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Nitrogen-Containing Dimeric *nor*-Multiflorane Triterpene from a *Turraea* sp.

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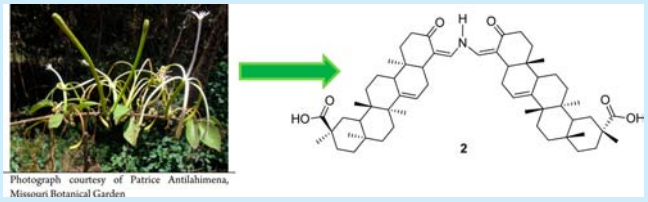
Supporting Information

ABSTRACT: The new triterpene turranoic acid (**1**) and the new N-containing *nor*-triterpene turraenine (**2**), along with triptocallic acid B (**3**) and esculentoic acid (**4**) were isolated from leaves of a *Turraea* sp. Compounds **1**−**3** showed weak to moderate in vitro antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain FCM29. Compound **1** also displayed weak cytotoxic activity against the nonsmall lung cancer cell line H522-T1 with an IC_{50} value of 16.4 μM.



Plants from the genus *Turraea* (family Meliaceae, order Rutales) have been extensively investigated due to their high content of bioactive limonoids[1−7] and triterpenoids such as turrapubesols[8−10] and pregnanes.[8,9,11,12] These compounds have been reported to have a wide range of biological activities including cytotoxic, insect antifeedant, and mosquito larvicidal activity. As part of a joint International Cooperative Biodiversity Group (ICBG) research program to search for new antimalarial and anticancer secondary metabolites from the natural resources of Madagascar, we selected a plant species from the genus *Turraea* for investigation. The genus *Turraea* contains approximately 85 species, about 60 of them found in tropical and southern Africa, one species in Australia, and 24 species in Madagascar.[13] In African ethnobotany, species of this genus have been used as an aphrodisiac and to treat wounds, parasites (bilharzias), abscesses, and impotence, and the ripe fruit and the bitter bark of *Turraea* species are used in Madagascar to treat throat problems.

A crude EtOH extract made from leaves of a Madagascar species of *Turraea* was selected for investigation since it demonstrated in vitro antiplasmodial activity at 3.9 μg/mL against the chloroquine-resistant strain FCM29 of *Plasmodium falciparum* during our preliminary screening. Bioassay-guided fractionation of this extract resulted in the isolation of turranoic acid (**1**), a new triterpenoid with a multiflorane skeleton, and turraenine (**2**), a new nitrogen-containing *nor*-multiflorane-type triterpene. Triptocallic acid B (**3**) and esculentoic acid (**4**) were also isolated (Figure 1). In this paper, we report the isolation and the structural elucidation of the new compounds **1** and **2** and the biological activity of all four compounds.

The EtOH extract was subjected to liquid−liquid partitioning followed by column chromatography over silica gel, RP-C18

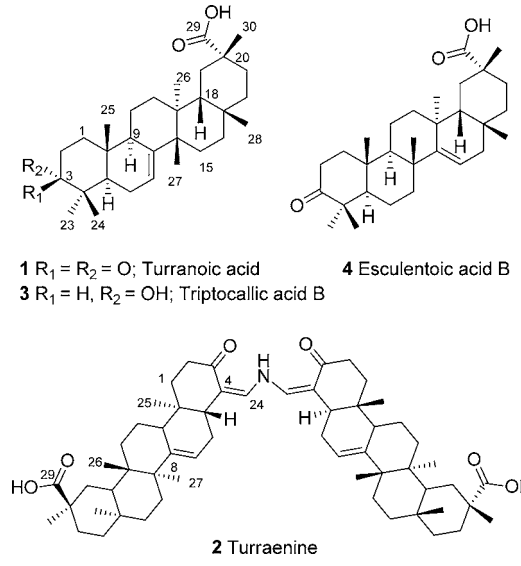


Figure 1. Structures of compounds**1**−**4**.

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silica gel, and Sephadex LH-20. Final crystallization afforded the four compounds **1**−**4**. Compounds **3** and **4** were identified as triptocallic acid B and esculentoic acid, respectively, based on the comparison of their spectroscopic data with those reported in the literature.[14,15]

The positive-ion high-resolution electrospray ionization (HRESI) mass spectrum of compound **1** displayed a protonated molecular ion peak at *m*/*z* 455.3517 corresponding to the molecular formula $C_{30}H_{47}O_3$ (required for $[M + H]^+$: *m*/*z* 455.3520). The IR spectrum showed a typical absorption band at 3443 cm^{-1} and strong absorptions at 1709 and 1732 cm^{-1} which were suggestive of hydroxyl, ketone carbonyl, and carboxyl functions. The ^{1}HNMR spectroscopic data of **1** (Table 1) exhibited seven methyl singlets (δ 0.93, 3H; 0.98, 6H;1.01, 3H; 1.03, 3H; 1.10, 3H; 1.23, 3H) and one olefin methine at δ 5.47 (dd, 6.4, 3.2 Hz) which was coupled to a methylene in a cyclohexene ring. The ^{13}C NMR data (Table 1) displayed 30 carbon resonances which were assigned to one ketone carbonyl (δ 217.2), one carboxylic acid (δ184.5), one olefin methine (δ 117.3), one sp^2-hybridized quaternary carbon (δ 145.6), 10 methylenes, three methines, six sp^3-hybridized quaternary carbons, and seven methyls by DEPT 135 experiments. The above NMR data were very similar to those of triptocallic acid B (**3**, Table 1), a multiflorane-type triterpene acid which was first isolated from a callus cell culture of *Trypterigium wilfordii* (Celastraceae).[14]

Table 1. ^{1}H NMR Data of Compound 1 and ^{13}C NMR Data of Compounds 1 and 3 in $CDCl_3$ (δ in ppm)[a]

	1		3
position	δ_H (multiplicity (m), *J* in Hz)	δ_C	δ_C
1	1.44, m	38.5	37.6
	2.00, m		
2	2.25, dt (14.5, 3.7)	35.0	27.6
	2.73, td (14.5, 5.5)		
3		217.2	79.3
4		47.8	39.2
5	1.64, dd (7.8, 6.5)	52.1	50.7
6	2.08, m	24.7	24.5
7	5.47, dd (6.4, 3.2)	117.3	117.6
8		145.6	145.7
9	2.12, m	48.1	48.3
10		35.3	35.5
11	1.52, m, 1.63, m	17.4	17.5
12	0.86 br d (13.8)	32.8	33.3
	2.00, td (13.8, 4.3)		
13		36.9	37.3
14		42.5	42.5
15	1.47−1.41[b]	29.4	30.9
16	2.00, m[b]	36.9	37.3
17		31.2	31.6
18	1.48, m	47.2	45.8
19	1.67, m	30.4	30.9
	2.37, br d (16.0)		
20		40.4	40.6
21	1.37−1.41[b]	29.4	29.5
22	1.37, m, 1.76, m	35.6	36.0
23	0.98, s	24.0	27.8
24	1.10, s	21.8	15.0
25	0.98, s	13.0	13.4
26	1.03, s	24.7	25.2
27	0.93, s	25.5	27.8
28	1.01, s	31.4	31.5
29		184.5	182.8
30	1.23, s	33.1	33.5

[a] ^{1}H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz. [b] Signals overlapped.

Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **3** indicated that the chemical shifts arising from the methyl, the methylene, and the methine groups were essentially the same except for the signals due to the A-ring. The ^{13}C NMR spectrum of **1** showed the presence of a signal at δ 217.2 (C-3) instead of the oxygen-bearing methine (δ 79.3) in **3**. The carbon chemical shifts of C-23 and C-24 shifted from 27.8 and 15.0 in **3** to 24.0 and 21.8, respectively, while the other signals remained almost the same.

In addition, the methylene signals at δ 2.25 dt (*J* = 14.5, 3.7 Hz, H-2a) and δ 2.73 dt (*J* = 14.5, 5.5 Hz, H-2b) in the proton spectrum of **1** corroborated the presence of a ketone carbonyl at C-3. Two-dimensional NMR data of **1** confirmed the assignment of the carbonyl to C-3, the olefin methine at C-7, the methyl groups at C-4, -10, -13, -14, -17, and -20, and the carboxyl group at C-29. The HSQC spectrum was used to assign the proton-bearing carbons (Table 1). The ^2J and ^3J couplings observed in the HMBC spectrum were then interpreted. The carbonyl carbon at C-3, the methyl group at C-10, and the location of the olefin methine (C-7) of the decalin were assigned by observing the long-range correlations from H-1 to C-3; CH_3-23 and −24 to C-3, C-4, and C-5; CH_3-25 to C-1, C-9, and C-5; and CH-7 to C-5, C-9, and C-14. The HMBC cross peaks between CH_3-26 and C-12, C-18, and C-27 on one hand and between CH_3-30 and C-29, C-19, and C-21 on the other hand indicated the presence of a carboxylic acid at C-29. In the same manner, the methyl groups at C-13 and C-14 were allocated. The relative and absolute configurations at C-20 and at other chiral centers were assigned by interpretation of the NOESY spectroscopic data and X-ray diffraction analysis of a single crystal of **1**. The X-ray structure of **1** showing anisotropic displacement ellipsoids at the 50% probability level is shown in Figure 2.[16] These findings confirmed the structure of **1** as multiflor-3-on-7-en-29-oic acid, named turranoic acid.

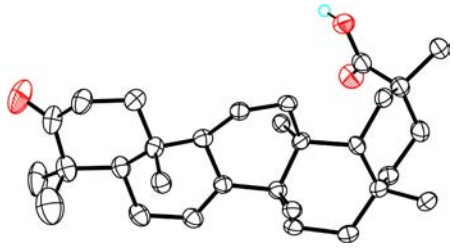

Figure 2. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **1**. One of two crystallographically independent molecules is shown. Aliphatic H atoms are omitted for clarity.

Compound **2** had the molecular formula $C_{58}H_{83}NO_6$ as determined by positive high-resolution ESIMS (observed *m*/*z* 890.6311, required for $C_{58}H_{84}NO_6$ $[M + H]^+$, 890.6293). Its ^{1}HNMR spectrum displayed a deshielded triplet resonance due to a hydrogen-bonded amine proton (δ14.0, t, *J* = 11.4 Hz, 1H), two 2H olefin methine signals (δ 6.46, d, *J* = 11.4, 2H, H-24 and -24′ and δ 5.40, brs, 2H, H-7 and -7′), and two sets of signals superposable on those of **1**. Excitation of the proton at δ

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14.0 by 1D TOCSY collapsed the 2H olefin methine signal at δ 6.46, indicating the presence of the partial structure =CHNHCH= in **2**.

The ^{13}C NMR spectrum of **2** showed signals for conjugated ketone carbonyl (δ 200.0, C-3) and carboxyl (δ 185.9) groups, signals due to olefin methine carbons (δ 114.0, C; 115.8, CH; 141.5, CH and 146.5, C), one of which was assignable to a methine attached via a heteroatom, and signals ascribable to a multiflorene-type triterpene dimer.

Inspection of the ^{1}H and ^{13}C NMR data (Table 2) of **2** revealed the presence of two sets of signals which were very

Table 2. ^{1}H and ^{13}C NMR Data for 2 in $CDCl_3$ (δ in ppm)[a]

position	δ_H (multiplicity (m), J in Hz)	δ_C
1,1′	2.49 m, 1.41−1.44[b]	34.9
2, 2′	1.40 m, 1.98 m	33.7
3,3′		200.0
4,4′		114.0
5,5′	2.39 dd (10.1, 5.6)	42.4
6,6′	1.30−1.38[b]	29.1
7,7′	5.40 br	115.8
8,8′		146.5
9,9′	2.11 brd (13.6)	44.8
10,10′		34.4
11,11′	1.48 m, 1.78 m	17.9
12,12′	0.90 m, 2.04 m	32.8
13,13′		36.7
14,14′		43.0
15,15′	1.68 m, 2.44 m	30.3
16,16′	1.36 m, 1.70 m	36.8
17,17		31.2
18,18′	1.49 brd (7.7)	47.2
19,19′	1.35−1.39[b]	29.2
20,20′		40.4
21,21′	1.35−1.39[b]	29.3
22,22′	1.38, m, 1.82, m	35.5
24,24′	6.46, d (11.4)	141.5
25,25′	0.67, s	11.6
26,26′	0.96, s	24.4
27,27′	0.89, s	25.1
28,28′	1.01, s	31.4
29,29′		185.9
30,30′	1.25, s	33.1
NH	14.0, t (11.4)	

[a] ^{1}H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz. [b] Signals overlapped.

similar to those of **1**. In addition, the IR spectroscopic data of **2** showed absorption bands superposable to those of **1**, suggesting that compound **2** also had ketone carbonyl, carboxyl, and olefin methine functions in its skeleton. A comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of **2** with those of **1** disclosed the absence of the two methyl signals at δ_H 0.98 (CH_3-23) and 1.10 (CH_3-24) of **1** in compound **2** and the presence instead of the olefin methine signal at δ 6.46. These data suggested that the CH_3-23 and -24 methyls were replaced by an N-bearing olefin methine which could be the dimerization site of two multiflorane-type triterpenes.

In order to assign the allocations of all functionalities present in **2**, to confirm the site of dimerization, and to elucidate its planar structure, an HMBC experiment was carried out. ^{2}J and ^{3}J long-range correlations between the hydrogen-bonded secondary amine proton at δ_H 14.0 and two olefin methine and quaternary carbons at δ_C 141.5 (C-24, C-24′) and 114.0 (C-4, C-4′) were observed. The ^{3}J long-range correlation between the two olefin protons at δ 6.46 (H-24, 24′) and their bearing carbons confirmed that the C-24 (24′) of the two units present in **2** were connected with the tertiary amine at δ_H14.0. Moreover, the presence of a ketone carbonyl at C-3 was evidenced by the cross-peaks observed from H-24 (24′) to the carbonyl at δ 200.0 and to the methine at C-5 (C-5′, δ 42.4). The double bond at C-7 (C-8), the carboxyl group at C-20, and the locations of the methyl groups were evidenced by interpretation of the HMBC spectroscopic data. The key correlations observed to support the structure of **2** are shown in Figure S3 (Supporting Information). The molecular formula of **2** required 18 degrees of unsaturation, 9 of which could be assigned to 23-*nor*-multiflora-7(8),4(24)-dien-3-on-29-oic acid. The remaining 9 were thus due to the second occurrence of the same monomer.

The relative configuration of **2** was determined by a NOESY experiment and by X-ray diffraction analysis of a single crystal obtained from a chloroform/methanol solution of **2**. The absolute configuration of **2** was assumed to be the same as the absolute configuration of **1**. The X-ray structure of **2** showing anisotropic displacement ellipsoids at the 50% probability is depicted in Figure 3. The structure of turraenine (**2**) was thus determined to be as shown in Figure 1.

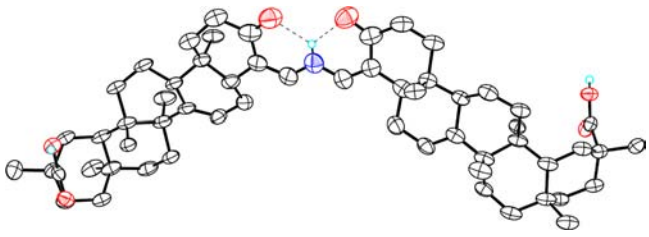

Figure 3. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **2**. Aliphatic H atoms and a methanol solvate are omitted for clarity.

The dimeric structure of turraenine immediately raised the question of whether it might be a simple artifact of the reaction of ammonia with a suitable aldehyde precursor, as observed in a study of acid hydrolysis of some 16-methylamino steroids.[17] This is considered to be highly unlikely for three reasons. In the first place, the required aldehyde precursor, 13-methyl-3,23-dioxo-24,26-bisnorolean-7-en-29-oic acid, is not a known natural product and would be relatively unstable. Second, no ammonia was used in the initial extraction process or in any subsequent purification step. Finally, compound **2** was shown to be present in the crude extract by direct ^{1}H NMR analysis, which clearly showed the presence of the triplet due to the hydrogen-bonded secondary amine (Figure S4, Supporting Information). When this signal was excited, the doublet signal due to the olefin methine (δ_H 6.46, d, J = 11.4) was collapsed.

Compound **2** is the first example known of a naturally occurring N-linked triterpene dimer. The biogenesis of **2** can be plausibly traced from **1**. Successive oxidation and decarboxylation of **1** would lead to a C-23 demethylated compound which could be oxidized to afford C-23 aldehyde **S3**. Schiff base formation with ammonia, followed by tautomerism and reaction with another molecule of aldehyde **S3** and loss of

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water, would give the dimerized compound **2** (Figure S5, Supporting Information).

All of the isolated compounds (**1**–**4**) were evaluated for antimalarial activity against the chloroquine-resistant strain FCM29 of *Plasmodium falciparum*. Turranoic acid (**1**) was the most active (IC_{50} 5.2 μM) among all compounds tested. The activities of triptocallic acid B (**3**) and turraenine (**2**) are comparable (16.4 and 16.6 μM, respectively) while esculentoic acid (**4**) was not active. Among the four compounds isolated, only compound **1** exhibited antiproliferative activity, albeit weak, against the A2780 human ovarian cancer cell line (IC_{50} 20 μM). Compound **1** was inactive against the H522-T1 nonsmall cell lung cancer cell line (IC_{50} > 20 μM) and showed weak activity against the human A2058 melanoma cancer cell line (IC_{50} = 16.4 μM).

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the isolation of compounds **1**–**4**; characterization data for compounds **1** and **2**; key HMBC correlations of **1** and **2** and NOESY correlations of **1**; single-crystal X-ray data for compounds **1** and **2**; ^{1}H NMR and 1D-TOCSY spectra of the crude ethanol extract of *Turraea* sp.; plausible biosynthetic pathway for **2**; ^{1}H, ^{13}C, DEPT 135, HSQC, and HMBC spectra of compounds **1** and **2**; 1D-TOCSY spectrum of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(16) Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 980410 (**1**) and no. CCDC 980561 (**2**). These data can be obtained free of charge on application to Cambridge Crystallographic Data Centre, 2 Union Road, Cambridge CB2 1EZ, UK [fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

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■ NOTE ADDED AFTER ASAP PUBLICATION

In the Supporting Information, Figure S5 was submitted in its uncorrected form. Figure S5 was replaced on April 29, 2014.