A Novel Tirucallane-type Triterpene and Sesquiterpene from *Trichilia maynasiana*

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One new tirucallane-type triterpene {3β, 24-dihydroxytirucallan-7,25-diene, 24-sulfate (**1**)}, one new sesquiterpene {7-*epi*-10-hydroxychabrol-1(2)-en-4,5-dione A (**2**)}, together with three known tirucallanes, and four aromadendranes were isolated from the leaves of *Trichilia maynasiana* C. DC.. Their structures were determined by means of NMR spectroscopy, mass spectrometric analysis, and chemical methods.

Keywords: *Trichilia maynasiana,* Meliaceae, Tirucallane, Aromadendrane, Seco-guaiane.

Species of *Trichilia* (Meliaceae) are found throughout tropical and subtropical regions of Africa and South America [1]. This genus has been the subject of a number of investigations, and various limonoids, tirucallane-type triterpenes, and sesquiterpenes have been identified [2a,b]. Species of the genus are used in the native regions for the treatment of many diseases such as asthma, hepatitis, cirrhosis and dysmenorrhea [3]. As part of an ongoing research program on Meliaceae plants, we performed a phytochemical study of the leaves of *T. maynasiana* C. DC., and we here describe the isolation and characterization of one new tirucallane-type triterpene (**1**) and a new 4,5-seco-guaiane (**2**), along with seven known compounds.

NMR and MS data of compound **1** demonstrated the molecular formula $C_{30}H_{48}O_4S$ (HRESIMS at *m/z* 523, 3440 [M+H]$^+$), which suggested the presence of a sulfate group in the molecule. Acid hydrolysis of **1** followed by treatment with $BaCl_2$, confirmed the presence of a sulfate group. The ESI-MS in positive ion mode showed the [M+H]$^+$ ion peak at *m/z* 523, and fragments by MS^n analysis at *m/z* 443 [M+H-80]$^+$) corresponding to the loss of the sulfate group. This information, along with the ^{13}C NMR spectrum allowed the determination of seven double bond equivalents. Resonances consistent with two double bonds (δ_C 119.0, 146.7, 149.0, and 113.0) were immediately identifiable from the NMR spectroscopic data of **1** (Experimental). The ^{1}H NMR spectrum of **1** demonstrated the presence of five tertiary methyls, a vinyl methyl, a secondary methyl, an oxymethine proton , and three vinyl protons. 1D TOCSY and COSY spectra suggested the presence in the molecule of four spin systems attributable to C-1—C-3, C-5—C-7, C-9—C-12, and C-15—C-24. Direct evidence of the substituent sites were derived from the HSQC and HMBC correlations, which also allowed the assignment of all the resonances in the ^{13}C NMR spectrum. Long-range correlations were observed between Me-18 and C-12, C-14, C-17, between H-17 and C-21, C-16, C-14, and between H-7 and C-9, C-8, C-14, Me-21 and C-17, C-21, C-22, H-24 and C-27, C-26, C-23, Me-27 and C-25, C-24. The α-orientation of H-9, H-5 and Me-30, and the β-orientation of Me-18 and Me-19 were indicated by ROE correlations. These results were confirmed by the chemical shifts of the carbons, which matched well with those of the related tyrucallane [4]. On the basis of these data, compound **1**
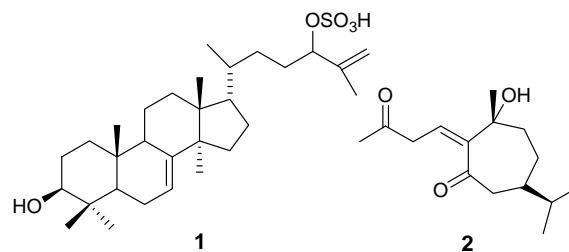

Figure 1: Compounds **1** and **2**.

was characterized as 3β,24-dihydroxy-tirucalla-7,25-diene, 24-sulfate.

The ^{13}C NMR spectrum of compound **2** showed signals for 15 carbons, including two carbonyl groups, one of them α,β unsaturated (δc 146.3, 152.0, 201.1 and 210.6). The HRESIMS of **2** showed a quasi-molecular ion at *m/z* 253.1799 [M+H]$^+$. From this and ^{13}C NMR data a total of eight hydrogen deficiencies were determined, one of which was a ring. The chemical shifts of **2** were obtained from the correlations observed in COSY, HSQC, and HMBC experiments. The HMBC spectrum showed correlations between the methyl signal at δ_H 2.10 and C-4, C-2; the methyl signal at δ_H 1.31 and C-2, C-9: the signal at δ_H 6.47 and C-5, C-4, C-6, C-10; and the signal at δ_H 2.35 and C-12, C-5, C-8, C-11, which locating the α,β unsaturated carbonyl group at C-1,C-2 and C-5, the carbonyl group at C-4, and the carbinol group at C-10. The relative stereochemistry of **2** was determined by 1D ROESY experiments and comparison with literature data [5]. Correlations were observed between H-8β at δ_H 1.90 and Me-14, H-11β, showing that the Me-14 group and isopropyl group at C-7 were in a β position. The structure established for **2** was 7-*epi*-10-hydroxychabrol-1(2)-en-dione.

The seven known compounds were identified as 3β-25-dihydroxytirucalla-7,23-diene [6], lochmolin F [7], butyrospermol [9], masticadienediol [7], 1,7-azulenediol, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl) [8], aromadendrane-4β,10α-diol [9] and spathulenol [9] by detailed NMR and MS analyses and comparison with literature data.

Experimental

General experimental procedures: Optical rotations, Rudolph Research Analytical Autopol IV polarimeter; NMR, Bruker DRX-600 spectrometer at 300 K. HR-ESIMS were acquired on a Q-TOF premier spectrometer (Waters-Milford) [10a,b].

Plant material: The leaves of *T. maynasiana* were collected in Mérida-Venezuela and identified by Ing. Juan Carmona. A voucher specimen number (N° 011) was deposited at the Herbarium MERF.

Extraction and isolation: The dried leaves of *T. maynasiana* (200 g) were powdered and exhaustively extracted using *n*-hexane, CHCl$_3$ and MeOH in an ASE 2000 extractor to yield 4.2, 6.2, 3.0 g of the respective residue. Part of the *n*-hexane (3.5 g) extract was separated using silica gel, eluting with *n*-hexane followed by increasing concentrations of CHCl$_3$ in *n*-hexane (between 1% and 100%) and MeOH in CHCl$_3$ (between 1% and 100%). Fractions of 25 mL were collected, analyzed by TLC and grouped into 11 fractions (A-K). Fraction D (270 mg) was subjected to RP-HPLC with MeOH-H$_2$O (95:5) as eluent to give pure 3β-25-dihydroxytirucalla-7,23-diene (1.2 mg, t_R 20 min). Fraction E (402 mg) was separated by RP-HPLC with MeOH-H$_2$O (9:1) as eluent to give pure spathulenol (1.5 mg, t_R 8 min), **1** (3.0 mg, t_R 28 min) and 3β-25-dihydroxytirucalla-7,23-diene (2 mg, t_R 32 min). Fraction I (84.7 mg) was further separated by RP-HPLC with MeOH-H$_2$O (17:8) as eluent to give aromadendrane-4β,10α-diol (1.0 mg, t_R 15 min). Fraction J (164 mg) was further separated by RP-HPLC with MeOH-H$_2$O (65:35) as eluent to give pure 1,7-azulenediol,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl) (2.9 mg, t_R 50 min).

Part of the CHCl$_3$ extract (5.0 g) was subjected to silica gel column chromatography eluting with CHCl$_3$, followed by increasing concentrations of MeOH in CHCl$_3$ (between 1% and 100%). Fractions of 25 mL were collected, analyzed by TLC and grouped into 13 fractions (Aa-Mm). Fraction Dd (610 mg) was subjected to RP-HPLC with MeOH-H$_2$O (95:5) as eluent to give pure butyrospermol (8.0 mg, t_R 40 min). Fraction Ee (1800 mg) was chromatographed over RP-HPLC with MeOH-H$_2$O (85:15) as eluent to yield pure compound **1** (1.6 mg, t_R 65 min), 3β-25-dihydroxytirucalla-7,23-diene (8.0 mg, t_R 72 min) and masticadienediol (2.2 mg, t_R 90 min). Fraction Hh (296.2 mg) was subjected to RP-HPLC with MeOH-H$_2$O (31:19) as mobile phase to yield compound **2** (1.1 mg, t_R 10 min) and aromadendrane-4β,10α-diol (1.6 mg, t_R 18 min). Fraction Ii (124.8 mg) was further fractionated by RP-HPLC with MeOH-H$_2$O (35:15) as mobile phase to yield lochmolin f (1.2 mg, t_R 11 min) and 1,7-azulenediol,

1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl) (3.2 mg, t_R 30 min).

Analysis of sulfate group: Briefly, a 1 mg aliquot of **2** was refluxed with 10% HCl (4 mL) for 4 h and extracted with Et$_2$O. An aliquot of the aqueous layer was treated with 70% BaCl$_2$ to give a white precipitate (BaSO$_4$).

3β, 24-dihydroxytirucallan-7,25-diene, 24-sulfate (1)

$[\alpha]_D^{25}$: -26.2 (*c* 0.13, MeOH).
^{1}H NMR (600 MHz, CD$_3$OD): 0.80 (3H,s, Me-19), 0.87 (3H,s, Me-18), 0.88 (3H, s, Me-28), 0.92 (1H, d, *J* = 6.0 Hz, H-21), 0.96 (3H, s, Me-29), 1.03 (3H, s, Me-30), 1.12 (1H, m, H-22), 1.18 (1H, m, H-1), 1.33 (2H, m, H-5, H-23), 1.43 (1H, m, H-20), 1.45 (1H, m, H-22), 1.48 (1H, m, H-12), 1.53 (1H, m, H-17), 1.56 (1H, m, H-15), 1.58 (1H, m, H-11), 1.67 (1H, m, H-12), 1.72 (1H, br s, H-27), 1.73 (1H, m, H-1), 1.83 (1H, br d *J*=14.0 , H-15), 1.85 (1H, m, H-2), 1.90 (1H, m, H-2), 1.91 (1H, br d, *J* = 6.0 Hz H-16), 1.95 (1H, br d, *J* = 6.0 Hz H-16), 2.01 (1H, m, H-6), 2.16 (1H, d *J*=8.0, H-6), 2.25 (1H, dd *J*=10.0 , and 5.0, H-9), 3.28 (1H, dd *J*=4.8 and 11.0, H-3), 5.31 (1H, br s, H-7), 4.19 (1H, d *J*=6.0 H-24), 4.90 (1H, d *J*=10.5 H-26), 4.94 (1H, m, H-26).
^{13}C NMR (600 MHz, CD$_3$OD): 13.5 (C-19), 15.6 (C-28), 17.0 (C-27), 18.8 (C-21), 19.4 (C-11), 22.0 (C-18), 25.3 (C-6), 26.8 (C-2), 27.0 (C-30), 28.0 (C-29), 28.0 (C-23), 33.0 (C-22), 35.0 (C-12), 35.2 (C-15), 36.4 (C-10), 37.0 (C-20), 37.8 (C-1), 40.0 (C-4), 45.0 (C-14), 50.0 (C-9), 52.0 (C-5), 52.2 (C-13), 54.0 (C-17), 79.0 (C-1), 90.0 (C-24), 113.0 (C-26), 119.0 (C-7), 146.7 (C-8), 149.0 (C-25),
ESIMS *m/z*: 523 [M + H]$^+$, 443 [(M +H) –80]$^-$,
HRESIMS *m/z*: 523.3440 [M+H]$^+$ (calcd for C$_{30}$H$_{50}$O$_5$S, 522.3379).

7-*epi*-10-hydroxychabrol-1(2)-en-4,5-dione A (2)

$[\alpha]_D^{25}$: +46.7 (*c* 0.11, MeOH).
^{1}H NMR (600 MHz, CD$_3$OD): 0.79 (1H, d, *J* = 6.5 Hz, H-12), 0.88 (1H, d, *J* = 6.5 Hz, H-13), 1.31 (1H, s, H-11), 1.43 (1H, s, H-14), 1.60 (1H, m, H-8), 1.90 (1H, m, H-8), 2.10 (1H, s, H-15), 2.35 (1H, dd, *J* = 6.0, 12.5 Hz, H-6), 2.31 (1H, d, *J* = 12.5 Hz, H-6), 2.38 (1H, m, H-7), 2.47 (1H, m, H-9), 2.60 (1H, m, H-9), 2.08 (1H, m, H-3), 6.47 (1H, m, H-2).
^{13}C NMR (600 MHz, CD$_3$OD): 20.5 (C-13), 20.9 (C-12), 25.6 (C-8), 27.6 (C-14), 29.7 (C-15), 36.3 (C-9), 33.0 (C-11), 37.8 (C-3), 42.3 (C-6), 44.1 (C-7), 69.4 (C-10), 146.3 (C-1), 152.0 (C-2), 201.6 (C-5), 210.1 (C-4).
ESIMS *m/z*: 253 [M + H]$^+$
HRESIMS *m/z*: 253.1795 [M+H]$^+$ (calcd for C$_{15}$H$_{24}$O$_3$, 252,1725).

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