Bioactive 12-Oleanene Triterpene and Secotriterpene Acids from *Maytenus undata*

Ilias Muhammad,[†,§] Khalid A. El Sayed,[†] Jaber S. Mossa,*[,†] Mansour S. Al-Said,[†] Farouk S. El-Feraly,[†] Alice M. Clark,[‡] Charles D. Hufford,[‡] Stephen Oh,[⊥] and Alejandro M. S. Mayer[⊥]

Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC) and Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi 38677, and Pharmacology Department, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street, Downers Grove, Illinois 60515

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The aerial parts of *Maytenus undata* yielded four new 12-oleanene and 3,4-seco-12-oleanene triterpene acids, namely, 3-oxo-11α-methoxyolean-12-ene-30-oic acid (**1**), 3-oxo-11α-hydroxyolean-12-ene-30-oic acid (**2**), 3-oxo-olean-9(11),12-diene-30-oic acid (**3**), and 3,4-seco-olean-4(23),12-diene-3,29-dioic acid (20-*epi*-koetjapic acid) (**5**), together with the known 3,11-dioxoolean-12-ene-30-oic acid (3-oxo-18*β*-glycyrrhetinic acid) (**4**), koetjapic acid (**6**), and the 12-oleanene artifact 3-oxo-11α-ethoxyolean-12-ene-30-oic acid (**7**). Koetjapic acid (**6**) inhibited the growth of *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Pseudomonas aeruginosa*, with an MIC range of 3.125−6.25 *μ*g/mL. The new 3,4-secotriterpene acid 20-*epi*-koetjapic acid (**5**) potently inhibited rat neonatal brain microglia phorbol ester-stimulated thromboxane B_2 (IC_{50} = 0.5 *μ*M) and superoxide anion (IC_{50} = 1.9 *μ*M) generation.

Plants of the genus *Maytenus*, Family Celastraceae, are widely used in folk medicine as antitumor, antiseptic, antiasthmatic and fertility-regulating agents, as well as for stomach problems and as sialogogues.[1,2] These plants display a diverse range of secondary metabolites, including triterpenes,[3,4] oligo-nicotinated sesquiterpenes and sesquiterpene pyridine alkaloids,[5,6] phenolic glucosides,[7,8] and agarofurans.[9] Many of these metabolites exhibited interesting biological effects, including antiinflammatory, analgesic and antipyretic,[10] antiulcerative,[11] antimicrobial,[12,13] antitumor,[14,15] anti-HIV,[16] insecticidal, and antifeedant activities.[9]

Maytenus undata (Thunb.) is a shrub or tree, 1.5−10 m high, widespread in tropical southern Africa and in south and southwestern Arabia.[17] This plant has not been recorded in Saudi Arabian folk medicine probably due to its rare occurrence, but the closely related species *Maytenus ovatus* is used as a decoction for stomach problems.[1] A bioautography (antibacterial) assay-guided fractionation of an ethanol extract of *M. undata* has led to the isolation of the antibacterial 12-oleanene and 3,4-seco-12-oleanene triterpene acids (**1**−**7**). The present study deals with their isolation, characterization, and bioactivities.

Results and Discussion

The HRFABMS of **1** displayed a pseudomolecular ion peak at *m*/*z* 485.3639 $[M + 1]^+$, suggesting the molecular formula $C_{31}H_{48}O_4$ and eight degrees of unsaturation. Its IR spectrum showed a broad absorption band at ν_{max} 3300−3560 cm^{-1}, indicating a carboxyl group. It also showed absorption bands consistent with the presence of a free carboxyl carbonyl and a ketone functionality.

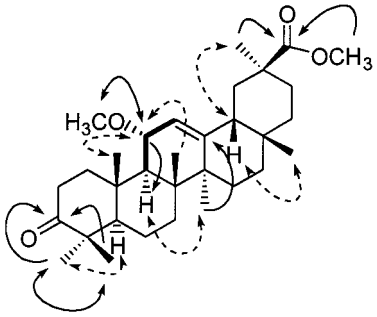

Figure 1. Important $^1H-^{13}C$-GHMBC (solid lines), $^1H-^1H$-NOESY (dotted lines), and COSY (bold lines) correlations of **1a**.

The ^{13}C and ^1H NMR spectra of **1** (Tables 1 and 2) were consistent with an olean-12-ene.[18−20] The ^{13}C NMR spectrum of **1** demonstrated the presence of a trisubstituted double bond (*δ* 122.8, *δ* 148.9), a ketone group (*δ* 218.6), a carboxylic acid group (*δ* 183.3), a methoxy group (*δ* 54.1), and seven methyl groups consistent with a 3-oxo-12-oleanene-30-oic acid carbon skeleton.[18−20] The oxygenated doublet of doublets at *δ* 3.94 (Table 2), which correlated to the methine carbon at *δ* 76.6 in the HETCOR spectrum, was assigned to H-11. This was based on the observed COSY coupling with the olefinic proton doublet resonating at *δ* 5.46 (H-12) and the proton doublet absorbing at *δ* 1.81 (H-9). The *β*-orientation of H-11 was suggested by the high $J_{9,11}$ value (9.3 Hz), indicating diaxial coupling, as well as by NOESY experiments on its methyl ester **1a** (vide infra). Upon methylation with CH_2N_2, **1** afforded ester **1a**. The gross structure of **1a** was established by complete spectral analyses (Tables 1 and 2). The $^1H-^{13}C$-GHMBC data of **1a** (Figure 1) supported the proposed structure. Thus, the ^3J-HMBC correlations between both C-23 and C-24 methyl protons and the ketone carbon resonating at *δ* 218.2 (Table 1) confirmed its location at C-3. The ^3J-HMBC correlations between C-23, C-24, and C-25 methyl protons and C-5 supported the assignments of ring A. The H-9 proton displayed ^3J-HMBC correlations with C-25 and

* To whom correspondence should be addressed. Tel: +966-1-467-7259. Fax: +966-1-467-7245. E-mail: mail to: fferaly@ksu.edu.sa.
† MAPPRC and Department of Pharmacognosy, King Saud University.
‡ National Center for Natural Products Research, Thad Cochran Research Center and Department of Pharmacognosy, University of Mississippi.
⊥ Pharmacology Department, Chicago College of Osteopathic Medicine, Midwestern University.
§ Current address: National Center for Natural Products Research, Thad Cochran Research Center, University of Mississippi.

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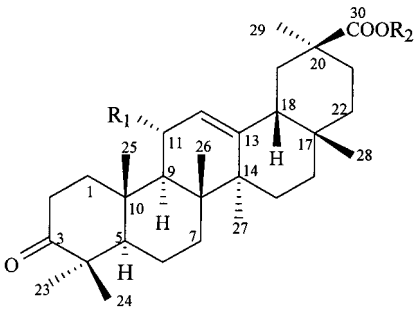
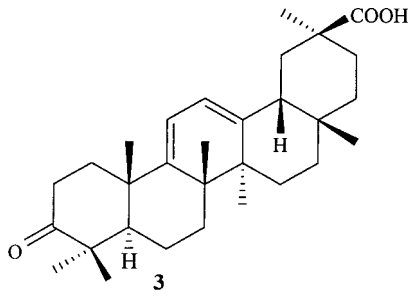


	R_1	R_2
1	OCH_3	H
1a	OCH_3	CH_3
2	OH	H
2a	OH	CH_3
4	=O	H
7	OCH_2CH_3	H



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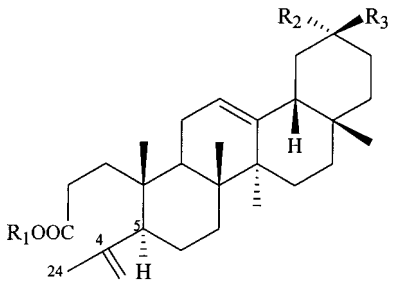

	R_1	R_2	R_3
5	H	COOH	CH_3
5a	CH_3	$COOCH_3$	CH_3
6	H	CH_3	COOH

C-26 methyl carbons as well as to the olefinic methine carbon at C-12, confirming the assignments of rings B and C. The ^3J-HMBC correlation between the C-27 methyl proton and the olefinic quaternary carbon C-13, in addition to the ^3J-HMBC correlation between the C-28 methyl protons and C-18, supported the assignments of ring D. In addition, the C-29 methyl protons displayed ^3J-HMBC correlations with the carbonyl ester C-30 and the methylene carbons C-19 and C-21, confirming the assignments of ring E.

The relative stereochemistry of **1** and **1a** was based on the NOESY data of the latter (Figure 1). Therefore, H-5, H-9, and C-23 were proved to be α-oriented, while H-11, H-18, H_3-25, H_3-26, and H_3-28 were on the β-side of the molecule. On the basis of the foregoing data, compound **1** was found to be the new triterpene 3-oxo-11α-methoxyolean-12-ene-30-oic acid.

Compound **2**, $C_{30}H_{46}O_4$, exhibited ^{13}C and ^1H NMR data (Tables 1 and 2) that were closely similar to **1**, but it lacked the methoxy group at C-11, which was hydroxylated instead. Methylation of **2**, using CH_2N_2, afforded ester **2a,** which exhibited spectral data (Tables 1 and 2) close to those of **1a,** except for the presence of the C-11 OMe group. Therefore, the identity of **2** was established as 3-oxo-11α-hydroxyolean-12-ene-30-oic acid, a new compound.

Compound **3**, $C_{30}H_{44}O_3$, completed the series, by having a conjugated diene system at $\Delta^{9,11}$ and Δ^{12} based on its ^{13}C and ^1H NMR data (Tables 1 and 3). Compound **3** was therefore identified as the new triterpene 3-oxoolean-9(11),12-diene-30-oic acid.

Compound **4** was found to be 3-oxoglycyrrhetic acid, also known as 3-oxo-18β-glycyrrhetinic acid. It had previously been reported as a semisynthetic derivative of 18β-glycyrrhetic acid,[21] and it was also used as a starting material during the synthesis of antiinflammatory glycyrrhetic acid derivatives.[22,23] Furthermore, 3-oxoglycyrrhetic acid has previously been isolated from *Glycyrrhiza uralensis*,[24] and its data were reported in the Chinese literature only. The ^{13}C NMR spectral data of **4** were indistinguishable from those of 3-oxo-18β-glycyrrhetinic acid, previously reported as a microbial metabolite of glycyrrhizic acid,[25] while complete unambiguous assignments of its ^1H NMR spectral data, with the aid of 2D NMR, are reported herein for the first time (Table 2).

The triterpenes **5** and **6** both analyzed for the formula $C_{30}H_{46}O_4$, corresponding to eight degrees of unsaturation. The ^{13}C and ^1H NMR data of **5** (Tables 1 and 3) and **6** suggested the presence of C-3,4-seco-olean-4,12-diene carbon skeleton.[18,20] The physical and spectral data of **6** were indistinguishable from those of the known triterpene koetjapic acid (3,4-seco-olean-4(23),20-diene-3,30-dioic acid) previously reported from the stems of *Sandoricum koetjapie* (Meliaceae).[26] Complete ^{13}C NMR data and relative stereochemical assignments for **6** were achieved with the aid of HMBC and NOESY experiments. It is worth noting that both chemical shift values of C-5 and C-9 reported for koetjapic acid (**6**)[26] were transposed and should be now reversed, based on the ^3J-HMBC correlation between H_2-23 and H_3-24 with C-5, as well as the ^3J-HMBC correlation between H-12 and H_3-26 with C-9.

Compound **5** formed the dimethyl ester **5a**, by treatment with CH_2N_2, and its ^{13}C and ^1H NMR data (Tables 1 and 3) suggested that it was the C-20 epimer of **6**. In contrast with the stereochemistry C-20 in **6**, the β-orientation of the C-30 methyl singlet of **5** was based on its NOESY correlations with the axial β-oriented H-18 and the β-oriented H_3-28 signals. Therefore, **5** was shown to be the new triterpene 3,4-seco-olean-4(23),12-diene-3,29-dioic acid (20-*epi*-koetjapic acid).

Compound **7**, $C_{32}H_{50}O_4$, displayed spectral data that were generally similar to those of **1**, but with an ethoxy group instead of the methoxy functionality. The ^1H NMR data of **7** (Table 3) displayed methyl doublet of doublets at δ 1.15, J = 7.1, 6.8 Hz, which was coupled to the geminally coupled methylene protons doublet of quartets at δ 3.62 and 3.36. This pattern was consistent with the presence of an ethoxy group attached to the chiral C-11, rather than as an ethyl ester group located on the C-29 carbonyl.[27] While compound **1** was detected by TLC in a fresh chloroformic extract of the plant material, compound **7** was not, and it appeared to be an artifact formed during the course of isolation. Hence, compound **7** was shown to be 3-oxo-11α-ethoxyolean-12-ene-30-oic acid, hitherto unreported from any source.

Triterpenes **1**−**3**, **5**, **6**, and **7** were tested for antimicrobial activities against a wide range of microorganisms (Table 4), using a modified microtiter-plate assay.[28] Koetjapic acid

Table 1. ^{13}C NMR Data of Compounds **1**, **1a**, **2**, **2a**, **3**, **5**, **5a**, and **7**[a]

C no.	**1**	**1a**	**2**[b]	**2a**	**3**	**5**[d]	**5a**	**7**
1	40.6, t	40.7, t	42.5, t	41.5, t	42.4, t	29.3, t	29.4, t	40.4, t
2	34.8, t	34.8, t	35.3, t	34.8, t	34.4, t	34.1, t	34.4, t	34.4, t
3	218.6, s	218.2, s	217.0, s	218.2, s	217.7, s	181.5, s	175.0, s	218.2, s
4	48.4, s	48.1, s	48.6, s	48.1, s	47.2, s	147.7, s	147.8, s	47.8, s
5	55.9, d	55.9, d	56.8, d	56.0, d	51.7, d	50.8, d	50.8, d	55.9, d
6	20.1, t	20.1, t	20.9, t	20.1, t	19.5, t	24.9, t	24.9, t	19.7, t
7	31.5, t	31.6, t	32.2, t	31.6, t	30.9, t	31.7, t	31.8, t	31.0, t
8	44.4, s	43.3, s	44.3, s	43.6, s	40.4, s	39.9, s	39.9, s	42.9, s
9	50.7, d	50.9, d	55.4, d	55.3, d	152.5, s	38.1, d	38.2, d	50.4, d
10	38.1, s	38.1, s	38.9, s	38.0, s	34.0, s	39.5, s	39.5, s	37.7, s
11	76.6, d	76.6, d	68.3, d	68.3, d	117.4[c], d	24.2, t	24.1, t	75.2, d
12	122.8, d	122.7, d	128.7, d	126.8, d	121.3[c], d	123.1, d	123.0, d	123.2, d
13	148.9, s	148.8, s	147.2, s	148.4, s	146.4, s	144.2, s	144.4, s	147.6, s
14	42.2, s	42.2, s	43.0, s	42.2, s	44.4, s	42.7, s	42.9, s	41.8, s
15	26.5, t	26.5, t	27.3, t	26.5, t	25.6, t	26.3, t	26.7, t	26.1, t
16	27.2, t	27.2, t	28.0, t	27.2, t	27.1, t	27.3, t	27.3, t	26.7, t
17	32.3, s	32.2, s	33.1, s	32.2, s	31.6, s	32.8, s	32.8, s	31.9, s
18	48.1, d	48.2, d	49.0, d	47.9, d	46.2, d	46.4, d	46.5, d	47.7, d
19	42.6, t	42.7, t	43.3, t	42.7, t	38.2, t	40.4, t	40.9, t	42.0, t
20	43.3, s	44.5, s	44.8, s	44.6, s	42.9, s	42.9, s	43.1, s	43.9, s
21	33.3, t	33.3, t	34.2, t	33.2, t	31.2, t	28.9, t	28.8, t	33.0, t
22	38.6, t	38.7, t	39.5, t	38.6, t	37.7, t	36.2, t	36.2, t	38.7, t
23	27.0, q	27.0, q	27.4, q	28.9, q	26.8, q	114.0, t	114.0, t	26.6, q
24	21.9, q	21.9, q	22.2, q	21.9, q	21.2, q	23.8, q	23.9, q	21.4, q
25	16.7, q	16.8, q	17.0, q	16.6, q	19.9, q	20.0, q	19.9, q	15.8, q
26	18.4, q	18.4, q	18.9, q	18.3, q	20.5, q	17.3, q	17.3, q	18.0, q
27	25.5, q	25.5, q	26.3, q	26.3, q	25.1, q	26.2, q	26.2, q	25.1, q
28	29.0, q	28.7, q	29.3, q	28.6, q	28.4, q	28.6, q	28.6, q	28.2, q
29	29.3, q	28.8, q	29.3, q	26.9, q	28.5, q	186.2, s	177.9, s	28.6, q
30	183.3, s	177.9, s	178.9, s	177.9, s	182.0, s	19.5, q	19.7, q	182.6, s
11-OMe	54.1, q	54.2, q						
11-OEt								61.7, t 15.4, q
3-OMe							52.0, q	
29-OMe							52.2, q	
30-OMe		52.0, q		52.1, q				

[a] In $CDCl_3$, 125 MHz. Carbon multiplicities were determined by DEPT135° experiments. [b] In acetone-d_6. [c] Assignments could be reversed.

Table 2. ^1H NMR Data of Compounds **1**, **1a**, **2**, **2a**, and **4**[a]

H no.	**1**	**1a**	**2**[b]	**2a**	**4**
1	2.29, m	2.24, m	2.59, ddd (13.2, 7.3, 3.9) 1.62, m	2.22, m	2.96, m
	1.62, m	1.62, m		1.43, m	1.42, m
2	2.49, ddd (15.7, 10.7, 7.3) 2.38, m	2.49, ddd (15.9, 10.8, 7.5) 2.35, m	2.52, m 2.31, ddd (15.7, 6.8, 3.6)	2.33, m 2.13, m	2.61, m 2.33, m
5	1.31, m	1.33, m	1.43, m	1.12, m	1.28, m
6	1.49, 2H, m	1.45, 2H, m	1.53, 2H, m	1.28, 2H, m	1.56, m
7	1.94, m 1.33, m	1.92, m 1.28, m	1.89, m 1.35, m	1.71, m, 1.07, m	2.02, m, 1.38, m
9	1.81, d (9.3)	1.76, d (9.4)	1.76, d (9.2)	1.41, m	2.44, s
11	3.94, dd (9.3, 3.1)	3.87, dd (9.4, 3.2)	4.25, dd (9.2, 3.0)	4.02, dd (8.9, 3.6)	
12	5.46, d (3.1)	5.41, d (3.2)	5.30, d (3.2)	5.10, brs	5.75, s
15	1.72, m, 1.01, m	1.67, m, 0.95, m	1.73, m, 1.03, m	1.52, m, 0.78, m	1.83, m, 1.22, m
16	1.98, m, 0.92, m	1.95, m, 0.78, m	2.05, m, 1.01 m	1.72, m, 0.67, m	2.03, m, 1.21, m
18	2.07, dd (13.0, 3.9)	1.93, m	1.99, dd (13.1, 3.9)	1.68,m	2.19, m
19	1.94, m, 1.63, m	1.88, m, 1.52, m	1.85, m, 1.68, m	1.85, m, 1.68, m	1.91, m, 1.68, m
21	1.50, m, 1.33, m	1.46, m, 1.33, m	1.53, m, 1.35, m	1.65, m, 1.36, m	1.75, m, 1.45, m
22	1.37, 2H, m	1.35, m, 1.20, m	1.37, 2H, m	1.13, m, 1.02, m	1.43, m
23	1.11, 3H, s	1.05, 3, s	1.07, 3H, s	0.87, 3H, s	1.10, 3H, s
24	1.06, 3H, s	1.00, 3H, s	1.04, 3H, s	0.83, 3H, s	1.06, 3H, s
25	1.21, 3H, s	1.11, 3H, s	1.21, 3H, s	0.96, 3H, s	1.27, 3H, s
26	1.05, 3H, s	0.96, 3H, s	1.10, 3H, s	0.82, 3H, s	1.17, 3H, s
27	1.22, 3H, s	1.18, 3H, s	1.26, 3H, s	0.99, 3H, s	1.38, 3H, s
28	0.82, 3H, s	0.74, 3H, s	0.80, 3H, s	0.56, 3H, s	0.85, 3H, s
29	1.15, 3H, s	1.09, 3H, s	1.15, 3H, s	0.90, 3H, s	1.22, 3H, s
11-OMe	3.26, 3H, s	3.21, 3H, s			
29-OMe		3.64, 3H, s		3.45, s	

[a] In $CDCl_3$, 500 MHz. Coupling constants (J) are in Hz. [b] In acetone-d_6.

(**6**) showed the most prominent antibacterial activity by inhibiting the growth of *S. aureus*, methicillin-resistant *S. aureus*, and *P. aeruginosa* with an MIC range of 3.125−12.50 μg/mL (Table 4). The antibacterial activities of related 12-oleanene, 2,3-seco-oleanene, and 3,4-seco-oleanene triterpenes have been previously reported.[18,19]

Table 5 illustrates the effect of compounds **1**−**5** on the release of neonatal rat brain microglia (BMΦ) superoxide

Table 3. ^{1}H NMR Data of Compounds **3**, **5**, **5a**, and **7**[a]

H no.	**3**	**5**	**5a**	**7**
1	1.91, m, 1.60, m	2.40, m 2.17, m	2.36, m, 2.12, m	2.36, m, 1.67, m
2	2.60, m 2.51, m	1.74, m 1.42, m	1.74, m 1.45, m	2.51, m, 2.41, m
5	1.51, m	2.03, m	1.80, m	1.38, m
6	1.60, 2H, m	1.75, m, 1.42, m	1.76, m 1.42, m	1.50, 2H, m
7	1.41, 2H, m	1.99, m, 1.50, m	1.98, m 1.52, m	1.95, m, 1.37, m
9		2.02, m	1.95, m	1.84, d (9.5)
11	5.65, s	1.75, 2H, m	1.75, 2H, m	4.02, dd (9.5, 2.9)
12	5.65, s	5.23, brs	5.23, brs	5.44, d (2.9)
15	1.90, m, 1.10, m	1.79, m, 1.03, m	1.77, m, 1.02, m	1.73, m, 1.01, m
16	1.97, m, 0.95, m	1.52, m, 1.28, m	1.53, m, 1.29, m	1.95, m, 0.91, m
18	2.19, brd (10.8)	2.23, m	2.17, m	2.04, m
19	1.43, 2H, m	2.17, m, 1.80, m	2.22, m, 1.80, m	1.93, m, 1.66, m
21	1.95, m, 1.75, m	2.06, dd (16.7, 3.7), 0.91, m	1.98, m, 0.93, m	1.50, m, 1.35, m
22	2.22, m, 1.88, m	1.93, 2H, m	1.76, 2H, m	1.38, 2H, m
23	1.12, 3H, s	4.87, brs, 4.67, brs	4.87, brs, 4.67, brs	1.10, 3H, s
24	1.08, 3H, s	1.75, 3H, brs	1.77, 3H, brs	1.06, 3H, s
25	0.98, 3H, m	0.94, 3H, s	0.85, 3H, s	1.21, 3H, s
26	1.16, 3H, m	1.02, 3H, s	1.20, 3H, s	1.05, 3H, s
27	1.27, 3H, s	1.19, 3H, s	1.02, 3H, s	1.22, 3H, s
28	0.88, 3H, s	0.86, 3H, s	0.93, 3H, s	0.81, 3H, s
29	1.21, 3H, s			1.15, 3H, s
30		1.24, 3H, s	1.21, 3H, s	
11-OEt				1.15, 3H, dd (7.1, 6.8) 3.62, dq (15.8, 6.9) 3.36, dq (15.6, 7.1)
3-OMe			3.64, 3H, s	
29-OMe			3.66, 3H, s	

[a] In $CDCl_3$, 500 MHz. Coupling constants (*J*) are in Hz.

Table 4. Antibacterial Activity of Compounds **1**−**3**, **5**, **6**, and **7**[a]

	MIC μg/mL		
compound	S. *aureus*	MR. *S. aureus*	P. *aeruginosa*
1	>10	>10	10
2	>6.25	>6.25	>6.25
3	>50.0	>50.0	6.25
5	>3.25	>6.25	6.25
6	>6.25	12.5	6.25
7	>12.5	50	12.5

[a] MIC values after 48 h of incubation at 37 °C.

Table 5. Antiinflammatory Activity of Compounds **1**−**5**[a]

compound	IC_{50} O_2^-, μM	IC_{50} TXB_2, μM	LDH_{50}, μM
1	15	5	>30
2	3.2	3.6	>1
3	>100	>100	>30
4	6.7	2.3	>10
5	1.9	0.5	>1

[a] Effect on rat neonatal BMΦ PMA [1 μM]-stimulated release of O_2^-, TXB_2, and LDH. Data shown corresponds to two independent experiments. The detailed experiment protocol used is described in the Experimental Section.

anion (O_2^-) and thromboxane B_2 (TXB_2), mediators thought to be involved in neuroinflammatory conditions[29] and lactate dehydrogenase (LDH), a marker for cell toxicity.[30] The new secotriterpene acid 20-*epi*-koetjapic acid (**5**) dose-dependently inhibited phorbol ester-stimulated neonatal BMΦ thromboxane B_2 (IC_{50} = 0.5 mM) and superoxide anion (IC_{50} = 1.9 μM) release (Table 5). LDH release was minimally above basal levels, thus suggesting that compound **5**'s effect on BMΦ O_2^- and TXB_2 release was of a pharmacological rather than of a toxicological nature. Compounds **1**, **2**, and **4** were less effective in inhibiting BMΦ thromboxane B_2, IC_{50} = 5, 3.6, and 2.3 μM, respectively, and superoxide anion generation, IC_{50} = 15, 3.2, and 6.7 mM, respectively (Table 5). Although less potent than compound **5**, compounds **1** and **4** are particularly interesting in view of their low lactate dehydrogenase release, indicating low toxicity to the BMΦ (Table 5). Despite its low toxicity, compound **3** did not show effects on both BMΦ superoxide anion and thromboxane B_2 release.

Experimental Section

General Experimental Procedure. Melting points (uncorrected) were recorded on an Electrothermal 9100 instrument. UV spectra were obtained in MeOH, using a Varian DMS 90 spectrophotometer, and IR spectra were taken as KBr disks/$CHCl_3$ solution on a Perkin-Elmer 5808 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or acetone-d_6, using TMS as internal standard, on Bruker AMX NMR spectrometers operating at 300 or 500 MHz for ^{1}H and 75 or 125 MHz for ^{13}C NMR. The MS spectra were measured using an E. I. Finnigan model 3200 (70 eV ionization potential) with the INCOS data system, an E. I. Finnigan model 4600 quadruple system, or a Shimadzu QP500 GC/mass spectrometer. Low-resolution chemical ionization mass spectra were obtained using a Finnigan instrument with isobutane or ammonia as the ionizing gases. HRMS were measured using a VG ZAB-SE mass spectrometer. Optical rotation values were recorded at ambient temperature, in $CHCl_3$, unless otherwise stated, using a Perkin-Elmer 241 MC polarimeter. TLC analyses were carried out on precoated silica gel G_{254} 1000 μm, with the following developing system: $CHCl_3$-$(CH_3)_2CO$−AcOH (90:10:0.1). For flash column chromatography, Si gel 60, 40 μm was used and $CHCl_3$−MeOH mixture as a solvent system. Centrifugal preparative TLC (CPTLC) was performed with a Chromatotron (Harrison Research Inc. model 7924), 1 or 4 mm Si gel G PF_{254} disk, using a flow rate of 3 mL/min. The isolated compounds were visualized using UV light (λ_{max} 254 nm) and 1% vanillin−H_2SO_4 spray reagent.

Plant Material. The aerial parts of *Maytenus undata* were collected in August 1995, near Abha, in the Southern Region of Saudi Arabia. The plant was identified at the College of Pharmacy, King Saud University, and a voucher specimen has been deposited at the Herbarium of Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University (MAPPRC 13328, 1995).

Extraction and Bioautography. The powdered air-dried aerial parts of *M. undata* (1.3 kg) were extracted with EtOH (95%, 3 × 2 L), and the combined extracts were evaporated under reduced pressure (yield 160 g). A portion of the anti-

microbially active EtOH extract was subjected to bioautography[31] on silica gel plates (5 × 10 cm, solvent system: $CHCl_3$−$(CH_3)_2CO$−AcOH, 90:10:0.1), using *Bacillus subtilis* (NCTC 10400) as a test organism. Two clear elongated inhibition zones with R_f values 0.20 and 0.55 were observed after 24 h of incubation. Hence, a portion of the active EtOH extract (50 g) was dissolved in CH_3CN (3 L), and the CH_3CN-soluble fraction (30 g) was partitioned with *n*-hexane (3 × 500 mL), after presaturation with each other. The combined *n*-hexane and CH_3CN fractions were separately filtered and dried (7.5 and 22 g, respectively). Antimicrobial screening of all fractions showed that the activity resided in the CH_3CN-soluble fraction.

Isolation of Triterpenes. A part of the active CH_3CN fraction (10 g) was flash-chromatographed on Si gel 60 (250 g) using $CHCl_3$−MeOH (99:1) as a solvent system to give several fractions of mixtures and semipure triterpenes that were pooled according to their TLC patterns. Mixtures were further subjected to repeated CC followed by CPTLC, using the system $CHCl_3$−$(CH_3)_2CO$−AcOH (90:10:0.1) to afford **1** (237 mg, R_f 0.30), **2** (219 mg, R_f 0.12), **3** (30 mg, R_f 0.58), **4** (29 mg, R_f 0.32), **5** (160 mg, R_f 0.18), **6** (105 mg, R_f 0.21), and **7** (210 mg, R_f 0.42).

3-Oxo-11α-methoxyolean-12-ene-30-oic acid (1): colorless powder, mp 83 °C ; $[\alpha]_D^{25}$ +200° (*c* 0.13, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 218 (3.18) nm; IR ν_{max} (KBr) 3420 (OH), 2985−2820, 1725 (C=O), 1700 (C=O) cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS [*m*/*z*] (% relative intensity): 484 $[M]^+$ (25); HRFABMS *m*/*z* 485.3639 $[M + 1]^+$ (calcd for $C_{31}H_{49}O_4$, 485.3633).

3-Oxo-11α-hydroxyolean-12-ene-30-oic acid (2): colorless powder, mp 205−207 °C; $[\alpha]_D^{25}$ +101° (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 210 (3.49) nm; IR ν_{max} (KBr) 3300−3500 (OH), 2950−2820, 1720 (C=O), 1700 (C=O), cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS [*m*/*z*] (% relative intensity) 470 $[M]^+$ (3); HRFABMS *m*/*z* 471.3470 $[M + 1]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3476).

3-Oxoolean-9(11),12-diene-30-oic acid (3): colorless powder, mp 259 °C; $[\alpha]_D^{25}$ +200° (*c* 0.11, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 210 (3.98), 235 (2.85) nm; IR ν_{max} (KBr) 3550−3400 (OH), 2990−2800, 1705 (C=O), cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 3; EIMS [*m*/*z*] (% relative intensity): 452 $[M]^+$ (66); HRFABMS *m*/*z* 453.3368 $[M + 1]^+$ (calcd for $C_{30}H_{45}O_3$, 453.3371).

3,11-Dioxoolean-12-ene-30-oic acid (4): colorless powder, mp 270 °C (dec); $[\alpha]_D^{25}$ +93° (*c* 0.06, $CHCl_3$) [lit.[22] mp 270° (recrystallized from MeOH) $[\alpha]_D^{25}$ +184° ($CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 210 (4.05), 250 (3.65) nm; IR ν_{max} (KBr) 3600−3400 (OH), 2950−2820, 1725 (C=O), 1680 (C=O), 1590 cm^{-1}; ^{13}C NMR data were in agreement with literature;[25] ^1H NMR, see Table 2; EIMS [*m*/*z*] (% relative intensity) 468 $[M]^+$ (15); HRFABMS *m*/*z* 469.3320 $[M + 1]^+$ (calcd for $C_{30}H_{45}O_4$, 469.3333).

3,4-Seco-olean-4(23),12-diene-3,29-dioic acid (5): colorless amorphous solid, mp 179 °C; $[\alpha]_D^{25}$ +32° (*c* 0.19, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 219 (3.30) nm; IR ν_{max} (KBr) 3600−3300 (OH), 2950−2800, 1720, 1690 (C=O), cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 3; CIMS [*m*/*z*] (% relative intensity) 471 $[M + H]^+$(100); HRFABMS *m*/*z* 471.3482 $[M + 1]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3476).

3,4-Seco-olean-4(23),12-diene-3,30-dioic acid (6): colorless amorphous solid, mp 315−318 °C; $[\alpha]_D^{25}$ +120° (*c* 0.11, MeOH) [lit.[26] mp 296−298 °C, $[\alpha]_D^{25}$ +114°; UV; IR; ^{13}C and ^1H NMR were in agreement with those reported for koetjapic acid;[26] CIMS [*m*/*z*] (% relative intensity) 471 $[M + H]^+$ (100); HRFABMS *m*/*z* 471.3480 $[M + 1]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3476).

3-Oxo-11α-ethoxyolean-12-ene-30-oic acid (7): colorless powder, mp 138 °C; $[\alpha]_D^{25}$ +81° (*c* 0.12, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 218 (3.21) nm; IR ν_{max} (KBr) 3450 (OH), 2950−2820, 1730 (C=O), 1700 (C=O), 1225 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 3; EIMS [*m*/*z*] (% relative intensity) 498 $[M]^+$ (35); HRFABMS *m*/*z* 499.3796 $[M + 1]^+$ (calcd for $C_{32}H_{51}O_4$, 499.3789).

Methylation of 1, 2, and 5. Compounds **1** (110 mg), **2** (100 mg), and **5** (61 mg) were separately methylated using ethereal CH_2N_2, which yielded compounds **1a** (41 mg, R_f 0.63), **2a** (29 mg, R_f 0.25), and **5a** (16 mg, R_f 0.88) after regular workup and purification using CPTLC (Si gel G PF_{254} 1 mm disk, system: $CHCl_3$−$(CH_3)_2CO$−AcOH (90:10:0.1)).

3-Oxo-11α-methoxyolean-12-ene-30-oic acid methyl ester (1a): colorless powder, mp 189−191 °C; $[\alpha]_D^{25}$ +14° (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 219 (3.27) nm; IR ν_{max} (KBr) 2990−2800, 1730 (C=O), 1700 (C=O), 1225 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS [*m*/*z*] (% relative intensity) 498 $[M]^+$ (18); HRFABMS *m*/*z* 499.3781 $[M + 1]^+$ (calcd for $C_{32}H_{51}O_4$, 499.3789).

3-Oxo-11α-hydroxyolean-12-ene-30-oic acid methyl ester (2a): viscous oil, $[\alpha]_D^{25}$ +84° (*c* 0.05, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 215 (3.58), 251 (2.92) nm; IR ν_{max} ($CHCl_3$) 2970−2800, 1730 (C=O) and 1695 (C=O) cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS [*m*/*z*] (% relative intensity) 484 $[M]^+$ (5); HRFABMS *m*/*z* 485.3649 $[M + 1]^+$ (calcd for $C_{31}H_{49}O_4$, 485.3633).

3,4-Seco-olean-4(23),12-diene-3,29-dioic acid dimethyl ester (5a): colorless powder, mp 179 °C; $[\alpha]_D^{25}$ +43° (*c* 0.05, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 202 (3.09), 287 (2.42) nm; IR ν_{max} (KBr) 2950−2856, 1720 (C=O) and 1690 (C=O) cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 3; EIMS [*m*/*z*] (% relative intensity) 498 $[M]^+$ (3); HRFABMS *m*/*z* 499.3780 $[M + 1]^+$ (calcd for $C_{32}H_{51}O_4$, 499.3789).

Antibacterial Assay. The preliminary antibacterial activities of the crude extracts/fractions were determined by using agar dilution assay,[32] and the MIC values of compounds **1**−**3**, **5**, **6**, and **7** were determined by using a modified microtiter plate assay protocol with a 96-well format plate, as recommended by the National Committee for Clinical Laboratory Standards.[28] The test organisms used are ATCC strains of *S. aureus* (# 6535), methicillin-resistant *S. aureus* (#33591), and *P. aeruginosa* (# 15442). Rifampin and DMSO were used as positive and negative controls, respectively.

Antiinflammatory Assay. BMΦ (2 × 10^5 cells) were seeded into each well of 24-well flat-bottom culture clusters and stimulated with bacterial lipopolysaccharide (LSP) (0.3 ng/mL) in Dulbecco's modified Eagle medium + 10% fetal bovine serum + penicillin + streptomycin for 17 h in a humidified 5% CO_2 incubator at 37 °C.[30] Media were then removed, and BMΦ was washed with warm (37 °C) Hanks' balanced salt solution (HBSS) and then incubated with compounds **1**−**5** (0.01−30 mM) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μM). All experimental treatments were run in triplicate and in a final volume of 1 mL. Seventy minutes after PMA stimulation, HBSS was aspirated from each well and O_2^-, TXB_2, and LDH release were determined as described elsewhere.[30] Table 5 shows the data for each compound from two representative experiments and are expressed as the compound's inhibitory concentration 50% (IC_{50}) for the measured mediator.

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