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Bioactive Dammarane-Type Saponins from *Operculina turpethum*

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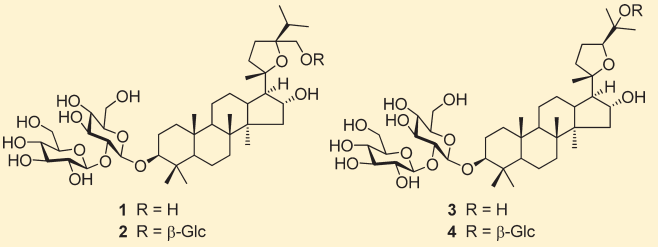
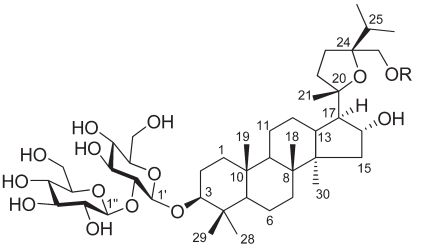
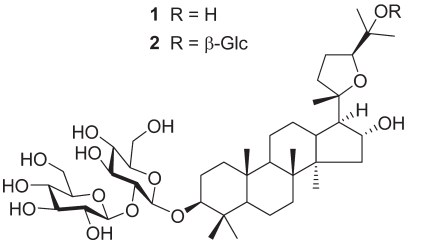
Supporting Information

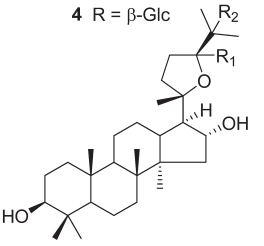
ABSTRACT: Four new dammarane-type saponins, operculinosides A–D (**1**–**4**), were isolated from the aerial parts of *Operculina turpethum*, of which **1** and **2** are the first two dammarane-type triterpenoids having an oxymethyl group at C-24. Their structures were determined by spectroscopic analysis and acid hydrolysis. The absolute configuration of operculinoside A (**1**) was confirmed by X-ray crystallography. Compounds **1** and **3** showed significant protective activities against D-galactosamine-induced toxicity in L-02 human hepatic cells.



Operculina is a genus of plants belonging to the family Convolvulaceae. The genus consists of about 25 species occurring in tropical areas worldwide.[1] *Operculina turpethum* (L.) Silva Manso (syn. *Ipomoea turpethum*), a perennial herbaceous vine, is distributed in southern mainland China, Southeast Asia, South Asia, the Pacific Islands, and Australia.[1] It is used as a Chinese medicine for the treatment of edema and as an astringent.[2] An extractive of its roots has been found to exhibit a wide range of bioactivities including hepatoprotective and anticlastogenic activities.[3] Resin glycosides were previously reported from this species,[4] which are known to be the constituents responsible for the purgative effect of many herbs in the family Convolvulaceae.[5,6] In a continuation of phytochemical studies on the medicinal plants growing in southern China, this plant was investigated and four new dammarane-type triterpenoid saponins, operculinosides A–D (**1**–**4**), have been isolated and their in vitro hepatoprotective activity was assessed. Herein, we report the isolation, structure elucidation, and in vitro hepatoprotective activity of these compounds.







RESULTS AND DISCUSSION

Operculinoside A (**1**) was obtained as colorless plates. Its molecular formula, $C_{43}H_{74}O_{14}$, was determined from NMR (^{1}H, ^{13}C, and DEPT) and ESIMS data and confirmed by HRESIMS. The ^{1}H NMR spectrum (Table 1) displayed readily recognizable signals for six tertiary methyl groups [δ 0.89 (s, H_3-18), 0.78 (s, H_3-19), 1.26 (s, H_3-21), 1.28 (s, H_3-28), 1.12 (s, H_3-29), and 1.32 (s, H_3-30)], two secondary methyl groups [0.97 (d, J = 7.0 Hz, H_3-26) and 0.99 (d, J = 7.0 Hz, H_3-27)], a tertiary oxymethylene [δ 3.63 and 3.84 (each 1H, d, J = 11.4 Hz, H_2-31)], two oxygenated methines [δ 3.33 (dd, J = 11.8, 4.5

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Table 1. ^{1}H NMR Data of Compounds 1−4 in Pyridine-d_5[a]

position	1	2	3	4
1α	0.75, m	0.75, m	0.73, m	0.74, m
1β	1.49, m	1.46, m	1.47, m	1.45, m
2α	2.24, m	2.23, m	2.24, dd (13.8, 3.2)	2.23, m
2β	1.85, m	1.84, m	1.83, m	1.84, m
3	3.33, dd (11.8, 4.5)	3.27, dd (11.7, 4.4)	3.30, dd (12.0, 4.4)	3.28, dd (11.8, 4.3)
5	0.65, dd (11.8, 1.3)	0.64, brd (11.7)	0.66, brd (11.6)	0.64, brd (11.5)
6α	1.45, m	1.43, m	1.44, m	1.44, m
6β	1.34, m	1.32, m	1.31, m	1.32, m
7α	1.50, m	1.42, m	1.52, m	1.48, m
7β	1.13, m	1.07, m	1.13, m	1.13, m
9	1.26, m	1.25, m	1.26, m	1.25, m
11α	1.43, m	1.41, m	1.42, m	1.42, m
11β	1.13, m	1.10, m	1.13, m	1.13, m
12α	1.60, m	1.53, m	1.62, m	1.63, m
12β	1.30, m	1.35, m	1.33, m	1.33, m
13	1.59, m	1.54, m	1.60, m	1.62, m
15α	1.43, dd (13.1, 1.3)	1.36, m	1.44, m	1.36, m
15β	2.02, dd (13.1, 9.2)	2.01, dd (13.5, 10.0)	2.02, dd (13.1, 9.0)	2.02, m
16	4.63, ddd (9.2, 5.5, 1.3)	4.42, m	4.68, br t (6.7)	4.46, m
17	2.19, m	2.13, dd (12.3, 6.1)	2.12, dd (11.7, 5.2)	2.11, dd (12.2, 6.1)
18	0.89, s	0.85, s	0.89, s	0.88, s
19	0.78, s	0.76, s	0.79, s	0.76, s
21	1.26, s	1.17, s	1.31, s	1.22, s
22α	1.82, m	1.75, m	1.70, m	1.66, m
22β	1.93, m	1.87, m	1.94, m	1.80, m
23α	1.82, m	1.74, m	1.83, m	1.84, m
23β	1.96, m	1.84, m	2.33, m	2.23, m
24			3.84, dd (9.9, 5.6)	4.03, t (7.1)
25	2.19, m	2.26, m		
26	0.97, d (7.0)	0.92, d (6.9)	1.23, s	1.43, s
27	0.99, d (7.0)	0.91, d (6.9)	1.52, s	1.43, s
28	1.28, s	1.27, s	1.29, s	1.24, s
29	1.12, s	1.11, s	1.10, s	1.10, s
30	1.32, s	1.29, s	1.29, s	1.25, s
31	3.63, d (11.4)	3.80, d (9.7)		
	3.84, d (11.4)	4.07, d (9.7)		
1′	4.94, d (7.6)	4.94, d (7.7)	4.95, d (7.6)	4.93, d (7.6)
2′	4.25, dd (9.2, 7.6)	4.28, dd (9.0, 7.7)	4.25, (9.0, 7.6)	4.25, dd (9.0, 7.6)
3′	4.32, t (9.2)	4.36, t (9.0)	4.33, t (9.0)	4.33, t (9.0)
4′	4.15, t (9.5)	4.17, t (9.0)	4.15, t (9.0)	4.15, t (9.0)
5′	3.95, ddd (9.5, 5.6, 2.4)	3.97, ddd (9.0, 5.6, 2.0)	3.92, ddd (9.0, 5.6, 2.0)	3.86−3.97, m
6′	4.36, dd (12.0, 5.6)	4.38, dd (11.6, 4.0)	4.36, dd (11.8, 5.6)	4.36, dd (11.8, 4.8)
	4.58, dd (12.0, 2.4)	4.59, dd (11.6, 2.0)	4.58, dd (11.8, 2.0)	4.58, dd (11.8, 2.4)
1″	5.38, d (7.8)	5.38, d (7.6)	5.38, d (7.8)	5.37, d (7.7)
2″	4.13, dd (9.3, 7.8)	4.15, dd (9.0, 7.6)	4.13, dd (9.0, 7.8)	4.13, dd (9.0, 7.7)
3″	4.24, t (9.1)	4.26, t (9.0)	4.24, t (9.0)	4.23, t (9.0)
4″	4.34, t (9.3)	4.30, t (9.0)	4.34, t (9.0)	4.30, t (9.1)
5″	3.92, ddd (9.6, 4.0, 3.1)	3.94, m	3.92, m	3.86−3.97, m
6″	4.46, dd (11.5, 4.0)	4.48, dd (11.6, 4.0)	4.46, dd (11.6, 4.0)	4.44−4.50, m
	4. 49, dd (11.5, 3.1)	4.52, dd (11.6, 3.0)	4.49, dd (11.6, 3.0)	4.44−4.50, m
1‴		4.80, d (7.7)		5.03, d (7.7)
2‴		4.02, dd (9.0, 7.7)		3.97, dd (9.0, 7.7)
3‴		4.23, t (9.0)		4.24, t (9.0)
4‴		4.28, t (9.0)		4.18, t (9.0)

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Table 1. Continued

position	1	2	3	4
5‴		3.89, m		3.86−3.97, m
6‴		4.38, dd (11.6, 4.0)		4.31, m
		4.47, dd (11.6, 2.0)		4.44−4.50, m

[a] Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses.

Hz, H-3) and 4.63 (ddd, J = 9.2, 5.5, 1.3 Hz, H-16)], and two anomeric protons [δ 4.94 (d, J = 7.6 Hz, H-1′) and 5.38 (d, J = 7.8 Hz, H-1″)]. The ^{13}C NMR spectrum (Table 2) gave 43 signals, of which 12 carbons were assignable to a disaccharide moiety and 31 carbons to the aglycone moiety. On the basis of DEPT and HSQC spectra, the carbons for the aglycone were identified as eight methyls, 10 methylenes, seven methines, and six quaternary carbons, of which a methylene [δ 63.2 (C-31)], two methines [δ 88.8 (C-3) and 73.3 (C-16)], and two quaternary carbons [δ 84.7 (C-20) and 88.2 (C-24)] are oxygenated. These NMR data suggested **1** as a C_{31}-dammarane-type saponin. Comparison of the ^{1}H and ^{13}C NMR data with those of closely related analogues[7−9] showed that **1** has the same sugar moiety as that in gynoside B[8] and an identical tetracyclic nucleus to that of triloculariol A 3-glucoside.[7] The α-orientation of the hydroxy group at C-16 was confirmed by an NOE interaction between H-16 and H_3-18 observed in the NOESY spectrum and the proton coupling pattern of H-16, which was different from those of the 16β-epimers.[7] However, the side chain at C-17 was found to be different from those of other analogues. The characteristic quaternary carbons at δ 84.7 (C-20) and 88.2 (C-24)[9] and the tertiary oxymethylene protons (δ_H 3.63 and 3.84) and carbon (δ_C 63.2) were indicative of the presence of a 20,24-epoxy-24-hydroxymethyl function in the side chain. This was supported by long-range correlations from both H_3-26 and H_3-27 to C-24, from H_3-21 and H-16 to C-20, and from the tertiary oxymethylene protons (H_2-31) to C-23, C-24, and C-25 in the HMBC spectrum. In order to determine the absolute configuration of the side chain, a single crystal was obtained by recrystallization in MeOH and subjected to X-ray diffraction analysis using Cu Kα radiation. The result (Figure 1) showed that both C-20 and C-24 have *S* configurations. Therefore, the structure of **1** was established as (20*S*,24*S*)-20,24-epoxy-3β,16α-dihydroxy-24-hydroxymethyldammarane 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Operculinoside B (**2**), obtained as an amorphous solid, was determined to have a molecular formula of $C_{49}H_{84}O_{19}$ by combined analysis of its HRESIMS and NMR data. By comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of **1**, this compound was found to have the same triterpenoid aglycone and disaccharide moiety as those in **1**, but an additional β-glucopyranosyl group. This was confirmed by acid hydrolysis of **2**, which yielded the same aglycone (**1a**) as that of **1** and D-glucose. The additional glucose moiety was deduced to be bound at C-31 via a glycosidic linkage from the downfield shift of the ^{13}C NMR resonance of C-31 relative to that in **1** (δ 73.0 in **2** vs δ 63.2 in **1**) and from long-range correlations of H-1‴ [δ 4.80 (d, J = 7.7 Hz)] with C-31 (δ 73.0) and H_2-31 [δ 3.80 and 4.07 (each 1H, d, J = 9.7 Hz)] with C-1‴ (δ 104.0) observed in the HMBC spectrum. Therefore, the structure of **2** was determined as (20*S*,24*S*)-20,24-epoxy-24-β-D-glucopyranosyloxymethyl-3β,16α-dihydroxydammarane 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Operculinoside C (**3**) gave a molecular formula of $C_{42}H_{72}O_{14}$ as determined from the ESIMS, HRESIMS, and NMR (^{1}H, ^{13}C, and DEPT) data. Analysis of its ^{1}H and ^{13}C NMR spectra in combination with the COSY, HSQC, and HMBC spectra indicated that **3** has a structure closely related to that of **1**, except for the side chain at C-17. Comparison of the ^{1}H and ^{13}C NMR data showed that the tertiary oxymethylene for C-31 in **1** is absent in **3**, with the oxygenated quaternary carbon for C-24 in **1** also replaced by an oxymethine [δ_H 3.84 (1H, dd, J = 9.9, 5.6 Hz), δ_C 85.6]. In addition, C-26 and C-27 appeared as tertiary methyl signals [δ_H 1.23 (3H, s), δ_C 26.2; δ_H 1.52 (3H, s), δ_C 28.8] in **3** instead of secondary methyls in **1**, and C-25 was an oxygenated quaternary carbon (δ_C 69.9) rather than a methine in **1**. These observations indicated that **3** has an ocotillol-type side chain at C-17.[8] The NOE cross-peak between H-24 and H_3-21 was not observed in the NOESY spectrum, and H-24 appeared as a double doublet at δ 3.84 (dd, J = 9.9, 5.6 Hz), suggesting that H-24 and H_3-21 are in a *trans* relationship.[10] Considering the co-occurrence of this compound with **1** and **2**, C-20 could be assigned with the *S* configuration on biogenetic grounds. Accordingly, C-24 was also assigned as *S* on the basis of the relative configuration of the tetrahydrofuran ring. Thus, **3** was determined to be (20*S*,24*S*)-20,24-epoxy-3β,16α,25-trihydroxydammarane 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside. The absolute configuration (20*S*, 24*S*) of the side chain in this compound is identical with that in gynoside A, a compound for which the structure was confirmed by X-ray analysis.[8]

Operculinoside D (**4**), an amorphous solid, was determined to have the molecular formula $C_{48}H_{82}O_{19}$ by combined analysis of HRESIMS and NMR data. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were similar to those of compound **3** except for the presence of proton and carbon resonances for an additional β-glucopyranose moiety. The downfield-shifted carbon signal of C-25 (δ 78.6) in the ^{13}C NMR spectrum and the presence of a correlation between H-1‴ [δ 5.03 (1H, d, J = 7.7 Hz)] and C-25 in the HMBC spectrum indicated that the third glucose moiety is attached to C-25. The H-24 proton signal appeared as a triplet at δ 4.03 (J = 7.1 Hz) in the ^{1}H NMR spectrum, in accordance with a *cis* relationship between Me-20 and H-24,[10] with a NOE interaction between H-24 and H_3-21 being absent in the NOESY spectrum. Acid hydrolysis of **3** and **4** yielded the same products, aglycone **3a** and D-glucose, indicating the aglycone of **4** to be identical with that of **3**. Therefore, the structure of compound **4** was determined as (20*S*,24*S*)-20,24-epoxy-25-β-D-glucopyranosyloxy-3β,16α-dihydroxydammarane 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

The occurrence of dammarane-type triterpenoids in the family Convolvulaceae was previously only reported from *Argyreia capitata*.[11] Compounds **1** and **2** are the first two dammarane-type triterpenoids having an oxymethyl group substituted at C-24.

The inhibitory effects of compounds **1**−**4** on D-galactosamine (D-GalN)-induced cytotoxicity in L-02 human embryo liver cells were assessed, because the hepatoprotective activity was reported

Table 2. ^{13}C NMR Data of Compounds 1−4, 1a, and 3a[a]

position	1	2	3	4	1a	3a
1	39.0, CH_2	39.0, CH_2	39.1, CH_2	39.0, CH_2	38.9, CH_2	38.9, CH_2
2	26.6, CH_2	26.7, CH_2	26.7, CH_2	26.7, CH_2	27.3, CH_2	27.3, CH_2
3	88.8, CH	88.9, CH	88.9, CH	88.8, CH	78.8, CH	78.8, CH
4	39.5, C	39.6, C	39.6, C	39.6, C	39.3, C	39.0, C
5	56.1, CH	56.2, CH	56.2, CH	56.1, CH	55.7, CH	55.7, CH
6	18.2, CH_2	18.3, CH_2	18.3, CH_2	18.3, CH_2	18.2, CH_2	18.2, CH_2
7	35.4, CH_2	35.4, CH_2	35.5, CH_2	35.4, CH_2	35.2, CH_2	35.2, CH_2
8	40.1, C	40.2, C	40.2, C	40.2, C	40.0, C	40.1, C
9	50.1, CH	50.2, CH	50.1, CH	50.2, CH	50.0, CH	50.0, CH
10	36.7, C	36.8, C	36.8, C	36.8, C	37.1, C	37.1, C
11	21.2, CH_2	21.2, CH_2	21.3, CH_2	21.3, CH_2	21.1, CH_2	21.2, CH_2
12	25.8, CH_2	25.4, CH_2	26.3, CH_2	25.8, CH_2	25.3, CH_2	25.7, CH_2
13	43.1, CH	42.9, CH	43.1, CH	42.3, CH	42.8, CH	42.7, CH
14	48.6, C	48.4, C	48.9, C	48.7, C	48.2, C	48.5, C
15	41.7, CH_2	40.2, CH_2	41.9, CH_2	40.7, CH_2	41.4, CH_2	41.2, CH_2
16	73.3, CH	73.9, CH	73.4, CH	74.0, CH	74.1, CH	74.2, CH
17	62.0, CH	62.5, CH	59.3, CH	60.2, CH	60.8, CH	58.9, CH
18	15.7, CH_3	15.9, CH_3	15.7, CH_3	15.9, CH_3	15.9, CH_3	15.8, CH_3
19	16.1, CH_3	16.1, CH_3	16.2, CH_3	16.1, CH_3	16.0, CH_3	16.0, CH_3
20	84.7, C	86.1, C	84.4, C	85.5, C	84.9, C	84.7, C
21	23.9, CH_3	22.8, CH_3	24.2, CH_3	23.8, CH_3	23.3, CH_3	22.9, CH_3
22	39.3, CH_2	39.4, CH_2	39.1, CH_2	38.7, CH_2	39.3, CH_2	39.0, CH_2
23	28.2, CH_2	29.2, CH_2	26.4, CH_2	26.9, CH_2	28.5, CH_2	26.1, CH_2
24	88.2, C	87.4, C	85.6, CH	83.6, CH	88.1, C	84.7, CH
25	32.5, CH	32.4, CH	69.9, C	78.6, C	32.3, CH	70.5, C
26	17.8, CH_3	18.4, CH_3	26.2, CH_3	22.7, CH_3	17.7, CH_3	25.4, CH_3
27	18.0, CH_3	18.0, CH_3	28.8, CH_3	22.3, CH_3	18.0, CH_3	28.2, CH_3
28	27.9, CH_3	28.0, CH_3	28.0, CH_3	28.0, CH_3	28.0, CH_3	28.0, CH_3
29	16.4, CH_3	16.5, CH_3	16.5, CH_3	16.5, CH_3	15.4, CH_3	15.4, CH_3
30	17.1, CH_3	17.5, CH_3	17.0, CH_3	18.0, CH_3	17.6, CH_3	17.5, CH_3
31	63.2, CH_2	73.0, CH_2			63.8, CH_2	
1′	104.9, CH	104.9, CH	105.0, CH	105.0, CH		
2′	83.1, CH	83.1, CH	83.3, CH	83.3, CH		
3′	78.1, CH	78.0, CH	78.2, CH	78.2, CH		
4′	71.4, CH	71.4, CH	71.5, CH	71.5, CH		
5′	77.8, CH	77.9, CH	78.0, CH	78.0, CH		
6′	62.6, CH_2	62.6, CH_2	62.6, CH_2	62.6, CH_2		
1″	105.8, CH	105.8, CH	105.9, CH	105.9, CH		
2″	76.8, CH	76.9, CH	77.0, CH	77.0, CH		
3″	77.7, CH	78.1, CH	78.2, CH	78.2, CH		
4″	71.4, CH	71.4, CH	69.9, CH	71.6, CH		
5″	78.0, CH	77.7, CH	77.8, CH	77.8, CH		
6″	62.5, CH_2	62.4, CH_2	62.7, CH_2	62.8, CH_2		
1‴		104.0, CH		98.5, CH		
2‴		74.9, CH		75.2, CH		
3‴		78.0, CH		78.0, CH		
4‴		70.7, CH		71.5, CH		
5‴		78.0, CH		78.4, CH		
6‴		62.0, CH_2		62.7, CH_2		

[a] Solvents: pyridine-d_5 for **1**−**4** and $CDCl_3$ for **1a** and **3a**.

earlier for a crude extract of *O. turpethum*.[3] The compounds were evaluated at concentrations of 3.13−12.5 μg/mL, using a previously described method.[12] As shown in Table 3, compound **3** exhibited more potent hepatoprotective activity than the

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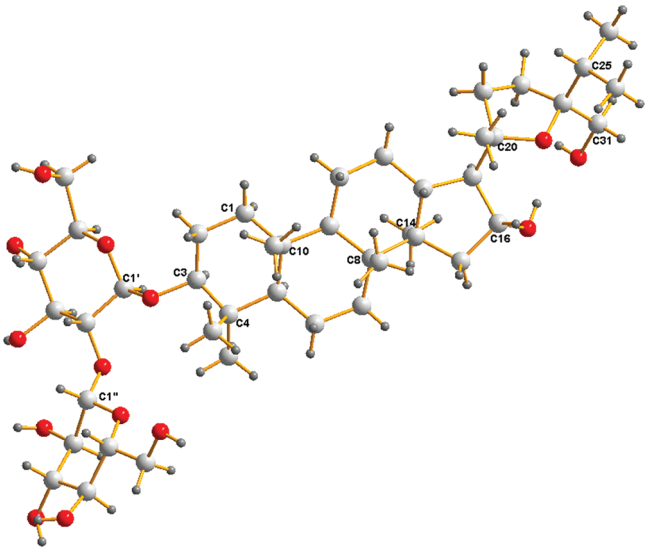

Figure 1. X-ray crystallographic structure of **1**.

Table 3. Protective Activity of Compounds 1−4 against D-GalN-Induced Toxicity in L-02 Cells[a]

compound	concentration (μg/mL)	OD_{570}	cell survival rate (% of blank)	inhibition (% of control)
blank		1.0 ± 0.067	100 ± 6.7	
control		0.66 ± 0.017	65.5 ± 1.7	0
1	12.5	0.82 ± 0.039[b]	81.6 ± 3.9[b]	46.7
	6.25	0.61 ± 0.045	60.4 ± 4.5	−14.7
	3.13	0.58 ± 0.008	57.6 ± 0.8	−22.8
2	12.5	0.64 ± 0.010	63.1 ± 1.0	−6.9
	6.25	0.70 ± 0.071	69.0 ± 7.1	10.4
	3.13	0.67 ± 0.031	66.7 ± 3.1	3.5
3	12.5	0.81 ± 0.124[b]	80.7 ± 12.3[b]	44.1
	6.25	0.90 ± 0.010[b]	89.5 ± 1.0[b]	69.5
	3.13	0.72 ± 0.007	71.3 ± 0.7	16.7
4	12.5	0.74 ± 0.014	73.1 ± 0.7	22.2
	6.25	0.68 ± 0.008	67.6 ± 0.8	6.3
	3.13	0.68 ± 0.020	67.4 ± 2.0	5.5
silybin[c]	6.25	0.76 ± 0.029[b]	75.2 ± 2.9[b]	28.2
	3.13	0.78 ± 0.039[b]	77.0 ± 3.9[b]	33.4
	1.56	0.69 ± 0.029	68.9 ± 2.9	10.1

[a] Data are expressed as means ± SD (n = 3). [b] $p < 0.05$, significantly different from the control by Student's t-test. [c] Positive control substance.

positive control, silybin.[13] The effect of compound **1** was dependent on concentration and exhibited 46.7% inhibition at 12.5 μg/mL, but appeared to enhance D-GalN-induced cytotoxicity at lower concentrations (−14.7% and −22.8% inhibitions at 6.25 and 3.13 μg/mL, respectively). The bisdesmoside **4**, a 25-*O*-β-D-glucoside of **3**, also showed some activity (22.2% inhibition) at the highest test concentration used. However, the bisdesmoside **2**, with an extra glucose residue at C-31 relative to **1**, was found to be inactive in this investigation. The activity profile of these compounds suggested that the additional glucose unit attached at the side chain may greatly affect the in vitro hepatoprotective activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Yanagimoto Seisakusho MD-S2 micromelting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH as solvent. The ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance-600 or a Bruker DRX-400 instrument using TMS as an internal standard. ESIMS were collected on an MDS SCIEX API 2000 LC/GC/MS instrument. HRESIMS data were obtained on an API QSTAR mass spectrometer. Preparative HPLC was performed with a Shimadzu LC-6A pump and a Shimadzu RID-10A refractive index detector using an XTerra prep MS C_{18} column (10 μm, 300 × 19 mm). For column chromatography, silica gel 60 (100−200 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), Develosil ODS (75 μm, Nomura Chemical Co., Ltd., Osaka, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used. For the in vitro hepatoprotective activity assay, silybin (99%, Tasly Pharmaceutical Co., Ltd.,Tianjin, People's Republic of China) was used as a positive control substance, which has recently been resolved into silybins A and B.[14]

Plant Material. *Operculina turpethum* was collected from Hengqin island, Zhuhai, Guangdong, People's Republic of China, in October 2008 and authenticated by Prof. Fuwu Xing, South China Botanical Garden, Chinese Academy of Sciences. A voucher specimen (743902) has been deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation. The powdered, dry aerial parts of *O. turpethum* (8.0 kg) were extracted with 95% EtOH (3 × 20 L, 48 h each) at room temperature. The extraction solution was concentrated under reduced pressure to give 1144 g of residue, which was suspended in water and then sequentially extracted with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH layer was evaporated under vacuum to yield a *n*-BuOH-soluble fraction (383 g). A portion (100 g) of this fraction was subjected to passage over a macroporus resin D101 column and eluted with H_2O and MeOH. The fraction (60 g) obtained by elution with MeOH was subjected to silica gel column chromatography eluted with a $CHCl_3$−MeOH mixture of increasing polarity (90:10 to 60:40), to yield 10 fractions (A−J). Fraction C (1.63 g), obtained on elution with $CHCl_3$−MeOH (80:20), was further applied to ODS column chromatography and eluted with MeOH−H_2O mixtures of decreasing polarities (70:30 to 90:10) to obtain four subfractions (C1−C4). Subfraction C2 (100 mg) was further separated by HPLC using 70% MeOH to afford **3** (31 mg). Subfraction C3 (200 mg) was separated by HPLC using 80% MeOH to provide **1** (68 mg). Fraction D (1.52 g), obtained on elution with $CHCl_3$−MeOH (80:20), was further subjected to ODS column chromatography using MeOH−H_2O (60:30 to 80:20) to obtain three subfractions (D1−D3). Subfraction D1 (253 mg) was purified by Sephadex LH-20 column chromatography using MeOH followed by HPLC using 65% MeOH to afford **4** (13 mg) and **2** (80 mg).

*Operculinoside A (**1**):* colorless plates (MeOH), mp 290−292 °C, $[\alpha]^{20}_D$ −1.0 (*c* 0.97, MeOH); ^{1}H NMR (600 MHz), see Table 1; ^{13}C NMR (150 MHz), see Table 2; positive ESIMS *m/z* 853 $[M + K]^+$, 837 $[M + Na]^+$; negative ESIMS *m/z* 813 $[M - H]^-$; HRESIMS *m/z* 873.5217 $[M + AcOH - H]^-$ (calcd for $C_{45}H_{77}O_{16}$, 873.5212).

X-ray crystal data of 1: $C_{43}H_{74}O_{14}$, M = 815.02, colorless plate, 0.3 × 0.2 × 0.08 mm^3, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 20.49390(10) Å, b = 29.4554(2) Å, c = 46.3360(3) Å, V = 27971.1(3) Å^3, Z = 4, D_c = 1.161 g/cm^3, F_{000} = 10656, goniometer Xcalibur, detector: Onyx (Nova), Cu Kα radiation, λ = 1.5418 Å, T = 150(2) K, $2\theta_{max}$ = 117.9°, 95 065 reflections collected, 40 132 unique (R_{int} = 0.0445). The structure was solved and refined using the programs SHELXS-97[15] and SHELXL-97,[16] respectively. The program X-Seed[17] was used as an interface to the SHELX programs and to prepare the figure. The program SQUEEZE was used to treat the disordered solvent molecules.[18]

Final GooF = 1.073, R1 = 0.1014, wR2 = 0.2683, *R* indices based on 28 494 reflections with $I > 2\sigma(I)$ (refinement on F^2), 3196 parameters, 3 restraints. *Lp* and absorption corrections were applied, $\mu = 0.701$ mm^{-1}. Absolute structure parameter Hooft = −0.09(7).[19] Crystallographic data for the structure of **1** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 836548. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Operculinoside B (***2***): amorphous powder, $[\alpha]^{20}_D$ −11.0 (*c* 0.79, MeOH); ^{1}H NMR (600 MHz), see Table 1; ^{13}C NMR (150 MHz), see Table 2; positive ESIMS *m/z* 1015 $[M + K]^+$, 999 $[M + Na]^+$; negative ESIMS *m/z* 975 $[M - H]^-$; HRESIMS *m/z* 1021.5601 $[M + AcOH - H]^-$ (calcd for $C_{50}H_{85}O_{21}$, 1021.5588).

Operculinoside C (***3***): colorless plates (MeOH), mp 287−289 °C, $[\alpha]^{20}_D$ +2.0 (*c* 0.29, MeOH); ^{1}H NMR (600 MHz), see Table 1; ^{13}C NMR (150 MHz), see Table 2; positive ESIMS *m/z* 839 $[M + K]^+$, 823 $[M + Na]^+$; negative ESIMS 799 $[M - H]^-$, 835 $[M + Cl]^-$; HRESIMS *m/z* 845.4860 $[M + AcOH - H]^-$ (calcd for $C_{43}H_{73}O_{16}$, 845.4904).

Operculinoside D (***4***): amorphous powder, $[\alpha]^{20}_D$ −7.0 (*c* 0.33, MeOH); ^{1}H NMR (600 MHz), see Table 1; ^{13}C NMR (150 MHz), see Table 2; positive ESIMS *m/z* 1001 $[M + K]^+$, 985 $[M + Na]^+$; negative ESIMS 961 $[M - H]^-$, 997 $[M + Cl]^-$; HRESIMS *m/z* 997.5063 $[M + Cl]^-$ (calcd for $C_{48}H_{82}O_{19}Cl$, 997.5144).

Acid Hydrolysis of Compounds 1−4. Operculinoside A (**1**) (32 mg) in 50 mL of 1 M HCl (1,4-dioxane−H_2O, 1:1) was heated under reflux for 8 h. After removal of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble portion was evaporated and subjected to ODS column chromatography using 90% MeOH to yield 11.5 mg of the triterpene **1a**. The water layer was neutralized with 5% NaOH and desalted (Sephadex LH-20, MeOH) to afford a sugar residue (6 mg). The sugar was confirmed to be D-glucose by comparison with an authentic sample on TLC [Merck Kieselgel 60GF254, EtOAc−MeOH−H_2O−AcOH (6.5:2.0:1.5:1.5), R_f = 0.40] and by measurement of its optical rotation ($[\alpha]^{20}_D$ + 69, *c* 0.55, H_2O). By the same method, **1a** (13.5 mg) was also obtained from **2** (50 mg), and **3a** (4.5 and 8.6 mg) was obtained from **3** (9 mg) and **4** (22 mg), respectively. The saccharides were all identified as D-glucose for **2**−**4**.

Compound ***1a***: amorphous powder, $[\alpha]^{20}_D$ +2.0 (*c* 0.29, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 (1H, m, H-16), 3.58 and 3.36 (each 1H, d, *J* = 11.5 Hz, H_2-31), 3.20 (1H, dd, *J* = 11.0, 4.7 Hz, H-3), 1.13, 1.10, 0.97, 0.91, 0.82, and 0.77 (each 3H, s, H_3-21, -28, -30, -29, -18, and -19), 0.91 (3H, d, *J* = 6.8 Hz, H-26), 0.90 (3H, d, *J* = 6.8 Hz, H-27); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; positive ESIMS *m/z* 491 $[M + H]^+$, 513 $[M + Na]^+$; negative ESIMS *m/z* 489 $[M - H]^-$, 525 $[M + Cl]^-$.

Compound ***3a***: amorphous powder, $[\alpha]^{20}_D$ +3.0 (*c* 0.10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (1H, m, H-16), 3.73 (1H, dd, *J* = 9.4, 5.8 Hz, H-24), 3.20 (1H, dd, *J* = 11.1, 4.7 Hz, H-3), 1.26, 1.14, 1.10, 1.09, 0.97, 0.91, 0.82, and 0.77 (each 3H, s, H_3-26, -21, -28, -27, -30, -29, -18, and -19); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; positive ESIMS *m/z* 499 $[M + Na]^+$; negative ESIMS *m/z* 475 $[M - H]^-$, 511 $[M + Cl]^-$.

Cell Culture Hepatoprotective Activity. Before examination for in vitro hepatoprotective activity, the cytotoxicity of the test compounds against the host cells, human embryo liver L-02 cells (CAS Shanghai Cell Bank), was assessed using a MTT colorimetric method.[20] Briefly, L-02 cells, maintained in RPMI-1640 medium plus 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin, and 0.3 mg/mL L-glutamine, were seeded into wells of 96-well microtiter plates at 2×10^4 cell/mL in 100 μL of culture medium and cultured for 24 h in a humidified atmosphere with 5% CO_2 at 37 °C. The medium in each well was removed. Serial dilution of test compounds in DMSO (1 mg/mL) was made in culture medium, and each serial solution (100 μL) was added to a well. The plates were incubated for 48 h. Then, 10 μL of 5 mg/mL MTT solution was added and incubated for further 4 h. The supernatant was removed, and 100 μL of DMSO was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA) at a wavelength of 570 nm. Cell viability was calculated according to the following formula: cell viability (%) = $OD_{(compound)}/OD_{(control)} \times 100$, and maximum nontoxic concentrations for compounds **1**−**4** and silybin (positive control) were determined to be 12.5, 100, 50, 100, and 6.25 μg/mL, respectively.

Next, the protective activity against D-GalN (Sigma)-induced cytotoxicity in L-02 cells was examined at concentrations of 3.13−12.5 μg/mL using the method described by Li et al.,[12] with minor modifications. Briefly, L-02 cells in 96-well microtiter plates were cultured for 24 h as described above. The medium in the wells was replaced by fresh medium containing the test compounds and silybin and incubated for 1 h. The cultured cells were then exposed to 5 mM D-GalN and cultured for 48 h. The medium without D-GalN and the test compounds was used as a blank, and that containing only D-GalN as a control. Cell viability was detected using the MTT colorimetric method as described above. Inhibition of compounds against D-GalN-induced cytotoxicity was obtained by the following formula: inhibition (%) = $[(OD_{(compound)} - OD_{(control)})/(OD_{(blank)} - OD_{(control)})] \times 100$. The activity data are shown in Table 3.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR spectra of compounds **1**−**4**, **1a**, and **3a**; HRESIMS of compounds **1**−**4**. These data are available free of charge via the Internet at http://pubs.acs.org.

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