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Thirteen Novel Cycloartane-Type Triterpenes from *Combretum quadrangulare*

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Thirteen novel cycloartane-type triterpenes were isolated from *Combretum quadrangulare*, a Vietnamese medicinal plant. The structures of the novel triterpenes were determined by spectroscopic methods as well as by chemical transformations. Among those compounds, quadrangularic acids F (**1**), G (**2**), and H (**4**) and 24-epiquadrangularic acid G (**3**) are the first examples of cycloartane-type triterpenes bearing carboxylic acid groups at both C-4 and C-20. Furthermore, norquadrangularic acid A (**13**) is the first example of a trinorcycloartane-type triterpene isolated from the genus *Combretum*.

Combretum species (Combretaceae) are widely used as folk medicine for the treatment of hepatitis, malaria, respiratory infections, and even cancer in different parts of Asia and Africa.[1] *Combretum quadrangulare* Kurz is an evergreen tree that grows widely in eastern Asia. Its seeds, leaves, and the stem bark are used in Vietnamese traditional medicine as an antipyretic, antidysenteric, and anthelmintic agent.[2] In the course of the chemical investigation on Vietnamese medicinal plants,[3,4] we have recently reported the isolation of seven novel cytotoxic cycloartane-type triterpenes from *C. quadrangulare*.[3] In this paper we describe the isolation and structure elucidation of 13 additional novel cycloartane-type triterpenes (**1**−**13**) from a MeOH extract of the leaves of *C. quadrangulare.*

Results and Discussion

Air-dried leaves of *C. quadrangulare* were extracted with MeOH at 80 °C. The dark-green MeOH extract showed a potent hepatoprotective effect on lipopolysaccharide-induced liver injury in D-galactosamine-sensitized mice in vivo.[5] Interestingly, the same extract also had a cytotoxic effect toward the liver-metastatic murine colon 26-L5 carcinoma in vitro, with an ED_{50} value of 75.9 μg/mL. Hence, the MeOH extract was further fractionated into 11 fractions by Si gel column chromatography. Repeated chromatography of fractions 8 and 9 on normal- and reversed-phase Si gel columns, together with preparative TLC, afforded 13 novel triterpenes, named quadrangularic acid F (**1**), quadrangularic acid G (**2**), 24-epiquadrangularic acid G (**3**), quadrangularic acid H (**4**), methyl quadrangularate I (**5**), quadrangularic acid J (**6**), quadrangularic acid K (**7**), quadrangularic acid L (**8**), 24-epiquadrangularic acid L (**9**), quadrangularic acid M (**10**), 24-epiquadrangularic acid M (**11**), 7β-hydroxy-23-deoxojessic acid (**12**), and norquadrangularic acid A (**13**).

Quadrangularic acid F (**1**) was obtained as a colorless amorphous solid, and its molecular formula was determined as $C_{31}H_{48}O_8$ by HRFABMS. Absorption bands at 3400 and 1700 cm^{-1} in the IR spectrum of **1** indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum of **1** displayed characteristic signals of a set of cyclopropane methylene protons (δ 0.76 and 0.48, both d, J = 4.5 Hz), two oxymethine protons (δ 5.36, dd, J = 12.0, 4.5 Hz; δ 3.77, br s), five tertiary methyls (δ 1.60, 1.56, 1.50, 1.34, 1.04), an ester methyl (δ 3.66), and two *trans*-olefinic protons (δ 6.18, d, J = 16.0 Hz; δ 6.07, dt, J = 16.0, 7.5 Hz), suggesting that **1** is a cycloartane-type triterpene bearing two hydroxyls and a *trans*-olefin. By treatment with diazomethane, **1** gave a dimethyl ester **1a**, indicating the presence of a free carboxylic acid group. The ^{1}H and ^{13}C NMR data of **1** were similar to those of methyl quadrangularate B (**14**),[3] except for the absence of an aldehyde signal and the appearance of an additional carbonyl signal (δ 177.8) in the ^{13}C NMR spectrum. Thus, **1** was considered to be the C-20 oxidized derivative of **14**. The presence of a carboxylic acid function at C-20 was further confirmed by the long-range correlations observed in the FG-pulsed HMBC spectrum (Figure 1).

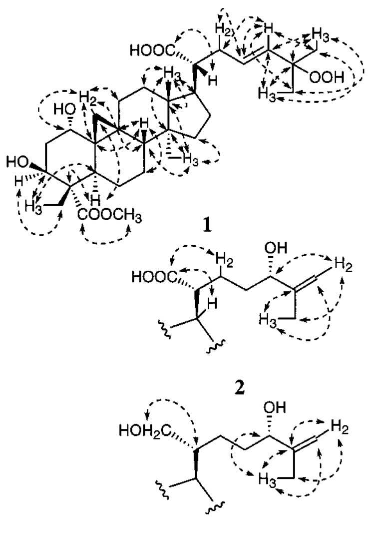

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Figure 1. Significant correlations observed in the FG-pulsed HMBC spectrum of compounds **1**, **2**, and **5**. Compounds **2** and **5** also showed the same significant correlations in rings A−D as compound **1**.

The stereochemistry of **1** was determined by NOE experiments and the analysis of coupling constants. The lack of any diaxial coupling of H-1 with H-2$_{ax}$ indicated that the hydroxyl group at C-1 is located in the axial position. This was also supported by the NOE enhancement from H-1 to H-19. The H-3 signal was observed as a double doublet due to diaxial (J = 12.0 Hz) and axial−equatorial

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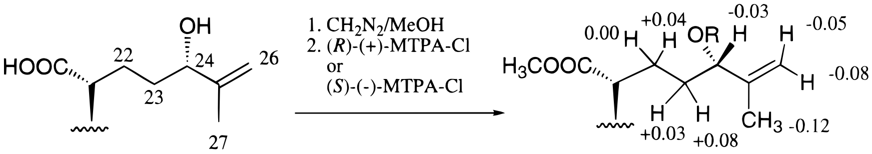
Scheme 1. $\Delta\delta^{RS}$ ($= \delta^R - \delta^S$) Values Obtained from the ^1H NMR Spectra of the MTPA Esters **2c** and **2d**



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2c R = (R)-MTPA
2d R = (S)-MTPA

coupling ($J = 4.5$ Hz), suggesting the axial nature of H-3. Irradiation of the methyl protons at δ 1.60 caused an NOE increase of H-19 and vice versa, indicating that the methyl group at C-4 should be in the β position, that is at C-29. Additionally, irradiation of H-3 gave enhancement of H-5, placing them in a 1,3-diaxial arrangement in a chair conformation. Finally, the structure of quadrangularic acid F, including the configuration at C-20, was confirmed to be **1** by sodium chlorite oxidation[6] of **14** to **1**.

Quadrangularic acid G (**2**), a colorless amorphous solid, was obtained as a monomethyl ester and was easily converted into dimethyl ester **2a** with diazomethane. The molecular formula of **2** ($C_{31}H_{48}O_7$) was calculated from the quasimolecular ion peak $[M + Na]^+$ at m/z 555.3272 in the HRFABMS. In the ^1H NMR spectrum of **2**, the signals of four tertiary methyls, an ester methyl, and two *exo*-olefinic protons were observed, in addition to the signals of two characteristic cyclopropane methylene protons. Furthermore, three signals of oxymethine protons at δ 5.33, 4.46, and 3.75 in the ^1H NMR spectrum suggested the presence of three hydroxyl groups, which was confirmed by acetylation of the dimethyl ester **2a** into a triacetate **2b**. Among the three hydroxyl groups, two were considered to be located at C-1 (δ 3.75) and C-3 (δ 5.33) by comparing the ^1H NMR spectrum with that of **1**. Detailed analysis of the $^1H-^1H$, $^1H-^{13}C$, and long-range $^1H-^{13}C$ COSY spectra indicated that the third hydroxyl group should be located at C-24 and that the ester (δ 178.1) and carboxylic acid (δ 178.6) groups were at C-4 and C-20, respectively (Figure 1). These and other long-range correlations established the planar structure of quadrangularic acid G (**2**).

The stereochemistry of rings A−D in **2** was determined to be the same as **1**, based on the coupling constants and the result of NOE experiments. The configuration at the chiral center C-24, on the other hand, was determined through the NMR study of MTPA esters of the dimethyl ester **2a**. In the ^1H NMR spectrum of the (R)-MTPA ester **2c**, H_2-26 and H_3-27 appeared shielded, whereas H_2-23 and H_2-22 were deshielded, in comparison to analogous data for (S)-MTPA ester **2d** (Scheme 1). Thus, H_2-26 and H_3-27 in the (R)-MTPA ester **2c** were more affected by the phenyl ring of the MTPA part; that is, the configuration at C-24 should be *S*.[7,8] The configuration at C-20 of **2** was assumed to be the same as that of **1** because both compounds were isolated from the same plant part.

24-Epiquadrangularic acid G (**3**), a colorless amorphous solid, showed the same molecular formula as **2** ($C_{31}H_{48}O_7$) in the HRFABMS. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **2**, except for a slight downfield shift of one of the olefinic protons (**3**, δ 5.31; **2**, δ 5.20) and small differences in the carbon chemical shifts from C-24 to C-27 (Table 1). Thus, **2** and **3** were considered to be epimers at C-24; that is, **3** has a 24*R* configuration. This was confirmed by the fact that their methyl esters **2a** and **3a** gave the same α,β-unsaturated ketone **4a** by MnO_2 oxidation.[9]

Quadrangularic acid H (**4**) was also obtained as a colorless amorphous solid, and the HRFABMS data suggested the molecular formula to be $C_{30}H_{44}O_7$. The ^1H and ^{13}C NMR spectra of **4** were similar to those of **2** and **3**, but they showed a new signal for a ketone−carbonyl at δ 201.2 and the disappearance of the signal assignable to the hydroxymethylene (C-24) found in **2** and **3**. In addition, correlations between the ketone carbon and the protons H_2-26 and H_3-27 were observed in the long-range $^1H-^{13}C$ COSY spectrum, suggesting that **4** should have a ketone group at C-24 instead of a hydroxyl group as in **2** and **3**. This was further confirmed by esterification of **4** with diazomethane to the α,β-unsaturated ketone **4a**.

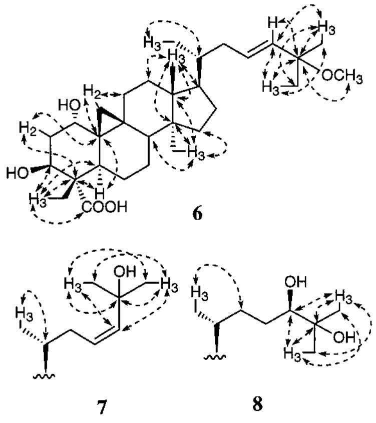
Quadrangularic acid I (**5**), a colorless amorphous solid, showed a quasimolecular ion at m/z 541.3506 in the HRFABMS, being consistent with the molecular formula $C_{31}H_{50}O_6$. The ^1H NMR spectrum of **5** was similar to those of **2** and **3**. Differences between **5** and **2** and **3** were apparent only in the signals due to the ring D side chain. In the ^1H and ^{13}C NMR spectra of **5**, the signals of the oxymethylene group appeared at δ_H 4.09 (dd, $J = 11.0, 3.0$ Hz), δ_H 3.85 (dd, $J = 11.0, 5.0$ Hz), and δ_C 62.0, instead of a signal for a carboxylic acid group at C-20 as in **2** and **3**. These data and the long-range correlations between H_2-21 and C-20 in the FG-pulsed HMBC spectrum (Figure 1) indicated that there should be a hydroxymethylene group at C-20 in **5**. The stereochemistry in rings A−D of **5** was determined by NOE difference experiments and found to be the same as **1**, while that of C-24 was concluded as *S* by comparing the ^1H and ^{13}C NMR data with those for **2** (24*S*) and **3** (24*R*) (Table 1 and Experimental Section).

Quadrangularic acid J (**6**), a colorless amorphous solid, was isolated as an acid and gave a monomethyl ester **6a** with diazomethane. The molecular formula of **6** was determined as $C_{31}H_{50}O_5$ by HRFABMS, and its IR spectrum showed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1700 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **6** showed almost identical signals to those of **1**, but the signals of a secondary methyl appeared at δ_H 0.96 (d, $J = 6.5$ Hz) and δ_C 18.6 instead of a signal due to a carboxylic acid group in **1**, suggesting that C-21 should be a methyl group. The quaternary carbon signal assignable to C-25 was shifted to δ 74.8, indicating the presence of a methoxyl group (δ_H 3.20, δ_C 50.1). These were further confirmed by the FG-pulsed HMBC spectrum (Figure 2). From these data and NOE difference experiments, the structure of quadrangularic acid J was determined as **6**.

Quadrangularic acid K (**7**) was isolated as a colorless amorphous solid, and its molecular formula was determined as $C_{30}H_{48}O_5$ by HRFABMS. The ^1H and ^{13}C NMR data (Table 1 and Experimental Section) of **7** were similar to those of **6**, and correlations observed in the long-range $^1H-^{13}C$ COSY spectrum (Figure 2) indicated that **7** has a hydroxyl group instead of a methoxyl group at C-25 as in **6**. In the ^1H NMR spectrum of **7**, the signals of two olefinic protons appeared as a broad singlet at δ 5.94, suggesting a cis configuration of the double bond. The cis nature of the double bond was further supported by comparison of

Table 1. ^{13}C NMR Data (100 MHz) of Compounds **1**−**13** in Pyridine-d_5

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	72.1	72.1	72.1	72.3	72.2	72.5	72.4	72.6	75.5	72.5	72.5	72.5	72.5
2	38.6	38.5	38.5	38.5	38.6	38.8	38.6	38.8	38.8	38.8	38.8	38.8	37.7
3	70.4	70.4	70.4	70.6	70.4	70.7	70.5	70.7	70.7	70.7	70.7	70.5	70.7
4	56.0	56.0	56.0	55.5	56.0	55.7	55.5	55.7	55.7	55.7	55.7	55.4	55.7
5	37.9	37.8	37.8	37.5	37.9	37.7	37.6	37.7	37.7	37.7	37.7	36.7	38.8
6	23.2	23.1	23.2	23.1	23.4	23.4	23.3	23.4	23.4	23.4	23.4	34.0	23.4
7	26.0	27.5	27.5	27.2	27.7	28.3	28.1	28.4	28.3	28.4	28.4	69.5	28.2
8	47.8	47.8	47.9	47.8	48.3	48.1	48.0	48.2	48.2	48.2	48.2	54.9	48.1
9	20.8	20.8	20.8	20.7	20.9	20.8	20.7	20.8	20.8	20.8	20.8	20.9	20.8
10	30.3	30.2	30.3	30.3	30.1	30.3	29.8	30.3	30.3	30.3	30.3	30.7	30.3
11	25.7	26.0	26.0	25.7	26.2	26.2	26.0	26.2	26.2	26.2	26.2	26.7	26.2
12	30.6	30.6	30.6	30.5	32.5	33.2	33.0	33.3	33.3	33.3	33.3	33.3	33.2
13	45.7	45.6	45.7	45.5	45.5	45.5	45.4	45.5	45.5	45.5	45.5	46.0	45.5
14	48.9	48.9	48.9	48.9	49.1	49.2	48.9	49.1	49.1	49.1	49.1	49.2	49.1
15	35.4	34.0	33.9	35.2	35.9	35.9	35.7	35.9	35.9	36.3	36.3	37.5	35.8
16	27.3	25.7	25.7	25.6	25.8	25.7	25.7	25.8	25.9	25.9	25.9	28.8	25.9
17	49.2	49.2	49.0	49.4	47.0	52.2	52.1	52.9	52.8	52.6	52.6	52.0	52.5
18	18.1	18.1	18.3	18.0	18.7	18.4	18.3	18.4	18.4	18.4	18.4	17.7	18.3
19	29.6	29.6	29.8	29.6	29.7	29.7	29.6	29.8	29.8	29.8	29.8	27.8	29.7
20	49.5	49.6	49.6	48.5	43.6	36.6	36.7	36.3	36.3	36.4	36.4	36.4	36.0
21	177.8	178.6	178.5	178.5	62.0	18.6	18.4	18.6	18.8	18.7	18.7	18.7	18.1
22	37.8	29.5	29.2	27.6	26.4	39.6	39.3	34.1	34.5	32.7	32.7	35.4	32.1
23	127.5	35.4	35.4	35.6	32.4	128.3	124.4	28.9	29.4	35.9	35.9	31.6	32.0
24	137.5	75.6	74.8	201.2	76.2	137.5	141.3	79.0	79.8	75.6	76.1	156.7	176.5
25	81.1	149.4	149.6	144.3	149.5	74.8	69.6	72.7	72.7	149.6	149.6	34.1	
25	25.3	110.6	110.0	124.6	110.2	26.5	30.6	26.1	26.1	110.0	110.4	22.0	
27	24.9	17.5	18.1	17.5	17.9	26.0	30.0	25.9	26.0	18.2	17.7	21.9	
28	178.1	178.1	178.1	180.1	178.1	180.0	180.0	180.0	180.0	180.1	180.1	179.9	180.0
29	9.4	9.4	9.5	9.6	9.5	9.7	9.6	9.7	9.7	9.8	9.8	9.7	9.7
30	19.4	19.4	19.4	19.3	19.7	19.4	19.3	19.5	19.5	19.5	19.5	19.0	19.4
31												106.6	
MeO-25						50.1							
MeO-28	51.4	51.4	51.4		51.5								



6

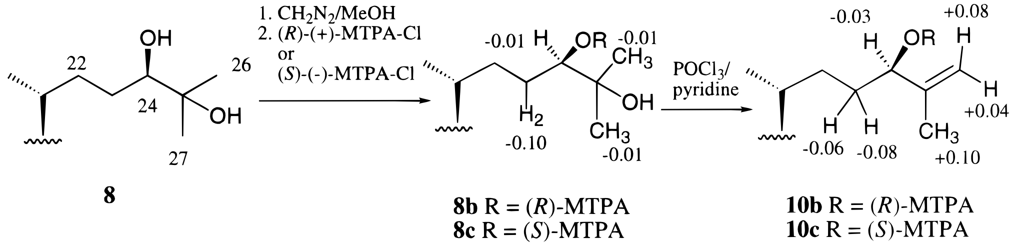
7 8

Figure 2. Significant correlations observed in the long-range $^1H-^{13}C$ COSY spectrum of compounds **6**, **7**, and **8**. Compounds **7** and **8** also showed the same significant correlations in rings A−D as compound **6**.

the chemical shifts of methyl ester **7a** in $CDCl_3$ (C-23, δ 125.5; C-24, δ 139.4) with those of (23*Z*)-3β-acetoxycycloart-23-en-25-ol, a compound having the same side chain (C-23, δ 125.6; C-24, δ 139.3) as **7**.[10]

Quadrangularic acid L (**8**), a colorless amorphous solid with the molecular formula $C_{30}H_{50}O_6$, showed hydroxyl and carbonyl group absorption in the IR spectrum. The ^1H NMR spectrum of **8**, analyzed with the aid of the $^1H-^1H$ COSY spectrum, showed signals due to a cyclopropane methylene, three oxymethines, five tertiary methyls, and a secondary methyl. Based on a comparison of the ^1H and ^{13}C NMR data with those of **7**, **8** was considered to be a C-24 hydroxyl derivative, which was confirmed by the long-range $^1H-^{13}C$ COSY spectrum (Figure 2). The orientation of the hydroxyl groups at C-1 and C-3 in **8** were concluded to be α and β, respectively, by comparison of the ^1H NMR data with those of **1**−**7**. The stereochemistry of the rest of the molecule was determined by NOE experiments. For the determination of the absolute configuration at C-24, the (*R*)-MTPA ester **8b** and the (*S*)-MTPA ester **8c** were prepared from the methyl ester **8a**. The $\Delta\delta^{RS}$ ($= \delta^R - \delta^S$) values of H_2-23, H_3-26, and H_3-27, however, were all negative, and thus the advanced Mosher's method[7] could not be applied. This could result from their unusual conformation due to the presence of the C-25 hydroxyl group. Thus, the MTPA esters **8b** and **8c** were dehydrated with $POCl_3$−pyridine[11] into the respective olefins, **10b** and **10c** (Scheme 2). In the case of **10b** and **10c**, H_2-26 and H_3-

Scheme 2. $\Delta\delta^{RS}$ ($= \delta^R - \delta^S$) Values Obtained from the MTPA Esters of Methyl Quadrangularate L (**8A**) and Methyl Quadrangularate M (**10A**)



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8b R = (*R*)-MTPA
8c R = (*S*)-MTPA

10b R = (*R*)-MTPA
10c R = (*S*)-MTPA

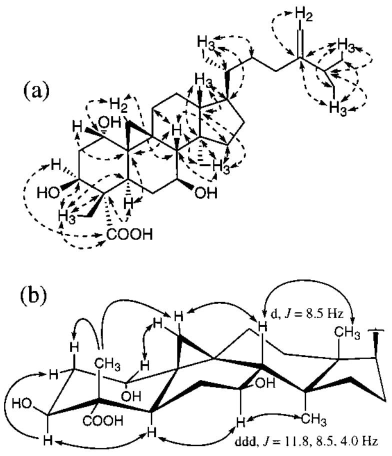


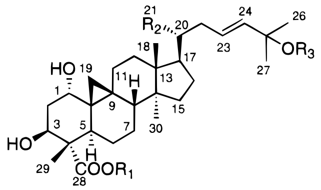
Figure 3. (a) Significant correlations observed in the FG-pulsed HMBC spectrum of compound **12**, and (b) NOEs observed in the NOE difference experiments of compound **12**.

27 of (*R*)-MTPA ester **10b** resonated downfield as compared to (*S*)-MTPA ester **10c**, while H_2-23 of **10b** resonated upfield when compared to **10c**. This indicated that H_2-23 in the (*R*)-MTPA ester **10b** was more affected by the phenyl ring of the MTPA part; that is, C-24 should have an *R* configuration.[7,8] The structure of quadrangularic acid L was thus determined as **8**.

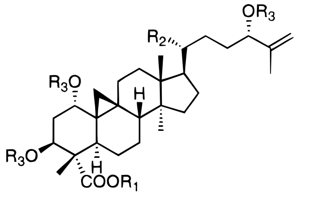
24-Epiquadrangularic acid L (**9**), a colorless amorphous solid, had the same molecular formula $C_{30}H_{50}O_6$ as **8**. The IR, ^1H NMR, and ^{13}C NMR spectra of **9** were almost the same as those of **8**, but a slight difference was found in the chemical shifts of H-24 (**9**, δ 3.71; **8**, δ 3.76) and C-24 (**9**, δ 79.8; **8**, δ 79.0). Analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectra indicated that **9** had the same planar structure as **8**, while the NOE spectra revealed the presence of the same stereochemistry on rings A–D as **8**. Thus, **9** was concluded to be the 24*S* epimer of **8**.

Quadrangularic acid M (**10**) and 24-epiquadrangularic acid M (**11**) were obtained as an epimeric mixture (**10**:**11** = 4:3 from the ^1H NMR spectrum), and their molecular formulas were determined as $C_{30}H_{48}O_5$ based on FABMS. Analysis of the ^1H and ^{13}C NMR spectra of the mixture through the $^1H-^1H$, $^1H-^{13}C$, and long-range $^1H-^{13}C$ COSY spectra enabled the assignment of the signals to each epimer. The assigned data of **10** and **11** differed only in the chemical shift of one of the olefinic protons (**10**, δ 5.27; **11**, δ 5.22) in the ^1H NMR spectrum, while in the ^{13}C NMR spectrum, they clearly differed at three signals, C-24, C-26, and C-27 (Table 1). Thus, they were considered to be epimers at C-24, and, by comparing their ^1H and ^{13}C NMR data with those of **2** and **3**, **10** and **11** were assigned with the 24*R* and 24*S* configuration, respectively. This was confirmed by the fact that methylation with diazomethane, followed by esterification with (*R*)-MTPA chloride, gave a mixture of **10b** and its 24*S* epimer (**10c**) (4:3).

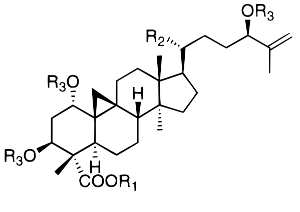
7β-Hydroxy-23-deoxojessic acid (**12**) was isolated as colorless crystals having a melting point of 219 °C, and its molecular formula was determined as $C_{31}H_{50}O_5$ by HRFABMS. The IR spectrum of **12** showed a broad absorption band at 3400 cm^{-1} and a sharp absorption band at 1700 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups, respectively. The ^1H and ^{13}C NMR data of **12** were similar to those of 23-deoxojessic acid[3] (**15**) except that **12** had one more hydroxyl group. The presence of three free hydroxyl groups was confirmed by acetylation of the methyl ester **12a** to a triacetate **12b**. The position of the additional hydroxyl group was determined to be at C-7 by $^1H-^1H$ COSY analysis and was further confirmed by the long-range correlations observed in the FG-pulsed HMBC spectrum (Figure 3a).



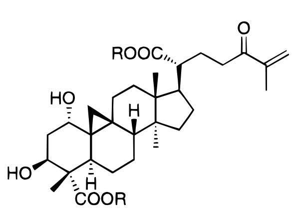
1 R_1 = Me, R_2 = COOH, R_3 = OH
1a R_1 = Me, R_2 = COOMe, R_3 = OH
6 R_1 = H, R_2 = R_3 = Me
6a R_1 = R_2 = R_3 = Me
14 R_1 = Me, R_2 = CHO, R_3 = OH



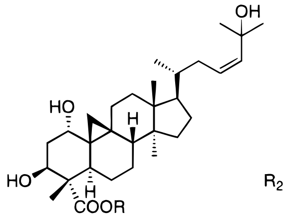
2 R_1 = Me, R_2 = COOH, R_3 = H
2a R_1 = Me, R_2 = COOMe, R_3 = H
2b R_1 = Me, R_2 = COOMe, R_3 = Ac
2c R_1 = Me, R_2 = COOMe, R_3 = (*R*)-MTPA
2d R_1 = Me, R_2 = COOMe, R_3 = (*S*)-MTPA
5 R_1 = Me, R_2 = CH_2OH, R_3 = H
11 R_1 = H, R_2 = Me, R_3 = H



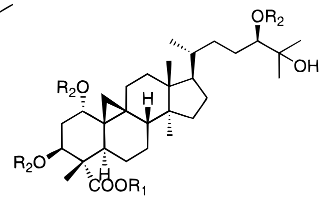
3 R_1 = Me, R_2 = COOH, R_3 = H
3a R_1 = Me, R_2 = COOMe, R_3 = H
10 R_1 = H, R_2 = Me, R_3 = H
10b R_1 = R_2 = Me, R_3 = (*R*)-MTPA)
10c R_1 = R_2 = Me, R_3 = (*S*)-MTPA)



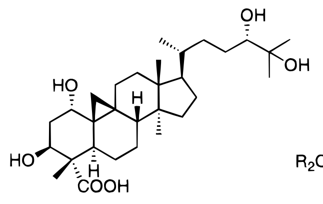
4 R =H
4a R = Me



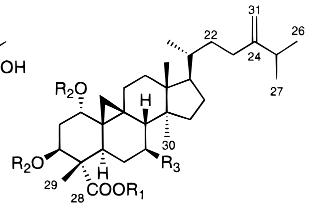
7 R = H
7a R = Me



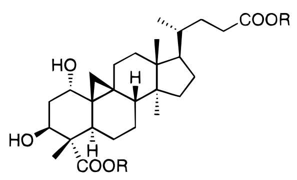
8 R_1 = R_2 = H
8a R_1 = Me, R_2 = H
8b R_1 = Me, R_2 = (*R*)-MTPA
8c R_1 = Me, R_2 = (*S*)-MTPA



9



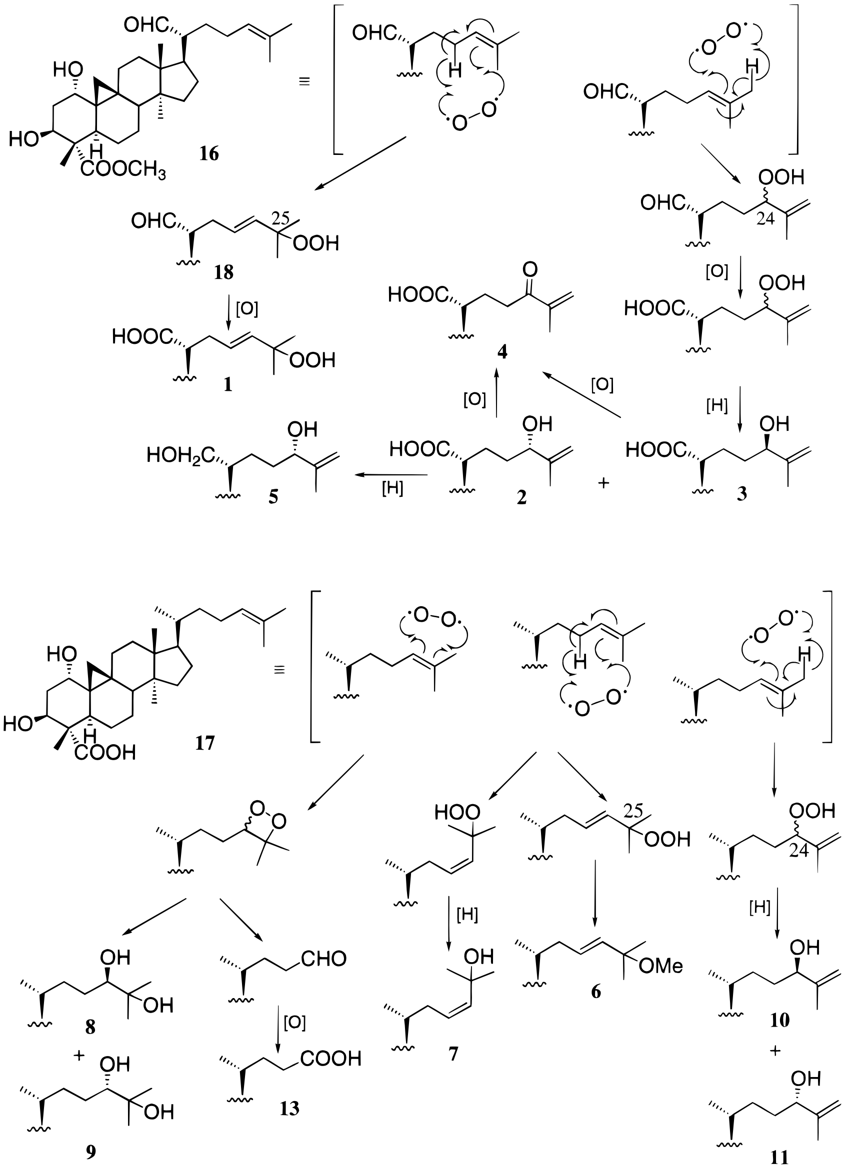
12 R_1 = R_2 = H, R_3 = OH
12a R_1 = Me, R_2 = H, R_3 = OH
12b R_1 = Me, R_2 = Ac, R_3 = OAc
15 R_1 = R_2 = H, R_3 = H_2



13 R = H
13a R = Me

The configurations of the hydroxyl groups at C-1 and C-3 were concluded to be α and β, respectively, on the basis of coupling constants of H-1 (br s) and H-3 (dd, J = 12.0, 4.5 Hz). The diaxial coupling of H-7 with H-6_{ax} (11.8 Hz) and H-8_{ax} (8.5 Hz) suggested the β configuration of OH-7. These were further confirmed by NOEs observed in NOE differ-

Scheme 3. Possible Biogenetic Pathway of **1**−**13** from the Hypothetical Precursor **16** or Mollic Acid (**17**)



ence experiments (Figure 3b). The cyclopropane methylene protons of 7β-hydroxycycloartane-type triterpenes have been reported to appear in the usual range; that is, δ 0.35−0.39 and δ 0.66−0.70.[12,13] It should be noted here that, contrary to previous reports, these protons in **12** were deshielded to resonate at δ 0.55 and 1.11.

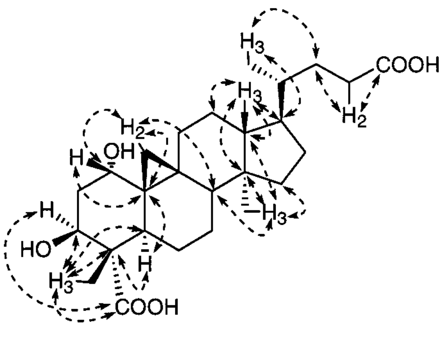


Figure 4. Significant correlations observed in the FG-pulsed HMBC spectrum of compound **13**.

Norquadrangularic acid A (**13**) was obtained as a colorless amorphous solid and gave a dimethyl ester **13a** by methylation with diazomethane. It showed a quasimolecular ion peak at m/z 485.2883 in HRFABMS corresponding to the molecular formula $C_{27}H_{42}O_6$. The IR spectrum of **13** suggested the presence of hydroxyl (3400 cm^{-1}) and carbonyl group (1700 cm^{-1}) absorptions. The ^1H NMR spectrum of **13** displayed the signals of two cyclopropane methylene protons at δ 0.82 and 0.54 (both d, J = 4.5 Hz) along with three tertiary methyls, one secondary methyl, and two oxymethine protons. These were identical to those of the other cycloartane-type triterpenes isolated from *C. quadrangulare*. The ^{13}C NMR spectrum of **13**, however, displayed only 27 carbon signals, and thus **13** was assigned as a trinorcycloartane-type triterpene. The ^{13}C NMR spectrum of **13** showed signals of two carbonyl carbons (δ 176.5 and 180.0) due to two free carboxylic acid groups. On the basis of the $^1H-^{13}C$ COSY spectrum and the long-range correlations observed in the FG-pulsed HMBC spectrum (Figure 4), the positions of the carboxylic acid groups were determined as C-23 and C-4. The positions of the two hydroxyl groups were determined as C-1α and C-3β by comparing the chemical shifts and the coupling constants of H-1 (δ 3.91, br s) and H-3 (δ 5.57, dd, J = 12.0, 4.5 Hz) with those of **1**−**12** and were consistent with the results of the NOE difference experiments. Accordingly, the structure of norquadrangularic acid A was established as **13**.

The cycloartane-type triterpenes (**1**−**13**) are all new and are characterized by the presence of 1α,3β-dihydroxy and 28-carboxyl groups, which seems to be typical for *Combretum* species among this class of compounds.[14−18] Previous literature reports reveal the presence of a carboxylic acid group either at C-4[14−18] or at C-20 in cycloartane-type triterpenes.[19−21] Compounds **1**−**4**, however, have two carboxylic acid groups at both C-4 and C-20. Furthermore, norquadrangularic acid A (**13**) is a trinorcycloartane-type triterpene. Previously, this type of triterpene was reported from *Wrightia tinctoria*[22] and *Euphorbia broteri*,[23] and the commercial drug "Cimicifuga Rhizoma",[24] but there is no previous report of their presence in *Combretum* species. In our previous work, we observed that few of the cycloartane-type triterpenes isolated from *C. quadrangulare* possessed potent cytotoxicity toward the liver-metastatic murine colon 26-L5 carcinoma cells.[3,28] The triterpenes **1**−**13**, however, showed only very weak cytotoxicity (ED_{50}: **5**, 29.4; **6**, 86.1; **7**, 82.6; **12**, 37.3; mixture of **10** and **11**, 88.4 μg/mL; others >100 μg/mL).

Most of the cycloartane-type triterpenes isolated from *C. quadrangulare* differ in the side chain attached to ring D. These compounds might be biosynthesized via photooxygenation of olefinic precursors such as **16** or mollic acid[14] (**17**) (Scheme 3). It is well-known that molecular oxygen reacts with olefins to form allylic hydroperoxides.[25−27] When the methyl proton of olefin **16** or **17** takes part in the reaction, a hydroperoxy group will be generated at C-24, which, on reduction, gives **2** and **3** (by **16**) or **10** and **11** (by **17**). If a methylene proton is involved, a hydroperoxide is generated at C-25. Then, **14** will be formed from **16**, which on oxidation gives **1** or on reduction gives methyl quadrangularate B (**18**).[2] By photooxygenation dioxetane may also be formed[25] and would lead to diols **8** and **9** through a reduction of the O−O bond or to trinorcycloartane **13** through a cleavage of O−O and C−C bonds.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-140 digital polarometer. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in KBr disks. NMR spectra were taken on a JEOL GX-400 spectrometer or a JEOL JNM-LA400WB spectrometer with tetramethylsilane (TMS) as the internal standard, and chemical shifts are expressed in δ values. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as a matrix. Column chromatography was performed with normal-phase (Fuji Silysia, BW-820 MH) or reversed-phase Si gel (Cosmosil $75C_{18}$-OPN, Nacalai Tesque Inc., Kyoto, Japan). Analytical and preparative TLC were carried out on precoated Merck Kieselgel $60F_{254}$ plates (0.25 or 0.50 mm thickness).

Plant Material. Leaves of *Combretum quadrangulare* Kurz were purchased at a local market at Ho Chi Minh City, Vietnam, in 1995. A voucher sample (TMPW 18999) is preserved in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Toyama, Japan, as a reference.

Extraction and Isolation. Air-dried leaves (2.65 kg) were extracted with MeOH (16L, 3 h × 3) at 80 °C. The filtrate was evaporated under reduced pressure to yield a dark green MeOH extract (610 g). A part of the MeOH extract (400 g) was chromatographed over Si gel with a $CHCl_3$−MeOH gradient system to give 11 fractions (fraction 1, 2% MeOH−$CHCl_3$ eluate, 6.1 g; fraction 2, 2% MeOH−$CHCl_3$ eluate, 11.3 g; fraction 3, 5% MeOH−$CHCl_3$ eluate, 20.4 g; fraction 4, 5% MeOH−$CHCl_3$ eluate, 15.0 g; fraction 5, 5% MeOH−$CHCl_3$ eluate, 39.4 g; fraction 6, 5% MeOH−$CHCl_3$ eluate, 23.2 g; fraction 7, 10% MeOH−$CHCl_3$ eluate, 10.2 g; fraction 8, 10% MeOH−$CHCl_3$ eluate, 12.9 g; fraction 9, 20% MeOH−$CHCl_3$ eluate, 23.2 g; fraction 10, 30% MeOH−$CHCl_3$ eluate, 45.7 g, and fraction 11, 50% MeOH−$CHCl_3$ eluate, 84.5 g).

Fractions 7 and 8 were combined (23.0 g) and chromatographed on a Cosmosil $75C_{18}$-OPN with H_2O−MeOH−CH_3CN (1:1:1) to give 12 subfractions. Further Si gel column chromatography and preparative TLC of subfraction 3 yielded quadrangularic acid F (**1**, 9.1 mg). Fraction 9 (20.0 g) was also applied on a Cosmosil $75C_{18}$−OPN column with H_2O−MeOH−CH_3CN (1:1:1) and eight subfractions were collected. Further Si gel column chromatography and preparative TLC of the subfractions 2−7 yielded the following compounds: fraction 2, norquadrangularic acid A (**13**, 20.6 mg), quadrangularic acid G (**2**, 120.2 mg), quadrangularic acid H (**4**, 15.5 mg), 24-epiquadrangularic acid L (**9**, 32.0 mg); fraction 3, 24-epiquadrangularic acid G (**3**, 74.3 mg), methyl quadrangularate I (**5**, 24.0 mg), quadrangularic acid L (**8**, 32.0 mg); fraction 5, a mixture of quadrangularic acid M (**10**) and 24-epiquadrangularic acid M (**11**) (63.3 mg); fraction 7, quadrangularic acid J (**6**, 73.0 mg), quadrangularic acid K (**7**, 15.5 mg), and 7β-hydroxy-23-deoxojessic acid (**12**, 62.1 mg).

Quadrangularic acid F (1): colorless amorphous solid; $[\alpha]^{25}_{D}$ +15.7° (*c* 0.03, MeOH); IR ν_{max} (KBr) 3400, 1720, 1440, 1250 cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.18 (1H, d, *J* = 16.0 Hz, H-24), 6.07 (1H, dt, *J* = 16.0, 6.0 Hz, H-23), 5.36 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 3.77 (1H, br s, H-1), 3.66 (3H, s, MeO-28), 3.23 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.40 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.19 (1H, ddd, *J* = 13.0, 12.0, 3.5 Hz, H-2), 1.60 (3H, s, H_3-29), 1.56 (3H, s, H_3-26), 1.50 (3H, s, H_3-27), 1.34 (3H, s, H_3-18), 1.04 (3H, s, H_3-30), 0.76 (1H, d, *J* = 4.5 Hz, H-19), 0.48 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 571.3239 (calcd for $C_{31}H_{48}O_8Na$ $[M + Na]^+$, 571.3247).

Quadrangularic acid G (2): colorless amorphous solid; $[\alpha]^{25}_{D}$ +73.4° (*c* 0.09, MeOH); IR ν_{max} (KBr) 3450, 1700, 1260, 1040 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.33 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 5.20 (1H, br s, H-26), 4.93 (1H, br s, H-26), 4.46 (1H, t, *J* = 5.0 Hz, H-24), 3.75 (1H, br s, H-1), 3.64 (3H, s, MeO-28), 3.21 (1H, dd, *J* = 12.5, 4.5 Hz, H-5), 2.66 (2H, m, H-17, H-11), 2.52 (1H, m, H-20), 2.37 (1H, dt, *J* = 13.0, 4.0 Hz, H-2), 2.17 (1H, ddd, *J* = 13.0, 12.0, 3.5 Hz, H-2), 1.89 (3H, s, H_3-27), 1.63 (1H, br t, *J* = 8.0 Hz, H-8), 1.58 (3H, s, H_3-29), 1.35 (3H, s, H_3-18), 1.03 (3H, s, H_3-30), 0.75 (1H, d, *J* = 4.5 Hz, H-19), 0.41 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 555.3272 (calcd for $C_{31}H_{48}O_7Na$ $[M + Na]^+$, 555.3298).

24-Epiquadrangularic acid G (3): colorless amorphous solid; $[\alpha]^{25}_{D}$ +103.5° (*c* 0.05, MeOH); IR ν_{max} (KBr) 3450, 1700, 1440, 1250 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.33 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 5.31 (1H, br s, H-26), 4.95 (1H, br s, H-26), 4.50 (1H, t, *J* = 5.0 Hz, H-24), 3.75 (1H, br s, H-1), 3.64 (3H, s, MeO-28), 3.21 (1H, dd, *J* = 12.5, 4.5 Hz, H-5), 2.72 (1H, m, H-17), 2.65 (1H, m, H-11), 2.52 (1H, m, H-20), 2.38 (1H, dt, *J* = 13.0, 4.0 Hz, H-2), 2.16 (1H, ddd, *J* = 13.0, 12.0, 3.5 Hz, H-2), 1.89 (3H, s, H_3-27), 1.58 (3H, s, H_3-29), 1.35 (3H, s, H_3-18), 1.03 (3H, s, H_3-30), 0.75 (1H, d, *J* = 4.5 Hz, H-19), 0.41 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 555.3272 (calcd for $C_{31}H_{48}O_7Na$ $[M + Na]^+$, 555.3298).

Quadrangularic acid H (4): colorless amorphous solid; $[\alpha]^{25}_{D}$ +14.3° (*c* 0.03, MeOH); IR ν_{max} (KBr) 3450, 1710, 1450, 1040 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.97 (1H, br s, H-26), 5.64 (1H, br s, H-26), 5.52 (1H, dd, *J* = 12.5, 4.5 Hz, H-3), 3.79 (1H, br s, H-1), 3.37 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.98 (2H, m, H_2-23), 2.65 (2H, m, H-11, H-20), 2.47 (2H, m, H-2, H-17), 2.24 (1H, ddd, *J* = 12.5, 12.0, 3.0 Hz, H-2), 1.86 (3H, s, H_3-27), 1.69 (3H, s, H_3-29), 1.35 (3H, s, H_3-18), 1.04 (3H, s, H_3-30), 0.80 (1H, d, *J* = 4.5 Hz, H-19), 0.42 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 539.2980 (calcd for $C_{30}H_{44}O_7Na$ $[M + Na]^+$, 539.2984).

Methyl quadrangularate I (5): colorless amorphous solid; $[\alpha]^{25}_{D}$ +137.0° (*c* 0.02, MeOH); IR ν_{max} (KBr) 3400, 1710, 1450 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.37 (1H, dd, *J* = 12.5, 4.5 Hz, H-3), 5.23 (1H, br s, H-26), 4.95 (1H, br s, H-26), 4.42 (1H, t, *J* = 5.0 Hz, H-24), 4.09 (1H, dd, *J* = 11.0, 3.0 Hz, H-21), 3.85

(1H, dd, *J* = 11.0, 5.0 Hz, H-21), 3.84 (1H, br s, H-1), 3.65 (3H, s, MeO-28), 3.24 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.75 (1H, ddd, *J* = 13.0, 8.0, 4.0, H-11), 2.42 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.21 (2H, m, H-2, H-17), 1.94 (3H, s, H_3-27), 1.61 (3H, s, H_3-29), 1.12 (3H, s, H_3-18), 1.01 (3H, s, H_3-30), 0.76 (1H, d, *J* = 4.5 Hz, H-19), 0.50 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 541.3502 (calcd for $C_{31}H_{50}O_6Na$ $[M + Na]^+$, 541.3506).

Quadrangularic acid J (6): colorless amorphous solid; $[\alpha]^{25}_D$ +8.4° (*c* 0.02, MeOH); IR ν_{max} (KBr) 3400, 1710, 1450 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.65 (1H, ddd, *J* = 15.5, 8.5, 6.0 Hz, H-23), 5.54 (2H, m, H-3, H-24), 3.90 (1H, br s, H-1), 3.40 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 3.20 (3H, s, MeO-25), 2.74 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.48 (1H, dt, *J* = 13.0, 4.0 Hz, H-2), 2.28 (2H, m, H-2, H-22), 1.72 (3H, s, H_3-29), 1.32 (6H, s, H_3-26, H_3-27), 1.05 (3H, s, H_3-18), 0.99 (3H, s, H_3-30), 0.96 (3H, d, *J* = 6.5 Hz, H_3-21), 0.84 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 525.3524 (calcd for $C_{31}H_{50}O_5Na$ $[M + Na]^+$, 525.3556).

Quadrangularic acid K (7): colorless amorphous solid; $[\alpha]^{25}_D$ +133.7° (*c* 0.03, MeOH); IR ν_{max} (KBr) 3450, 1700, 1460, 1380 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.94 (2H, m, H-23, H-24), 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 3.92 (1H, br s, H-1), 3.41 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.74 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.52 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.29 (2H, m, H-2, H-23), 1.73 (3H, s, H_3-29), 1.55 (6H, s, H_3-26, H_3-27), 1.04 (3H, s, H_3-18), 0.98 (3H, s, H_3-30), 0.95 (3H, d, *J* = 6.5 Hz, H_3-21), 0.83 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 511.3392 (calcd for $C_{30}H_{48}O_5Na$ $[M + Na]^+$, 511.3400).

Quadrangularic acid L (8): colorless amorphous solid; $[\alpha]^{25}_D$ +100.4° (*c* 0.03, MeOH); IR ν_{max} (KBr) 3450, 1700, 1370, 1040 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.56 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 3.91 (1H, br s, H-1), 3.76 (1H, dd, *J* = 8.0, 2.5 Hz, H-24), 3.42 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.75 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.50 (1H, ddd, *J* = 12.5, 4.5, 4.0 Hz, H-2), 2.29 (1H, ddd, *J* = 12.5, 12.0, 2.0 Hz, H-2), 1.73 (3H, s, H_3-29), 1.54 (3H, s, H_3-26), 1.52 (3H, s, H_3-27), 1.06 (3H, s, H_3-18), 1.00 (3H, d, *J* = 6.5 Hz, H_3-21), 0.97 (3H, s, H_3-30), 0.83 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 529.3476 (calcd for $C_{30}H_{50}O_6Na$ $[M + Na]^+$, 529.3495).

24-Epiquadrangularic acid L (9): colorless amorphous solid; $[\alpha]^{25}_D$ +76.2° (*c* 0.08, MeOH); IR ν_{max} (KBr) 3450, 1700, 1470, 1380, 1050 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 3.92 (1H, br s, H-1), 3.71 (1H, dd, *J* = 10.0, 2.0 Hz, H-24), 3.43 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.75 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.50 (1H, ddd, *J* = 12.5, 4.5, 4.0 Hz, H-2), 2.30 (1H, ddd, *J* = 12.5, 12.0, 2.0 Hz, H-2), 1.74 (3H, s, H_3-29), 1.55 (3H, s, H_3-26), 1.52 (3H, s, H_3-27), 1.05 (3H, s, H_3-18), 1.01 (3H, d, *J* = 6.5 Hz, H_3-21), 0.98 (3H, s, H_3-30), 0.84 (1H, d, *J* = 4.5 Hz, H-19), 0.56 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 529.3512 (calcd for $C_{30}H_{50}O_6Na$ $[M + Na]^+$, 529.3505).

Quadrangularic acid M (10): ^1H NMR (pyridine-d_5) δ 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 5.27 (1H, br s, H-26), 4.97 (1H, br s, H-26), 4.36 (1H, t, *J* = 6.0 Hz, H-24), 3.92 (1H, br s, H-1), 3.43 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.76 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.50 (1H, ddd, *J* = 12.5, 4.5, 4.0 Hz, H-2), 2.30 (1H, ddd, *J* = 12.5, 12.0, 2.0 Hz, H-2), 1.92 (3H, s, H_3-27), 1.74 (3H, s, H_3-29), 1.06 (3H, s, H_3-18), 0.99 (3H, s, H_3-30), 0.97 (3H, d, *J* = 6.5 Hz, H_3-21), 0.84 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19).

24-Epiquadrangularic acid M (11): ^1H NMR (pyridine-d_5) δ 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 5.22 (1H, br s, H-26), 4.97 (1H, br s, H-26), 4.36 (1H, t, *J* = 6.0 Hz, H-24), 3.92 (1H, br s, H-1), 3.43 (1H, dd, *J* = 12.0, 4.5 Hz, H-5), 2.76 (1H, ddd, *J* = 13.0, 9.0, 8.0 Hz, H-11), 2.50 (1H, ddd, *J* = 12.5, 4.5, 4.0 Hz, H-2), 2.30 (1H, ddd, *J* = 12.5, 12.0, 2.0 Hz, H-2), 1.93 (3H, s, H_3-27), 1.74 (3H, s, H_3-29), 1.06 (3H, s, H_3-18), 0.99 (3H, s, H_3-30), 0.97 (3H, d, *J* = 6.5 Hz, H_3-21), 0.84 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19).

7β-Hydroxy-23-deoxojessic acid (12): colorless crystals; mp 219 °C; $[\alpha]^{25}_D$ +80.9° (*c* 0.07, MeOH); IR ν_{max} (KBr) 3400, 1700, 1470, 1380 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.60 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 4.86 (1H, br s, H-31), 4.85 (1H, br s, H-31), 4.12 (1H, ddd, *J* = 11.0, 8.5, 4.0 Hz, H-7), 4.00 (1H, br s, H-1), 3.71 (1H, dd, *J* = 12.5, 4.5 Hz, H-5), 2.63 (1H, ddd, *J* = 13.0, 8.0, 4.0 Hz, H-11), 2.54 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.34 (1H, ddd, *J* = 13.0, 12.0, 3.5 Hz, H-2), 2.10 (1H, d, *J* = 8.5 Hz, H-8), 1.77 (3H, s, H_3-29), 1.31 (3H, s, H_3-30), 1.15 (3H, s, H_3-18), 1.11 (1H, d, *J* = 4.5 Hz, H-19), 1.06 (3H, d, *J* = 7.0 Hz, H_3-26), 1.05 (3H, d, *J* = 7.0 Hz, H_3-27), 0.98 (3H, d, *J* = 5.0 Hz, H_3-21), 0.55 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 525.3593 (calcd for $C_{31}H_{50}O_5Na$ $[M + Na]^+$, 525.3556).

Norquadrangularic acid A (13): colorless amorphous solid; $[\alpha]^{25}_D$ +200.6° (*c* 0.01, MeOH); IR ν_{max} (KBr) 3400, 1710, 1550, 1470 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 3.91 (1H, br s, H-1), 3.43 (1H, dd, *J* = 11.5, 4.5 Hz, H-5), 2.74 (1H, m, H-11), 2.62 (1H, m, H-23), 2.52 (1H, m, H-23), 2.50 (1H, m, H-2), 2.30 (1H, ddd, *J* = 12.5, 11.0, 2.0 Hz, H-2), 2.10 (1H, m, H-22), 1.92 (1H, m, H-7), 1.74 (3H, s, H_3-29), 1.03 (3H, s, H_3-18), 0.98 (3H, s, H_3-30), 0.95 (3H, d, *J* = 5.0 Hz, H_3-21), 0.82 (1H, d, *J* = 4.5 Hz, H-19), 0.54 (1H, d, *J* = 4.5 Hz, H-19); HRFABMS *m/z* 485.2883 (calcd for $C_{27}H_{42}O_6Na$ $[M + Na]^+$, 485.2879).

Oxidation of Methyl Quadrangularate B (14) to Quadrangularic acid F (1). To a stirred solution of **14** (2 mg, 3.75 μmol) in a mixture of CH_3CN (0.5 mL), aqueous NaH_2PO_4 (0.1 mg/mL, 0.5 mL), 30% H_2O_2 (40 μL), and an aqueous solution of $NaClO_2$ (0.4 mg/mL, 125 μL) were added dropwise at 10 °C, and the mixture was stirred for 2 h at 10 °C. After Na_2SO_3 (1 mg) was added, the mixture was subjected to preparative TLC with 20% MeOH−$CHCl_3$ to yield **1** (1.2 mg, 58.4%).

Oxidation of Dimethyl Quadrangularate G (2a) and Dimethyl 24-Epiquadrangularate (3a) to Dimethyl Quadrangularate H (4a). To a stirred solution of **2a** (10 mg) in $CHCl_3$ (2 mL), MnO_2 (200 mg) was added, and the mixture was stirred for 24 h at room temperature. The precipitate was filtered off, and the filtrate was purified by preparative TLC with MeOH−$CHCl_3$ (1:9) to give **4a** (2.7 mg, 26.8%). By the same procedure, **3a** (2.0 mg) also gave **4a** (0.4 mg, 20.0%).

Preparation of (*R*)- and (*S*)-MTPA Esters of Dimethyl Quadrangularate G (2a) and Methyl Quadrangularate L (8a). To a solution of **2a** (10 mg) in $CHCl_3$ (0.5 mL) and pyridine (0.5 mL), (*R*)-MTPA-Cl (100 μL) was added, and the mixture was stirred overnight at room temperature. The reaction mixture was then directly purified by preparative TLC with MeOH−$CHCl_3$ (1:19) to give (*R*)-MTPA ester **2c** (15.4 mg; 70.4%). By the same procedure, the (*S*)-MTPA ester **2d** (14.4 mg, 65.9%) and the (*R*)- and (*S*)-MTPA esters of **8a**, **8b** (3.7 mg, 47.8%), and **8c** (3.5 mg, 38.9%) were prepared.

(*R*)-MTPA ester of dimethyl quadrangularate G (2c): colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 7.59−7.30 (15H, m, Ph-H × 3), 5.69 (1H, dd, *J* = 12.5, 4.5 Hz, H-3), 5.27 (1H, t, *J* = 5.5 Hz, H-24), 5.10 (1H, br s, H-26), 4.87 (1H, br s, H-26), 4.78 (1H, br s, H-1), 3.58 (3H, s, MeO-21), 3.49 (3H, s, MeO-28), 3.60, 3.47, 3.40 (each 3H, s, OMe × 3), 2.45 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.41 (1H, dd, *J* = 12.0, 4.0 Hz, H-5), 2.19 (1H, td, *J* = 10.0, 3.0 Hz, H-22), 2.00 (1H, br t, *J* = 10.0 Hz, H-22), 1.90 (1H, ddd, *J* = 13.0, 12.5, 3.5 Hz, H-2), 1.60 (1H, m, H-23), 1.50 (3H, s, H_3-27), 1.48 (1H, m, H-23), 1.08 (3H, s, H_3-29), 0.87 (3H, s, H_3-18), 0.73 (1H, d, *J* = 4.5 Hz, H-19), 0.53 (1H, d, *J* = 4.5 Hz, H-19), 0.52 (3H, s, H_3-30); HRFABMS *m/z* 1217.4624 (calcd for $C_{62}H_{71}F_9O_{13}Na$ $[M + Na]^+$, 1217.4649).

(*S*)-MTPA ester of dimethyl quadrangularate G (2d): colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 7.59−7.30 (15H, m, Ph-H × 3), 5.63 (1H, dd, *J* = 12.5, 4.5 Hz, H-3), 5.30 (1H, t, *J* = 5.5 Hz, H-24), 4.99 (1H, br s, H-26), 4.92 (1H, br s, H-26), 4.77 (1H, br s, H-1), 3.57 (3H, s, MeO-21), 3.56 (3H, s, MeO-28), 3.50, 3.45, 3.38 (each 3H, s, OMe × 3), 2.49 (1H, dd, *J* = 12.0, 4.0 Hz, H-5), 2.35 (1H, ddd, *J* = 13.0, 4.5, 4.0 Hz, H-2), 2.15 (1H, td, *J* = 10.0, 3.0 Hz, H-22), 2.00 (1H, br t, *J* = 10.0 Hz, H-22), 1.77 (1H, ddd, *J* = 13.0, 12.5, 3.5 Hz, H-2), 1.60 (3H, s, H_3-27), 1.57 (1H, m, H-23), 1.40 (1H, m, H-23), 1.10 (3H, s, H_3-29), 0.88 (3H, s, H_3-18), 0.76 (1H, d, *J* = 4.5 Hz, H-19), 0.72 (3H, s, H_3-30), 0.52 (1H, d, J = 4.5 Hz, H-19); HRFABMS m/z 1217.4657 (calcd for $C_{62}H_{71}F_9O_{13}Na$ $[M + Na]^+$, 1217.4649).

(*R*)-MTPA ester of methyl quadrangularate L (8b): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.62−7.31 (15H, m, Ph-H × 3), 5.71 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.89 (1H, dd, J = 9.0, 2.5 Hz, H-24), 4.81 (1H, br s, H-1), 3.50 (3H, s, MeO-28), 3.62, 3.50, 3.41 (each 3H, s, OMe × 3), 2.46 (1H, ddd, J = 12.5, 4.5, 4.0 Hz, H-2), 2.42 (1H, dd, J = 12.0, 4.5 Hz, H-5), 1.92 (1H, dd, J = 12.5, 12.0, 2.0 Hz, H-2), 1.50 (2H, m, H_2-23), 1.19 (3H, s, H_3-29), 1.10 (3H, s, H_3-26), 1.10 (3H, s, H_3-27), 0.79 (3H, s, H_3-18), 0.76 (1H, d, J = 4.5 Hz, H-19), 0.68 (3H, d, J = 6.5 Hz, H_3-21), 0.55 (1H, d, J = 4.5 Hz, H-19), 0.53 (3H, s, H_3-30); HRFABMS m/z 1191.4825 (calcd for $C_{61}H_{73}F_9O_{12}Na$ $[M + Na]^+$, 1191.4856).

(*S*)-MTPA ester of methyl quadrangularate L (8c): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.57−7.31 (15H, m, Ph-H × 3), 5.65 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.91 (1H, dd, J = 9.0, 2.5 Hz, H-24), 4.80 (1H, br s, H-1), 3.56 (3H, s, MeO-28), 3.53, 3.50, 3.39 (each 3H, s, OMe × 3), 2.42 (1H, dd, J = 12.0, 4.5 Hz, H-5), 2.36 (1H, ddd, J = 12.5, 4.5, 4.0 Hz, H-2), 1.95 (1H, td, J = 11.0, 3.5 Hz, H-11), 1.80 (1H, ddd, J = 12.5, 12.0, 3.5 Hz, H-2), 1.60 (2H, m, H_2-23), 1.19 (3H, s, H_3-29), 1.11 (3H, s, H_3-26), 1.11 (3H, s, H_3-27), 0.84 (3H, s, H_3-18), 0.77 (1H, d, J = 4.5 Hz, H-19), 0.75 (3H, d, J = 6.5 Hz, H_3-21), 0.74 (3H, s, H_3-30), 0.55 (1H, d, J = 4.5 Hz, H-19); HRFABMS m/z 1191.4840 (calcd for $C_{61}H_{73}F_9O_{12}Na$ $[M + Na]^+$, 1191.4856).

Dehydration of MTPA Esters 8b and 8c with $POCl_3$. To a solution of **8b** (1.0 mg) in pyridine (100 μL), $POCl_3$ (20 μL) was added, and the mixture was stirred overnight at room temperature. After the reaction mixture was poured in ice-cold water (5 mL), the mixture was extracted with $CHCl_3$ (5 mL × 3). The $CHCl_3$ extract was washed with water, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to yield **10b** (0.4 mg, 40.9%). By a similar procedure **10c** (2.0 mg, 84.0%) was prepared from **8c** (2.4 mg).

(*R*)-MTPA ester of methyl quadrangularate M (10b): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.68−7.36 (15H, m, Ph-H × 3), 5.76 (1H, dd, J = 12.0, 4.5 Hz, H-3), 5.38 (1H, dd, J = 10.0, 2.0 Hz, H-24), 5.02 (1H, br s, H-26), 4.94 (1H, br s, H-26), 4.86 (1H, br s, H-1), 3.55 (3H, s, MeO-28), 3.68, 3.53, 3.47 (each 3H, s, OMe × 3), 2.53 (1H, ddd, J = 12.5, 4.5, 4.0 Hz, H-2), 2.49 (1H, dd, J = 12.0, 4.5 Hz, H-5), 1.99 (1H, dd, J = 12.5, 12.0, 2.0 Hz, H-2), 1.74 (1H, m, H-23), 1.71 (3H, s, H_3-27), 1.50 (1H, s, H-23), 1.17 (3H, s, H_3-29), 0.84 (3H, s, H_3-18), 0.79 (1H, d, J = 4.5 Hz, H-19), 0.77 (3H, d, J = 6.5 Hz, H_3-21), 0.61 (1H, d, J = 4.5 Hz, H-19), 0.56 (3H, s, H_3-30); HRFABMS m/z 1173.4735 (calcd for $C_{61}H_{71}F_9O_{11}Na$ $[M + Na]^+$, 1173.4750).

(*S*)-MTPA ester of methyl quadrangularate M (10c): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.60−7.38 (15H, m, Ph-H × 3), 5.71 (1H, dd, J = 12.0, 4.5 Hz, H-3), 5.34 (1H, dd, J = 10.0, 2.0 Hz, H-24), 4.94 (1H, br s, H-26), 4.90 (1H, br s, H-26), 4.86 (1H, br s, H-1), 3.63 (3H, s, MeO-28), 3.60, 3.56, 3.45 (each 3H, s, OMe × 3), 2.56 (1H, dd, J = 12.0, 4.5 Hz, H-5), 2.43 (1H, ddd, J = 12.5, 4.5, 4.0 Hz, H-2), 1.83 (1H, dd, J = 12.5, 12.0, 2.0 Hz, H-2), 1.82 (1H, m, H-23), 1.61 (3H, s, H_3-27), 1.56 (1H, m, H-23), 1.17 (3H, s, H_3-29), 0.88 (3H, s, H_3-18), 0.86 (3H, d, J = 6.5 Hz, H_3-21), 0.83 (1H, d, J = 4.5 Hz, H-19), 0.81 (3H, s, H_3-30), 0.63 (1H, d, J = 4.5 Hz, H-19); HRFABMS m/z 1173.4744 (calcd for $C_{61}H_{71}F_9O_{11}Na$ $[M + Na]^+$, 1173.4750).

Cytotoxic Assay. Cellular viability in the presence and absence of experimental agents were determined using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (Sigma, St. Louis, MO) assays, as described previously.[29]

Supporting Information Available: ^{1}H and ^{13}C NMR data of **1a**−**4a**, **6a**−**8a**, **12a**, **13a**, **2b**, and **12b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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