

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

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Supporting Information Placeholder

ABSTRACT: The new triterpene turraoic 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 non-small lung cancer cell line H522-T1 with an IC₅₀ 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: turraoic 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. 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 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}

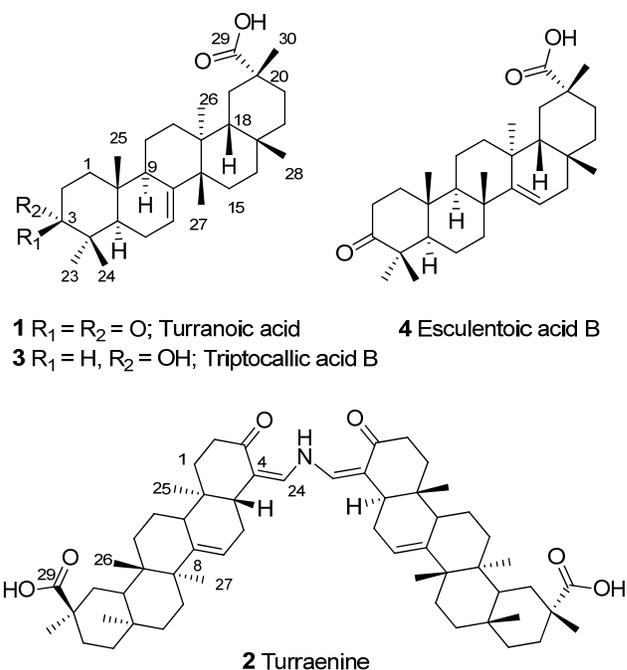


Figure 1. Structures of compounds **1–4**

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 C₃₀H₄₇O₃ (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 ¹H NMR 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 ¹³C NMR data (Table 1)

displayed 30 carbon resonances which were assigned to one ketone carbonyl (δ 217.2), one carboxylic acid (δ 184.5), one olefin methine (δ 117.3), one sp^2 hybridized quaternary carbon (δ 145.6), ten methylenes, three methines, six sp^3 hybridized quaternary carbons and seven methyls by DEPT 135 experiment. 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 *Tripterigium wilfordii* (Celastraceae).¹⁴

Comparison of the ^1H 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-one-7-ene-29-oic acid, named turranic acid.

Compound **2** had the molecular formula $\text{C}_{58}\text{H}_{83}\text{NO}_6$ as determined by positive high resolution ESIMS (observed m/z 890.6311, required for $\text{C}_{58}\text{H}_{84}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 890.6293). Its ^1H NMR spectrum displayed a deshielded triplet resonance due to a hydrogen-bonded amine proton (δ 14.0, t, $J=11.4$ Hz, 1H), two 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 14.0 by 1D TOCSY collapsed the 2H olefin methine signal at δ 6.46, indicating the presence of the partial structure $=\text{CH}-\text{NH}-\text{CH}=\text{in } 2$.

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 multiflorane-type triterpene dimer.

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

position	1 δ_{H} (multiplicity (m), J in Hz)	δ_{C}	3 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 ^1H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz.

^b Signals overlapped.

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

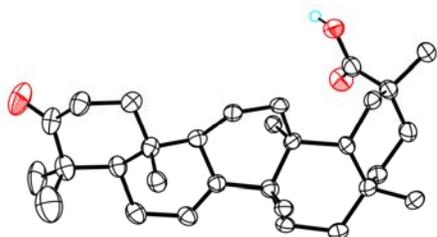


Figure 2. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **1**. One of two crystallographically independent molecules is shown. Aliphatic H-atoms are omitted for clarity.

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. 2J and 3J 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 3J 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_{H} 14.0. 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 S4. The molecular formula of **2** required 18 degrees of unsaturation, 9 of which could be assigned to 23-nor-multiflora-7(8),4(24)-diene-3-one-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.

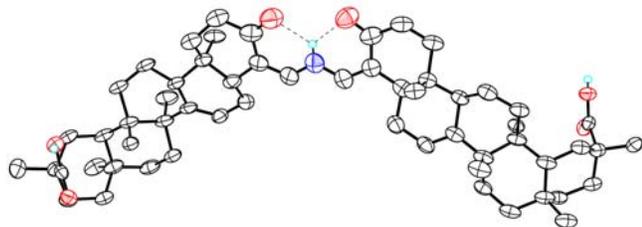


Figure 3. Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **2**. Aliphatic H-atoms and a methanol solvate are omitted for clarity.

The dimeric structure of turraenine immediately raised the question of whether it might be a simple artefact 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

Table 2. ^1H 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 ^1H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz.

^b Signals overlapped

a known natural product and would be relatively unstable. Secondly, 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 ^1H NMR analysis, which clearly showed the presence of the triplet due to the hydrogen-bonded secondary amine (Figure S4). 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 water would give the dimerized compound **2** (Figure S5).

All of the isolated compounds (**1-4**) were evaluated for anti-malarial activity against the chloroquine-resistant strain FCM29 of *Plasmodium falciparum*. Turraoic acid (**1**) was the most active (IC₅₀ 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 (IC₅₀ 20 μM). Compound **1** was inactive against the H522-T1 non-small cell lung cancer cell line (IC₅₀ > 20 μM), and showed weak activity against the human A2058 melanoma cancer cell line (IC₅₀ = 16.4 μM).

ASSOCIATED CONTENT

Supporting Information

Experimental details for the isolation of compounds **1 – 4**; characterization data for compounds **1 – 2**; Key HMBC correlations of **1** and **2** and NOESY correlations of **1**; single-crystal X-ray data for compounds **1** and **2**; ¹H NMR and 1D-TOCSY spectra of the crude ethanol extract of *Turraea* sp.; plausible biosynthetic pathway for **2**; ¹H, ¹³C, 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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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / [†]These authors contributed equally.

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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