JOURNAL OF NATURAL PRODUCTS

Article

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Isoflavones and Rotenoids from the Leaves of *Millettia oblata* ssp. *teitensis*

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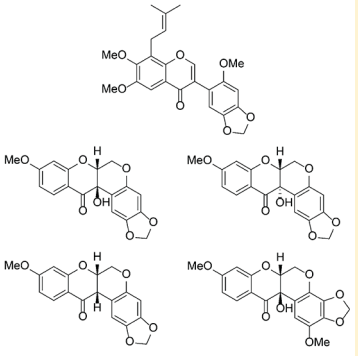
Supporting Information

ABSTRACT: A new isoflavone, 8-prenylmilldrone (**1**), and four new rotenoids, oblarotenoids A–D (**2**–**5**), along with nine known compounds (**6**–**14**), were isolated from the CH_2Cl_2/CH_3OH (1:1) extract of the leaves of *Millettia oblata* ssp. *teitensis* by chromatographic separation. The purified compounds were identified by NMR spectroscopic and mass spectrometric analyses, whereas the absolute configurations of the rotenoids were established on the basis of chiroptical data and in some cases by single-crystal X-ray crystallography. Maximaisoflavone J (**11**) and oblarotenoid C (**4**) showed weak activity against the human breast cancer cell line MDA-MB-231 with IC_{50} values of 33.3 and 93.8 μM, respectively.



The genus *Millettia* (family Leguminosae, subfamily Papilionoideae) contains over 200 species that are distributed in the tropical and subtropical regions of Africa, Asia, and Australia.[1,2] The majority of these plants, which are typically trees, shrubs, or liana, are known for traditional medicinal applications.[1] *Millettia nitida* var. *hirsutissima* and *M. speciosa* are, for example, used in Chinese folk medicine to alleviate dysmenorrhea and rheumatic pain and to treat paralysis.[3,4] In Cameroon, the concoction of the roots and stem bark of *M. griffoniana* is employed orally for the treatment of boils, insect bites, inflammation, amenorrhea, sterility, and menopausal syndromes.[5,6] The roots of *M. usaramensis* are used as an antidote against snake bite,[7] while *M. oblata* is used to treat stomachache and as a remedy for cough, swollen body, and bladder problems in Kenya and Tanzania.[1]

The genus *Millettia* is rich in flavonoids including chalcones, isoflavones, and rotenoids, some of which show valuable antiplasmodial and cytotoxic activities.[7,8] In a previous study, we reported isoflavones, rotenoids, and a triterpene from the stem bark of *M. oblata* ssp. *teitensis*.[8] Herein, the isolation and characterization of a new isoflavone and four new rotenoids, along with nine known compounds, are reported from the leaves of the same plant. Selected compounds were evaluated for cytotoxicity against the MDA-MB-231 human breast cancer cell line and for translation inhibitory activity.

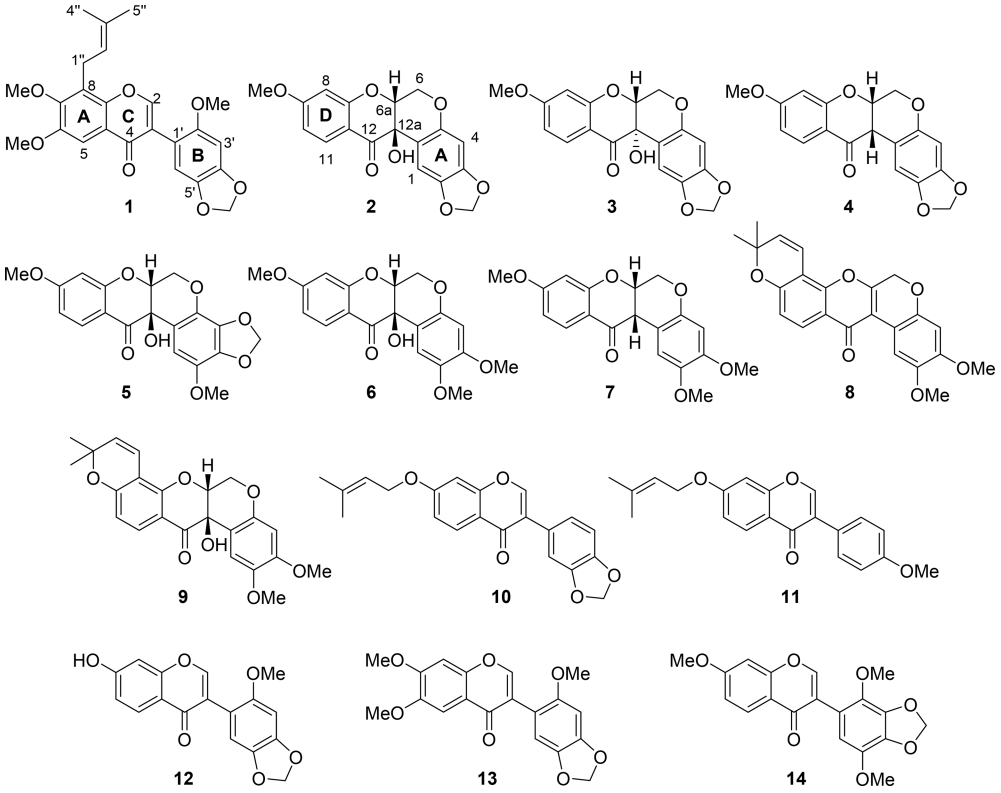
RESULTS AND DISCUSSION

The air-dried and ground leaves of *M. oblata* ssp. *teitensis* were extracted with CH_2Cl_2/CH_3OH (1:1) to afford a dark green crude extract. Chromatographic separation of this extract gave a new isoflavone (**1**), four new rotenoids (**2**–**5**), the known rotenoids 12a-hydroxymunduserone (**6**),[9] munduserone (**7**),[9] 6a,12a-dehydrodeguelin (**8**),[10,11] and tephrosin (**9**),[9−11] and the known isoflavones maximaisoflavone B (**10**),[8,12] maximaisoflavone J (**11**),[8] maximaisoflavone G (**12**),[13] milldurone (**13**),[14−16] and 7,2′,5′-trimethoxy-3′,4′-methylenedioxyisoflavone (**14**).[17] Among the known compounds, the rotenoids 6a,12a-dehydrodeguelin (**8**) and tephrosin (**9**) demonstrated cytotoxic activities against HepG2, C26, LL2, and B16 cancer cell lines, with IC_{50} values of 0.56–15.95 and 1.35–9.85 μM,

Received: March 28, 2017
Published: June 30, 2017

DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060−2066

Chart 1



respectively.[18] Strong inhibitory effects on NO production and significant insecticidal activity were also reported for **8** and **9**, with the latter being a common property of rotenoids.[19,20] Moreover, the *Neorautanenia mitis* extract containing 12a-hydroxyrotenoids showed strong acaricidal and insecticidal activities.[21]

Compound **1** was obtained as a colorless, amorphous solid, whose molecular formula was deduced as $C_{24}H_{24}O_7$ based on HRESIMS ($[M + H]^+$ *m/z* 425.1609, calcd 425.1601) and NMR analyses (Table 1). A singlet at δ_H 7.97 (H-2), in the ^{1}H NMR spectrum, and the ^{13}C NMR signals at δ_C 154.2 (C-2), 121.4 (C-3), and 175.9 (C-4) were consistent with an isoflavone core structure that was corroborated by its UV spectrum (λ_{max} at 280 and 305 nm). The NMR data (Table 1) further indicated the presence of a *C*-prenyl, a methylenedioxy, and three methoxy groups. The only aromatic proton in the A-ring was assigned to H-5 (δ_H 7.57) due to its HMBC correlations (Table 1) to C-4 (δ_C 175.9), C-6 (δ_C 150.9), C-7 (δ_C 151.8), C-4a (δ_C 120.8), and C-8a (δ_C 150.0). Attachment of the prenyl group to C-8 was indicated by the HMBC correlations of CH_2-1″ (δ_H 3.59) to C-8 (δ_C 124.7) and C-7 (δ_C 151.8). One of the methoxy groups (δ_H 3.95, δ_C 56.0) exhibited an NOE to H-5 (7.57), suggesting its placement at C-6 (δ_C 150.9). Another methoxy group (δ_H 3.92, δ_C 61.2) was placed at C-7 based on its HMBC correlation to C-7 (δ_C 151.8). The deshielded ^{13}C NMR chemical shift (δ_C 61.2) of the latter methoxy functionality confirms di-*ortho* alkoxy substitution.[22] Two *para*-oriented aromatic proton singlets at δ_H 6.63 and 6.82 were assigned to H-3′ and H-6′, respectively, with a methoxy group (δ_H 3.74, δ_C 56.9) being positioned at C-2′ (δ_C 153.0) and a methylenedioxy functionality at C-4′ (δ_C 148.4) and C-5′ (δ_C 141.2) of the B-ring based on the HMBC correlations shown in Table 1 and the NOE correlation of 2′-OCH_3 (δ_H 3.74) and H-3′ (δ_H 6.63). On the basis of the above spectroscopic data, the new compound **1**, 8-prenylmilldurone, was characterized as 8-(3,3-dimethylallyl)-6,7,2′-trimethoxy-4′,5′-methylenedioxyisoflavone. It is structurally closely related

Table 1. ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR Spectroscopic Data for 1 Acquired in $CDCl_3$ at 25 °C [δ_C, δ_H, Multiplicity (*J* in Hz), HMBC]

position	δ_C, type	δ_H (m, *J* in Hz)	HMBC
2	154.2, CH	7.97 (s)	C-3, C-4, C-1′, C-8a
3	121.4, C		
4	175.9, C		
4a	120.8, C		
5	103.9, CH	7.57 (s)	C-4, C-6, C-7, C-4a, C-8a
6	150.9, C		
7	151.8, C		
8	124.7, C		
8a	150.0, C		
1′	113.1, C		
2′	153.0, C		
3′	95.5, CH	6.63 (s)	C-1′, C-2′, C-4′, C-5′
4′	148.4, C		
5′	141.2, C		
6′	111.2, CH	6.82 (s)	C-3, C-2′, C-4′, C-5′
1″	23.0, CH_2	3.59 (d, 7.2)	C-7, C-8, C-8a, C-2″, C-3″
2″	121.6, CH	5.22 (m)	C-4″, C-5″
3″	132.6, C		
4″	17.9, CH_3	1.83 (s)	C-2″, C-3″, C-5″
5″	25.8, CH_3	1.69 (s)	C-2″, C-3″, C-4″
6-OCH_3	56.0, CH_3	3.95 (s)	C-6
7-OCH_3	61.2, CH_3	3.92 (s)	C-7
2′-OCH_3	56.9, CH_3	3.74 (s)	C-2′
OCH_2O	101.4, CH_2	5.97 (s)	C-4′, C-5′

DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060−2066

Table 2. ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR Spectroscopic Data for 2 and 3 Acquired in $CDCl_3$ at 25 °C [δ_C, δ_H (Multiplicity, J in Hz), HMBC]

	2			3		
position	δ_C	δ_H (m, J in Hz)	HMBC	δ_C	δ_H (m, J in Hz)	HMBC
1	105.8, CH	6.52 (s)	C-2, C-3, C-4a, C-12a	109.3, CH	7.71 (s)	C-2, C-3, C-4a, C-12a
2	142.3, C			142.4, C		
3	149.3, C			149.5, C		
4	99.1, CH	6.46 (s)	C-2, C-4a, C-12b	98.5, CH	6.42 (s)	C-2, C-3, C-4a, C-12b
4a	149.4, C			150.7, C		
6	63.9, CH_2	4.59 (dd, 2.4, 12.0)	C-4a, C-6a, C-12a	61.6, CH_2	4.37 (dd, 4.0, 9.6)	C-4a, C-6a, C-12a
		4.47 (br d, 12.0)	C-6a		4.44 (dd, 9.6, 11.2)	C-6a, C-12a
6a	75.9, CH	4.57 (t, 2.4)	C-12a, C-12b	76.6, CH	4.63 (dd, 4.0, 11.2)	C-6, C-12a
7a	162.5, C			161.5, C		
8	100.7, CH	6.38 (d, 2.4)	C-10, C-7a, C-11a	100.5, CH	6.50 (d, 2.4)	C-9, C-10, C-7a, C-11a
9	167.1, C			166.1, C		
10	111.1, CH	6.59 (dd, 2.4, 8.8)	C-8, C-11a	111.1, CH	6.69 (dd, 2.4, 8.8)	C-8, C-11a
11	129.4, CH	7.84 (d, 8.8)	C-9, C-12, C-7a	130.6, CH	7.93 (d, 8.8)	C-9, C-12, C-7a
11a	111.0, C			113.6, C		
12	191.2, C			187.0, C		
12a	67.7, C			66.6, C		
12b	109.7, C			110.5, C		
9-OCH_3	55.7, CH_3	3.79 (s)	C-9	55.8, CH_3	3.86 (s)	C-9
OCH_2O	101.3, CH_2	5.85 (d, 1.2)	C-2, C-3	101.5, CH_2	5.95 (s)	C-2, C-3
		5.83 (d, 1.2)				
12a–OH		4.43 (s)	C-12, C-6a, C-12a		2.59 (s)	C-6a, C-12b

to milldurone (**13**), an isoflavone that was isolated from the seeds of *M. dura*[14] and from the leaves of *Ateleia glazioviana*.[15]

Compound **2** was isolated as a colorless solid (UV λ_{max} 250, 269 nm) and assigned the molecular formula $C_{18}H_{14}O_7$ based on HRESIMS ($[M + Na]^+$ m/z 365.0645, calcd 365.0637) and NMR analyses (Table 2). The NMR data indicated a 12a-hydroxyrotenoid skeleton[22−24] with a methoxy (δ_H 3.79, δ_C 55.7) and a methylenedioxy (δ_H 5.83; 5.85, δ_C 101.3) group as substituents. The presence of a hydroxy group at C-12a was confirmed by the HMBC correlations of the hydroxy proton to C-12 (δ_C 191.2), C-6a (δ_C 75.9), and C-12a (δ_C 67.7). The D-ring possesses three mutually coupled aromatic protons, H-11 (δ_H 7.84), H-10 (δ_H 6.59), and H-8 (δ_H 6.38) (Table 2). The HMBC correlations of H-11 with C-9 (δ_C 167.1) and C-12 (δ_C 191.2) are consistent with this ring being oxygenated at C-9 (δ_C 167.1), which is corroborated by the HMBC correlation of the methoxy protons (δ_H 3.79) to this carbon. The A-ring is 2,3-disubstituted, as revealed by the aromatic singlets H-1 (δ_H 6.52) and H-4 (δ_H 6.46), placing the methylenedioxy group at C-2 and C-3. Thus, **2** was identified as 12a-hydroxy-9-methoxy-2,3-methylenedioxyrotenoid. The equatorial orientation of H-6a (δ_H 4.57) was established on the basis of the small J values ($J_{6a,6}$ = 2.4 and <1 Hz) with the C-6 diastereotopic protons (δ_H 4.47 and 4.59). This, along with the chemical shift of H-1 (δ_H 6.51), suggests a *cis*-B/C ring junction.[25,26] The negative specific rotation ($[\alpha]^{20}_D$ −38.3) and the negative Cotton effect at 325 nm, in the ECD spectrum (Figure 1), are in agreement with a (6a*R*,12a*R*) absolute configuration of **2**.[27] The *cis*-B/C ring fusion and the (6a*R*,12a*R*) absolute configuration were further corroborated by the computed electronic circular dichroism (ECD) spectra (Figure 1) of the calculated (B3LYP/6-311G) global energy minimum structures (Figure 2). This new compound, oblarotenoid A (**2**), was therefore characterized as (6a*R*,12a*R*)-12a-hydroxy-9-methoxy-2,3-methylenedioxyrotenoid.

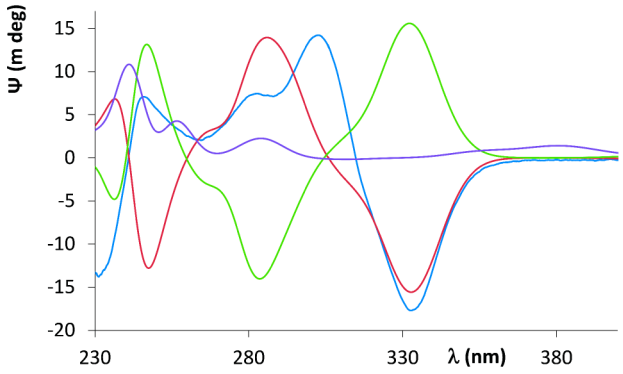


Figure 1. ECD spectra: Experimental **2** (blue), calculated for *cis*-fused (6a*R*,12a*R*)-**2** (red), calculated for *cis*-fused (6a*S*,12a*S*)-**2** (green), and calculated for *trans*-fused (6a*R*,12a*S*)-**2** (violet).

12a-Hydroxymunduserone (**6**), a rotenoid having an identical oxygenation pattern to **2**, has been reported to be dextrorotatory, $[\alpha]^{20}_D$ +33.3,[28] yet assigned a (6a*R*,12a*R*) absolute configuration, based on the observation of a negative ECD Cotton effect at 320 nm.[29] We have isolated the dextrorotatory form, $[\alpha]^{20}_D$ +53.3, of compound **6**, and it also showed a negative Cotton effect at 330 nm. The ECD spectra of compounds **2** and **6** are similar and are consistent with that previously proposed[27] and computationally predicted here for the (6a*R*,12a*R*) enantiomer. However, these compounds have specific rotations of opposite signs, $[\alpha]^{20}_D$ −38.3 (**2**) and $[\alpha]^{20}_D$ +33.3 (**6**),[28] indicating that the ECD and the specific rotation data may not always be complementary. The uncertainty associated with the use of the sign of the specific rotation in assigning the absolute configuration of rotenoids is further indicated by the examples of gliricidol and 2-methoxygliricidol. These 12a-hydroxyrotenoids are dextrorotatory[30] and were assigned a (6a*R*,12a*R*) configuration, although a negative rotation has been associated with almost all

DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060−2066

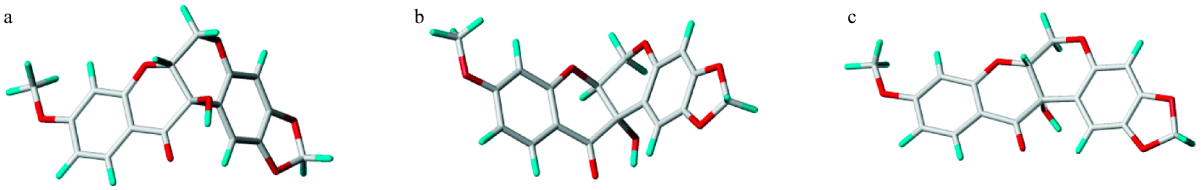


Figure 2. Calculated (B3LYP/6-311G) global energy minimum structures of (a) (6a*R*,12a*R*)-**2**, (b) of (6a*S*,12a*S*)-**2**, and (c) of (6a*R*,12a*S*)-**2**.

known (6a*R*,12a*R*)-configured 12a-hydroxyrotenoids.[27] Hence, we propose that the Cotton effect at 300−330 nm may be a better tool for the assignment of the absolute configuration of 12a-hydroxyrotenoids. The Cotton effect at 330−360 nm, due to an n → π transition, has also been proposed to be diagnostic in determining the absolute configuration of rotenoids; however, this band is generally weak[26] and is often not observed,[29] limiting its applicability.

Compound **3** was isolated as colorless crystals and showed UV absorption maxima at 285 and 300 nm. HRESIMS analysis did not show a molecular ion but rather a fragment ion at *m*/*z* 325.0702 for $[M + H - H_2O]^+$ accounting for the molecular formula, $C_{18}H_{14}O_6$. The H-6α (δ_H 4.37), H-6β (δ_H 4.44), and H-6a (δ_H 4.63) as well as C-6 (δ_C 61.6), C-6a (δ_C 76.6), C-12a (δ_C 66.6), and C-12 (δ_C 187.0) NMR signals of **3** are consistent with a 12a-hydroxyrotenoid skeleton[12,22,23] having a methoxy (δ_H 3.86, δ_C 55.8) and a methylenedioxy (δ_H 5.95, δ_C 101.5) group as substituents. Similar to **2**, the ^{1}H NMR spectrum of **3** displayed signals for three mutually coupled aromatic protons at δ_H 7.93 (d, *J* = 8.8 Hz, H-11), 6.69 (dd, *J* = 2.4, 8.8 Hz, H-10), and 6.50 (*d*, *J* = 2.4 Hz, H-8) of the D-ring. The methoxy group was located at C-9 (δ_C 166.1) based on its NOE to H-8 (δ_H 6.50) and HMBC correlation with C-9, similar to those observed for **2**. In ring A, two *para*-oriented aromatic singlets at δ_H 7.71 and 6.42 were assigned to H-1 and H-4, respectively, which, therefore, allowed the placement of the methylenedioxy group at C-2/C-3 (δ_C 142.4/149.5).

This compound is a stereoisomer of **2** having the same core 12a-hydroxy-9-methoxy-2,3-methylenedioxyrotenoid structure. One apparent distinction is that the chemical shift value for H-1 (δ_H 7.71) in compound **3** is strongly deshielded compared to that of **2** and other rotenoids with a *cis*-B/C junction. This observation and the large coupling constant between H-6a and one of the C-6 protons (*J* = 11.2 Hz, 1,2-diaxial) suggested that **3** possesses a *trans*-orientation at the B/C-ring junction, which could imply either a (6a*R*,12a*S*) or a (6a*S*,12a*R*) absolute configuration.[12,22,23] The ECD spectrum of **3** (Figure 3) showed a positive Cotton effect at ca. 340 nm and a negative one at ca. 300 nm, which together with the positive specific rotation, $[\alpha]^{20}_D$ +130, reflected a (6a*R*,12*S*) absolute configuration.[27] This is also similar to the computed ECD spectrum for the (6a*R*,12*S*)-configured isomer (Figure 1). Therefore, compound **3**, oblarotenoid B, was characterized as (6a*R*,12a*S*)-12a-hydroxy-9-methoxy-2,3-methylenedioxyrotenoid.

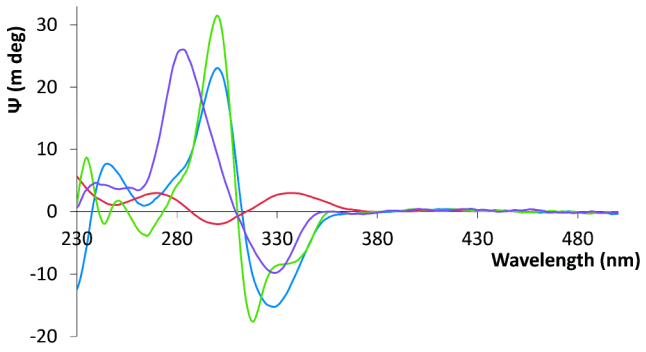


Figure 3. ECD spectra of the rotenoids **2** (blue), **3** (red), **4** (green), and **5** (violet).

Compound **4** was obtained as needles (CH_3OH), with UV absorption maxima at 285 and 300 nm, and assigned the molecular formula $C_{18}H_{14}O_6$ based on HRESIMS ($[M + H]^+$ *m*/*z* 327.0865, calcd 327.0869) and NMR data (Table 3). Its ^{1}H NMR spectrum resembled those of **2** and **3**, displaying four mutually coupled aliphatic protons, H-6α (δ_H 4.59), H-6β (δ_H 4.16), H-6a (δ_H 4.92), and H-12a (δ_H 3.80), consistent with a rotenoid skeleton.[9] Whereas the A- and D-rings of **4** have an identical substitution pattern to those of **2** and **3**, its C-12a is not oxygenated. The chemical shift of H-1 (δ_H 6.73) is consistent with a *cis*-B/C ring junction.[25−27] It should be noted that the 9-methoxy-2,3-methylenedioxyrotenoid core structure of this compound has previously been reported as a synthesized product,[31] however, without any NMR data to support the structural proposal. The negative and positive ECD Cotton effect (Figure 3) at 340 and 300 nm, respectively, suggested a (6a*S*,12a*S*) absolute configuration. Although a negative specific rotation has previously been proposed for this configuration,[27] **4** showed an $[\alpha]^{20}_D$ of +38.3 despite its 100% enantiomeric purity, as established by chiral HPLC analysis. The absolute configuration (6a*S*,12a*S*)-**4** was confirmed by single-crystal X-ray crystallography (Figure 4). The above analyses suggest that ECD should be preferred above optical rotation for the determination of the absolute configuration of rotenoids. Thus, the structure of compound **4**, oblarotenoid C, was defined as (6a*S*,12a*S*)-9-methoxy-2,3-methylenedioxyrotenoid.

Compound **5** was obtained as a colorless solid. It showed UV absorption maxima at 245, 260, and 280 nm and was assigned the molecular formula $C_{19}H_{16}O_8$ based on HRESIMS ($[M + H]^+$ *m*/*z* 373.0918, calcd 373.0924) and NMR (Table 3) analyses. The three mutually coupled aliphatic protons H-6α (δ_H 4.67), H-6β (δ_H 4.53), and H-6a (δ_H 4.59) were similar to those of **2** and **3**, indicative of a 12a-hydroxyrotenoid skeleton. The broad singlet at δ_H 4.40 was assigned to OH-12a, based on its HMBC correlations to C-12, C-6a, and C-12a. The position of CH_3O-9 (δ_H 3.80, δ_C 55.8), similar to those of **2**−**4**, was determined based on its NOE with H-8 (δ_H 6.38), HMBC correlation to C-9 (δ_C 167.2), and the coupling pattern of the three mutually coupled aromatic protons H-8, H-10, and H-11 of the D-ring (Table 3). The A-ring of **5** possesses only one singlet proton, H-1 (δ_H 6.25), unusual for a rotenoid, with its position having been determined based on its HMBC correlation to C-12a (δ_C 67.4). The placement of the methylenedioxy group was determined to be at C-3/C-4 based on the HMBC correlation of H-1 (δ_H 6.25) to C-2 (δ_C 138.8), the carbon that also showed an HMBC cross-peak to the CH_3O-2 protons (δ_H 3.74) and had a chemical shift

DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060−2066

Table 3. ^{1}H (799.87 MHz) and ^{13}C (201.25 MHz) NMR Spectroscopic Data for 4 and 5 Acquired in $CDCl_3$ at 25 °C [δ_C, δ_H, (Multiplicity, J in Hz), HMBC]

	4			5		
position	δ_C	δ_H (m, J in Hz)	HMBC	δ_C	δ_H (m, J in Hz)	HMBC
1	106.9, CH	6.73 (s)	C-2, C-3, C-4a, C-12a, C-12b	105.6, CH	6.25 (s)	C-2, C-3, C-4a, C-12a
2	142.3, C			138.8, C		
3	147.8, C			137.9, C		
4	98.8, CH	6.43 (s)	C-2, C-3, C-4a, C-12b	135.9, C		
4a	148.3, C			133.6, C		
6	66.3, CH_2	4.16 (br d, 12.0)	C-12, C-6a, C-12a	63.9, CH_2	4.53 (d, 12.0)	C-6a
		4.59 (dd, 3.2, 12.0)	C-12, C-6a, C-12a		4.67 (dd, 2.4, 12.0)	C-4a, C-6a, C-12a
6a	72.1, CH	4.92 (t, 3.2, 7.2)	C-12, C-6, C-12b	76.1, CH	4.59 (dd, 1.6, 2.4)	C-12b
7a	162.7, C			162.6, C		
8	100.6, CH	6.42 (d, 2.4)	C-7a, C-9, C-10, C-11a	100.8, CH	6.38 (d, 2.4)	C-10, C-7a, C-11a
9	166.5, C			167.2, C		
10	110.7, CH	6.57 (dd, 2.4, 8.8)	C-8, C-11a	111.3, CH	6.61 (dd, 2.4, 8.8)	C-11a
11	129.4, CH	7.86 (d, 8.8)	C-9, C-12, C-7a	129.3, CH	7.85 (d, 8.8)	C-9, C-12, C-7a
11a	112.7, C			111.1, C		
12	189.1, C			190.9, C		
12a	44.9, CH	3.80 (d, 7.2)	C-12, C-4a, C-12b	67.4, C		
12b	105.7, C			113.0, C		
2-OCH_3				56.9, CH_3	3.74 (s)	C-2
9-OCH_3	55.7, CH_3	3.79 (s)	C-9	55.8, CH_3	3.80 (s)	C-9
OCH_2O	101.2, CH_2	5.81 (d, 1.6)	C-2, C-3	102.6, CH_2	6.02 (d, 1.3)	C-3, C-4
		5.86 (d, 1.6)			5.98 (d, 1.3)	
12a-OH					4.40 (br s)	C-12, C-6a, C-12a

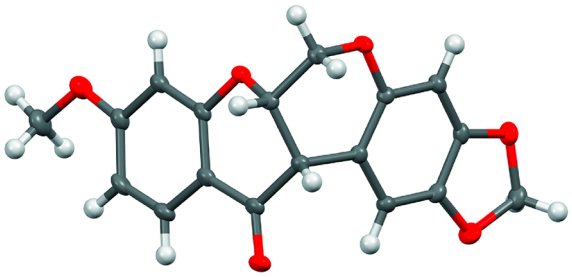


Figure 4. Single-crystal X-ray structure of compound **4**.

compatible with the proposed structure.[22] The chemical shift of H-1 (δ_H 6.25) and the small $J_{6,6a}$ (1.6, 2.4 Hz) suggested a *cis*-B/C ring junction, whereas the absolute configuration was determined as 6a*R*,12a*R* based on the negative Cotton effect at 327 nm in the ECD spectrum (Figure 3). The structure of compound **5**, oblarotenoid D, was therefore characterized as (6a*R*,12a*R*)-12a-hydroxy-2,9-dimethoxy-3,4-methylenedioxyrotenoid and possesses an unprecedented A-ring oxygenation pattern. The co-occurrence of isoflavone **14**, with a B-ring oxygenation pattern comparable to that of the A-ring of rotenoid **5**, suggests that **5** may be biosynthesized from **14**.

As part of our ongoing investigation of East African medicinal plants in search for novel antitumor natural products, we tested some of the compounds isolated from the leaves of *M. oblata* ssp. *teitensis* against the breast cancer cell line MDA-MB-231. Maximaisoflavone J (**11**) and oblarotenoid C (**4**) showed moderate, IC_{50} 33.3 μM, and low, IC_{50} 93.8 μM, cytotoxicity, respectively. Maximaisoflavone J (**11**), which has a C-4′ methoxy substituent, was almost 5 times more active than maximaisoflavone B (**10**, IC_{50} 153.6 μM), which possesses a methylenedioxy group at C-3′/4′. Compounds **1** (IC_{50} > 235 μM) and **14** (IC_{50} 167.5 μM) were inactive. The isolated constituents were also tested for in vitro translation inhibition using Krebs-II translation extracts programmed with a bicistronic firefly-HCV IRES-Renilla mRNA construct, to monitor cap-dependent as well as cap-independent translation;[32] however, none of the compounds showed significant translation inhibitory activity.

In conclusion, one new isoflavone **1** and four new rotenoids **2**–**5** were isolated from the leaves of *M. oblata* ssp. *teitensis*. Oblarotenoid D (**5**) has an unusual A-ring oxygenation pattern and is likely biosynthesized from the isoflavone **14** through oxidation of its OCH_3-2′ and subsequent cyclization. Using X-ray crystallography, we have demonstrated the higher reliability of ECD as compared to optical rotation data for determination of the absolute configuration of rotenoids. Among the isolated compounds, maximaisoflavone J (**11**) and oblarotenoid C (**4**) showed moderate and low activities, respectively, against the MDA-MB-231 human breast cancer cell line.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on a Büchi B-545 melting point apparatus. Optical rotations were measured on a PerkinElmer 341-LC, ECD spectra on a Jasco J-715 spectropolarimeter, and UV spectra on a Specord S600 (Analytik Jena AG) or a Molecular Devices SpectraMax M2 spectrophotometer. NMR spectra were acquired on a Bruker Avance III HD 800 MHz NMR spectrometer, and the spectra were processed with the MestReNova 10.0 software, using the solvent residual signal as chemical shift reference. LC-MS(ESI) spectra were acquired on a PerkinElmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source connected to a Gemini 5 mm RPC_{18} 110 Å column, applying a H_2O/MeCN (80:20 to 20:80) gradient in the presence of 0.2% HCOOH, with a separation time of 8 min. The HRMS analysis (Q-TOF-MS with a lockmass-ESI source) was done by Stenhagen Analys Lab AB, Gothenburg. TLC analyses were carried out on Merck precoated silica gel 60 F_{254} plates. Preparative TLC was done on 20 × 20 cm glass plates, precoated with silica gel 60F_{254} having 0.25 to 1 mm thickness. Column chromatography was run on silica gel 60 Å (70–230 mesh), whereas gel filtration was performed

DOI: 10.1021/acs.jnatprod.7b00255
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on Sephadex LH-20. Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd.) software and an RP C_8 Kromasil (250 mm × 55 mm) column eluting with various H_2O–CH_3OH gradients. Enantiomeric purity of oblarotenoid A (**2**) and oblarotenoid C (**4**) were determined using chiral HPLC chromatography with a Varian 9012Q solvent delivery system coupled to a Varian 9050 UV/vis detector, a Chiralpack AD (amylose tris) column (250 mm × 4.6 mm), and an *n*-hexane/2-propanol (19:1) eluent mixture with a 1 mL/min flow rate. X-ray data were acquired using an Agilent SuperNova Dual diffractometer with Atlas detector at T = 123.0(1) K and mirror-monochromatized Cu Kα radiation (λ = 1.541 84 Å).

Plant Material. The leaves of *Millettia oblata* ssp. *teitensis* were collected from Ngangao Forest, Taita Hill, Taita County, Kenya, in July 2014. The plant was identified by Mr. Patrick Chalo Mutiso of the School of Biological Sciences, University of Nairobi, Kenya, where a voucher specimen (TD-04/2014) was deposited.

Extraction and Isolation. The air-dried and ground leaves of *M. oblata* ssp. *teitensis* (1.6 kg) were extracted by percolation with CH_2Cl_2/CH_3OH (1:1), 4 × 3 L for 24 h, yielding 120 g of dark green crude extract following evaporation of the solvents using a rotary evaporator. A 110 g portion of the crude extract was subjected to column chromatography (silica gel 60, 500 g) using *n*-hexane and increasing amounts of EtOAc to give a total of 125 fractions, ca. 250 mL each. Fractions 31–35, eluted with 3% EtOAc in *n*-hexane, were combined and subjected to column chromatography on Sephadex LH-20 with CH_2Cl_2/CH_3OH (1:1) eluent, yielding maximaisoflavone B (**10**, 4.2 mg). The separation of fractions 38–44 by column chromatography over silica gel, eluted with 3% EtOAc in *n*-hexane, yielded maximaisoflavone J (**11**, 8.4 mg). Fractions 46–54, eluted with 5% EtOAc in *n*-hexane, were combined and subjected to column chromatography on Sephadex LH-20 with CH_2Cl_2/CH_3OH (1:1) eluent to give oblarotenoid C (**4**, 33.4 mg, needle-like crystals from CH_3OH). The mother liquid of the above crystallization was purified on reversed-phase preparative HPLC with a CH_3OH/H_2O gradient (60:40 to 90:10) to give oblarotenoid A (**2**, 6.3 mg) and additional amounts of **4** (2.1 mg). Fractions 55–62, eluting with 5% EtOAc in *n*-hexane, were subjected to column chromatography on Sephadex LH-20 using CH_2Cl_2/CH_3OH (1:1) eluent to afford 6a,12a-dehydrodeguelin (**8**, 53.1 mg). Fractions 67–71, eluted with 7% EtOAc in *n*-hexane, were combined and separated by column chromatography on Sephadex LH-20 with CH_2Cl_2/CH_3OH (1:1) as eluent to give 8-prenylmilldurone (**1**, 17.9 mg) as a colorless solid and two major subfractions. The first subfraction was subjected to reversed-phase preparative HPLC with a CH_3OH/H_2O (60:40 to 90:10) gradient to give munduserone (**7**, 5.4 mg) and tephrosin (**9**, 9.1 mg). The second subfraction was separated on reversed-phase preparative HPLC with CH_3OH/H_2O (60:40 to 90:10) to afford oblarotenoid B (**3**, 4.0 mg), oblarotenoid A (**2**, 7.6 mg), and oblarotenoid C (**5**, 1.4 mg). Fractions 74–77, eluted with 9% EtOAc in *n*-hexane, were combined and separated on Sephadex LH-20 eluting with CH_2Cl_2/CH_3OH (1:1) to give 8-prenylmilldurone (**1**, 3.0 mg) and a mixture of two additional compounds. 12a-Hydroxymunduserone (**6**, 4.3 mg, colorless solid) and 7,2′,5′-trimethoxy-3′,4′-methylenedioxyisoflavone (**14**, 23.5 mg) were separated using column chromatography on silica gel with an *n*-hexane/EtOAc (8:2 to 7:3) gradient. 2′,6,7-Trimethoxy-4′,5′-methylenedioxyisoflavone (**13**, 7.0 mg), a colorless, amorphous solid, was isolated from the combined fractions 85–90, eluting with 10–12% EtOAc in *n*-hexane, using Sephadex LH-20 and CH_2Cl_2/CH_3OH (1:1) as eluent. Maximaisoflavone G (**12**, 98.7 mg), a colorless solid, was precipitated from fractions 95–104, eluted with 20% EtOAc in *n*-hexane.

*8-Prenylmilldurone (**1**):* colorless solid; mp 183–185 °C; UV (CH_3OH) λ_{max} (log ε) 305 (3.88), 280 (3.56) nm. ^1H and ^{13}C NMR data, see Table 1 and Figures S2–S8 (Supporting Information); HRESIMS $[M + H]^+$ m/z 425.1609 (calcd for $C_{24}H_{25}O_7$ 425.1601).

*Oblarotenoid A (**2**):* colorless solid; mp 128–130 °C; $[\alpha]^{20}_D$ −38.3; UV (CH_3OH) λ_{max} 250, 269 nm; ECD (CH_3OH): 300 (23.0833), 327 (−15.1694) nm; ^1H and ^{13}C NMR data, see Table 2 and Figures S9–S15 (Supporting Information); HRESIMS $[M + Na]^+$ m/z 365.0645 (calcd for $C_{18}H_{14}O_7Na$ 365.0637).

*Oblarotenoid B (**3**):* colorless solid; mp >205 °C; $[\alpha]^{20}_D$ +130.3; UV (CH_3OH) λ_{max} (log ε) 285 (3.40) and 300 (3.45) nm; ECD (CH_3OH) 340 (2.9294), 300 (−2.0102) nm; ^1H and ^{13}C NMR data, see Table 2 and Figures S16–S22 (Supporting Information); HRESIMS $[M + H - H_2O]^+$ m/z 325.0702 (calcd for $C_{18}H_{13}O_6$ 325.0667).

*Oblarotenoid C (**4**):* needles (from CH_3OH); mp 193–195 °C; $[\alpha]^{20}_D$ +38.3; UV λ_{max} (log ε) 285 (3.49), 300 (3.51) nm. ECD (CH_3OH) 300 (31.4803), 340 (−7.8223) nm; ^1H and ^{13}C NMR data, see Table 3 and Figures S23–S28 (Supporting Information); HRESIMS $[M + H]^+$ m/z 327.0865 (calcd for $C_{18}H_{15}O_6$ 327.0869).

*Oblarotenoid D (**5**):* colorless solid; mp 132–134 °C; $[\alpha]^{20}_D$ −9.04; UV λ_{max} (log ε) 245 (3.04), 260 (3.08), 280 (3.01) nm; ECD (CH_3OH) 285 (25.29), 327 (−9.66) nm; ^1H and ^{13}C NMR data, see Table 3 and Figures S29–S37 (Supporting Information); HRESIMS $[M + H]^+$ m/z 373.0918 (calcd for $C_{19}H_{17}O_8$ 373.0924) and $[M + Na]^+$ m/z 395.0698 (calcd for $C_{19}H_{16}O_8$ 395.0742).

Cytotoxicity Assays. Following a published procedure,[7,33] MDA-MB-231 human breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in humidified 5% CO_2. Cells were seeded in 96-well plates at optimal cell density (10^4 cells per well) to ensure exponential growth for the duration of the assay. After a 24 h preincubation period, the medium was replaced with experimental medium containing the appropriate compound concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). Following 72 h of incubation, cell viability was measured using Alamar Blue (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least six times. Statistical analyses were performed using a two-tailed Student's t test. $P < 0.05$ was considered to be statistically significant.

Translation Inhibitory Assay. A previously developed assay[32] was used to measure translation inhibitory activity. The compounds were suspended at a concentration of 10 mM in DMSO and subsequently diluted to 200 μM in H_2O. They were tested at a final concentration of 20 μM in Krebs-2 translation extracts programmed with a bicistronic Firefly-HCV IRES-Renilla luciferase mRNA construct. Translation reactions were incubated at 30 °C for 60 min, at which point the luciferase activities were measured. Compounds that inhibit only FF would be considered cap-dependent translation inhibitors, compounds that inhibit expression of Ren only would be inhibitors of HCV IRES translation, while compounds that inhibit both FF and Ren would likely be translation elongation inhibitors. None of the compounds were observed to display significant inhibition of translation.

Computation. Conformations were optimized at the B3LYP/6-311G** level of theory[34,35] without any restrictions. The ECD spectra were computed using the time-dependent DFT[36,37] algorithm as implemented in the GAUSSIAN 09 software package[38] using the 6-31G* basis set. For ECD calculations 15 singlet and 15 triplet states were solved, obtained with the keyword TD (N_{states} = 15, 50–50). All GAUSSIAN results were analyzed and the spectra displayed using the software SpecDis 1.62.[39] Conformations were weighted according to their Boltzmann distribution. Molecules were displayed using SYBYL-X 2.1.1.[40]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00255.

NMR, MS, and CD spectra (PDF)
Crystallographic data (CIF)

DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060–2066

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Mr. P. C. Mutiso of the Herbarium, Botany Department, University of Nairobi, for the identification of the plant species for this study. T.D. thanks the German Academic Exchange Service (DAAD) for a Ph.D. scholarship, offered through the Natural Products Research Network for Eastern and Central Africa (NAPRECA). T.D. is also grateful to the Swedish International Development Cooperation Agency (SIDA) for covering the costs of his research visit to Sweden. Financial support from the Swedish Research Council (2016-05857), the Academy of Finland (K.R.'s grant nos. 263256, 265328, and 292746), the University of Jyväskylä, and the Royal Society of Arts and Sciences in Göteborg is gratefully acknowledged.

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DOI: 10.1021/acs.jnatprod.7b00255
J. Nat. Prod. 2017, 80, 2060−2066