequently treated with 1 N HCl over 24 h to afford compound **6**. Compound **6** was then subjected to a photodimerization reaction to give compound **7**, which was coupled with **5** to give compound **8**. Finally, removal of the TBDMS protecting groups of **8** afforded compound **1** in 80% yield.[8] The overall yield of **1** was 10.8%.

In a separate sequence, compound **9** was obtained *via* the esterification of compound **7** with methanol in the presence of N^1-[(ethylimino)methylene]-N^3,N^3-dimethylpropane-1,3-diamine hydrochloride (EDCI) and *N*,*N*-dimethylpyridin-4-amine (DMAP). The TBDMS protecting groups in **9** were then removed with tetrabutylammonium fluoride (Bu_4NF) to give **10**. Finally, the acetylation of **10** provided compound **11**. The stereochemistry of **11** was confirmed by X-ray analysis (Fig. 2). The results indicated that compound **6** had undergone topochemically controlled α-dimerization to form the corresponding head-to-tail adduct **7**.

Compound **2**§ was isolated as a colorless oil. Its HRESIMS (negative ion mode) gave a pseudomolecular ion peak for $[M - H]^-$ at *m*/*z* 867.2357, corresponding to the molecular formula $C_{42}H_{44}O_{20}$. The NMR data of **2** were similar to those of **1**, with similar resonance structures corresponding to the two maltol groups, two 4-hydroxyphenyl rings, two β-glucose moieties, and a four-membered ring deduced by interpretation of the 2D NMR spectra of **2** (Table 1). The ^{1}H–^{1}H COSY correlations of H-7″ (δ_H 3.83) with H-8″ (δ_H 3.97) and H-8‴ (δ_H 3.95), and of H-7‴ (δ_H 4.10) with H-8″ and H-8‴ implied the presence of a four-membered ring similar to that of compound **1**, which suggested that **2** may be a diastereoisomer of **1**. The relative configuration of the four-membered ring was determined using the NOESY spectrum of compound **2**. The NOESY correlation of H-7″ with H-7‴ indicated that they existed in the *cis*-form. Similarly, the NOESY correlation of H-8″ with H-8‴, also indicated that *t* H-8″ and H-8‴ existed in the *cis*-form. In addition, the NOESY correlations of H-8″ and H-2‴ (δ_H 6.93) with H-8‴ and H-2″ (δ_H 6.93) implied that H-8″/H-7‴ and H-8‴/H-7″ were in the *trans*-form, respectively. Therefore, the structure of compound **2** was deduced as being (7″α, 8″β, 7‴α, 8‴β)-diinnovanoside A, and named diinnovanoside B.

Nitric oxide (NO) plays an important role in the inflammatory process.[9] The isolates from the current study were therefore tested for their inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages using methods that have been previously reported in the literature.[10] Compounds **2** and **3** showed potent inhibitory activities against the production of NO

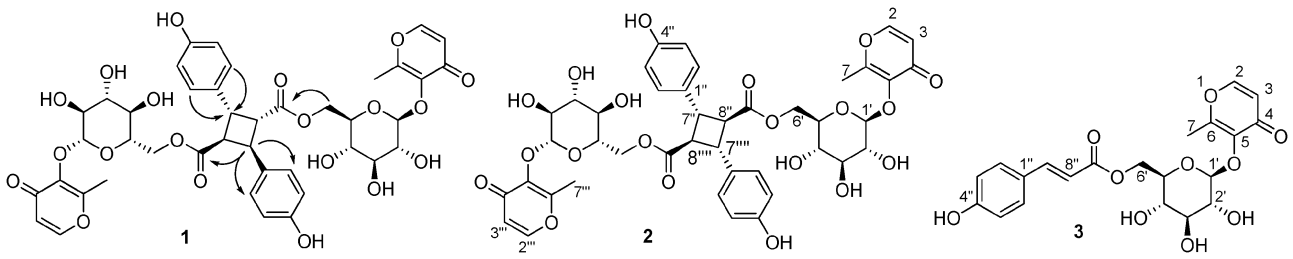
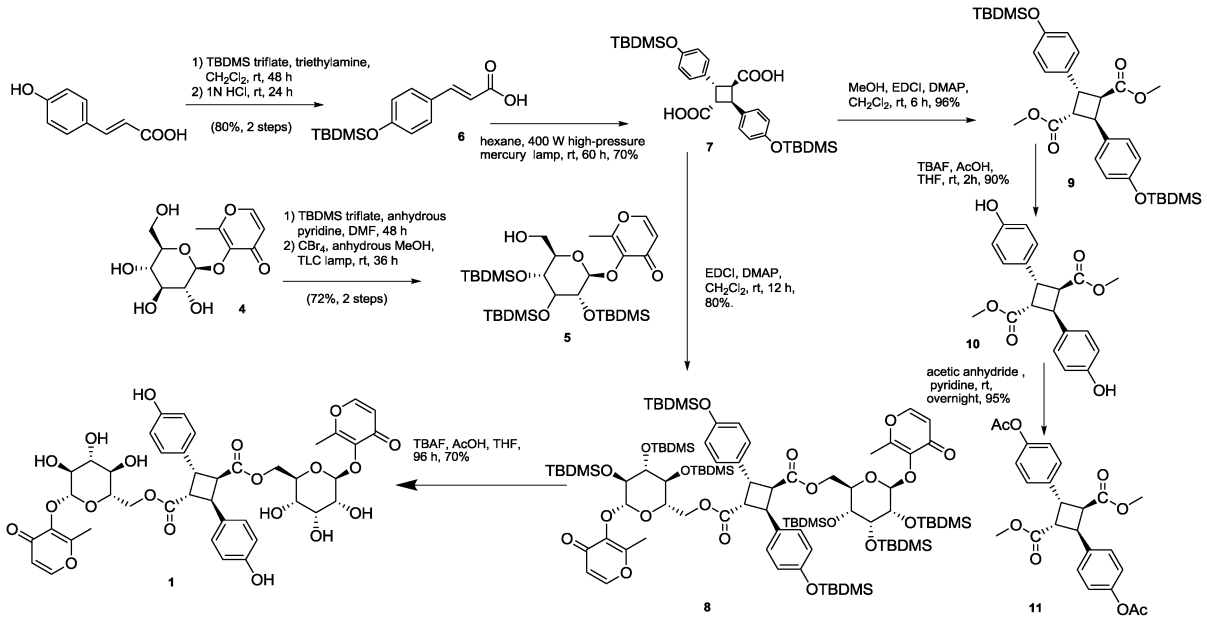


Fig. 1 Structures of compounds **1–3** and key HMBC correlations of compound **1** (H → C).



Scheme 1 Synthesis of compounds **1** and **11**.

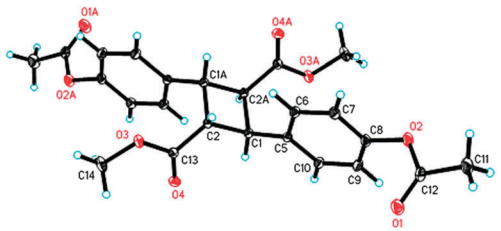


Fig. 2 Single-crystal X-ray diffraction of compound **11**.

with IC_{50} values of 0.284 and 0.068 μM, respectively (aminoguanidine, positive control, IC_{50} 0.048 μM).

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Notes and references

§ Diinnovanoside A (**1**): colorless oil (MeOH); UV (MeOH): 259 (2.41), 231 (2.82); $[\alpha]_D^{20}$ −47 (*c* 0.12, CH_3OH); IR ν_{max}^{KBr} cm^{-1}: 3392, 2896, 1731, 1645, 1615, 1517, 1445, 1255, 1198, 1068, 837 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; negative HR-ESIMS, found 867.2354, calcd 867.2348 for $C_{42}H_{43}O_{20}$ $[M - H]^-$. Diinnovanoside B (**2**): colorless oil (MeOH); UV (MeOH): 256 (2.58), 230 (3.34); $[\alpha]_D^{20}$ −48 (*c* 0.08, CH_3OH); IR ν_{max}^{KBr} cm^{-1}: 3419, 2900, 1732, 1646, 1615, 1517, 1455, 1255, 1197, 1066, 838 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; negative HR-ESIMS, found 867.2354, calcd 867.2348 for $C_{42}H_{43}O_{20}$ $[M - H]^-$.

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