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CYTOTOXIC CYCLOARTANE-TYPE TRITERPENES FROM *COMBRETUM QUADRANGULARE*

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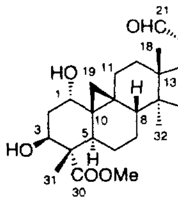
Abstract: Seven novel cycloartane-type triterpenes were isolated from *Combretum quadrangulare*, and their structures were elucidated on the basis of spectral analysis. All these compounds were tested for their cytotoxicity against murine colon 26-L5 carcinoma cells. Methyl quadrangularate B (**2**) and methyl quadrangularate D (**4**) exhibited potent cytotoxicity having ED_{50} values 9.54 and 5.42 μM, respectively.

Combretum species (Combretaceae) are widely used as folk medicine for the treatment of hepatitis, malaria, respiratory infection and even cancer in different parts of Asia and Africa.[1] With a view to isolate biologically active compounds from *C. quadrangulare*, grown wildly in Vietnam and prized for its antipyretics, antidysentery and anthelmintic activities,[2] chemical analysis of leaves of *C. quadrangulare* was undertaken. Its MeOH extract showed strong cell proliferative activity against murine colon 26-L5 carcinoma cells. Fractionation guided by proliferation assay with colon 26-L5 cells led us to isolate seven novel cycloartane-type triterpenes. Here we would like to communicate the isolation and cytotoxicity of the novel triterpenes.

Isolation and structure

Air dried leaves of *Combretum quadrangulare* Kurz (2.65 kg) were extracted with MeOH (10L, 3h, × 3) at 80°C. After evaporation under reduced pressure a dark green MeOH extract (610 g) was obtained. Repeated column chromatography on normal and reversed-phase silica gel, together with preparative TLC, afforded seven novel triterpenes, *i.e.*, methyl quadrangularate A—D (**1—4**), 23-deoxojessic acid (**6**), methyl O^1-acetyl-23-deoxojessic acid (**7**) and quadrangularic acid E (**9**),[3] along with a known cycloartane 4β,14α-dimethyl-5α-ergosta-9β,19-cyclo-24(24¹)-en-3β-hydroxy-4α-carboxylic acid (**10**).[4]



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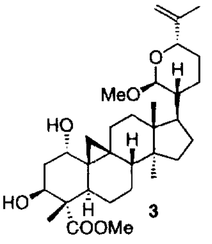

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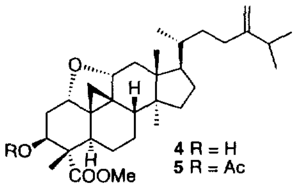

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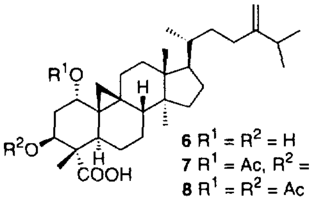
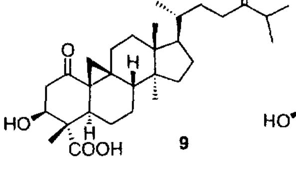


6 R¹ = R² = H
7 R¹ = Ac, R² = H
8 R¹ = R² = Ac



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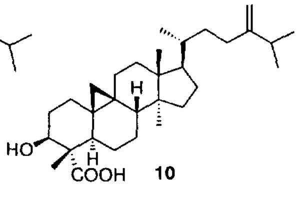

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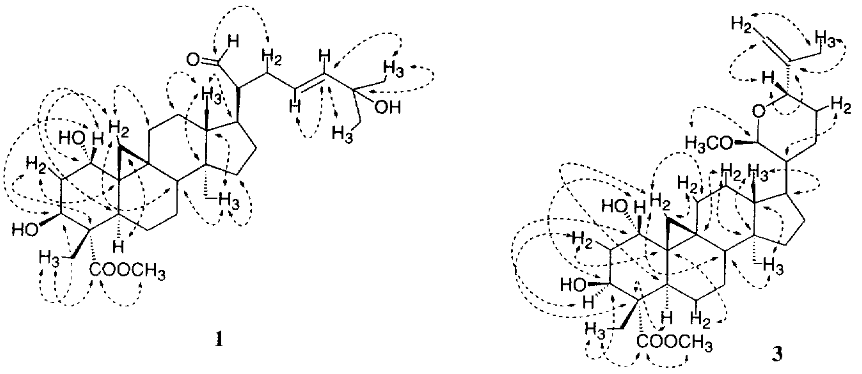
Methyl quadrangularate A (**1**) showed a molecular ion peak at *m/z* 516.3442 in HRMS, corresponding to the molecular formula $C_{31}H_{48}O_6$. Broad IR absorption bands at 3450 cm^{-1} and 1715 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^1H NMR spectrum of **1** displayed two coupled doublets at δ 0.74 and 0.44 (J = 4.5 Hz), characteristic to a methylene group on a cyclopropane. In addition, the signals of five tertiary methyls, two oxygenated methines, one aldehyde group and one *trans*-olefin were observed in the ^1H NMR spectrum, suggesting that **1** should be a cycloartane-type triterpene bearing an aldehyde and a *trans*-olefin. The ^1H and ^{13}C NMR spectra of **1** were closely related with those of mollic acid,[5] except the difference of the signals in the side chain on D-ring. Two olefinic protons at δ 5.89 (dt, J = 16.0, 6.5 Hz) and 5.97 (d, J = 16.0 Hz) in the ^1H NMR spectrum of **1** indicated that the position of the olefin group differ from that of mollic acid which have only one olefinic proton. Furthermore, appearance of the signals due to an oxygenated quaternary carbon at δ 69.5 and upfield shift of the signals of geminal methyls suggested the presence of a hydroxy group at C-25. This observation, together with the ^1H-^1H COSY correlation, indicated the position of the olefin to be C-23(24). In addition, the ^1H-^1H COSY spectrum indicated that the aldehyde group should be located at C-21, which was supported by the long-range ^1H-^{13}C COSY correlation (Fig. 1). The stereochemistry of 1-OH was determined as α because the 1-H signal appears as broad singlet, while the coupling constants of 3-H (dd, J = 12, 5.5 Hz) indicated that the 3-OH group should be in β position. In the difference NOE experiments irradiation of the methyl protons at δ 1.59 caused an NOE increase at H-19 and *vice versa*, indicating that 31-methyl group should be in β position. Finally the structure of methyl quadrangularate A was elucidated as **1**, which was confirmed by the fact that reduction of **2** gave **1**.

Methyl quadrangularate B (**2**) has molecular formula $C_{31}H_{48}O_7$, one more oxygen atom than that of **1**. The ^1H and ^{13}C NMR spectra of **2** were almost identical to those of **1** (Table 1). However the downfield shift of 24-H (**1**, δ 5.97; **2**, δ 6.05) and upfield shift of 23-H (**1**, δ 5.89; **2**, δ 5.78), together with the downfield shift of C-25 (**1**, δ 69.5; **2**, δ 80.9), suggested the presence of a hydroperoxy group instead of a hydroxy group in **1**.[6,7] The structure of **2** was determined by NOE experiments and X-ray analysis (Fig. 2).[8] This also confirmed the structure of **1** because reduction of **2** with triphenylphosphine[9] gave **1**.

Molecular formula of methyl qudrangularate C (**3**) was deduced as $C_{32}H_{50}O_6$ by the positive ion FABHRMS. Its IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and carbonyl groups (1720 cm^{-1}). The ^1H NMR spectrum showed signals of two cyclopropane methylene protons, three tertiary methyls, two oxygenated methine and a methyl ester, suggesting that **3** should also be a cycloartane-type triterpene. The ^1H and ^{13}C NMR spectra (Table 1 and ref. 3) indicated that the rings A—D of **3** was the same as those of **1** and that the aldehyde group in **3** was absent. The signals due to two oxygenated methines [δ_H 4.90 (br s), δ_C 100.1; δ_H 4.22 (br d, J= 11.0 Hz), δ_C 71.27] suggested the presence of a hemiacetal pyran ring on the side chain.[10] The ^1H NMR spectrum, together with the ^1H-^1H COSY, ^1H-^{13}C COSY and long-range ^1H-^{13}C COSY correlations (Fig. 1), fully supported the existence of tetrahydropyrane ring with isopropenyl group. Based on these evidences, the structure of methyl



1 **3**

Fig. 1. Significant correlations observed in the long-range ^1H-^{13}C COSY spectrum of **1** and **3**.

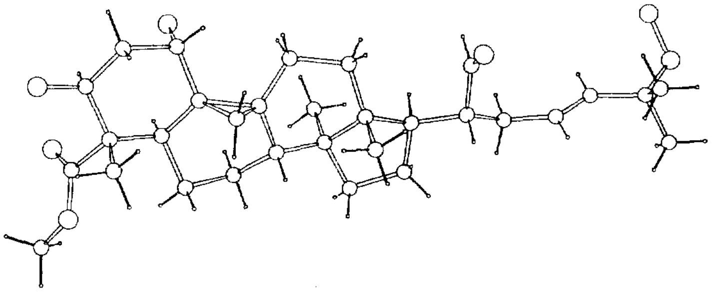


Fig. 2. X-ray structure of methyl quadrangularate B (**2**).

quadrangularate C was elucidated as **3**. The stereochemistry of rings A—D was determined by NOE experiment to be the same as that of **1**. While the relative stereochemistry of tetrahedropyran ring was established to be *equatorial* 21-H and *axial* 24-H from the coupling pattern of 21-H (br s) and 24-H (br d, *J* = 11.0 Hz). Stereochemistry of C-20 was considered as same as **1** and **2** because all the compounds were obtained from the same extract.

Table 1. ^{13}C NMR data (100 MHz) of cycloartanes **1—10** in pyridine-d_5

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	72.1	72.8	72.4	80.4	79.4	72.5	76.5	75.6	208.7	32.2
2	38.6	38.6	38.7	37.0	37.0	38.8	35.0	31.0	49.0	30.8
3	70.4	70.1	70.5	71.7	74.6	70.7	70.3	74.2	73.6	75.3
4	55.9	55.9	56.1	51.6	50.1	55.6	55.1	52.8	54.7	55.4
5	37.7	37.7	38.0	37.3	37.9	37.7	38.9	39.1	44.7	44.6
6	23.0	23.0	23.3	19.0	19.9	23.4	22.8	22.4	22.0	23.5
7	25.8	25.8	27.2	22.1	22.9	28.3	28.2	28.2	28.0	28.3
8	47.2	47.4	48.2	38.8	38.6	48.1	46.3	46.2	43.8	47.8
9	20.6	20.6	20.8	32.9	33.0	20.8	20.8	21.4	28.1	19.9
10	30.3	30.3	30.2	37.5	37.2	30.3	30.0	30.0	37.1	25.7
11	25.5	25.5	27.2	84.4	84.7	26.6	26.4	26.3	27.8	26.6
12	31.6	31.6	31.6	39.6	39.7	33.2	33.0	33.0	33.2	33.1
13	45.4	45.5	45.9	47.5	47.6	45.6	45.2	45.4	45.4	45.5
14	48.9	48.9	49.1	49.5	49.5	49.1	49.2	49.1	49.5	49.0
15	35.3	35.3	35.9	28.4	28.4	35.9	35.5	35.5	34.6	35.6
16	26.8	26.8	25.8	34.0	33.9	25.8	25.1	24.9	23.5	25.9
17	47.5	47.3	46.9	51.3	51.3	52.5	52.5	52.5	52.2	52.5
18	19.2	19.1	19.1	15.5	15.6	18.3	17.9	17.9	18.4	18.2
19	29.4	29.3	29.9	30.6	30.0	29.7	28.0	28.0	28.2	29.8
20	55.9	55.8	42.8	36.3	36.3	36.5	36.4	36.4	36.4	36.4
21	205.5	205.4	100.1	18.7	18.9	18.5	18.5	18.5	16.9	18.5
22	32.3	32.6	38.8	35.3	35.3	35.3	35.2	35.3	35.3	35.3
23	122.8	138.0	24.7	31.6	31.6	31.6	31.7	31.7	31.6	31.6
24	142.4	126.5	71.2	156.6	156.7	156.7	156.7	156.7	156.7	156.7
25	69.5	80.9	146.8	34.0	34.1	34.0	34.0	34.1	34.0	34.0
26	30.5	25.2	110.4	22.0	22.2	22.0	22.1	22.1	21.9	22.1
27	30.5	24.9	19.0	21.9	22.0	21.9	22.0	22.0	21.9	22.0
28				106.6	106.7	106.6	106.6	106.7	106.6	106.6
30	176.1	178.1	178.1	178.5	177.1	179.9	179.5	177.9	179.0	179.8
31	9.4	9.4	9.5	13.6	13.2	9.7	9.6	10.5	10.6	10.4
32	19.0	19.1	19.1	18.8	19.0	19.4	19.1	19.1	18.5	19.4
COOMe	51.4	51.0	51.6	51.7	52.1					
OMe			53.9							
OAc					20.5		21.1	21.1		
					170.5		170.1	170.4		
OAc								20.0		
								170.0		

Methyl quadrangularate D (**4**), $C_{32}H_{50}O_4$, showed hydroxy and carbonyl absorptions in the IR spectrum. Its ^{1}H NMR signal indicated the presence of three oxygenated methines, two terminal methylene protons, three secondary methyls and three tertiary methyl groups along with characteristic signals of cyclopropane methylenes. The downfield chemical shift of cyclopropane methylene proton (δ 1.35 and 0.71) suggested that there should be presence of an oxygen substituent at C-11[11] which was further confirmed by ^{1}H-^{1}H COSY spectrum and HMBC correlations (Fig. 3). The downfield shift of both carbon and proton signals of C-1 and C-11 of **4**, as compare to cycloartane-type triterpene bearing hydroxy groups in the positions,[11] suggested the presence of ether linkage between C-1 and C-11. It was further supported by the fact that by an acetylation with acetic anhydride and pyridine **4** gave only monoacetate **5**. The stereochemistry was deduced by the difference NOE experiments and coupling constants. In the NOE experiments, NOEs were observed between H-11 and H_3-18, between H-8 and H_3-18, between H-19 and H-1, between H-19 and H-11 and between H_3-31 and H-1, suggesting that all the protons, H_3-31, H_3-18, H-19, H-11 and H-1, are in β position (Fig. 4). The coupling constants of H-1 (dd, J = 11.0, 6.0 Hz) and H-3 (dd, J = 4.5, 2.5 Hz) as well as NOE results suggested that A-ring should be in boat conformation and hydroxy group at C-3 in β-orientation. On these evidences the structure of methyl quadrangularate D was determined as **4**, which has a very interesting ether linkage between C-1 and C-11. To the best of our knowledge, it is the first report with such linkage between C-1 to C-11 in cycloartanol series.

23-Deoxojessic acid (**6**) showed the presence of hydroxy and carbonyl groups in the IR spectrum and its FABHRMS indicated the molecular formula should be $C_{31}H_{50}O_4$. The ^{1}H NMR spectrum of **6** showed characteristic signals of cyclopropane methylene protons, three tertiary methyls, two oxygenated methines and two terminal olefin protons. These signals, and also the ^{13}C NMR data (Table 1), were similar to those of jessic acid[12] except the absence of keto group at C-23. Thus **6** was concluded as 23-deoxojessic acid.

Compound **7**, $C_{33}H_{52}O_5$, was isolated as colorless amorphous solid and the ^{1}H and ^{13}C NMR spectra showed additional signal of an acetyl group than **6**. The position of the acetyl group was deterimined as C-1 by the long-range ^{13}C-^{1}H COSY correlations. Hydrolysis of **7** gave **6** and acetylation of both **6** and **7** gave the same diacetate derivative **8**. Thus **7** was elucidated as methyl O^1-acetyl-23-deoxojessic acid.

Quadrangularic acid E (**9**) showed molecular ion peak at m/z 484.3580 corresponding to the molecular formula $C_{31}H_{48}O_4$. Its ^{1}H NMR spectrum demonstrated the presence of only one oxygenated methine proton, three secondary methyls, three tertiary methyls and two terminal olefin protons. Both of the characteristic cyclopropane methylene protons of **10** were found to be highly deshilded as compared to the rest at δ 1.30 and 1.02 (both d, J = 4.5 Hz). One additional keto signal at δ 208.7 was found in the ^{13}C NMR spectrum instead of a hydroxyl-bearing carbon as found in **1**—**8**. The position of the keto group was assigned at C-1 and a hydroxy group at C-3 by the HMBC correlations and the stereochemistry of the ring were determined by NOE experiments, accordingly the structure of quadrangularic acid E was determined as **9**.

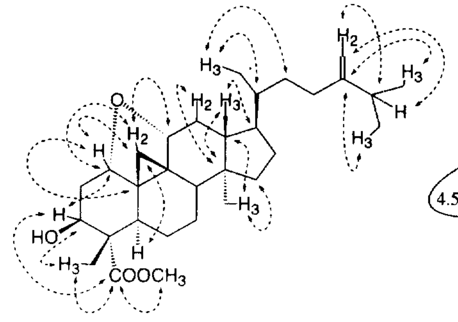


Fig. 3. Significant correlations observed in the HMBC spectrum of **4**.

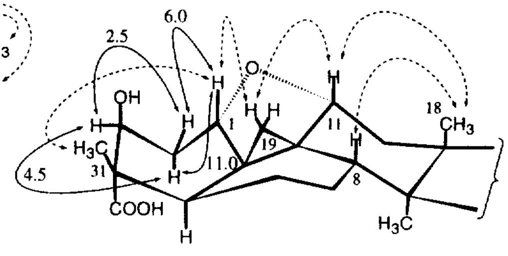


Fig. 4. NOE's observed in the difference NOE experiment of **4** (---) and coupling constant of A-ring protons (—).

Cytotoxicity

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 (MTT Sigma, St. Louis, MO, USA) assays.[13] Among the isolated compounds, only **2**—**4** showed the strong cytotoxicity towards murine colon 26-L5 carcinoma cells having ED$_{50}$ values less than 20 μM. The hydroperoxide group in the terpene skeleton seemed to enhance the cytotoxicity. Compound **4** having ether linkage between C-1 to C-11 showed the strongest cytotoxicity, but its activity dramatically decreased after acetylation. Rest of the compounds had moderate tumor cell proliferation activity. Regarding with the previous literature on the cytotoxicity of cycloartanes, only two compounds, 9,19-cycloart-23-ene-3β-24-diol (ED$_{50}$, 7.5 μM) and 9,19-cycloart-23-ene-3β-25-diol, were reported to have cytotoxicity, and a simple cycloartane such as cycloartanol had no effect upto 233 μM against Ehrlich ascites tumor cells.[14] It is therefore interesting to note that in the present study we have isolated a cycloartane, methyl quadrangutarate D (**4**), which had strong proliferation activity in murine colon 26-L5 carcinoma with an ED$_{50}$ value of 5.42 μM. The simplest cycloartane **10**, which has only one hydroxy group and a terminal olefin, had an ED$_{50}$ value of 62.38 μM. These results led us to conclude that the carboxylic acid group at C-4 and the terminal olefin in the cycloartanol skeleton seemed to be responsible for the tumor cell proliferation activity. The hydroxy group at C-1 have no any significant role for the proliferation activity but the hydroxy group at C-3 in boat conformation, *i.e.* methyl quadrangularate D (**4**), play key role for the cytotoxicity.

Table 2. Cytotoxicity of cycloartanes **1**—**10** against murine colon 26-L5

Cycloartanes	ED$_{50}$ (μM)^{a}
Methyl quadrangularate A (**1**)	43.79
Methyl quadrangularate B (**2**)	9.54
Methyl quadrangularate C (**3**)	18.11
Methyl quadrangularate D (**4**)	5.42
Methyl *O*^{3}-acetylquadrangularate D (**5**)	77.77
23-Deoxojessic acid (**6**)	49.56
Methyl *O*^{1}-acetyl-23-deoxojessic acid (**7**)	57.65
O^{1,3}-Diacetyl-23-deoxojessic acid (**8**)	52.54
Quadrangularic acid E (**9**)	58.59
4β,14α-Dimethyl-5α-ergosta-9β,19-cyclo-24(24^{1})-en-3β-hydroxy-4α-carboxylic acid (**10**)	62.38

[a] ED$_{50}$ values were calculated from the mean of data from six determinations.

References and Notes

1. Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666.
2. WHO Regional Office for the Western Pacific Malina, and Institute of Material Medica Hanoi, In *Medicinal Plants in Vietnam*, Science and Technology Publishing House: Hanoi, 1990, pp. 119.
3. **Characterization Data:**

   Methyl quadrangularate A (**1**): colorless amorphous solid; $[\alpha]_D^{25}$ +43.7° (c = 0.76, MeOH); EIHRMS *m/z*: 516.3442 [calcd for $C_{31}H_{48}O_6$ $(M)^+$, 516.3443]; IR ($CHCl_3$) ν_{max} 3450, 1715, 1460, 1380, 1260, 1090, 1050, 1010 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 9.61 (1H, d, J = 5.5 Hz, H-21), 5.97 (1H, d, J = 16 Hz, H-24), 5.89 (1H, dt, J = 16, 6.5 Hz, H-23), 5.35 (1H, dd, J = 12, 5.5, H-3), 3.82 (1H, br s, H-1), 3.23 (1H, dd, J = 12.5, 4.5 Hz, H-5), 3.66 (3H, s, COOMe), 2.40 (1H, ddd J = 13, 4, 3.5 Hz, H-2), 2.34 (2H, dd, J = 9, 6.5 Hz, H-22), 2.20 (1H, m, H-2), 1.59 (3H, s, H_3-31), 1.49 (3H, s, H_3-26), 1.49 (3H, s, H_3-27), 1.08 (3H, s, H_3-18), 0.98 (3H, s, H_3-32), 0.74 (1H, d, J = 4.5 Hz, H-19), 0.44 (1H, d, J = 4.5 Hz, H-19).

   Methyl quadrangularate B (**2**): colorless crystals; mp 190 °C; $[\alpha]_D^{25}$ +53.4° (c = 0.79, MeOH); FABHRMS *m/z*: 555.3315 [calcd for $C_{31}H_{48}O_7Na$ $(M+Na)^+$, 555.3312]; IR (KBr) ν_{max} 3500, 1710, 1450, 1380, 1040, 990, 750 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 9.63 (1H, d, J = 5.5 Hz, H-21), 6.05 (1H, d, J = 16 Hz, H-24), 5.78 (1H, dt, J = 16, 6.5 Hz, H-23), 5.37 (1H, dd, J = 12, 5.5 Hz, H-3), 3.85 (1H, br s, H-1), 3.23 (1H, dd, J = 12.5, 4.5 Hz, H-5),

3.66 (3H, s, COOMe), 2.41 (1H, ddd *J* = 13, 4, 3.5 Hz, H-2), 2.34 (2H, dd, *J* = 9, 6.5 Hz, H-22), 2.20 (1H, m, H-2), 1.60 (3H, s, H_3-31), 1.53 (3H, s, H_3-26), 1.51 (3H, s, H_3-27), 1.08 (3H, s, H_3-18), 0.98 (3H, s, H_3-32), 0.74 (1H, d, *J* = 4.5 Hz, H-19), 0.44 (1H, d, *J* = 4.5 Hz, H-19).

Methyl quadrangularate C (**3**): colorless amorphous solid; $[\alpha]_D^{25}$ +37.2° (*c* = 0.33, $CHCl_3$); FABHRMS *m/z* 531.3672 [calcd for $C_{32}H_{51}O_6(M+H)^+$, 531.3674]; IR ($CHCl_3$) ν_{max} 3400, 1720, 1450, 1230, 1110, 1030 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.33 (1H, dd, *J* = 12, 4.5 Hz, H-3), 5.18 (1H, br s, H-26), 4.92 (1H, br s, H-26), 4.90 (3H, br s, H_3-21), 4.22 (1H, br d, *J* = 11 Hz, H-24), 3.85 (1H, br s, H-1), 3.65 (3H, s, COOMe), 3.35 (3H, s, OMe), 3.23 (1H, dd, *J* = 12, 4.5 Hz, H-5), 2.42 (1H, ddd, *J* = 13, 4, 4 Hz, H-2), 2.22 (1H, ddd, *J* = 14, 13, 3.5 Hz, H-2), 1.85 (3H, s, H_3-27), 1.62 (3H, s, H_3-31), 1.04 (3H, s, H_3-18), 0.99 (3H, s, H_3-32), 0.76 (1H, d, *J* = 4.5 Hz, H-19), 0.52 (1H, d, *J* = 4.5 Hz, H-19).

Methyl quadrangularate D (**4**): colorless amorphous solid; $[\alpha]_D^{25}$ +57.4° (*c* = 0.39, MeOH); FABHRMS *m/z* 499.3814 [calcd for $C_{32}H_{51}O_4(M+H)^+$, 499.3801]; IR ($CHCl_3$) ν_{max} 3400, 1720, 1460, 1380, 1240 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 4.95 (1H, dd, *J* = 4.5, 2.5 Hz, H-3), 4.87 (1H, br s, H-28), 4.84 (1H, br s, H-28), 4.27 (1H, dd, *J* = 11, 6 Hz, H-1), 3.93 (1H, dd, *J* = 9.5, 6 Hz, H-11), 3.67 (3H, s, COOMe), 2.62 (1H, dd, *J* = 11.5, 3 Hz, H-5), 2.60 (1H, ddd, *J* = 12.5, 6, 2.5 Hz, H-2), 2.40 (1H, ddd, *J* = 12.5, 11, 5 Hz, H-2), 2.30 (1H, dd, *J* = 14, 4 Hz, H-12), 2.14 (1H, dd, *J* = 10, 5.5 Hz, H-8), 1.86 (1H, dd, *J* = 14, 6 Hz, H-12), 1.55 (3H, s, H_3-31), 1.37 (1H, d, *J* = 4.5 Hz, H-19), 1.06 (3H, s, H_3-32), 1.07 (3H, d, *J* = 7 Hz, H_3-21), 1.06 (3H, d, *J* = 7 Hz, H_3-26), 0.93 (3H, d, *J* = 6.5 Hz, H_3-27), 0.90 (3H, s, H_3-18), 0.71 (1H, d, *J* = 4.5 Hz, H-19)

23-Deoxojessic acid (**6**): colorless amorphous solid; $[\alpha]_D^{25}$ +75.4° (*c* = 0.24, MeOH); FABHRMS *m/z* 486.3663 [calcd for $C_{31}H_{50}O_4$ M^+, 486.3672]; IR ($CHCl_3$) ν_{max} 3400, 1700, 1470, 1380, 1260 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.57 (1H, dd, *J* = 12.0, 4.5 Hz, H-3), 4.87 (1H, br s, H-28), 4.86 (1H, br s, H-28), 3.92 (1H, br s, H-1), 3.44 (1H, dd. *J* = 12, 4.5 Hz, H-5), 2.76 (1H, ddd, *J* = 14, 10, 7 Hz, H-11), 2.50 (1H, ddd, *J* = 13.5, 4, 3.5 Hz, H-2), 2.32 (1H, m, H-2), 2.20 (1H, ddd, *J* = 14.5, 10, 3.5 Hz, H-22), 1.98 (1H, ddd, *J* = 14.5, 10, 5 Hz, H-22), 1.74 (3H, s, H_3-31), 1.07 (3H, d, *J* = 7 Hz, H_3-26), 1.06(3H, d, *J* = 7 Hz, H_3-27), 1.06 (3H, s, H_3-18), 1.01 (3H, s, H_3-32), 0.96 (3H, d, *J* = 6 Hz, H_3-18), 0.85 (1H, d, *J* = 4.5 Hz, H-19), 0.56 (1H, d, *J* = 4.5 Hz, H-19).

Methyl *O*^{1}-acetyl-23-deoxojessic acid (**7**): colorless amorphous solid; $[\alpha]_D^{25}$ +55.8° (*c* = 0.11, MeOH); FABHRMS *m/z* 551.3703 [calcd for $C_{33}H_{52}O_5Na$ $(M+Na)^+$, 551.3704]; IR ($CHCl_3$) ν_{max} 3400, 1700, 1470, 1380, 1250 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.16 (1H, dd, *J* = 12, 4.5 Hz, H-3), 5.02 (1H, br s, H-1), 4.87 (1H, br s, H-28), 4.86 (1H, br s, H-28), 3.11 (1H, dd, *J* = 12, 4.5 Hz, H-5), 2.55 (1H, ddd, *J* = 13.5, 4, 3.5 Hz, H-2), 2.24 (1H, m, H-2), 2.06 (3H, s, OAc), 1.67 (3H, s, H_3-31), 1.07 (3H, d, *J* = 7 Hz, H_3-26), 1.06 (3H, d, *J* = 7 Hz, H_3-27), 1.01 (3H, s, H_3-32), 0.97 (3H, s, H_3-18), 0.93 (3H, d, *J* = 6 Hz, H_3-21), 0.87 (1H, d, *J* = 4.5 Hz, H-19), 0.55 (1H, d, *J* = 4.5 Hz, H-19).

Quadrangulararic acid E (**9**): colorless amorphous solid; $[\alpha]_D^{25}$ +18.2° (*c* = 0.20, MeOH); FABHRMS *m/z* 484.3580 [calcd for $C_{31}H_{48}O_4$ M^+, 484.3575]; IR ($CHCl_3$) ν_{max} 3400, 1700, 1460, 1370, 1255, 1190 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.20 (1H, dd, *J* = 10.5, 7 Hz, H-3), 4.87 (1H, br s, H-28), 4.85 (1H, br s, H-28), 3.17 (2H, br d, *J* = 8 Hz, H_2-2), 2.90 (1H, dd, *J* = 12, 4 Hz, H-5), 2.57 (1H, ddd, *J* = 15, 8, 5 Hz, H-11), 1.76 (3H, s, H_3-31), 1.30 (1H, d, *J* = 4.5 Hz, H-19), 1.07 (3H, d, *J* = 6.5 Hz, H_3-26), 1.06 (3H, d, *J* = 6.5 Hz, H_3-27), 1.02 (1H, d, *J* = 4.5 Hz, H-19), 1.01 (3H, s, H_3-32), 0.93 (3H, s, H_3-18), 0.92 (3H, d, *J* = 6.5 Hz, H_3-21).

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