Cytotoxic Compounds from *Mundulea chapelieri* from the Madagascar Rainforest[1]

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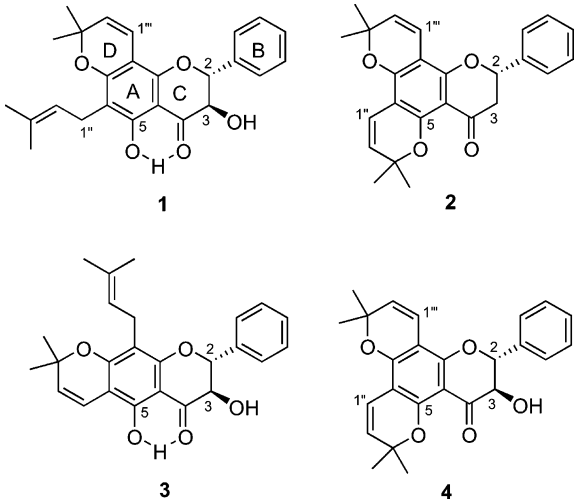
Bioassay-guided fractionation of methanolic extracts of *Mundulea chapelieri* resulted in the isolation of two new flavonoids, isomundulinol (**1**) and 3-deoxy-MS-II (**2**), in addition to the eight known flavonoids 8-(3,3-dimethylallyl)-5,7-dimethoxyflavanone, MS-II, mundulinol, mundulone, munetone, rotenolone, rotenone, and tephrosin, and one known sesquiterpenoid, 8α-acetoxyelemol. The structures of the new flavonoids **1** and **2** were determined by 1D and 2D NMR experiments. All the isolated compounds were tested for cytotoxicity against the A2780 human ovarian cancer cell line; rotenolone and rotenone were the most potent compounds isolated, with IC_{50} values of 0.5 and 0.7 μg/mL, respectively.

In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, we obtained separate extracts of the roots, bark, wood, and leaves and flowers of the plant *Mundulea chapelieri* (Baill.) R. Viguier ex Du Puy & Labat (Fabaceae) from Madagascar. *Mundulea* (DC.) Benth. in Miquel is a genus of 12 species,[2] 11 of which are endemic to Madagascar and the 12th occurs in Madagascar, Africa, and Southeast Asia. The genus is quite similar to, and questionably distinct from, *Tephrosia* Pers., which has about 250 species and is pantropical in distribution. *M. chapelieri* has not been chemically studied before, but other species of the genus have been investigated and have been found to contain several flavonoids and related compounds.[3−10] Various biological properties have been reported for compounds from *Mundulea*, including cancer chemopreventive activity,[11−14] antibacterial activity,[15] and insecticidal properties.[16−18]

The extracts of the bark and of the leaves and flowers of *M. chapelieri* proved to be active in the A2780 ovarian cancer cytotoxicity assay, with IC_{50} values of 11 and 25 μg/mL respectively, so they were selected for fractionation. Bioassay-guided fractionation of these extracts led to the isolation of two new prenylated flavonoids, isomundulinol (**1**) and 3-deoxy-MS-II (**2**), in addition to nine known compounds. The structures of the known natural products were identified as the aromatic compounds mundulinol (**3**),[19,20] MS-II (**4**),[21] 8-(3,3-dimethylallyl)-5,7-dimethoxyflavanone (**5**),[22] rotenolone (**6**),[23] rotenone (**7**),[24] tephrosin (**8**),[5] mundulone (**9**),[4] and munetone (**10**),[4] and the sesquiterpenoid 8α-acetoxyelemol (**11**).[25]

Compound **1** was obtained as a pale yellow amorphous solid and had a molecular formula of $C_{25}H_{26}O_5$ based on HRFABMS. The UV data (λ_{max} 274, 315, and 364 nm) suggested the presence of a flavanol skeleton.[26] The ^{1}H NMR spectrum of **1** showed the presence of one chelated hydroxyl (δ_H 11.50, s), one hydroxyl doublet (δ_H 3.51, d, J = 1.4 Hz), a multiplet (δ_H 7.42−7.57, m) due to a mono-



substituted benzene ring, one doublet (δ_H 5.05, d, J = 11.7 Hz), and one doublet of doublets (δ_H 4.50, dd, J = 11.7, 1.4 Hz), assigned to two oxymethines (Table 1). The ^{1}H NMR spectrum of **1** also showed signals for an olefinic proton at δ_H 5.19 (t, J = 7.1 Hz), two methylene protons at δ_H 3.25 (d, J = 7.1 Hz), and a pair of methyl singlets at δ_H 1.68 (s) and 1.79 (s), which corresponded to a 3-methyl-2-butenyl group. In addition, a pair of *cis*-coupled olefinic doublets (δ_H 6.51, d, J = 10.1 Hz; 5.47, d, J = 10.1 Hz) and a pair of methyl singlets at δ_H 1.42 (s) and 1.46 (s) implied the presence of a *gem*-dimethyldihydropyran ring. A comparison of both the ^{1}H and ^{13}C NMR data of **1** with the reported data for mundulinol (**3**), which has the same molecular formula as **1**, indicated that chemical shifts for all the protons and carbons were similar. Both rings B and C of **1** were the same as those of **3**. Ring D, the *gem*-dimethyldihydropyran ring, of **3** was linear to rings A and C with the 3-methyl-2-butenyl group located at C-8. On the other hand, as an isomer of **3**, the substitution at C-6 of **1** must be a 3-methyl-2-butenyl group and a C_5 group at C-8 must be cyclized with the hydroxyl group at C-7 to form a *gem*-dimethyldihydropyran ring. In the HMBC spectrum of **1** (Figure 1), the deshielded chelated hydroxyl signal (δ_H 11.50, s) correlated with three quaternary carbons (δ_C 100.1, C-4a; 160.7, C-5 and 110.3, C-6). H_2-1″ (δ_H 3.25, d,

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Table 1. NMR Spectral Data[a] of **1** and **2**

	1		2	
position	^{13}C[b]	^{1}H[c] (mult. J = Hz)	^{13}C[b]	^{1}H[c] (mult. J = Hz)
2	83.2	5.05 (d, 11.7)	79.0	5.39 (dd, 13.3, 3.3)
3	72.5	4.50 (dd, 11.7, 1.4)	46.0	2.77 (dd, 16.5, 3.3)
				2.96 (dd, 16.5, 13.3)
4	195.7		188.7	
4a	100.1		105.5	
5	160.7		154.2	
6	110.3		104.6	
7	160.5		155.9	
8	102.1		102.3	
8a	154.9		157.5	
1′	136.5		139.1	
2′, 6′	127.4	7.42−7.57 (m)	126.0	7.36−7.46 (m)
3′, 5′	128.6	7.42−7.57 (m)	128.7	7.36−7.46 (m)
4′	129.2	7.42−7.57 (m)	128.5	7.36−7.46 (m)
1″	20.9	3.25 (d, 7.1)	115.9	6.60 (d, 10.1)
2″	122.0	5.19 (t, 7.1)	126.2	5.50 (d, 10.1)
3″	131.6		77.8[d]	
4″	25.8	1.68 (s)	27.9	1.48 (s)
5″	17.9	1.79 (s)	28.1[e]	1.52 (s)
1‴	115.6	6.51 (d, 10.1)	116.3	6.57 (d, 10.1)
2‴	126.5	5.47 (d, 10.1)	126.6	5.46 (d, 10.1)
3‴	78.3		77.9[d]	
4‴	28.3	1.42 (s)	28.2[e]	1.44 (s)
5‴	28.6	1.46 (s)	28.5	1.45 (s)
OH-3		3.51 (d, 1.4)		
OH-5		11.50 (s)		

[a] Assignments based on COSY, HMQC, and HMBC. [b] Chemical shifts (δ) in ppm. [c] Chemical shifts (δ) in ppm. s: singlet; d: doublet; t: triplet; m: multiplet. [d,e] Interchangeable.

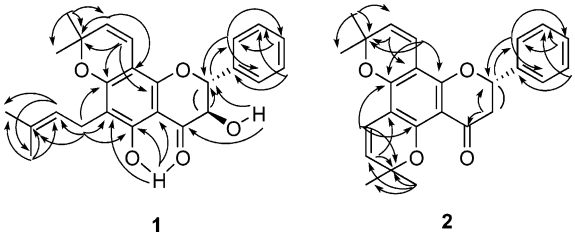

Figure 1. Key HMBC (⌒) and COSY (∩) correlations of **1** and **2**.

J = 7.1 Hz) showed correlations with C-5 (δ_C 160.7), C-6 (δ_C 110.3), C-7 (δ_C 160.5), C-2″ (δ_C 122.0), and C-3″ (δ_C 131.6), while H-1‴ (δ_H 6.51, d, J = 10.1 Hz) exhibited correlations with C-7 (δ_C 160.5), C-8 (δ_C 102.1), C-8a (δ_C 154.9), C-2‴ (δ_C 126.5), and C-3‴ (δ_C 78.3). These correlations confirmed the proposed structure of **1**, which was given the trivial name isomundulinol. The absolute configuration of isomundulinol (**1**) was deduced from analysis of its CD spectrum and by comparison with published data for lupinifoninol.[27] Negative and positive Cotton effects were observed at 302 and 325 nm, respectively. In addition, the $J_{2,3}$ value (11.7 Hz) between H-2 and H-3 was indicative of equatorial substituents at C-2 and C-3. The above data suggested the absolute configuration of isomundulinol (**1**) to be 2*R*, 3*R*.

Compound **2** was also obtained as a pale yellow amorphous solid and had the molecular formula $C_{25}H_{24}O_4$ based on HRFABMS. The analysis of the ^{1}H NMR spectrum of **2** revealed the presence of two *gem*-dimethyldihydropyran rings, one monosubstituted benzene ring, one oxymethine, and one methylene group. The ^{1}H and ^{13}C NMR spectra of **2** had features similar to those of the known compound MS-II (**4**). The NMR data (see Table 1 and Figure 1) together with the molecular formula suggested that **2** was the dehydroxylated analogue of **4**. The COSY spectrum of **2** exhibited a correlation between the oxymethine (δ_H 5.39, dd, J = 13.3, 3.3 Hz, H-2) and the methylene (δ_H 2.77, dd, J = 16.5, 3.3 Hz and 2.96, dd, J = 16.5, 13.3 Hz, H_2-3), and connectivities from H-3 to C-4 (H-2′ and H-6′ to C-2; H-1″ to C-5, C-7; H-1‴ to C-7, C-8a) were established by HMBC correlations. The CD spectrum of **2** exhibited a positive Cotton effect in the region 340−380 nm due to an n−π* transition and a negative Cotton effect in the region (265−310 nm) due to a π−π* transition, which is characteristic of 2*S* flavanones.[28] Furthermore, the large coupling constant ($J_{2ax,3ax}$ = 13.3 Hz) between H-2 and H-3ax suggested that the 2-phenyl ring exists in the equatorial position. Thus, the structure of **2** was elucidated as 3-deoxy-MS-II.

Table 2. Cytotoxicities of Compounds **1**−**11**[a]

compound	IC_{50} (μg/mL)
isomundulinol (**1**)	18
3-deoxy-MS-II (**2**)	33
mundulinol (**3**)	25
MS-II (**4**)	20
8-(3,3-dimethylallyl)-5,7-dimethoxyflavanone (**5**)	23
rotenolone (**6**)	0.5
rotenone (**7**)	0.7
tephrosin (**8**)	9.1
mundulone (**9**)	13
munetone (**10**)	20
8α-acetoxyelemol (**11**)	17

[a] Concentration of each compound that inhibited 50% of the growth of the A2780 human ovarian cell line according to the procedure described,[29] with actinomycin D (IC_{50} 1−3 ng/mL) as the positive control.

All the isolated compounds were tested against the A2780 human ovarian cancer cell line; the results are shown in Table 2. It was found that most of the compounds were inactive, with IC_{50} values in the range 20−33 μg/mL, while rotenolone and rotenone, which have pyrano−pyrano junctions and furano rings, showed good cytotoxic activity with IC_{50} values of 0.5 and 0.7 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. The chemical shifts are given in δ (ppm) with TMS (tetramethylsilane) as internal reference, and coupling constants are reported in Hz. A Horizon flash chromatograph from BioTage Inc. was used for flash column chromatography. HPLC was performed on a Shimadzu LC-10AT instrument on a Varian Dynamax C_{18} column (250 × 10 mm).

Cytotoxicity Bioassays. The A2780 ovarian cancer cell line cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[29]

Plant Material. The samples of *Mundulea chapelieri* (Fabaceae) were collected in January 2002, at an elevation of 850 m and coordinates 17°17′25″ S; 48°40′30″ E, at Ambodisakoana (10 km from Vohimenakely, outside of the Zahamena National Park) in the province of Toamasina in eastern Madagascar. The collection was made by Stephan Rakotonandrasana, Fidy Ratovoson, Ignace Rakotozafy, Richard Razakamalala, and Lucien Randrianjanaka. The voucher specimen is S. Rakotonandrasana et al. 624, and duplicates are deposited at Centre National d'Application et des Recherches Pharmaceutiques in Antananarivo, Madagascar (CNARP); Missouri Botanical Garden, St. Louis, Missouri (MO); Museum National d'Histoire Naturelle Herbarium in Paris, France (P); and Parc de Tsimbazaza Herbarium in Antananarivo, Madagascar (TAN).

Extract Preparation. The bark (506.8 g) of *M. chapelieri* was dried, ground, and extracted with MeOH at room tem-

perature for 24 h. Removal of the solvent under vacuum at 50 °C yielded extract MG1405 (5.8 g). Similar treatment of the combined leaves and flowers (321.9 g) gave extract MG1407 (18.4 g).

Extraction and Isolation. Extract MG1405 (0.12 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 10 mL) and extracted with *n*-hexane (3 × 10 mL). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 10 mL). The CH_2Cl_2 extract was found to be cytotoxic. The CH_2Cl_2 fraction (25 mg) was chromatographed on a Horizon flash chromatograph over C_{18} Si gel using H_2O−MeOH (40:60 to 0:100) to furnish eight fractions (1−8), of which fraction 2 (5.6 mg) was the most active. Fraction 2 on reversed-phase HPLC with the mobile phase MeOH−H_2O (70:30) yielded the three compounds rotenolone (t_R 21.5 min; 0.4 mg), rotenone (t_R 27.5 min, 0.3 mg), and tephrosin (t_R 31 min; 0.6 mg), while fractions 3 and 5 yielded mundulone (1.2 mg) and munetone (1 mg), respectively.

Extract MG1407 (1.0 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 50 mL) and extracted with *n*-hexane (3 × 50 mL). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 50 mL). The *n*-hexane extract were found to be more active than the CH_2Cl_2 and MeOH extracts. The *n*-hexane extract (375 mg) was chromatographed on a Horizon flash chromatograph over C_{18} Si gel using H_2O−MeOH (50:50 to 0:100) to furnish nine fractions (1−9), of which fractions 2 (17.3 mg), 3 (7.5 mg), 6 (29.7 mg), and 7 (6.0 mg) were weakly active. 8-(3,3-Dimethylallyl)-5,7-dimethoxyflavanone (R_f 0.3, 1 mg) and 8α-acetoxyelemol (R_f 0.25, 5 mg) were obtained from fractions 2 and 3 using preparative TLC developed with CH_2Cl_2. Fractions 6 and 7 on reversed-phase HPLC with the mobile phase MeOH−H_2O (90:10) yielded the four compounds **1** (t_R 21.5 min; 1 mg), **2** (t_R 20 min, 1.5 mg), mundulinol (**3**) (t_R 22 min, 2 mg), and MS-II (**4**) (t_R 15 min; 2 mg). The structures of the known compounds were identified by comparison of their spectral data with literature values.[4,5,19−25]

Isomundulinol (1): pale yellow amorphous solid; $[\alpha]_D$ −26.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 274 (4.22), 315 (3.73), 364 (3.24) nm; IR ν_{max} 3350, 2916, 2849, 1632, 1593, 1135, 1119 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; CD (MeOH, *c* 0.02) $[\theta]_{325}$ +0.9, $[\theta]_{302}$ −2.6, $[\theta]_{258}$ +0.9, $[\theta]_{248}$ +0.7, $[\theta]_{225}$ +2.7; HRFABMS *m*/*z* 407.1832 $[M + H]^+$ (calcd for $C_{25}H_{27}O_5$, 407.1858).

3-Deoxy-MS-II (2): pale yellow amorphous solid; $[\alpha]_D$ −5.2° (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 269 (4.23), 315 (sh), 361 (3.09) nm; IR ν_{max} 2916, 2849, 1633, 1590, 1258, 1085, 1009, 790 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; CD (MeOH, *c* 0.02) $[\theta]_{375}$ +0.8, $[\theta]_{360}$ +0.7, $[\theta]_{355}$ +0.5, $[\theta]_{340}$ +0.2, $[\theta]_{310}$ −2.1, $[\theta]_{265}$ −2.3, $[\theta]_{255}$ −1.7, $[\theta]_{225}$ +3.0, $[\theta]_{220}$ +3.5, $[\theta]_{210}$ +2.4; HRFABMS *m*/*z* 389.1742 $[M + H]^+$ (calcd for $C_{25}H_{25}O_4$, 389.1753).

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Supporting Information Available: ^1H NMR spectra for compounds **1** and **2** and structures of all isolated compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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