Quadranosides I−V, New Triterpene Glucosides from the Seeds of *Combretum quadrangulare*

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Five new triterpene glucosides, quadranosides I−V (**1**−**5**), have been isolated from a MeOH extract of the seeds of *Combretum quadrangulare*, together with 13 known compounds. The structures of compounds **1**−**5** were elucidated on the basis of spectroscopic analysis. Among the new triterpene glucosides, three compounds (**1**, **2**, **5**) showed significant hepatoprotective effects against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

Combretum species (Combretaceae) are widely used in folk medicine for the treatment of hepatitis, malaria, respiratory infections, and cancer in different parts of Asia and Africa.[1] *Combretum quadrangulare* is a tree indigenous to eastern Asia that is commonly known as "Tram bau" in Vietnam. The seeds, leaves, and stem bark of the plant have been used in Vietnamese folk medicine as an antipyretic, antidysenteric, and antihepatitis agent. The seeds are administered orally together with ripe bananas as an anthelmintic for ascariasis and oxyuriasis.[2] Previous chemical investigations have been undertaken only on the leaves and flowers of *C. quadrangulare*.[3−5] In our continuing study on hepatoprotective natural products,[6] it was found that a MeOH extract of the seeds of *C. quadrangulare* exhibited potent hepatoprotective activity on D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes. Purification of the MeOH extract by passage over Si gel followed by preparative TLC has afforded five new triterpene glucosides, quadranosides I−V (**1**−**5**, Chart 1), along with 13 known compounds (**13**−**18**). In this paper, we report the isolation and structure elucidation of these new triterpene glucosides together with their hepatoprotective activity.

Results and Discussion

The dried seeds of *C. quadrangulare* were extracted with MeOH, and evaporation under reduced pressure yielded a light brown MeOH extract, which showed a potent hepatoprotective effect on D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes (43.3% inhibition of cell death at 100 μg/mL). The MeOH extract was then subjected to column chromatography over Sephadex LH-20, Cosmosil 75C_{18}-OPN, and Si gel followed by preparative TLC, to afford five new triterpene glucosides (**1**−**5**), together with 13 known compounds, 19α-hydroxyasiatic acid (**6**),[7] nigaichigoside F1 (**7**),[7] arjungenin (**8**),[8] arjunglucoside I (**9**),[8] pinfaensin (**10**),[9] 2α,3β,23-trihydroxyurs-12,19-dien-28-oic acid β-D-glucopyranosyl ester (**11**),[10] 5-methoxy-(−)-isolariciresinol (**12**),[11] 5-methoxy-9-β-xylopyranosyl-(−)-isolariciresinol (**13**),[11] (+)-gallocatechin (**14**),[12] (−)-epicatechin (**15**),[12] β-sitosterol glucoside (**16**), gallic acid (**17**), and methyl gallate (**18**).

Quadranoside I (**1**) was isolated as a colorless amorphous solid. The molecular ion peak at *m*/*z* 673.3923 in its HRFABMS suggested the molecular formula to be $C_{36}H_{58}O_{10}$. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}), carbonyl (1720 cm^{-1}), and olefinic (1640 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** displayed signals corresponding to six tertiary methyls (δ_H 1.04, 1.44, 1.59,1.74, 1.76, 1.78), two exo-olefinic protons (δ_H 4.79, 4.89), and oxygenated methine and methylene protons ascribable to a sugar unit. The ^{13}C NMR spectrum, on the other hand, showed 36 carbon signals including 6 primary, 10 secondary, 13 tertiary, and 7 quaternary carbons, suggesting **1** to be a triterpene monoglycoside. The ^{1}H and ^{13}C NMR data (Table 1), assigned to the aglycon moiety from its ^{1}H−^{1}H COSY and FG-pulsed HMQC spectra, suggested that the aglycon is a lupane-type triterpene bearing three hydroxyls and a carboxyl group (C-28).[8] Two oxymethine protons at δ_H 3.41 and 4.27 showed correlations in the ^{1}H−^{1}H COSY spectrum, suggesting their vicinal arrangement. Furthermore, both of the protons had long-range correlations, in the FG-pulsed HMBC spectrum, with a quaternary carbon at δ_C 40.8 assigned to C-4. This indicated that the position of the two hydroxyl groups should be at C-2 and C-3. Similarly, the position of the third hydroxyl group was determined to be at C-6 from the ^{1}H−^{1}H COSY and the FG-pulsed HMBC spectra (Table 1). The sugar moiety of **1** was determined to be a glucose unit based on the coupling constants of each proton and the ^{13}C NMR chemical shifts (δ_C 62.2, 71.2, 74.3, 78.8, 79.4, 95.5). The chemical shifts of the anomeric proton (δ_H 6.40, d, J = 7.9 Hz) and carbon (δ_C 95.5) revealed that the glucose was attached to the carboxyl group (C-28). This was confirmed by a long-range correlation between the anomeric proton and the carboxyl carbon (δ_C 176.5) in the FG-pulsed HMBC spectrum. Accordingly, the planar structure of quadranoside I was determined as **1**.

The stereochemistry of **1** was determined by analysis of its coupling constants and ROESY data. The coupling constant (9.5 Hz) between H-3 and H-2 indicated the hydroxyl groups to have a 2α,3β-orientation, which was further supported by the ROESY correlation between H-3 and H-5 (Figure 1). The broad singlet nature of H-6 suggested the hydroxyl group at C-6 should be β-oriented, which was further supported by the intense cross-peak between H-6 and H_3-23 in the ROESY spectrum. Thus, the

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Chart 1

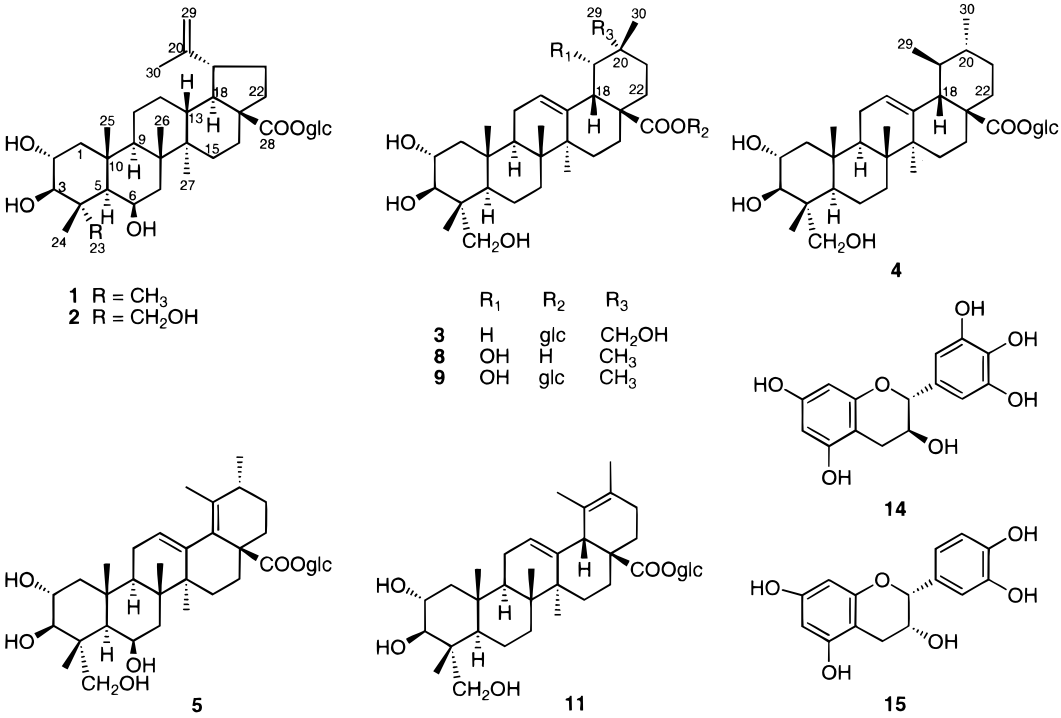

Table 1. ^{1}H and ^{13}C NMR Data and HMBC Correlations of **1** and **2** in C_5D_5N[a]

	1			**2**		
position	^{13}C	^{1}H	HMBC[b]	^{13}C	^{1}H	HMBC[b]
1	50.4	1.41 m, 2.37 dd (12.3, 4.5)		50.4	1.41 m, 2.42 dd (12.3, 4.4)	
2	69.0	4.27 m	4	69.3	4.42 d (9.7)	3
3	84.2	3.41 d (9.5)	2, 4, 23	78.2	4.25 m	2, 4, 23, 24
4	40.8			44.5		
5	56.7	1.10 m	10, 25	49.1	1.92 m	3, 4, 6, 10, 24, 25
6	67.9	4.80 br s		67.8	5.06 br s	8, 10
7	42.4	1.92 m, 1.71 m	5, 14	42.1	1.91 m	
8	40.8			43.0		
9	51.8	1.61 m	5, 26	51.8	1.72 m	7, 14
10	38.7			38.4		
11	21.4	1.21 m, 1.61 m		21.4	1.64 m, 1.79 m	10
12	26.2	1.93 m		26.1	1.18 m, 1.93 m	
13	37.5	2.87 td (12.0, 3.5)		37.4	2.84 td (11.8, 2.4)	
14	43.1			40.7		
15	30.3	1.42 m, 2.20 m	8	30.2	1.23 m, 2.17 m	
16	32.3	1.52 m, 2.68 td (12.5, 3.0)		32.2	1.46 m, 2.63 m	
17	57.0			56.9		
18	50.0	1.78 m		49.9	1.74 m	
19	47.5	3.46 m	30	47.4	3.42 td (10.7, 4.6)	29
20	150.9			150.8		
21	30.8	2.13 m		30.8	1.41 m, 2.10 m	
22	36.9	1.52 m, 2.20 m		36.8	2.16 m, 1.47 m	
23	28.8	1.74 s	3, 5, 24	66.0	4.06 d (10.4), 4.40 d (10.4)	3, 4, 5, 24
24	19.1	1.44 s	3, 4, 5	15.7	1.72 s	3, 4, 5
25	19.4	1.59 s	1, 5, 9, 10	19.4	1.73 s	1, 5, 9, 10
26	17.1	1.78 s	7, 9	17.0	1.80 s	8, 9, 14
27	15.2	1.04 s	13, 14, 15	15.1	0.95 s	8, 13, 14, 15
28	174.9			174.9		
29	110.1	4.75 br s, 4.89 d (2.2)	19, 30	110.1	4.74 br s, 4.88 br s	19, 30
30	19.5	1.76 s	19, 20, 29	19.7	1.65 s	19, 20, 29
GLC						
1′	95.5	6.40 d (7.9)	28	95.4	6.39 d (8.2)	28
2′	74.3	4.16 dd (8.9, 7.9)	1′, 3′	74.2	4.15 dd (8.9, 8.2)	1′, 3′
3′	78.8	4.27 dd (9.0, 8.9)	2′, 4′	78.7	4.27 dd (9.0, 8.9)	2′, 4′
4′	71.2	4.36 dd (10.0, 9.0)	3′	71.0	4.36 dd (12.8, 9.0)	3′, 5′, 6′
5′	79.4	4.02 ddd (10.0, 5.0, 2.4)		79.3	4.03 ddd (12.8, 4.0, 3.7)	3′
6′	62.2	4.38 dd (12.0, 5.0), 4.44 dd (12.0, 2.4)		62.0	4.40 m, 4.43 m	

[a] *J* values (in Hz) in parentheses. [b] ^{13}C NMR signal correlating with ^{1}H resonance.

structure of quadranoside I was determined to be 2α,3β,6β-trihydroxylup-20(29)-en-28-oic acid β-glucopyranosyl ester (**1**).

Quadranoside II (**2**) was also isolated as a colorless amorphous solid, and its molecular formula was determined to be $C_{36}H_{58}O_{11}$ by HRFABMS. The IR spectrum

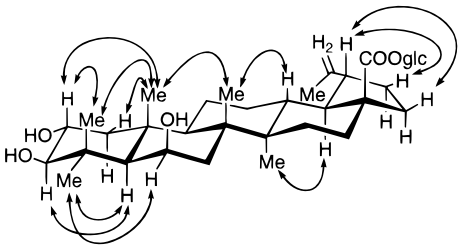

Figure 1. Significant correlations observed in the ROESY spectrum of **1**.

indicated the presence of hydroxyl (3400 cm^{-1}), carbonyl (1720 cm^{-1}), and olefinic (1640, 890 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1** except for the presence of one oxymethylene group (δ_H 4.06, 4.40; δ_C 66.0) instead of the methyl group in **1** (δ_H 1.74; δ_C 28.8). The position of the new oxymethylene group was determined to be at C-4α (i.e., C-23), on the basis of the long-range correlations between the oxymethylene protons and C-3, C-4, C-5, and C-24 in the FG-pulsed HMBC spectrum (Table 1) and the ROESY correlations between the oxymethylene protons and H-6. Thus, the structure of quadranoside II was determined to be 2α,3*β*,6*β*,23-tetrahydroxylup-20(29)-en-28-oic acid *β*-glucopyranosyl ester (**2**).

Quadranoside III (**3**) was obtained as a colorless amorphous solid, and its molecular formula was determined to be $C_{36}H_{58}O_{11}$ by HRFABMS. The IR spectrum of **3** also indicated the presence of hydroxyl, carbonyl, and olefinic groups. The ^1H NMR spectrum of **3** displayed signals corresponding to an olefinic proton (δ_H 5.46, br t, J = 3.0 Hz) and five tertiary methyls (δ_H 1.01, 1.08, 1.10, 1.17, 1.18). Additionally an anomeric proton was also observed in its ^1H NMR spectrum (δ_H 6.36, d, J = 8.0 Hz). These spectral data suggested that **3** was also a triterpene monoglycoside. This was supported by the ^{13}C NMR spectrum, which showed 36 carbon signals. The chemical shifts of the olefinic carbons (δ_C 122.9 and 144.3) suggested that **3** should have an oleanane skeleton with a double bond at C-12(13).[8] The carbon signals corresponding to ring A, including the two oxymethine groups (δ_C 68.9, 78.2) and an oxymethylene group (δ_C 66.5), were identical with those of **2,** suggesting the presence of three hydroxyl groups at C-2, C-3, and C-23 (i.e., with **3** having the same ring A as **2**). This was supported by the FG-pulsed HMBC spectrum, which showed long-range correlations between the quaternary carbon C-4 and H-2, H-3, and H_2-23 (Table 2). In addition, the ^1H and ^{13}C NMR spectra of **3** indicated the presence of one more oxymethylene group (δ_H 3.52, 2H, br s; δ_C 73.9, t). The oxymethylene protons had HMBC correlations with C-19, C-20, and C-21 and the methyl carbon at δ_C 19.7 (C-30), suggesting the position of the second oxymethylene group to be at C-20. The sugar moiety was determined to be a glucose attached to C-28 by comparing the ^1H and ^{13}C NMR data with those of **1** and **2**. From this evidence the planar structure of quadranoside III was determined as **3**. The stereochemistry of **3** was determined by the ROESY spectrum (Figure 2). The intense cross-peaks between H_3-24 and H-2, between H-2 and H_3-25, and between H-3 and H-5 led the relative configuration of ring A to be determined as OH-2α, OH-3*β*, and CH_2OH-4α. Furthermore, the cross-peak between H-18 and H_3-30 indicated that CH_2OH-20 should be α (C-29). From these data, quadranoside III was concluded to be 2α,3*β*,23,29-tetrahydroxyolean-12-en-28-oic acid *β*-glucopyranosyl ester (**3**).

Quadranoside IV (**4**), having the molecular formula $C_{36}H_{58}O_{10}$, showed an $[\alpha]^{25}_D$ of +13.1° (*c* 0.137, MeOH). The IR spectrum of **4** showed the bands at 3350 and 1725 cm^{-1} corresponding to hydroxyl and carbonyl group absorption, respectively. The ^1H NMR spectrum of **4** (Table 2) displayed signals corresponding to four tertiary methyls (δ_H 1.06, 1.10, 1.11, 1.19), two secondary methyls (δ_H 0.87, 0.97), an olefinic proton (δ_H 5.44), and an anomeric proton (δ_H 6.27, d, J = 8.0 Hz). The ^{13}C NMR spectrum showed 36 carbon signals including 6 primary, 10 secondary, 13 tertiary, and 7 quaternary carbons, which led to the conclusion that **4** is also a triterpene monoglycoside. The presence of two secondary methyl groups (δ_C 17.4, 21.3) and the chemical shifts of the olefinic carbons (δ_C 126.1, 138.5) suggested that **4** is an ursane-type triterpene with a double bond at C-12(13).[8] The signals at δ_C 66.5, 68.9, and 78.2 were almost identical to those of **3**, indicating the presence of three hydroxyl groups at C-2, C-3, and C-23. In the FG-pulsed HMBC spectrum, both the methine protons H-2 and H-3 showed long-range correlations with C-4 and the oxymethylene protons showed correlations with C-3, C-4, and C-5 and the methyl carbon (C-24). Furthermore, the sugar moiety attached to the carboxyl group was concluded to be a glucose by comparing its ^1H and ^{13}C NMR data with those of **1**−**3**. Thus, the planar structure of **4** was evident, and the relative stereochemistry of ring A was found to be identical with that of **3**, i.e., OH-2α, OH-3*β*, and OH-23, from the ROESY spectrum. The configurations of the methyl groups at C-19 and C-20 were also determined to be 19*β* and 20α, from the ROESY correlations between H-18 and H_3-29 and between H-18 and H-20. Thus, the structure of quadranoside IV was concluded to be 2α,3*β*,23-trihydroxyurs-12-en-28-oic acid *β*-glucopyranosyl ester (**4**).

Quadranoside V (**5**) was isolated as a colorless amorphous solid having an $[\alpha]_D^{25}$ of +116.8° (*c* 0.128, MeOH). The molecular formula of **5** was determined to be $C_{36}H_{56}O_{11}$ by HRFABMS. The IR spectrum of **5** indicated the presence of hydroxyl and carbonyl groups, and its UV spectrum showed the absorption of a heteroannular diene at 220 nm.[13] The ^1H NMR spectrum of **5** showed signals similar to **4** except for the absence of the H-18 resonance and the appearance of an additional oxymethine signal at δ_H 5.06 (br s). The ^{13}C NMR spectra of **4** and **5** were also similar, but the spectrum of **5** was characterized by the presence of the signals of one additional oxygenated carbon at δ_C 67.4 and two olefinic carbons at δ_C 134.5 and 135.9, along with the disappearance of signals of two methine carbons [δ_C 53.3 (C-18) and δ 39.3 (C-19)] and one methylene carbon [δ 18.5 (C-6)] in **4**. Thus, the spectral data of **5** were consistent with the compound being an ursane-type triterpene having one more hydroxyl group than **4** and a heteroannular diene. The position of the additional hydroxyl group was determined to be at C-6 and the extra double bond to be at C-18(19) from the FG-pulsed HMBC spectrum (Table 2). The stereochemistry of **5** was determined to be identical with that of **4** from its ROESY spectrum. The additional hydroxyl group at C-6 was determined to be *β* because H-6 appeared as a broad singlet, by analogy with the same signal in **1** and **2**. Thus, the structure of quadranoside V was concluded to be 2α,3*β*,6*β*,23-tetrahydroxyurs-12,18-dien-28-oic acid *β*-glucopyranosyl ester (**5**).

Compounds **1**−**5** all bear a 2α,3*β*-dihydroxyl functionality even though they belong to three different (lupane-, oleanane-, and ursane-type) triterpene classes. Ursane-type triterpenes with a heteroannular diene at C-12(13) and C-18(19) are very rare,[14] and **5** is the first example of an ursane-type triterpene bearing a heteroannular diene from a *Combretum* species. Furthermore, among the known

Table 2. ^{1}H and ^{13}C NMR Data and HMBC Correlations of **3**−**5** in C_5D_5N[a]

	3			**4**			**5**		
position	^{13}C	^{1}H	HMBC[b]	^{13}C	^{1}H	HMBC[b]	^{13}C	^{1}H	HMBC[b]
1	47.8	1.35 m, 2.28 (dd 12.4, 3.4)		48.0	1.39 m, 2.30 dd (12.4, 4.1)	2, 3, 5, 9, 10	50.8	1.54 m, 2.45 dd (12.3, 4.3)	
2	68.9	4.24 m	3, 4	68.9	4.24 m	3, 4	69.2	4.44 m	3, 4
3	78.2	4.21 m	2, 4, 5, 24	78.2	4.28 m	2, 4	78.2	4.25 d (9.4)	2, 4, 23
4	42.2			43.6			44.5		
5	48.2	1.78 m		48.1	1.82 m	4, 10, 24, 25	48.4	2.04 m	4, 10, 24, 25
6	18.5	1.45 m, 1.68 m		18.5	1.69 m		67.4	5.06 br s	
7	32.0	1.34 m, 1.68 m		33.2	0.87 m, 1.36 m		42.5	1.92 m, 2.01 m	
8	40.0			40.2			38.9		
9	47.9	1.82 m		47.9	1.84 m	1, 8, 10, 25, 26	48.9	1.90 m	26
10	38.4			38.3			38.1		
11	24.0	2.12 m		23.8	2.06 m		23.9	2.24 m, 2.33 m	
12	122.9	5.46 br t (3.0)		126.1	5.44 br t (3.1)	14	127.0	5.77 br t (3.1)	
13	144.3			138.5			138.3		
14	43.6			42.5			45.5		
15	28.3	1.12 m, 2.37 m		28.6	1.10 m, 2.43 td (13.0,4.4)		29.1	1.21 m, 2.58 m	
16	23.4	1.97 m, 2.14 m		24.6	1.98 m		35.4	1.49 m, 2.55 m	
17	47.4			48.3			50.0		
18	41.1	3.31 dd (14.3, 5.0)		53.3	2.51 d (11.2)	12, 13, 14, 17, 19	134.1		
19	40.9	1.42 m, 1.84 m		39.3	1.37 m		135.9		
20	36.4			39.1	0.86 m		34.6	2.14 m	
21	28.9	1.72 m, 1.93 m		30.8	1.28 m		26.4	1.24 m, 2.06 m	
22	32.8	1.26 m, 1.84 m		36.8	1.70 m, 1.88 m		31.0	1.68 m, 2.17 m	
23	66.5	3.70 d (10.5), 4.20 m	3, 4, 5, 24	66.5	3.70 d (10.4), 4.23 d (10.4)	3, 4, 5, 24	66.1	4.04 d (10.4) 4.40 d (10.4)	3, 5, 24
24	14.4	1.07 s	3, 23, 4, 5	14.4	1.06 s	3, 4, 5, 23	16.0	1.73 s	3, 4, 5, 23
25	17.6	1.10 s	1, 5, 9, 10	17.6	1.11 s	1, 5, 9, 10	19.8	1.82 s	1, 5, 9, 10
26	17.5	1.17 s	7, 8, 9, 14	17.8	1.19 s	7, 8, 9, 14	20.6	1.72 s	8, 9, 14, 15
27	26.1	1.18 s	13, 15	23.8	1.10 s	8, 13, 14, 15	22.2	1.04 s	7, 8, 13, 14, 15
28	176.5			176.2			174.7		
29	73.7	3.52 s (2H)	19, 20, 21, 30	17.4	0.91 d (5.9)	18, 19, 20	19.6	1.80 s	18, 19, 20
30	19.7	1.08 s	19, 20, 21, 29	21.3	0.87 br s	19, 20, 21	18.7	1.02 d (7.0)	19, 20, 21
glc									
1′	95.7	6.36 d (8.0)	28	95.7	6.27 d (8.0)	28	95.9	6.31 d (8.2)	28
2′	74.1	4.21 m	1′, 3′	74.0	4.21 m	1′, 3′	74.2	4.13 dd (8.4, 8.2)	1′, 3′
3′	78.9	4.29 t (8.8)	2′, 4′	78.9	4.28 dd (9.0, 8.0)	2′, 4′	78.9	4.24 dd (8.4, 8.0)	2′
4′	71.1	4.38 dd (9.3, 8.8)	3′, 6′	71.2	4.39 dd (9.3, 9.0)	3′	71.2	4.32 dd (9.4, 8.0)	5′, 6′
5′	79.3	4.03 m		79.2	4.01 m		79.1	3.94 ddd (9.4, 4.0, 3.1)	
6′	62.2	4.42 m, 4.47 m		62.3	4.40 m, 4.45 m		62.3	4.35 m, 4.43 m	

[a] *J* values (in Hz) in parentheses. [b] ^{13}C NMR signal correlating with ^{1}H resonance.

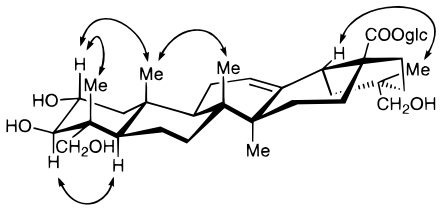

Figure 2. Significant correlations observed in the ROESY spectrum of **3**.

compounds, **8**−**13** have been isolated for the first time from *C. quadrangulare*.

The hepatoprotective effects of the isolated compounds were examined on D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes.[15] Of the new saponins, compounds **1**, **2**, and **5** showed hepatoprotective activity at 25−200 μM in a concentration-dependent manner, while **4** did not show any hepatoprotective activity, and **3** was much less active. At a 50 μM concentration, **1**, **2**, and **5** showed 37.6, 40.9, and 67.5% inhibition against cell death, respectively, while a positive control, silibinin, revealed 61.2% inhibition. Among the known compounds, five (**8**, **9**, **11**, **14**, and **15**) showed significant hepatoprotective activity against D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. These triterpene glucosides and catechins therefore contribute to the hepatoprotective activity of the MeOH extract observed for *C. quadrangulare* seeds.

Experimental Section

General Experimental Procedures. Optical rotations were determined in MeOH on a JASCO DIP 140 digital polarimeter at 25 °C. The UV spectrum was taken in MeOH solution on a Shimadzu UV-160A UV−visible spectrophotometer. IR spectra were recorded in KBr disks on a Shimadzu IR-408 spectrophotometer. NMR spectra were recorded in C_5D_5N containing TMS as internal standard on a JEOL JNM-GX400 spectrometer. Mass spectra were obtained on a JEOL JMS-SX102A spectrometer using glycerol as a matrix.

Plant Material. Seeds of *C. quadrangulare* Kurz were collected in Ho Chi Minh City, Vietnam, in January 1998. A voucher sample (TMPW 19000) is preserved in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. The dried seeds (2.25 kg) of *C. quadrangulare* were extracted with MeOH (7 L, 3 h × 2) at 80 °C, followed by removal of the solvent under reduced pressure, to yield a dried MeOH extract (748 g). The MeOH extract (700 g) was dissolved in H_2O to give a water-soluble

extract (507 g) and a residue (180 g). The water-soluble extract (400 g) was subjected to Sephadex LH-20 column chromatography with a H_2O−MeOH gradient system to afford seven fractions (fraction 1, H_2O eluate, 47.0 g; fraction 2, H_2O eluate, 22.0 g; fraction 3, 75% H_2O−MeOH eluate, 23.0 g; fraction 4, 75% H_2O−MeOH eluate, 10.6 g; fraction 5, 50% H_2O−MeOH eluate, 8.0 g; fraction 6, 25% H_2O−MeOH eluate, 45.0 g; fraction 7, MeOH eluate, 133 g).

Fraction 2 (20 g) was chromatographed on Cosmosil 75C_{18}-OPN with a H_2O−MeOH gradient system to give eight subfractions. Further Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1) of subfraction 8 yielded quadranoside I (**1**, 5.0 mg), nigaichigoside F1 (**7**, 14.7 mg), and β-sitosterol glucoside (**16**, 30.0 mg).

Fraction 3 (20 g) was applied on a Si gel column with $CHCl_3$−MeOH−H_2O (14:6:1), and nine subfractions were collected. Further Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1) of subfractions 2 and 3 yielded 19α-hydroxyasiatic acid (**6**, 3.6 mg) and arjunglucoside I (**9**, 38 mg), and nigaichigoside F1 (**7**, 3.6 mg) and pinfaensin (**10**, 4.5 mg), respectively. On the other hand, Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1; and then EtOAc−AcOH−H_2O, 100:16:13) of subfraction 5 gave quadranoside II (**2**, 29.6 mg), nigaichigoside F1 (**7**, 4.2 mg), and 5-methoxy-(−)-isolariciresinol (**12**, 6.4 mg).

Fraction 4 (10 g) was also chromatographed on a Si gel column with $CHCl_3$−MeOH−H_2O (14:6:1) to afford eight subfractions. Further Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1.5) of subfractions 2, 4, and 5 yielded the following compounds: subfraction 2, nigaichigoside F1 (**7**, 8.8 mg) and methyl gallate (**18**, 29.1 mg); subfraction 4, nigaichigoside F1 (**7**, 33.6 mg); subfraction 5, quadranosides II (**2**, 71.5 mg) and III (**3**, 8.8 mg). Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1; and then EtOAc−AcOH−H_2O, 100:16:13) of subfraction 3 gave gallic acid (**17**, 7.5 mg) and 5-methoxy-9-β-xylopyranosyl-(−)-isolariciresinol (**13**, 4.6 mg).

Fraction 5 (8.0 g) was again chromatographed on Si gel with $CHCl_3$−MeOH−H_2O (14:6:1), and nine subfractions were collected. Further Cosmosil 75C_{18}-OPN column chromatography (MeOH−MeCN−H_2O, 1:1:2) and preparative TLC (MeOH−MeCN−H_2O, 1:1:1) of subfractions 2−4, 6, and 7 afforded the following compounds: subfraction 2, arjungenin (**8**, 5.6 mg); subfraction 3, (+)-gallocatechin (**14**, 3.4 mg) and (−)-epicatechin (**15**, 10 mg); subfraction 4, quadranoside IV (**4**, 27.3 mg) and 2α,3β,23-trihydroxyursa-12,19-dien-28-oic acid β-D-glucopyranosyl ester (**11**, 19.6 mg); subfraction 6, quadranosides III (**3**, 9.5 mg) and V (**5**, 8.9 mg); subfraction 7, quadranoside V (**5**, 17.0 mg).

Quadranoside I (**1**)**:** colorless amorphous solid; $[\alpha]_D^{25}$ +5.7^o (*c* 0.153, MeOH); IR (KBr) ν_{max} 3400, 1725, 1640, 1070, 890 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 673.3923 [calcd for $C_{36}H_{58}O_{10}Na$ (M + Na)$^+$, 673.3927].

Quadranoside II (**2**)**:** colorless amorphous solid; $[\alpha]_D^{25}$ +43.4^o (*c* 0.153, MeOH); IR (KBr) ν_{max} 3350, 1720, 1640, 890 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 689.3904 [calcd for $C_{36}H_{58}O_{11}Na$ (M + Na)$^+$, 689.3876].

Quadranoside III (**3**)**:** colorless amorphous solid; $[\alpha]_D^{25}$ + 26.8^o (*c* 0.073, MeOH); IR (KBr) ν_{max} 3400, 1728, 1640 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 689.3864 [calcd for $C_{36}H_{58}O_{11}Na$ (M + Na)$^+$; 689.3877].

Quadranoside IV (**4**)**:** colorless amorphous solid; $[\alpha]_D^{25}$ + 13.1^o (*c* 0.137, MeOH); IR (KBr) ν_{max} 3350, 1725, 1640 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 673.3913 [calcd for $C_{36}H_{58}O_{10}Na$ (M + Na)$^+$, 673.3928].

Quadranoside V (**5**)**:** colorless amorphous solid; $[\alpha]_D^{25}$ + 116.8^o (*c* 0.128, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.05) nm; IR (KBr) ν_{max} 3350, 1725, 1640 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 687.3710 [calcd for $C_{36}H_{56}O_{11}Na$ (M + Na)$^+$, 687.3721].

TNF-α-Induced Cell Death in Primary Cultured Mouse Hepatocytes. Mouse liver parenchymal cells were isolated by a modified collagenase perfusion method as previously reported,[15] with the viability exceeding 90% determined in a trypan blue exclusion test. The isolated hepatocytes were suspended in Williams' E medium supplemented with 10% calf serum, 100 IU/mL penicillin G, 100 μg/mL streptomycin, 100 μM dexamethasone, and 50 ng/mL insulin and inoculated in a 96-well plastic plate (10^5 cells/well). After preincubation for 2 h, the medium was replaced with fresh medium containing D-GalN (0.5 mM) and test samples at various concentrations. Thirty minutes later, TNF-α (100 ng/mL) was added to each well, and the hepatocyte viability was assessed 18 h thereafter by a determination of the MTT colorimetric reaction. Compounds **1** (71.2%, 29.7%, 37.6%, 6.3%), **2** (83.6%, 46.1%, 40.9%, 32.0%), **3** (35.5%, 17.8%, 8.0%, 15.1%), and **5** (80.1%, 77.7%, 67.5%, 59.3%) showed inhibition against cell death at 200, 100, 50, and 25 μM, respectively, while **4** showed no hepatoprotective activity at the concentrations. Among the known compounds, **8** (46.2%, 37.0%, 31.8%), **9** (32.7%, 31.5%, 10.1%), **11** (15.0%, 25%, 8.3%), **14** (33.5%, 30.3%, 19.7%), and **15** (98.9%, 58.1%, 38.0%) showed inhibition at 200, 100, and 50 μM, respectively. The clinically used silibinin was used as a positive control and showed inhibition rates of 70.8%, 96.5%, 61.2%, and 38.1% at the same concentrations.

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