Glycosides from the Methanol Extract of *Notopterygium incisum*

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Abstract

Five new (**1**–**5**) and twelve known (**6**–**17**) different types of glycosides together with a known sesquiterpene triol (**18**) were isolated from the methanol extract of the rhizomes of *Notopterygium incisum*. The new structures were elucidated by means of spectroscopic and chemical methods to be pregn-5-en-3*β*,20(*S*)-diol-3-*O*-*bis*-*β*-D-glucopyranosyl-(1→2,1→6)-*β*-D-glucopyranoside (**1**), oleuropeic aldehyde 8-*O*-*β*-D-glucopyranoside (**2**), 2(*R*)-(3,4-dimethoxyphenyl)-propane-1,3-diol-1-*O*-*β*-D-glucopyranoside (**3**), eudesman-3*α*,4*α*,11-triol-11-*O*-*β*-D-glucopyranoside (**4**), and marmesin-11-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**). The absolute configuration of the aglycone in compound **3** was assigned by application of Klyne's rule.

Key words

Notopterygium incisum · Umbelliferae · structure elucidation · glycosides

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The traditional Chinese medicine "Qianghuo" is composed of the rhizomes of two species of *Notopterygium* (Umbelliferae), *N. incisum* Ting ex H.T. Chang and *N. forbesii* De Boiss [1]. Chemically, only a few polar constituents (coumarin glycosides, amino acids, chlorogenic acid, ferulic acid, and daucosterol) were previously characterized from this herb medicine [2–6]. In an earlier work on the chloroform extract of the rhizomes of *N. incisum*, a number of lipophilic constituents (including antiproliferative furocoumarins) have been identified [7]. After being exhaustively extracted with chloroform, the rhizomes were further extracted with methanol. The methanol extract was found to show a preliminary cytotoxic effect against the human MCF-7 breast cancer cell line. During a reinvestigation of polar constituents from this plant and in a continuation of our ongoing project towards the discovery of novel antitumor agents from natural products [8], seventeen (**1**–**17**) different types of glycosides and a polar sesquiterpene hedytriol (**18**) [9] (**Fig. 1**) were obtained from the methanol extract. These include a new pregnane glycoside (**1**), a new monoterpenoid glycoside (**2**), a new phenylpropanoid glycoside (**3**), a new eudesmane glycoside (**4**), and a new furanocou-

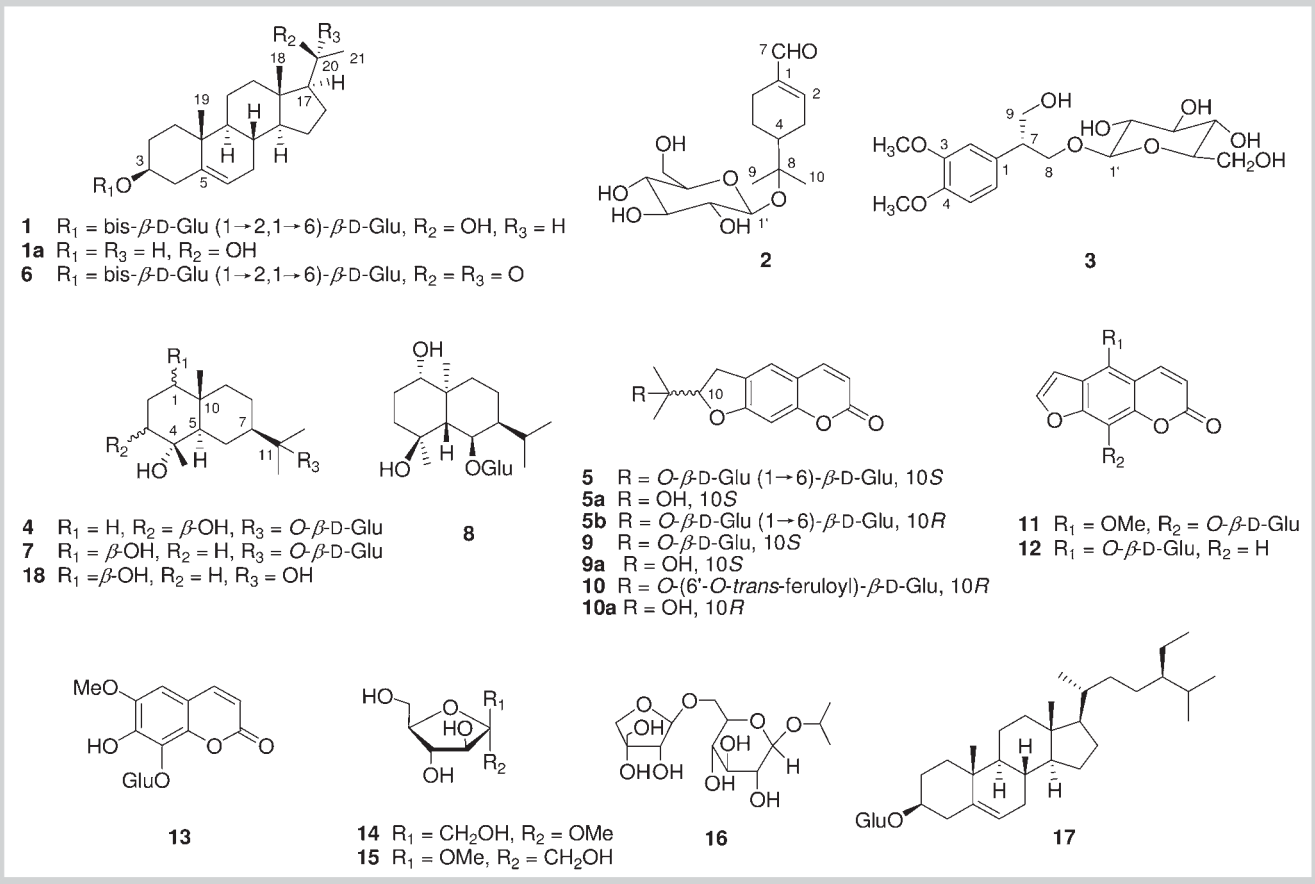

Fig. 1 Chemical structures of compounds **1**–**18**.

Table 1 ^{1}H NMR (500 MHz, *J* in Hz) and ^{13}C NMR (125 MHz) data of compounds **1** and **6**.

No.	1[a]		6[a]		No.	1[a]		6[a]	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1	1.72 br d, 13.1	37.2	1.69 br d, 12.8	37.1	1′	5.10 d, 7.8	100.7	5.11 d, 7.6	100.7
	0.95 m		0.92 m		2′	4.17 dd, 9.0, 7.8	83.3	4.18 dd, 8.7, 8.4	83.3
2	2.16 m	29.9	2.16 br d, 11.0	29.8	3′	4.38 dd[b]	77.4	4.39 dd[b]	77.4
	1.82 m		1.82 m		4′	4.30 dd, 9.4, 8.9	71.0[e]	4.30 dd, 9.3, 9.0	71.1[e]
3	3.92 m	79.1	3.91 m	79.0	5′	4.06 m	76.9	4.07 m	76.8
4	2.84 dd, 13.2, 1.2	39.0	2.84 dd, 13.4, 1.2	39.0	6′	4.81 br d, 11.0	69.6	4.82 br d, 11.0	69.7
	2.69 dd, 13.2, 12.1		2.69 dd, 13.4, 10.8			4.39 dd[b]		4.41 dd, 11.0, 4.6	
5	–	140.6	–	140.7	1″	5.29 d, 7.7	105.2	5.31 d, 7.6	105.1
6	5.46 br s	121.7	5.44 br s	121.4	2″	4.06 dd[c]	75.9	4.08 dd[c]	75.8
7	1.96 m	31.9	1.91 m	31.7	3″	4.22 dd[d]	77.9[f]	4.22 dd[d]	77.9[f]
	1.55 m		1.52 m		4″	4.23 dd[d]	70.9[e]	4.23 dd[d]	71.0[e]
8	1.39 m	31.5	1.32 m	31.6	5″	3.92 m	77.9[f]	3.91 m	77.9[f]
9	0.89 m	50.0	0.88 m	49.8	6″	4.51 br d, 11.0	62.2[g]	4.52 br d, 11.8	62.2[g]
10	–	36.7	–	36.6		4.36 dd[b]		4.38 dd[b]	
11	1.39 m	20.8	1.43 m	21.0	1‴	5.05 d, 7.8	105.2	5.07 d, 7.6	105.1
			1.26 m		2‴	4.06 dd[c]	74.8	4.08 dd[c]	74.7
12	1.88, m	38.8	1.91 m	38.5	3‴	4.22 dd[d]	78.1[f]	4.22 dd[d]	78.1[f]
	1.06 m		1.23 m		4‴	4.23 dd[d]	71.2[e]	4.23 dd[d]	71.3[e]
13	–	41.3	–	43.5	5‴	3.92 m	77.7[f]	3.91 m	77.6[f]
14	0.95 m	56.6	0.92 m	56.5	6‴	4.51 br d, 11.0	62.4[g]	4.52 br d, 11.8	62.4[g]
15	1.57 m	24.2	1.49 m	24.3		4.36 dd[b]		4.38 dd[b]	
	1.09 m		1.08 m						
16	2.16 m	26.5	2.28 m	22.8					
	1.88 m		1.54 m						
17	1.51 m	59.0	2.42 t, 8.8	63.3					
18	0.71 s	12.3	0.57 s	12.9					
19	1.02 s	19.4	0.97 s	19.2					
20	3.92 m	68.9	–	208.0					
21	1.43 d, 6.0	24.4	2.06 s	31.0					

[a] Recorded in C_5D_5N. [b–d] Signals were overlapped within the same superscript in the same column. [e–g] Assignments may be interchangeable within the same superscript in the same column

marin glycoside (**5**). In this paper, we report the isolation and structure elucidation of the new compounds and evaluation of their cytotoxic effects on a small panel of human cancer cell lines. Comparing their MS and NMR data and their physical properties with the literature, or by direct comparison with authentic samples, the known compounds were identified as pregn-5-en-3*β*-ol-20-one-3-*O*-*bis*-*β*-D-glucopyranosyl-(l → 2,1 → 6)-*β*-D-glucopyranoside (**6**) [10], celerioside E (**7**) [11], ananosmoside A (also named pumilaside A) (**8**) [12, 13], marmesinin (**9**) [14], 6′-*O*-*trans*-feruloyl-nodakenin (**10**) [2], 5-methoxy-8-*O*-*β*-D-glucosyloxypsoralene (**11**) [15], bergaptol-*O*-*β*-D-glucopyranoside (**12**) [2], fraxin (**13**) [16], methyl *α*-D-fructofuranoside (**14**) [17], methyl *β*-D-fructofuranoside (**15**) [17], 2-{(6-*O*-[*β*-D-apiofuranosyl]-*β*-D-glucopyranosyl)oxy}propane (**16**) [18], *β*-daucosterol (**17**), and hedytriol (**18**) [9].

The molecular weight of compound **1** and its chemical formula $C_{39}H_{64}O_{17}$ were determined from the positive mode HR-ESIMS. The ^{1}H NMR spectrum (**Table 1**) of **1** displayed signals assignable to two methyl singlets at *δ* 0.71 (3H, s) and 1.02 (3H, s), one methyl doublet at 1.43 (3H, d, *J* = 6.0 Hz), three anomeric protons at *δ* 5.05 (1H, d), 5.10 (1H, d), and 5.29 (1H, d), and an olefinic proton resonating at *δ* 5.46 (1H, br s). The ^{13}C and DEPT NMR spectra (**Table 1**) of **1** revealed twenty-one carbon signals classified as three sp^3 methyls, eight sp^3 methylenes, six sp^3 (two oxygenated), and one sp^2 methines, two sp^3 and one sp^2 quaternary carbons in addition to eighteen carbon signals attributed to three glucopyranosyl units. These NMR data showed that **1** has general features very similar to those of pregn-5-en-3*β*-ol-20-one-3-*O*-*bis*-*β*-D-glucopyranosyl-(l → 2,1 → 6)-*β*-D-glucopyranoside (**6**), a known compound previously obtained from an apocynaceous plant *Nerium odorum* [10]; however, its ^{1}H and ^{13}C NMR data were not completely assigned until this study (**Table 1**). The most obvious difference between these two compounds was that a methyl singlet at *δ* 2.06 in **6** was replaced by one methyl doublet at *δ* 1.43 (Me-21) in **1**, indicating that the ketone carbonyl group at C-20 of **6** was reduced to a hydroxyl group in **1**. This secondary hydroxyl group at C-20 was supported by the HMBC correlations between H-20 (*δ* 3.92) and C-16 (*δ* 26.5)/C-17 (*δ* 59.0), between Me-21 (*δ* 1.43) and C-20 (*δ* 68.9)/C-17 (*δ* 59.0).

The glycosidic linkage position at C-3 was determined by the HMBC NMR experiment. A clear ^{3}J correlation was found between the anomeric proton H-1′ resonating at *δ* 5.10 and C-3 at *δ* 79.1. The interglycosidic linkage positions (1 → 2, 1 → 6) were unambiguously confirmed by HMBC correlations from H-1″ (*δ* 5.29) to C-2′ (*δ* 83.3) and H-1‴ (*δ* 5.05) to C-6′ (*δ* 69.6). The observed coupling constants of the anomeric protons [H-1′ (*J* = 7.8 Hz), H-1″ (*J* = 7.8 Hz), H-1‴ (*J* = 7.7 Hz)] were characteristic for *β*-glucosidic linkage in glucopyranosyl units. In addition, acid hydrolysis of **1** with 2 N HCl yielded a monosaccharide and the aglycone (**1a**) (Supporting Information). The sugar was ascertained as D-glucose by direct comparison with an authentic sample according to HPLC analysis and optical rotation detection. The *S* absolute configuration at C-20 in **1a** was determined by comparing the proton chemical shift (in $CDCl_3$) of Me-21 (*δ* 1.22, see Supporting

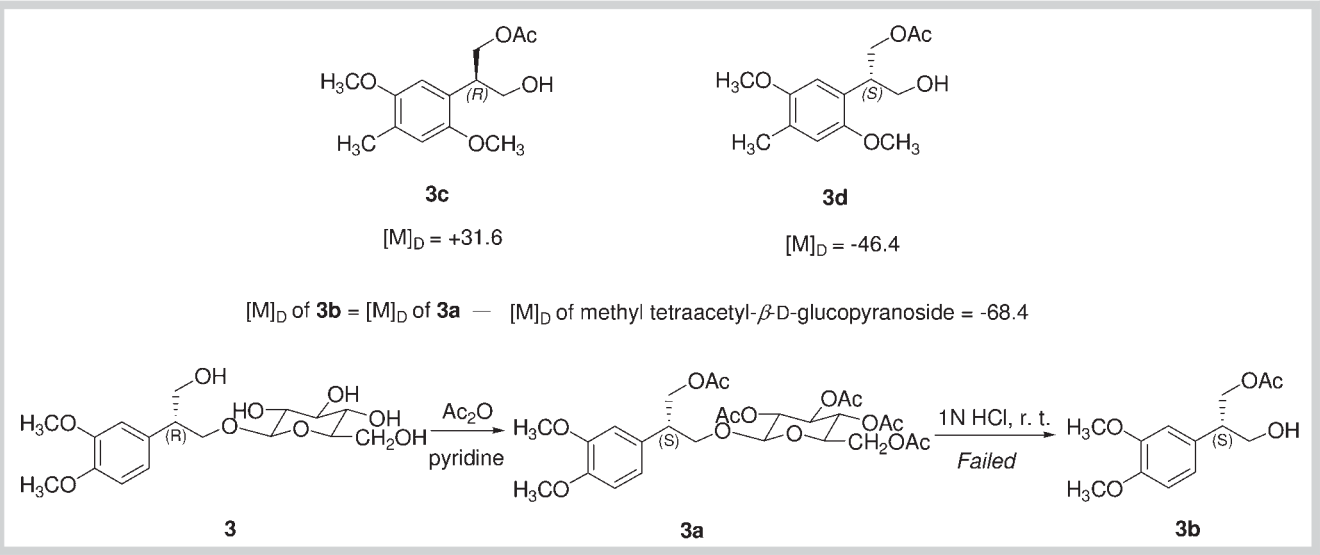

Fig. 2 Application of Klyne's rule to compound **3**.

Information) with those of C-20 epimers of pregn-5-en-3*β*,20-diol [20(*R*): *δ* 1.14; but 20(*S*): *δ* 1.21] [19]. Consequently, **1** was elucidated to be pregn-5-en-3*β*,20(*S*)-diol-3-*O*-*bis*-*β*-D-glucopyranosyl-(1 → 2,1 → 6)-*β*-D-glucopyranoside.

Compound **2** exhibited an $[M + Na]^+$ ion peak at *m/z* 353.1572 in the positive mode HR-ESIMS, indicating its molecular formula to be $C_{16}H_{26}O_7$. The ^{13}C and DEPT NMR spectra of **2** exhibited ten carbon signals assignable to a monoterpene moiety in addition to six carbon signals attributed to a glucopyranosyl unit. The ^1H NMR spectrum of **2** revealed the presence of two tertiary methyl groups [*δ* 1.23, 1.26 (each 3H, s)], an olefinic proton [*δ* 6.94 (1H, brdd, *J* = 2.7, 2.3 Hz)], and an aldehyde proton [*δ* 9.38 (1H, s)] in the aglycone moiety, which was further deduced to be oleuropeic aldehyde (a menthane-type monoterpene) by a spin system ($-CH_2CH_2CHCH_2CH=$) in its COSY NMR spectrum. Thus, compound **2** was determined to be oleuropeic aldehyde 8-*O*-*β*-D-glucopyranoside. The bulky substituent at C-4 was in an equatorial orientation due to the large coupling constant (*J* = 11.3 Hz) between H-4$_{ax}$ and H-3$_{ax}$. An attempt to obtain the aglycone of **2** by hydrolysis with 2 N HCl failed probably due to sample decomposition, and the absolute configuration at C-4 hence remains unknown.

The molecular formula $C_{17}H_{26}O_9$ of compound **3** was determined by HR-ESIMS, which gave an $[M + Na]^+$ ion peak at *m/z* 397.1452. Detailed analyses of ^1H, ^{13}C NMR data of **3** with the aid of COSY and HSQC NMR experiments established the presence of a benzene ring with an ABX system [*δ* 6.94 (1H, brs), 6.88 (1H, brd, *J* = 8.0 Hz), 6.84 (1H, d, *J* = 8.0 Hz)], two methoxyl groups [*δ* 3.83, 3.80 (each 3H, s)], one 1,3-propanediol group, together with a glucopyranosyl unit. The above data indicated that **3** was a (1,3,4-trisubstituted phenyl)propanoid glucoside. In the HMBC NMR spectrum of **3**, the anomeric proton H-1′ at *δ* 4.31 exhibited a correlation with C-8 (*δ* 72.2), whereas H-7 at *δ* 3.03 showed correlations with C-1 (*δ* 135.1), C-2 (*δ* 113.6), and C-6 (*δ* 113.1), respectively. The *β*-orientation of the glucosidic linkage was also deduced from the characteristic coupling constant (*J* = 7.9 Hz) of the anomeric proton.

The determination of the absolute configuration at C-7 has been challenging. Acetylation of **3** with anhydrous Ac_2O in pyridine gave a pentaacetate derivative [**3a**: $[\alpha]_D^{20} = -23.0$ (*c* 0.15, $CHCl_3$)]. However, we failed to obtain the desired aglycone (**3b**) by acid hydrolysis in the presence of 2 N HCl. Significantly, enantiomers of 9-acetoxy-7-(2,5-dimethoxy-4-methylphenyl)propan-8-ol [(7*R*)-**3c**: $[\alpha]_D = +11.8$; (7*S*)-**3 d**: $[\alpha]_D = -17.3$] have been previously reported in a synthetic study [20]. The absolute configuration at C-7 in **3a** could be deduced by the application of Klyne's rule [21]. As shown in **Fig. 2**, the calculated $[M]_D$ (−68.4) of **3b** (subtracting the $[M]_D$ of methyl tetraacetyl-*β*-D-glucopyranoside (−65.9) [22] from the calculated $[M]_D$ −134.3 of **3a**) was in good correspondence to the 7*S* configuration. Therefore, compound **3** was assigned as 2(*R*)-(3,4-dimethoxyphenyl)-propane-1,3-diol-1-*O*-*β*-D-glucopyranoside.

The positive mode HR-ESIMS of compound **4** showed an $[M + Na]^+$ ion peak at *m/z* 441.2468, corresponding to the molecular formula $C_{21}H_{38}O_8$. The assignments of ^1H and ^{13}C NMR data of **4** were made by a combination of 1D and 2D NMR techniques (COSY, HSQC, and HMBC). These NMR data showed general features very similar to celerioside E (**7**), a eudesmane glucoside previously isolated from the polar extract of fruits of *Apium graveolens* L. (Umbelliferae) [11]. The major difference between these two compounds was that the secondary hydroxyl group at C-1 in **7** was relocated at C-3 in **4** of ring A, which was confirmed by HMBC correlations of Me-14 (*δ* 1.08) with C-3 (*δ* 75.7), C-4 (*δ* 74.4), and C-5 (*δ* 48.1), and correlations of Me-15 (*δ* 0.91) with C-1 (*δ* 34.9), C-5 (*δ* 48.1), C-9 (*δ* 45.9), and C-10 (*δ* 35.2). A clear ^3J HMBC correlation was also observed between the anomeric proton H-1′ at *δ* 4.46 (1H, d, *J* = 7.7 Hz) and C-11 at *δ* 81.6, indicating that C-11 was the glycosidic linkage position. The relative stereochemistry at C-3, C-4, C-5, C-7, and C-10 in **4** was characterized through analyses of the coupling patterns of the protons bonded to the cyclohexane ring and the NOE correlations in the NOESY NMR experiment. The small coupling constant (*br s*) found for H-3 resonating at *δ* 3.51 indicated that it is in an equatorial orientation. Clear NOE correlations were observed between Me-14 (*δ* 1.08) and Me-15 (*δ* 0.91), between Me-14 and H-3 (*δ* 3.51), between H$_{ax}$-9 (*δ* 1.24) and H-5 (*δ* 1.57), as well as between H-5 and H-7 (*δ* 1.55). As indirect evidence, the ^{13}C NMR data of C-6 (*δ* 22.1), C-7 (*δ* 49.6), C-8 (*δ* 24.3), C-11 (*δ* 81.6), C-12 (*δ* 23.5),

and C-13 (δ 25.5) of compound **4** resembled those of **7**, indicating that these two compounds have the same configuration at C-7. Consequently, compound **4** was determined to be eudesman-3α,4α,11- triol-11-*O*-β-D-glucopyranoside.

The ^{1}H and ^{13}C NMR data revealed that **5** is structurally related to the known coumarin glucosides marmesinin (**9**) [14] and decuroside I (**5b**) [23]. Similar to **5b**, the carbon chemical shift (δ 62.6) [15] of C-6′ in **9** was shifted downfield to δ 68.3 in **5**, indicating that the terminal glucopyranosyl unit is linked to C-6′. Acid hydrolysis of compound **5** gave D-glucose. Meanwhile, the measured optical rotation ($[\alpha]_D^{24}$ = + 15.6) of the aglycone (**5a**) was consistent with the previous reported data for marmesin (lit. [24]: $[\alpha]_D$ = + 23) rather than nodakenetin (lit. [2]: $[\alpha]_D$ = − 26.0). Interestingly, marmesin was previously isolated as a major component from the chloroform extract of this plant [7]. Therefore, the structure of **5** was established as marmesin-11-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside.

The known glucosides **6**–**8, 13, 16**, and the polyhydroxylated sesquiterpene **18** were isolated for the first time from the genus *Notopterygium*. Until now, phytochemical investigation of the rhizomes of *N. incisum* has been accomplished in our group. The results would give a broad spectrum of naturally occurring compounds from *N. incisum*. In agreement with our previous findings (especially coumarins and sesquiterpenoids) from the chloroform extract [7], six coumarin glucosides (**5, 9**–**13**) and three sesquiterpenoid glucosides (**4, 7, 8**) were isolated from the methanol extract in this study. In the case of compounds **1, 2, 5, 9**, and **10**, we extensively proved the sugar moiety to be β-D-glucopyranoside (Supporting Information). In accordance to this result and with the assumption of a common biosynthetic pathway for the rest new and known glycosides, the sugar unit should also be D-glucose.

All the isolates (except **2** and **17**) were evaluated for their *in vitro* cytotoxic effects against a small panel of human cancer cell lines (SNU739, NUGC-3, MCF-7, SH-SY5Y) using the CellTiter Glo™ luminescent cell viability assay. But none of them appear to be active (IC_{50} > 100 µM). It is worthy of note that the inactivity of furanocoumarin glucosides (**5, 9**–**12**) against MCF-7 cells might support our previous hypothesis that a lipophilic side chain bearing a free hydroxyl is essential for the cytotoxic effect of linear furanocoumarins [8].

Materials and Methods

For general experimental procedure, collection, and identification of the plant material, see a preceding paper [7]. For extraction and isolation of compounds **1**–**18** from the methanol extract of the rhizomes of *N. incisum*, see Supporting Information.

Pregn-5-en-3β,20(S)-diol-3-O-bis-β-D-glucopyranosyl-(1 → 2,1 → 6)-β-D-glucopyranoside (**1**): White amorphous powder; $[\alpha]_D^{24}$ − 30.4 (*c* 0.85, MeOH); IR ν_{max}^{KBr} · cm^{-1}: 3359 (*br*), 2929, 2857, 1653, 1074; ^{1}H NMR and ^{13}C NMR data, see **Table 1**. LR-ESIMS: *m*/*z* 827 $[M + Na]^+$, 1631 $[2M + Na]^+$, 863 $[M + CH_3COO^-]^-$, 1607 $[2M - H]^-$; HR-ESIMS: *m*/*z* 827.4046 $[M + Na]^+$ (calcd. for $C_{39}H_{64}O_{17}Na$: 827.4036, Δ = − 1.2 ppm)

Oleuropeic aldehyde 8-O-β-D-glucopyranoside (**2**): Colorless oil; $[\alpha]_D^{24}$ − 2.0 (*c* 0.13, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 9.38 (1H, s, CHO), 6.94 (1H, brdd, *J* = 2.7, 2.3 Hz, H-2), 4.49 (1H, d, *J* = 7.8 Hz, H-1′), 3.81 (1H, dd, *J* = 11.9, 2.2 Hz, H_a-6′), 3.64 (1H, dd, *J* = 11.9, 5.4 Hz, H_b-6′), 3.36 (1H, dd, *J* = 9.0, 8.8 Hz, H-5′), 3.28 (1H, dd, *J* = 9.0, 8.7 Hz, H-4′), 3.24 (1H, m, H-3′), 3.14 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 2.59 (1H, brd, *J* = 19.2 Hz, H_a-3), 2.43 (1H, m, H_a-6), 2.23 (1H, brdd, *J* = 19.1, 11.3 Hz, H_b-3), 2.14 (1H, m, H_a-5), 2.01 (1H, m, H_b-6), 1.80 (1H, m, H-4), 1.28 (3H, s, Me-9), 1.26 (3H, s, Me-10), 1.21 (1H, m, H_b-5). ^{13}C NMR (125 MHz, CD_3OD): δ 142.7 (C-1), 153.9 (C-2), 29.4 (C-3), 45.1 (C-4), 23.1 (C-5), 23.7 (C-6), 195.9 (C-7), 80.4 (C-8), 24.7 (C-9), 23.4 (C-10), 98.5 (C-1′), 75.3 (C-2′), 78.3 (C-3′), 71.8 (C-4′), 77.5 (C-5′), 62.9 (C-6′). LR-ESIMS: *m*/*z* 353 $[M + Na]^+$, 683 $[2M + Na]^+$; HR-ESIMS: *m*/*z* 353.1572 $[M + Na]^+$ (calcd. for $C_{16}H_{26}O_7Na$: 353.1571, Δ = − 0.3 ppm).

2(R)-(3,4-Dimethoxyphenyl)-1,3-propanediol-1-O-β-D-glucopyranoside (**3**): Colorless oil; $[\alpha]_D^{24}$ − 18.2 (*c* 0.79, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 6.94 (1H, brs, H-2), 6.88 (1H, brd, *J* = 8.0 Hz, H-6), 6.84 (1H, d, *J* = 8.0 Hz, H-5), 4.31 (1H, d, *J* = 7.9 Hz, H-1′), 4.13 (1H, dd, *J* = 10.0, 6.0 Hz, H_a-8), 3.89 (1H, dd, *J* = 11.5, 6.5 Hz, H_a- 9), 3.86 (1H, brd, *J* = 12.0 Hz, H_a-6′), 3.84 (1H, dd, *J* = 10.0, 6.5 Hz, H_b-8), 3.83 (3H, s, OMe), 3.80 (3H, s, OMe), 3.78 (1H, dd, *J* = 11.5, 6.5 Hz, H_b-9), 3.67 (1H, dd, *J* = 12.0, 5.4 Hz, H_b-6′), 3.34 (1H, dd, *J* = 8.3, 6.5 Hz, H-3′), 3.27 (2H, m, H-4′, H-5′), 3.20 (1H, dd, *J* = 8.5, 8.0 Hz, H-2′), 3.03 (1H, quint, *J* = 6.0 Hz, H-7). ^{13}C NMR (125 MHz, CD_3OD): δ 135.1 (C-1), 113.6 (C-2), 149.3 (C-3), 150.3 (C-4), 121.7 (C-5), 113.1 (C-6), 49.0 (C-7), 72.2 (C-8), 64.8 (C-9), 56.5 (OMe × 2), 104.7 (C-1′), 75.1 (C-2′), 78.2 (C-3′), 71.7 (C-4′), 78.0 (C-5′), 62.8 (C-6′). LR-ESIMS: *m*/*z* 397 $[M + Na]^+$, 771 $[2M + Na]^+$, 409 $[M + Cl]^-$, 783 $[2M + Cl]^-$; HR-ESIMS: *m*/*z* 397.1452 $[M + Na]^+$ (calcd. for $C_{17}H_{26}O_9Na$: 397.1469, Δ = + 4.4 ppm).

Eudesman-3α,4α,11-triol-11-O-β-D-glucopyranoside (**4**): White amorphous powder; $[\alpha]_D^{24}$ − 3.3 (*c* 0.33, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.46 (1H, d, *J* = 7.7 Hz, H-1′), 3.85 (1H, brd, *J* = 11.9 Hz, H_a-6′), 3.59 (1H, dd, *J* = 11.9, 5.6 Hz, H_b-6′), 3.51 (1H, brs, H-3), 3.35 (1H, dd, *J* = 8.9, 8.0 Hz, H-3′), 3.22 (2H, m, H-4′, H-5′), 3.13 (1H, dd, *J* = 8.9, 7.7 Hz, H-2′), 2.14 (1H, brd, *J* = 12.3 Hz, H_a-6), 1.84 (1H, brdd, *J* = 14.5, 13.8 Hz, H_a-2), 1.68 (1H, brd, *J* = 14.5 Hz, H_b-2), 1.59 (1H, m, H_a-8), 1.57 (1H, dd, overlapped, H-5), 1.55 (1H, m, H-7), 1.53 (1H, m, H_a-1), 1.41 (1H, brd, *J* = 11.7 Hz, H_a-9), 1.28 (1H, m, H_b-8), 1.21 (1H, m, H_b-9), 1.05 (1H, m, H_b-1), 1.02 (1H, ddd, overlapped, H_b-6), 1.26, 1.21, 1.08, 0.91 (each 3H, s, Me-13, Me-12, Me-14, Me-15, respectively). ^{13}C NMR (125 MHz, CD_3OD): δ 34.9 (C-1), 26.7 (C-2), 75.7 (C-3), 74.4 (C-4), 48.1 (C-5), 22.1 (C-6), 49.6 (C-7), 24.3 (C-8), 45.9 (C-9), 35.2 (C-10), 81.6 (C-11), 23.5 (C-12), 25.5 (C-13), 21.7 (C-14), 18.9 (C-15), 98.4 (C-1′), 75.3 (C-2′), 78.2 (C-3′), 72.0 (C-4′), 77.7 (C-5′), 63.0 (C-6′). LR-ESIMS: *m*/*z* 441 $[M + Na]^+$, 859 $[2M + Na]^+$, 453 $[M + Cl]^-$; HR-ESIMS: *m*/*z* 441.2468 $[M + Na]^+$ (calcd. for $C_{21}H_{38}O_8Na$: 441.2459, Δ = − 2.1 ppm).

Marmesin-11-O-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**5**): White amorphous powder; $[\alpha]_D^{24}$ − 33.7 (*c* 0.43, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (1H, d, *J* = 9.5 Hz, H-4), 7.49 (1H, s, H-5), 6.82 (1H, s, H-8), 6.21 (1H, d, *J* = 9.5 Hz, H-3), 4.86 (1H, dd, *J* = 9.0, 8.5 Hz, H-10), 4.42 (1H, d, *J* = 8.0 Hz, H-1″), 4.22 (1H, d, *J* = 7.5 Hz, H-1′), 3.79 (1H, brd, *J* = 11.0 Hz, H_a-6″), 3.67 (1H, brd, *J* = 11.5 Hz, H_a-6′), 3.52 (1H, dd, *J* = 11.0, 6.5 Hz, H_b-6″), 3.43 (1H, dd, *J* = 11.5, 4.5 Hz, H_b-6′), 3.29 (1H, dd, *J* = 16.0, 8.0 Hz, H_a- 9), 3.19 (1H, dd, *J* = 16.0, 8.5 Hz, H_b- 9), 3.15 (2H, m, H-3′, H-3″), 3.04 (4H, m, H-4′, H-5′, H-4″, H-5″), 2.93 (1H, dd, *J* = 8.0, 8.0 Hz, H-2″), 2.87 (1H, dd, *J* = 8.0, 7.5 Hz, H-2′), 1.25 (3H, s, Me-12), 1.22 (3H, s, Me-13). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.5 (C-2), 111.3 (C-3), 144.7 (C-4), 112.2 (C-4a), 124.0 (C-5), 125.6 (C-6), 163.1 (C-7), 96.8 (C-8), 155.0 (C-8a), 28.9 (C-9), 90.0 (C-10), 77.1 (C-11), 22.9 (C-12), 21.8 (C-13), 97.2 (C-1′), 73.5 (C-2′), 76.7 (C-3′), 70.1 (C-4′), 75.6 (C-5′), 68.3 (C-6′), 103.2 (C-1″), 73.4 (C-2″), 76.9 (C-3″), 69.9 (C-4″), 76.6 (C-5″), 61.1 (C-6″). LR-ESIMS: *m*/*z* 593

$[M + Na]^+$, 1163 $[2M + Na]^+$, 569 $[M - H]^-$, 1139 $[2M - H]^-$; HR-ESIMS: *m*/*z* 593.1821 $[M + Na]^+$ (calcd. for $C_{26}H_{34}O_{14}Na$: 593.1841, $\Delta = +3.3$ ppm).

Pregn-5-en-3β-ol-20-one-3-O-bis-β-D-glucopyranosyl-(1 → 2,1 → 6)-β-D-glucopyranoside (**6**): White amorphous powder; ^1H NMR and ^{13}C NMR data, see ▶ **Table 1**. LR-ESIMS: *m*/*z* = 825 $[M + Na]^+$; HR-ESIMS: *m*/*z* 825.3831 $[M + Na]^+$ (calcd. for $C_{39}H_{62}O_{17}Na$: 825.3879, $\Delta = +5.8$ ppm).

Supporting information

The extraction and isolation of compounds **1**–**18**, details on the acid hydrolysis of compounds **1**, **2**, **5**, **9**, **10**, sugar analysis, acetylation of **3**, full assignment of NMR data for compounds **3a**, **7**, **11**, and the NMR and MS spectra of compounds **1**–**7** and **11** are available as Supporting Information.

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Conflict of Interest

We declare that we have no conflict of interest.

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