# 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 $\mu$M.


Photograph courtesy of Patrice Antilahimena, Missouri Botanical Garden
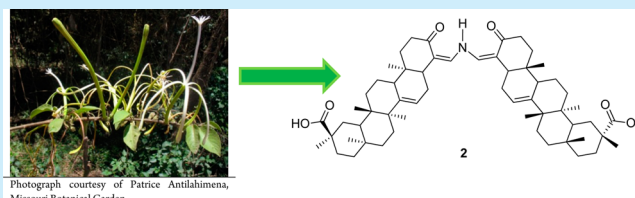
Plants from the genus *Turraea* (family Meliaceae, order Rutales) have been extensively investigated due to their high content of bioactive limonoids[1–7] and triterpenoids such as turrapubesols[8–10] and pregnanes.[8,9,11,12] These compounds have been reported to have a wide range of biological activities 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 $\mu$g/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
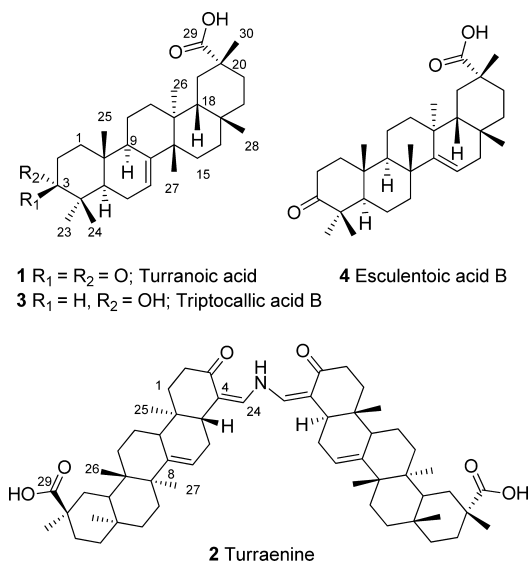

**Figure 1.** Structures of compounds **1**–**4**.

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 ($\delta$ 0.93, 3H; 0.98, 6H; 1.01, 3H; 1.03, 3H; 1.10, 3H; 1.23, 3H) and one olefin methine at $\delta$ 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 ($\delta$ 217.2), one carboxylic acid ($\delta$184.5), one olefin methine ($\delta$ 117.3), one sp$^2$-hybridized quaternary carbon ($\delta$ 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]

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 $\delta$ 217.2 (C-3) instead of the oxygen-bearing methine ($\delta$ 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 $\delta$ 2.25 dt ($J$ = 14.5, 3.7 Hz, H-2a) and $\delta$ 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.

**Table 1. $^1$H NMR Data of Compound 1 and $^{13}$C NMR Data of Compounds 1 and 3 in CDCl$_3$ ($\delta$ in ppm)$^a$**

| | 1 | | 3 |
| position | $\delta_H$ (multiplicity (m), $J$ in Hz) | $\delta_C$ | $\delta_C$ |
|---|---|---|---|
| 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.
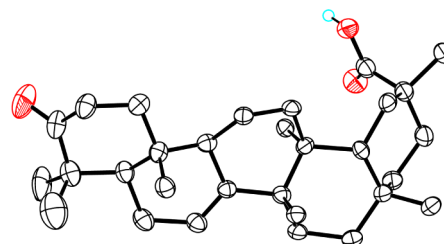


**Figure 2.** Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **1**. One of two crystallographically independent molecules is shown. Aliphatic H atoms are omitted for clarity.

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 ($\delta$14.0, t, $J$ = 11.4 Hz, 1H), two 2H olefin methine signals ($\delta$ 6.46, d, $J$ = 11.4, 2H, H-24 and -24′ and $\delta$ 5.40, brs, 2H, H-7 and -7′), and two sets of signals superposable on those of **1**. Excitation of the proton at $\delta$

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$_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 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.
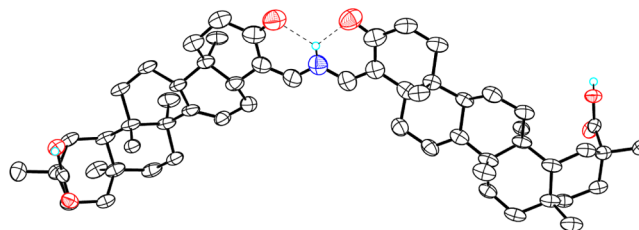


**Figure 3.** Displacement ellipsoid drawing (50% probability) of the single-crystal X-ray structure of **2**. Aliphatic H atoms and a methanol solvate are omitted for clarity.

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

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 $\mu$M) among all compounds tested. The activities of triptocallic acid B (**3**) and turraenine (**2**) are comparable (16.4 and 16.6 $\mu$M, 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 $\mu$M). Compound **1** was inactive against the H522-T1 nonsmall cell lung cancer cell line ($IC_{50}$ > 20 $\mu$M) and showed weak activity against the human A2058 melanoma cancer cell line ($IC_{50}$ = 16.4 $\mu$M).

## ■ 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.

## ■ ACKNOWLEDGMENTS

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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.