Methyl Quadrangularates A—D and Related Triterpenes from *Combretum quadrangulare*

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From the MeOH extract of leaves of *Combretum quadrangulare*, fifteen new cycloartane-type triterpenes, methyl quadrangularates A—D (1—4) and N—P (8, 6, 12), methyl 24-epiquadrangularate C (5), quadrangularic acid E (9), 23-deoxojessic acid (10), 1-0-acetyl-23-deoxojessic acid (11), quadrangularol A (7) and B (13) and norquadrangularic acids B (14) and C (15) were isolated together with two known cycloartane-type triterpenes, methyl 23-deoxojessate (16) and 4β,14α-dimethyl-5α-ergosta-9β,19-cyclo-24(31)-en-3β-hydroxy-4α-carboxylic acid (17). Betulinic acid (18), β-sitosterol (19), kamatakenin (20), isokaempferide (21), 5,7,4′-trihydroxy-3,3′-dimethoxyflavone (22) and 5,4′-di hydroxy-3,7,3′-trimethoxy flavone (23) were also obtained from the same extract. The structures of the new compounds were elucidated on the basis of spectral analysis and chemical conversions. All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells, and the cycloartane-type triterpenes showed various degrees of cytotoxicity, whereas all the flavonoids possessed strong cytotoxicity with ED50 values equal to or less than 6 μM.

Key words: *Combretum quadrangulare*; cycloartane-type triterpene; cytotoxicity; flavonoid; murine colon 26-L5.
Thus, the compound reported by Ganzera et al. should have the structural formula 2, i.e., methyl quadrangularate B.

Methyl quadrangularate C (3) and its 24-epimer, methyl 24-epiquadrangularate C (5), were obtained as an epimeric mixture (1:1 from 1H-NMR spectrum), and only the former one succeeded in achieving a pure form as a colorless amorphous solid. The molecular formula of 3 was deduced to be C32H50O6 by HR-FAB-MS. The additional signals of two oxymethines in the 1H- and 13C-NMR spectra of 3 [δH 4.90 (br s), δC 100.1; δH 4.22 (br d, J = 11.0 Hz), δC 71.2] as compared to those of 1 and 2, suggested the presence of a pyran ring on the ring D side chain.18 The existence of the pyran ring with a methoxyl and an isopropenyl group was further confirmed by analysis of the 1H- and 13C-NMR spectra of 3 [δH 4.90 (br s), δC 100.1; δH 4.22 (br d, J = 11.0 Hz), δC 71.2] as compared to those of 1 and 2, suggested the presence of a pyran ring on the ring D side chain.18 The existence of the pyran ring with a methoxyl and an isopropenyl group was further confirmed by analysis of the 1H- and 13C-NMR spectra of the mixture, by the 1H-1H COSY, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, enabled us to assign the signals of 5, which showed only small differences with 3 in the chemical shifts of H-24 (5, δ 4.26; 3, δ 4.22), H-26 (5, δ 5.14, 4.94; 3, δ 5.18, 4.92) and a methoxyl group (5, δ 3.45; 3, δ 3.35). Methyl quadrangularate O (6), with the molecular formula C31H48O6, showed similar 1H- and 13C-NMR spectra to those of 3, except for the absence of a signal corresponding to the C-21 methoxyl group. The upfield shift of C-21 of 6 (δ 93.0) as compared to 3 (δ 100.1) suggested the presence of a free hydroxyl group at C-21 in 6.

The stereochemistry of rings A—D of 3, 5 and 6 was found to be the same as that of 1 based on coupling constants and NOE experiments. The relative stereochemistry of the pyran ring of 3 and 6 was established to be equatorial H-21 (axial OMe) and axial H-24 from the coupling pattern of H-21 (br s) and H-24 (br d, J = 11.0 Hz). This was further supported by the NOE experiment in which irradiation of
methoxyl protons of 3 caused an NOE increase at H-24, placing them on a 1,3-diaxial arrangement in a chair conformation. Considering a biogenetic pathway (Chart 2), there should be two possible epimeric relations of 5 to 3; either at C-21 or at C-24. The identical chemical shift and coupling pattern of H-21 of 3 and 5 led us to the conclusion that they should be epimers at C-24. The pyran ring of 5 was considered to be in a boat conformation because of strong 1,3-diaxial repulsion between the methoxyl group at C-21 and the isopropenyl group at C-24. This was further supported by the coupling constant of H-24 (J = 11.0 Hz) due to diaxial coupling with H-23, in a boat conformation. From these data, the structure of methyl quadrangularate C, methyl 24-epiquadrangularate D, and methyl 24-epiquadrangularate O were determined to be 3, 5, and 6, respectively.

Methyl quadrangularate D (4) was isolated as a colorless amorphous solid having the molecular formula C31H50O4. The 1H- and 13C-NMR spectra of 4 showed the presence of three oxymethines (δH 4.95, 4.27, 3.93; δC 71.7, 80.4, 84.4), an exo-olefin (δH 4.87, 4.84; δC 106.6, t, δC 156.6, s), an ester methyl (δH 3.67; δC 51.7), three secondary methyls and a cyclopropane ring (δH 1.37, 0.71). The signal of the exo-olefin suggested that 4 might have the same side chain of ring D as 7β-hydroxy-23-deoxojessic acid,7 which was confirmed by the HMBC spectrum (Fig. 2). The downfield shift of the cyclopropane methylene protons from the usual range suggested the presence of an oxygen substituent at C-11,19) which was further confirmed by the 1H-1H COSY and HMBC spectra in which H-19 had long-range correlations with both C-1 and C-11. Moreover, a downfield shift of both

![Fig. 1. Significant Correlations Observed in the Long-Range 1H-13C COSY Spectrum of 2 and FG-Pulsed HMBC Spectra of 6, 8, and 12](image)

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Table 1. 13C-NMR Data (100 MHz) of Novel Cycloartane-Type Triterpenes in Pyridine-d5

1. Measured in methanol-d6. 2) Identical to the data of reference 9. 3) Signals of acetyl groups appeared at δ 170.5 and 20.5. 4) Signals of acetyl group were at δ 170.1 and 21.1. 5) Signals of acetyl groups appeared at δ 170.4, 21.1 and 170.0, 20.0.
carbon signals, C-1 and C-11, of 4 compared with those of 1—3 and cycloartenyl-type triterpenes bearing a hydroxyl group either at C-1 or C-11,19 suggested that there may be ether linkage between C-1 and C-11. This was further supported by the fact that on acetylation 4 gave only monoacetyl 4a; the possibility that due to steric hindrance the 1-OH group did not take part in acetylation should be ruled out because 23-deoxojessic acid (10) having the same 1-OH group as 4 easily gave a diacetate under the same conditions (see Experimental). From these data, we concluded that there should be an ether linkage between C-1 and C-11, which was further supported by the compositions of 4 and its monoacetyl 4a from HR-FAB-MS spectra.

In the NOE experiments, NOEs were observed between H-11 and H3-18, between H-8 and H3-18, between H-19 and H-1, between H-19 and H-11 and between H2-29 and H-1, suggesting that all the protons, H2-29, H3-18, H-19, H-11 and H-1, are in the β position. These NOEs and the coupling constants of H-1 (dd, J = 11.0, 6.0 Hz) and H-3 (dd, J = 4.5, 2.5 Hz) suggested that the A ring should be in a boat conformation and the hydroxyl group at C-3 in the β-orientation. Accordingly, the structure of methyl quadrangularate D was determined to be 4.

Quadrangularol A (7) was isolated as a colorless amorphous solid having the molecular formula C31H48O4. The IR spectrum of 7 indicated the presence of a hydroxyl group (3400 cm⁻¹). The 1H-NMR spectrum displayed signals corresponding to two olefinic protons (δ 5.94, s, 2H), two oxymethylene protons (δ 4.28, 3.89, both d, J = 10.2 Hz), two oxymethylene protons (δ 5.08, 3.85), five tertiary methyls (δ 1.54, 1.54, 1.22, 1.06, 0.97), one secondary methyl (δ 0.96) and two highly shielded cyclopropane methylene protons (δ 0.77, 0.52, both d, J = 4.5 Hz). Both the 1H- and 13C-NMR data of 7 were similar to those of 1—6, having a 1,3-dihydroxyoctaene skeleton. However, the absence of a signal corresponding to the carboxylic acid group and the presence of signals of an oxymethylene group suggested that 7 should have an oxymethylene group at C-4 instead of the carboxylic acid group, which was further confirmed by the field gradient-pulsed (FG-pulsed) HMBC spectrum (Fig. 3).

The stereochemistry of rings A—D of 7 was determined to be the same as 1 by the rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectrum. The correlation between H3-29 and H-19 in the ROESY spectrum indicated that the methyl group at C-4 should be β. The broad singlet of two olefinic protons at δ 5.94 was identical with those of quadrangularic acid J (25, δ 5.94)7 and (23Z)-3β-acetoxy-cycloart-23-en-25-ol (δ 5.60)20 having a cis-23(24) double bond, suggesting the cis configuration of the olefinic group of 7. The cis nature of the double bond was further supported by similar chemical shifts of the olefinic carbons of 7 [δ 124.4 (C-23), 141.6 (C-24), in C5D5N] with those of 25 [δ 124.4 (C-23), 141.3 (C-24), in C5D5N] and (23Z)-3β-acetoxy-cycloart-23-en-25-ol [δ 125.6 (C-23), 139.3 (C-24), in CDCl3].21 Thus, the structure of quadrangularol A was concluded to be 7.

Methyl quadrangularate N (8), [α]D²⁰ +70.8° (c=0.11, MeOH), showed a pseudomolecular ion at m/z 537.3189 corresponding to the molecular formula C31H48O4 in HR-FAB-MS. The 1H-NMR spectrum of 8 was similar to that of quadrangularic acid H (26),7 but the 1H-NMR spectrum of 8 had one more aldehydic proton at δ 9.59 than that of 26, suggesting the presence of an aldehydeic group at C-20 instead of a carboxylic acid group. This was confirmed by the FG-pulsed HMBC spectrum (Fig. 1). Accordingly, the structure of methyl quadrangularate N was concluded to be 8, because oxidation with sodium chlorate,22 followed by esterification with diazomethane, gave dimethyl quadrangularate H (26a).7 Quadrangularic acid E (9), [α]D²⁰ +18.2° (c=0.20, MeOH), had the molecular formula C31H48O4. The 1H-NMR spectrum of 9 demonstrated the presence of only one oxymethylene proton at δ 5.20 (t, J = 8.0 Hz). Both of the cyclopropane methylene protons of 9 were found to be highly deshielded (δ 1.30 and 1.02) as compared to the other cycloartane-type triterpenes isolated from C. quadrangulare. The disappearance of the oxymethylene proton corresponding to H-1 in the 1H-NMR spectrum and the presence of a new ketone signal at δ 208.7 in the 13C-NMR spectrum suggested the presence of a ketone group at C-1. This was further supported by the significant correlation between C-1 and H-3 in the FG-pulsed HMBC spectrum (Fig. 2). The stereochemistry of 9 was also determined by the NOE experiments; NOEs between H-3 and H-5 and between H2-29 and H-19 indicated the OH-3 and H2-29 to be β. Due to the presence of a ketone group at C-1, ring-A has a distorted chair conformation. Finally, the structure of quadrangularic acid E was con-
23-Deoxojessic acid (10) was the major cycloartane-type triterpene isolated from the leaves of C. quadrangularare. The molecular formula of 10 was calculated to be C_{30}H_{48}O_{4} by HR-FAB-MS. The $^1$H- and $^{13}$C-NMR data of 10 were found to be similar to those of jessic acid (27), except for a new signal due to a methylene group ($\delta_{HH} 2.20, 1.98$; $\delta_{C} 31.6$) instead of the signal of a ketone carbonyl carbon. Thus, 10 was presumed to be as 23-deoxojessic acid, which was confirmed by the HMBC spectrum. A monocetyl derivative of 10, i.e., 1-O-acetyl-23-deoxojessic acid (11), was also obtained as a colorless amorphous solid having the molecular formula C_{32}H_{52}O_{5}. The $^1$H- and $^{13}$C-NMR spectra of 11 showed additional signals of an acetyl group ($\delta_{HH} 2.06$; $\delta_{C} 170.1, 21.1$). The downfield shift of H-1 at $\delta$ 5.02 suggested the location of the acetyl group at C-1, which was confirmed by the long-range $^1$H-$^{13}$C COSY spectrum. Furthermore, the structure of 1-O-acetyl-23-deoxojessic acid was confirmed to be 11 by the fact that acetylation of both 10 and 11 gave the same diacetate 11a.

Methyl quadrangularate P (12) was obtained as a colorless amorphous solid with $[\alpha]_{D}$^{25} +163.5° ($c$=0.03, MeOH) and its molecular formula was determined to be C_{32}H_{52}O_{6} by HR-FAB-MS. The $^1$H- and $^{13}$C-NMR spectra of 12 were found to be almost identical to those of methyl 23-deoxojessate (16), except for an additional signal of an acetyl group at C-1, which was confirmed by the FG-pulsed HMBC spectrum (Fig. 1). The long-range correlations between the oxymethylene protons and C-17 and C-22 and between H-17 and the oxymethylene carbon in the FG-pulsed HMBC spectrum (Fig. 1) confirmed the oxymethylene group to be C-21. The stereochemistry of 12 was concluded to be the same as that of 1 by the analysis of NOE difference spectra. Thus, the structure of methyl quadrangularate P was concluded to be 12.

Quadrangularol B (13), having the molecular formula C_{30}H_{48}O_{5} was isolated as a colorless amorphous solid. The absorption bands at 3400 and 1700 cm$^{-1}$ in the IR spectrum suggested the presence of hydroxyl and carbonyl groups, respectively. The $^1$H- and $^{13}$C-NMR spectra of 13 were almost identical to those of 7 except for some difference in the ring D side chain. The $^1$H- and $^{13}$C-NMR signals of the exo-olefin appeared relatively downfield ($\delta_{HH}$ 6.02, 5.73; $\delta_{C}$ 124.6, 144.8) instead of those of C-23(24) cis-olefin in 7. Moreover, an additional signal of a ketone carbonyl carbon appeared at $\delta$ 202.2 in the $^{13}$C-NMR spectrum of 13. These data suggested the presence of an $\alpha, \beta$-unsaturated ketone group as in 8. The signal of a vinyllic methyl at $\delta$ 1.92 in the $^1$H-NMR spectrum suggested the position of the exo-olefin should be at C-25(26) and the ketone group at C-24, which was further confirmed by the FG-pulsed HMBC spectrum (Fig. 3). The stereochemistry of 13 was determined to be the same as 7 by the ROESY spectrum. Thus, the structure of quadrangularol B was concluded to be 13.

Norquadrangularic acid B (14) was isolated as a colorless amorphous solid having $[\alpha]_{D}$^{25} +77.7° ($c$=0.15, MeOH). Its HR-FAB-MS showed a quasimolecular ion at m/z 495.3410 consistent with the molecular formula C_{30}H_{48}O_{4}. Its IR spectrum suggested the presence of hydroxyl and carbonyl groups. The $^1$H-NMR spectrum displayed signals of two oxymethylene protons at $\delta$ 4.06 and 3.87 (both dd, $J$=10.7, 3.1 Hz) instead of that of the secondary methyl; also, the $^{13}$C-NMR spectrum showed the presence of an oxymethylene carbon instead of a methyl carbon. The long-range $^1$H-$^{13}$C COSY spectrum. Furthermore, the structure of 1-O-acetyl-23-deoxojessic acid was confirmed to be 11 by the fact that acetylation of both 10 and 11 gave the same diacetate 11a.

Norquadrangularic acid C (15), having the molecular formula C_{32}H_{52}O_{5} showed absorption bands at 3400 (OH) and 1710 cm$^{-1}$ (CO) in its IR spectrum. The $^1$H- and $^{13}$C-NMR spectra of 15 were almost identical to those of 14 except for differences in the exo-olefinic group. The new signals of two oxymethylene protons at $\delta$ 4.03 and 3.92 (both $J$=10.5 Hz) in 15, instead of olefinic protons in 14, suggested the presence of an oxymethylene group at C-24, while the new oxygenated quaternary carbon signal at $\delta$ 78.0 was assigned to C-24 from the FG-pulsed HMBC spectrum (Fig. 4).
phase (Fuji Silysia BW-820MH) or reversed-phase silica gel (Nacalai Tesque).

In this paper, we have reported the isolation and structures of fifteen new and two known cycloartane-type triterpenes. Among the isolated cycloartane-type triterpenes 1—3, 5, 6 and 8 differed only in ring D side chain, and thus they would be biosynthesized from a common hypothetical precursor 28 via photooxygenation of an olefin (Chart 2). 23-Dexojessic acid (10), a major cycloartane-type triterpene in C. quadrangularare leaves, may be derived from either 24 or cycloartenol (29) through alkylation at C-24, 23 or cycloartenol (29) through alkylation at C-24, 23 or cycloartenol (29) through alkylation at C-24,

All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells. Cycloartane-type triterpenes showed various degrees of cytotoxicity, and only 2, 3 and 4 had ED_{50} values less than 20 μM. Methyl quadrangularate D (4), having an ether linkage between C-1 to C-11, showed the strongest cytotoxicity with an ED_{50} value of 5.42 μM, while all four flavonoids possessed strong cytotoxicity with ED_{50} values equal to or less than 6 μM (see Experimental).

Experimental

Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in KBr disks. Electron impact-MS (EI-MS) and FAB-MS measurements were performed on a JEOL JMS-700 spectrometer with m-nitrobenzylalcohol (NBA) or glycerol as matrices. NMR spectra were taken on a JEOL GX-400 spectrometer or JEOL JNM-GX400 spectrometer with tetramethylsilane (TMS) as the internal standard, and chemical shifts are expressed in δ values. Column chromatography was performed with normal-phase (Fuji Silysia BW-820MH) or reversed-phase silica gel (Nacalai Tesque Cosmosil 75C_{18}-OPN). TLC and preparative TLC were carried out on pre-coated Merck Kieselgel 60 F_{254} plates (0.25 and 0.50 mm).

Plant Material

Leaves of C. quadrangularare Kurz were purchased at the local market at Ho Chi Minh City, Vietnam in 1995. The voucher sample (TMPW 18999) is preserved in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation

Air dried leaves (2.65 kg) were extracted with MeOH and the MeOH extract (400 g) was chromatographed on a silica gel column and partitioned into eleven fractions. By preparative TLC with MeOH–CHCl3 (1:1) fractions 4, 11 and 22, 19 (10.1 mg), 18 (6.5 mg) and 4 (63.6 mg) were isolated, respectively. Combined fractions 23—28 afforded a mixture of two flavonoids (1.3 g), and a part of the mixture (100 mg) was separated by preparative TLC with MeOH–CHCl3 (1:9) to give fractions 20 (36.4 mg) and 23 (50.2 mg).

Fraction 3 (18.0 g) was chromatographed over a silica gel column with an EtOAc–hexane solvent system and thirty-three fractions were collected. By preparative TLC with MeOH–CHCl3 (1:19) of subfractions 4, 11 and 22, subfraction 3 afforded a mixture of two flavonoids (1.3 g), and a part of the mixture (100 mg) was separated by preparative TLC with MeOH–CHCl3 (1:9) to give fractions 20 (36.4 mg) and 23 (50.2 mg).

Fraction 4 (12.0 g) was separated into fourteen subfractions by a Cosmosil 75C_{18}-OPN column with H$_2$O–MeOH–CH$_3$CN (1:1:1). Two flavonoids, 21 (19.2 mg) and 22 (23.8 mg), were obtained from subfractions 2 and 5, respectively, as a precipitate. Reversed-phase preparative TLC using H$_2$O–CH$_3$CN–MeOH (1:1:1) on subfraction 3 afforded fractions 10 (9.0 mg) and 17 (20.1 mg), and similar treatment on subfraction 10 afforded fraction 16 (10.2 mg).

Fraction 5 (20.0 g) was also applied on a silica gel column and eluted with a MeOH–CHCl$_3$ solvent system to give five subfractions. Further normal- and reversed-phase silica gel column chromatography and preparative TLC of the subfractions yielded the following compounds: subfraction 1: 2 (13.2 mg), 8 (9.8 mg), 9 (7.2 mg), 11 (14.2 mg), 12 (10.2 mg), 16 (17.1 mg); subfraction 3: 5 (67.1 mg); subfraction 4: 1 (89.2 mg), 2 (117.3 mg), 3 (56.3 mg), 6 (32.1 mg), 9 (7.8 mg); subfraction 5: 7 (8.5 mg).

Fraction 6 (22.0 g) was divided into acetone-soluble (19.41 g) and acetone-insoluble (1.74 g) portions. A part of the acetone-insoluble portion (150 mg) was purified by reversed-phase preparative TLC with H$_2$O–CH$_3$CN–MeOH (1:1:1) to afford fraction 10 (130.7 mg). The acetone-insoluble portion, on the other hand, was further fractioned into five subfractions by a Cosmosil 75C_{18}-OPN column with H$_2$O–MeOH–CH$_3$CN (1:1:1). Subfraction 5 on evaporation gave fraction 10 (1.0 g) as a precipitate. Further silica gel column chromatography and preparative TLC on subfraction 4 afforded fractions 10 (82.8 mg) and 14 (34.1 mg).

The combined fractions 7 and 8 were (23.0 g) chromatographed over Cosmosil 75C_{18}-OPN with H$_2$O–MeOH–CH$_3$CN (1:1:1) to give twelve sub-
fractions. Further preparative TLC of subfraction 8 (CHCl₃–CH₃CN–MeOH, 10:3:1) gave 13 (12.0 mg).

Fraction 9 (20.0 g) was also applied to a Cosmosil 75C₅–OPN column with H₂O–MeOH–CH₃CN (1:1:1), and eight subfractions were collected. Further silica gel column chromatography and preparative TLC of subfraction 3 yielded 15 (48.6 mg).

Methyl Quadrangularate A (1): Colorless amorphous solid. ¹H-NMR (pyridine-d₅) δ: 9.61 (1H, d, J=5.5 Hz, H-21), 5.97 (1H, d, J=16.0 Hz, H-24), 5.89 (1H, d, J=16.0, 6.5 Hz, H-23), 5.35 (1H, dd, J=12.0, 4.5, H-3), 3.82 (1H, br s, H-1), 3.66 (3H, s, 28-OMe), 3.23 (1H, dd, J=12.5, 4.5 Hz, H-5), 2.40 (1H, ddd, J=13.0, 4.5, 3.5 Hz, H-2), 2.34 (2H, dd, J=9.0, 6.5 Hz, H-22), 2.20 (1H, m, H-2), 1.59 (3H, s, H-29), 1.49 (3H, s, H-26), 1.49 (3H, s, H-27), 1.08 (3H, s, H-18), 0.98 (3H, s, H-30), 0.74 (1H, d, J=4.5 Hz, H-19), 0.44 (1H, d, J=4.5 Hz, H-19). ¹H-NMR (methanol-d₅) δ: 9.44 (1H, d, J=5.5 Hz, H-21), 5.26 (1H, d, J=16.0 Hz, H-23), 5.49 (1H, dd, J=16.0, 6.5 Hz, H-23), 4.49 (1H, dd, J=12.0, 4.5 Hz, H-3), 3.68 (3H, s, 28-OMe), 3.51 (1H, br s, H-1), 2.55 (1H, dd, J=12.5, 4.5 Hz, H-5), 2.32 (3H, m, H-11, H-17, H-20), 2.01 (1H, m, H-16), 1.84 (1H, ddd, J=13.0, 4.5, 3.5 Hz, H-2), 1.76 (1H, ddd, J=13.0, 12.0, 3.5 Hz, H-2), 1.22 (3H, s, H-26), 1.21 (3H, s, H-27), 1.07 (3H, s, H-29), 1.01 (3H, s, H-18), 1.01 (3H, s, H-30), 0.69 (1H, d, J=4.5 Hz, H-19), 0.46 (1H, d, J=4.5 Hz, H-19). ¹³C-NMR: Table 1.

IR (CHCl₃) cm⁻¹: 3450, 1715, 1460, 1380, 1260, 1090, 1050, 1010. HR-ELMS m/z: 516.3442 [Caled for C₃₁H₄₈O₆: 516.3443 (M⁺)]. [α]D²⁰ +43.7° (c=0.76, MeOH).

Methyl Quadrangularate B (2): Colorless crystals, mp 190 °C. ¹H-NMR (pyridine-d₅) δ: 9.63 (1H, d, J=5.5 Hz, H-21), 6.05 (1H, d, J=16.0 Hz, H-24), 5.78 (1H, dt, J=16.0, 6.5 Hz, H-23), 5.37 (1H, dd, J=12.0, 4.5 Hz, H-3), 3.85 (1H, br s, H-1), 3.66 (3H, s, 28-OMe), 3.23 (1H, dd, J=12.5, 4.5 Hz, H-5), 2.41 (1H, ddd, J=13.0, 4.5, 3.5 Hz, H-2), 2.34 (2H, dd, J=9.0, 6.5 Hz, H-22), 2.20 (1H, m, H-2), 1.60 (3H, s, H-29), 1.53 (3H, s, H-26), 1.51 (3H, s, H-27), 1.08 (3H, s, H-28), 0.98 (3H, s, H-30), 0.74 (1H, d, J=4.5 Hz, H-19), 0.44 (1H, d, J=4.5 Hz, H-19). ¹H-NMR (methanol-d₅) δ: 9.45 (1H, d, J=5.5 Hz, H-21), 5.62 (1H, d, J=16.0 Hz, H-24), 5.51 (1H, dt, J=16.0, 6.5 Hz, H-23), 4.49 (1H, dd, J=12.0, 4.5 Hz, H-3), 3.68 (3H, s, 28-OMe), 3.51 (1H, br s, H-1), 2.55 (1H, dd, J=12.5, 4.5 Hz, H-5), 2.32 (3H, m, H-11, H-17, H-20), 2.01 (1H, m, H-16), 1.84 (1H, ddd, J=13.0, 4.5, 3.5 Hz, H-2), 1.76 (1H, ddd, J=13.0, 12.0, 3.5 Hz, H-2), 1.24 (3H, s, H-26), 1.23 (3H, s, H-27), 1.07 (3H, s, H-28), 1.00 (3H, s, H-18), 1.00 (3H, s, H-30), 0.69 (1H, d, J=4.5 Hz, H-19), 0.46 (1H, d, J=4.5 Hz, H-19). ¹³C-NMR: Table 1.

IR (KBr) cm⁻¹: 3500, 1710, 1450, 1380, 1040, 990, 750. HR-FAB-MS m/z: 555.3315 [Caled for C₃₁H₄₈NaO₆: 555.3312 (M⁺+Na⁺)]. [α]D²⁰ +53.4° (c=0.79, MeOH).

Methyl Quadrangularate C (3): Colorless amorphous solid. ¹H-NMR (pyridine-d₅) δ: 5.33 (1H, dd, J=12.0, 4.5 Hz, H-3), 5.18 (1H, brs, H-26), 4.92 (1H, brs, H-26), 4.90 (1H, brs, H-21), 4.22 (1H, brd, J=11.0 Hz, H-24), 3.85 (1H, brs, H-1), 3.65 (3H, s, 28-OMe), 3.35 (3H, s, 21-OMe), 3.23 (1H, dd, J=12.0, 4.5 Hz, H-5), 2.75 (1H, m, H-11), 2.42 (1H, ddd, J=13.0, 4.5, 4.0 Hz, H-2), 2.22 (1H, ddd, J=13.0, 12.0, 3.5 Hz, H-2), 1.85 (3H, s, H-27), 1.62 (3H, s, H-28), 1.04 (3H, s, H-18), 0.99 (3H, s, H-30), 0.76 (1H, d, J=4.5 Hz, H-19), 0.52 (1H, d, J=4.5 Hz, H-19). ¹³C-NMR: Table 1.
IR (CHCl₃) cm⁻¹: 3400, 1720, 1450, 1230, 1110, 1030. HR-FAB-MS m/z: 531.3672 [Calced for C$_{32}$H$_{49}$O$_{3}$: 531.3664 (M+Na)]. [M]$^+$/2 + 37.2° (c=0.33, MeOH).

Methyl Quadrangular D (4): Colorless amorphous solid. 1H-NMR (pyridine-d$_5$) δ: 5.73 (1H, br, H-21), 2.54 (6H, s, H-3-22), 2.57 (1H, br, H-25), 2.28 (1H, m, H-23), 0.99 (3H, s, H-30), 0.92 (3H, d, J=6.5 Hz, H-28), 0.88 (1H, d, J=4.5 Hz, H-19), 0.52 (1H, d, J=4.5 Hz, H-19). 13C-NMR: Table 1. (KBr) cm⁻¹: 1210, 1168, 1050, 1000, 1040. HR-FAB-MS m/z: 539.3731 [Calced for C$_{32}$H$_{49}$NaO$_{3}$: 539.3724 (M+Na)]. [M]$^+$/2 + 163.5° (c=0.03, MeOH).

Methyl Quadrangular P (12): Colorless amorphous solid. 1H-NMR (pyridine-d$_5$) δ: 5.36 (1H, dd, J=12.0, 4.5 Hz, H-3), 4.91 (1H, br, H-21), 2.68 (1H, d, J=4.5 Hz, H-28), 2.06 (3H, s, 1-OAc), 1.87 (3H, s, H-30), 1.78 (1H, d, J=7.0 Hz, H-27), 1.07 (3H, s, H-30), 0.93 (3H, d, J=6.0 Hz, H-21), 0.79 (3H, d, J=6.5 Hz, H-19). 13C-NMR: Table 1. (KBr) cm⁻¹: 2300, 1700, 1450, 1350, 1250. HR-FAB-MS m/z: 539.3451 [Calced for C$_{32}$H$_{49}$NaO$_{3}$: 539.3450 (M+Na)]. [M]$^+$/2 + 152.9° (c=0.04, MeOH).

Norquadrangularic Acid B (14): Colorless amorphous solid. 1H-NMR (pyridine-d$_5$) δ: 5.27 (1H, td, J=11.0, 4.5 Hz, H-3), 4.87 (1H, bs, H-31), 4.85 (1H, bs, H-39), 3.99 (1H, bs, H-3), 2.31 (1H, m, H-21), 2.95 (1H, d, J=6.5 Hz, H-19), 1.81 (1H, br, H-25), 1.82 (1H, m, H-23), 0.66 (3H, s, H-30), 0.90 (3H, d, J=6.5 Hz, H-28). 13C-NMR: Table 1. (KBr) cm⁻¹: 1440, 1140, 1080, 1030. HR-FAB-MS m/z: 495.3401 [Calced for C$_{26}$H$_{39}$O$_{7}$Na: 495.3405 (M+Na)]. [M]$^+$/2 + 77.7° (c=0.15, MeOH).

Reduction of Methyl Quadrangular A (2) to Methyl Quadrangular A (1): To a stirred solution of 2.5 mg in CHCl₃ (0.5 ml) at 0°C, a solution of Ph$_3$P (3.1 mg) in CH$_2$Cl$_2$ (0.5 ml) was added. After stirring for 30 min under the same condition, the mixture was directly subjected to preparative TLC (MeOH:H$_2$O:CH$_2$Cl$_2$=1:2:2) to give 1 (4.2 mg, 83%).

Acetylation of 4, 10, and 11: A solution of 4 (8.0 mg) in dry pyridine (0.3 ml) and acetic anhydride (0.3 ml) was stirred overnight at room temperature. After aqueous work-up, the reaction mixture was extracted with CHCl$_3$ (5 × 3 ml), and the CHCl$_3$ extract was washed with water, dried over anhydrous MgSO$_4$ and evaporated under reduced pressure to yield an acetate 4a (7.6 mg, 87%). By the same procedure, 11 was converted into 11a.
H-30), 0.93 (3H, d, J=7.0 Hz, H-21), 0.87 (3H, s, H-18), 0.65 (1H, d, J=4.5 Hz, H-19). 13C-NMR: Table 1. HR-EI-MS m/z: 593.3820 [Calcd for C35H54NaO6: 540.3815 (M)]

H-1, 4.87 (1H, br s, H-28), 4.85 (1H, br s, H-29), 3.11 (1H, dd, J=13.5, 4.5 Hz, H-19). 13C-NMR: Table 1. HR-FAB-MS m/z: 593.3779 [Calcd for C34H52O5: 540.3813 (M+Na)]

**Conversion of Methyl Quadrangularate N (8) to Dimethyl Quadrangularate H (26a)** To a stirred solution of 5 (2.1 mg) in a mixture of CH3CN (0.5 ml), aqueous NaH2PO4 (0.1 mg/ml, 0.5 ml) and 30% H2O2 (40 µl), an aqueous solution of NaClO3 (0.4 mg/ml, 125 µl) was added dropwise at 10 °C, and the mixture was stirred for 2 h at 10 °C. After Na2SO4 (1 mg) was added, the mixture was evaporated and dissolved in MeOH and filtered. The filtrate was then treated with excess CH2N2, followed purification with preparative TLC, to give 26a (0.4 mg, 17%).

**Cytotoxic Assay** Cellular viability in the presence and absence of experimental agents was determined using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) assays as described previously.20 The cytotoxicity of isolated compounds towards murine colon 26-L5 carcinoma cells are as follows. ED50 value (µm): 1, 43.8; 2, 95.3; 3, 18.9; 4, 5.4; 6, 52.6; 7, 49.7; 8, 61.9; 49; 10, 49.6; 11, 57.7; 12, 59.7; 13, 17.6; 14, 65.9; 15, >100; 16, 65.7; 17, 62.4; 18, 55.8; 19, >100; 20, 3.0; 21, 1.8; 22, 5.2; 23, 4.5.

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References and Notes