Wortmannilactones A−D, 22-Membered Triene Macrolides from *Talaromyces wortmannii*

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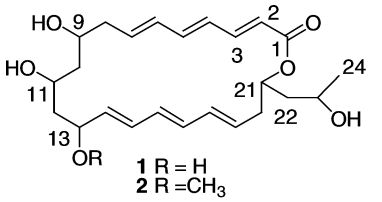
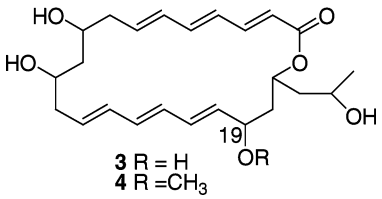
The fungus *Talaromyces wortmannii*, isolated from a soil sample collected in China's Yunnan province, produced four novel 22-membered macrolides, namely, wortmannilactones A−D (**1**−**4**). Structures **1**−**4** were elucidated by extensive 1D and 2D NMR and MS spectral analyses. Compounds **1**−**4** exhibited in vitro cytotoxic activity against several human cancer cell lines with IC_{50} values ranging from 28.7 to 130.5 μM.

Soil filamentous fungi are recognized as a prolific source of biologically active natural products.[1,2] In our program of searching for bioactive substances from soil-derived fungi and evaluating their drug potential, an isolate of the fungus *Talaromyces wortmannii* was obtained from soil collected from Xishuangbanna, Yunnan province, China. HPLC−UV analysis of the crude organic extract of the culture broth indicated the presence of four compounds with characteristic UV absorptions. Follow-up fractionation of the crude extract, guided by analytical HPLC, subsequently yielded four novel 22-membered macrolides, which we designated wortmannilactones A−D (**1**−**4**).





A literature survey revealed that a number of 24- or 22-membered macrolides, such as macrolactins A−M, have previously been reported from a deep-sea bacterium,[3] from a marine *Bacillus* sp. PP 19-H3,[4] from the marine *Bacillus* sp. So26,[5] and from a culture broth of *Actinomadura* sp.[6] Macrolatin A has been reported to show selective antibacterial activity and cytotoxicity against B16-F10 murine melanoma cancer cells (IC_{50} = 3.5 μg/mL) and antiviral activities against *Herpes simplex* type I and type II (IC_{50} = 5.0 and 8.3 μg/mL, respectively) and HIV,[3] as well as squalene synthase inhibitory activity.[7] Swinholide-A,[8] an antifungal compound isolated from a red sea sponge, was another 22-membered macrolide related to compounds **1**−**4**; the dolabelides,[9] 22-membered macrolides from the sea hare *Dolabella auricularia* (Aplysiidae), are cytotoxic.

T. wortmannii was cultured in 20 500 mL Erlemnmeyer flasks, each containing 100 g of solid rice medium at 27 °C for 14 days. The solid culture was extracted with ethyl acetate, and the extract was separated by sequential chromatography using a normal-phase silica gel flash column (eluted with $CHCl_3$/MeOH), Sephadex LH-20 (eluted with MeOH), and reversed-phase HPLC (Phenomenex ODS, 20 × 250 mm, eluted with 35% CH_3CN/H_2O), to afford compounds **1** (62.0 mg), **2** (8.1 mg), **3** (11.2 mg), and **4** (6.1 mg).

Wortmannilactone A (**1**) was obtained as an amorphous solid with molecular formula $C_{24}H_{34}O_6$ by high-resolution FABMS (*m*/*z* 441.2247 $[M + Na]^+$, calcd for $C_{24}H_{34}O_6Na$, Δ 0.8 mmu error), requiring eight degrees of unsaturation. The UV spectrum of **1** in MeOH exhibited absorption bands at 225, 261, and 271 nm, indicating the presence of a triene moiety.[10] The IR spectrum showed hydroxyl (3519 cm^{-1}) and conjugated carbonyl (1695 cm^{-1}) absorptions. ^{13}C NMR and DEPT spectra confirmed the presence of 24 carbons, including a conjugated carbonyl (δ 165.9), 5 oxymethines (δ 62.8, 64.8, 66.3, 68.0, and 70.0), 12 olefinic methines (δ 120.1−145.2), 5 methylenes (δ 37.2, 42.8, 43.3, 47.1, and 47.6), and a methyl group (δ 24.3).^1H NMR (DMSO-d_6) and HMQC spectra of **1** displayed 34 proton signals, including a methyl doublet at δ 1.05 (J = 6.0 Hz), 5 oxymethine protons at δ 3.27, 3.60, 3.85, 4.12, and 4.99, and 12 olefinic protons at δ 5.53−7.01. The protons of **1** connected to their respective carbons were assigned unambiguously via the HMQC spectrum. Four hydroxyl protons that were not attached to carbon atoms were readily discerned, resonating at δ 4.36, 4.47, 4.53, and 4.59.

The planar structure of **1** was established by straightforward analysis of COSY, HMQC, and HMBC correlations. All of the proton signals in **1** were well-resolved, with the exception that the signals due to H-6 and H-15 overlapped. The COSY spectrum suggested three contiguous substructures from C-2 to C-6, from C-7 to C-15, and from C-16 to C-24 (terminal methyl group). Four OH groups attached to C-9, C-11, C-13, and C-23 were firmly assigned on the basis of the COSY correlations observed between OH-9 (δ 4.53)/H-9, OH-11 (δ 4.36)/H-11, OH-13 (δ 4.59)/H-13, and OH-23 (δ 4.47)/H-23, respectively. The HMBC correlations observed from H-3/C-1, H-5/C-7, and H-14/C-16 joined the carbonyl group at C-1 (δ 165.9) and the three substructures, completing the linear structure from C-1 to C-24. The carbonyl and two sets of triene moieties accounted for seven degrees of unsaturation, requiring one additional ring. The relatively downfield chemical shift of the remaining oxymethine proton (H-21) appearing at δ 4.99, as well as the C-21 resonating at δ 70.0, implied that **1** has an ester linkage between C-1 and C-21, which was confirmed by the HMBC correlation from H-21 to C-1. The four hydroxyl groups located at C-9, C-11, C-13, and C-23 were also confirmed by the observed HMBC correlations.

The six double bonds in the two triene moieties were each assigned the *E* geometry on the basis of the well-resolved proton−proton coupling constants (J = 15.0 Hz). However, at this stage the stereochemistry of **1** at the five stereogenic centers (C-9, C-11,

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Table 1. Inhibitory Effects of Wortmannilactones on Cancer Cell Lines (IC_{50}, μM)[a]

compd	HCT-5	HCT-115	A549	MDA-MB-231	K562
1	28.7	35.0	76.5	107.6	65.7
2	32.4	41.7	104.2	95.6	115.7
3	56.3	52.4	118.5	120.4	130.5
4	46.3	44.7	115.7	111.1	122.6

[a] Cisplatin, as the positive control substance, had an IC_{50} value of 13.3 μM against HCT-5 cell line.

C-13, C-21, and C-23) remained unassigned. Thus, the structure of wortmannilactone A was assigned as **1**.

Wortmannilactone B (**2**) had the molecular formula $C_{25}H_{36}O_6$, as determined by HRFABMS, 14 mass units greater than that of **1**, which is consistent with a methoxy derivative of **1**. The UV and ^{1}H and ^{13}C NMR spectra of **2** showed considerable similarities to those of **1**, except that the presence of a methoxy group (δ_H 3.07, δ_C 55.1) was apparent. On the basis of COSY, HMQC, and HMBC analyses, all ^{1}H and ^{13}C NMR signals of **2** were assigned (see the Experimental Section). The only difference was the presence of a methoxy group in **2** rather than the hydroxyl group at C-13 in **1**. The COSY correlations for H-12/H-13/H-14 and HMBC correlation from δ_H 3.07 (OCH_3) to C-13 (δ 78.9) demonstrated the methoxy group to be at C-13. The stereochemistry for **2** was assumed to be the same as that of **1**, on the basis of their close ^{1}H and ^{13}C NMR splitting patterns and chemical shifts.

Wortmannilactone C (**3**) was shown to have the same molecular formula as **1** (HRFABMS). The UV and ^{1}H and ^{13}C NMR data of **3** were similar to those of **1**. A series of 2D NMR experiments (COSY, HMQC, and HMBC) were performed, which allowed assignment of all ^{1}H and ^{13}C signals. Analyses of the COSY and HMBC spectra led to identification of the same substructures in **3** from C-1 to C-12 and from C-20 to C-24 and the 22-membered ester linkage between C-1 and C-21 that were found in **1**. Compound **3** was found to possess a second triene moiety at C-13−C-18 and a hydroxyl group at C-19. The four hydroxyl groups in **3** resonating at δ 4.61, 4.50, 4.68, and 4.38 were assigned to 9-OH, 11-OH, 19-OH, and 23-OH, respectively, on the basis of the COSY correlations of 9-OH/H-9 (δ 3.73), 11-OH/H-11 (δ 3.49), 19-OH/H-19 (δ 3.88), and 23-OH/H-23 (δ 3.54). The cyclic ester functionality was established by HMBC correlation from H-21 (δ 5.14) to C-1 (δ 166.0). The locations of the two triene moieties and four hydroxyls in **3** were also confirmed by the observed HMBC correlations. Hence, the structure of wortmannilactone C was assigned as **3**.

Wortmannilactone D (**4**), $C_{25}H_{36}O_6$, showed ^{1}H and ^{13}C NMR spectra similar to those of **3**. The spectra of **4** differed from those of **3** in that **4** had a methoxy group attached at C-19 instead of a hydroxyl group. In comparison with that of **3**, the C-19 signal (δ 80.8) in **4** shifted downfield 9.9 ppm. Analyses of 2D NMR (COSY, HMQC) data supported this proposal, and the HMBC correlation between δ 3.06 (OCH_3) and C-19 confirmed the methoxy group at C-19. Compound **4** was assumed to have the same stereochemistry as **3** on the basis of their close ^{1}H and ^{13}C NMR splitting patterns and chemical shifts.

Attempts to obtain single crystals of **1**−**4** in several solvents have been unsuccessful. Many macrolides whose stereostructures have been determined have been elucidated by chemical degradation and total synthesis, because of their noncrystalline properties.[11,12]

Compounds **1**−**4** were subsequently screened for cytotoxic activity against a panel of human cancer cell lines (HCT-5, HCT-115, A549, MDA-MB-231, and K562). The IC_{50} values of compounds **1**−**4** range from 28.7 to 130.5 μM (Table 1).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were measured with a Nicolet Magna-IR 550 instrument. UV spectra were recorded with a Pharmacia Ultrospec 2100 Pro instrument. Mass spectra were obtained using a Waters LCMS ZQ 2000 (ESI mode) or Bruker Daltonics Apex II mass spectrometer (FAB mode). 1D and 2D NMR spectra were measured on a Varian Inova-500 spectrometer using standard Varian pulse sequences. HPLC was performed using a Waters HPLC system equipped with a photodiode array (PDA) detector.

Fungal Material. The fungus was isolated from a soil sample collected in Xishuangbanna of Yunnan Province, China. The strain was identified as *Talaromyces wortmannii* according to Pitt's description[13] on the basis of standard biological and physiological tests and taxonomical determination by one of the authors (M.L.). The strain was deposited in the China General Microbiological Culture Collection with accession number CGMCC No. 1224.

Fermentation and Isolation of Wortmannilactones. Fermentation was a two-stage process. A stock culture of *T. wortmannii* was inoculated into a 500 mL Erlenmeyer flask containing 80 mL of seed medium consisting of 2.0% starch, 1.0% glucose, 0.2% soybean meal, 0.6% malt extract, 0.3%yeast extracts, 0.2% NaCl, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.2% $CaCO_3$ (pH 7.0 before sterilization). The flask was incubated on a rotary shaker at 27 °C, at 220 rpm, for 72 h. The seed culture (4 mL) was used to inoculate 100 g of solid medium consisting of 97.5% rice and 2.5% soybean meal in a 500 mL Erlenmeyer flask (×20). The flasks were still-cultured for 14 days at 27 °C.

The solid culture (2 kg) was then extracted with ethyl acetate (2.0 L). The ethyl acetate layer was evaporated under reduced pressure to yield a residue (40 g). The residue was applied on a silica gel flash column and eluted with $CHCl_3$/MeOH ((10:0)−(0:10)) to give 20 fractions, each of which was analyzed by HPLC−UV. Fractions 5−8, containing compounds **1** and **3**, were combined and chromatographed on a Sephadex LH-20 column with MeOH as eluent. Further separation was achieved via preparative RP-HPLC to afford **1** (62.0 mg) and **3** (11.2 mg). Fractions 10−12 containing compounds **2** and **4** were combined and similarly separated by Sephadex LH-20 chromatography and preparative RP-HPLC to yield compounds **2** (8.1 mg) and **4** (6.1 mg). Compounds **1**−**4** were eluted from a preparative RP-HPLC column at 15.4, 20.5, 17.8, and 22.3 min, respectively. Analytical HPLC was conducted using a Chromasil ODS column (4.6 × 250 mm, 10 μm), with 30% CH_3CN/H_2O as eluent at a flow rate of 0.8 mL/min and UV detection at 271 nm. Preparative RP-HPLC was conducted using a Phenomenex ODS column (20 × 250 mm, 10 μm), with 35% CH_3CN/H_2O as eluent at a flow rate of 6 mL/min and UV detection at 271 nm.

Cytotoxicity Testing. The cytotoxicity assays were carried out in triplicate against cancer cell lines HCT-5, HCT-115 (colon cancer), A549 (lung cancer), MDA-MB-231 (breast cancer), and K562 (leucocythemia), according to a published method.[14] The IC_{50} value was defined as the concentration necessary to inhibit the cell growth to 50% of the control. Cisplatin was used as a positive control substance.

Wortmannilactone A (1): amorphous powder; $[\alpha]_D^{20} = -52.0$ (c = 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.80), 261 (4.91), 271 nm (4.95); IR (KBr) ν_{max} 3519, 1696 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) 7.01 (1H, dd, J = 15.0, 11.0 Hz, H-3), 6.38 (1H, dd, J = 15.0, 11.0 Hz, H-5), 6.23 (1H, dd, J = 15.0, 11.0 Hz, H-4), 6.12 (2H, m, H-6, 15), 6.10 (1H, m, H-17), 5.99 (1H, dd, J = 15.0, 11.0 Hz, H-16), 5.92 (1H, dd, J = 15.0, 11.0 Hz, H-18), 5.88 (1H, ddd, J = 15.0, 11.0, 5.0 Hz, H-7), 5.73 (1H, d, J = 15.0 Hz, H-2), 5.59 (1H, dt, J = 15.0, 7.5 Hz, H-19), 5.53 (1H, dd, J = 15.0, 7.5 Hz, H-14), 4.99 (1H, m, H-21), 4.59 (1H, d, J = 4.5 Hz, 13-OH), 4.53 (1H, d, J = 6.0 Hz, 9-OH), 4.47 (1H, d, J = 4.5 Hz, 23-OH), 4.36 (1H, d, J = 5.0 Hz, 11-OH), 4.12 (1H, m, H-13), 3.85 (1H, m, H-9), 3.60 (1H, m, H-23), 3.27 (1H, m, H-11), 2.55 (1H, m, H-8β), 2.42 (1H, m, H-20β), 2.19 (1H, m, H-20α), 2.06 (1H, m, H-8α), 1.68 (1H, m, H-22β), 1.58 (1H, m, H-12β), 1.56 (1H, m, H-22α), 1.51 (1H, m, H-10β), 1.34 (1H, m, H-10α), 1.18 (1H, m, H-12α), 1.05 (3H, d, J = 6.0 Hz, H-24); ^{13}C NMR (125 MHz, DMSO-d_6) 165.9 (Cq-1), 145.2 (CH-3), 140.9 (CH-5), 137.6 (CH-14), 136.7 (CH-7), 135.7 (CH-18), 132.1 (CH-16), 130.7 (CH-6), 130.5 (CH-17), 129.1 (CH-15), 127.8 (CH-4), 127.2 (CH-19), 120.1 (CH-2), 70.0 (CH-21), 68.0 (CH-13), 66.3 (CH-9), 64.8 (CH-11), 62.8 (CH-23), 47.6 (CH_2-10), 47.1 (CH_2-12), 43.3 (CH_2-8), 42.8 (CH_2-22), 37.2 (CH_2-20), 24.3(CH_3-24); ESIMS (+) m/z 441 [M + Na]$^+$; ESIMS (−) m/z 417 [M − H]$^-$; HRFABMS (+) m/z 441.2247 [M + Na]$^+$ (calcd 441.2239 for $C_{24}H_{34}O_6Na$).

Wortmannilactone B (2): amorphous powder; $[\alpha]_D^{20} = -35.0$ (c = 0.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.83), 261 (4.92), 271 nm (4.98); IR (KBr) ν_{max} 3520, 1696 cm^{-1}; ^{1}H NMR (500 MHz,

DMSO-d_6) 6.99 (1H, dd, J = 15.0, 11.0 Hz, H-3), 6.24 (3H, m, H-4, 5, 16), 6.20 (1H, m, H-15), 6.15 (1H, m, H-17), 5.95 (1H, dd, J = 15.0, 11.0 Hz, H-6), 5.85 (2H, m, H-7, 18), 5.74 (1H, d, J = 15.0 Hz, H-2), 5.70 (1H, dd J = 15.0, 11.0 Hz, H-19), 5.33 (1H, dd, J = 15.0, 7.5 Hz, H-14), 5.01 (1H, m, H-21), 4.55 (1H, d, J = 4.5 Hz, 9-OH), 4.53 (1H, d, J = 5.0 Hz, 23-OH), 4.36 (1H, d, J = 5.0 Hz, 11-OH), 3.89 (1H, m, H-9), 3.71 (1H, m, H-13), 3.60 (1H, m, H-23), 3.11 (1H, m, H-11), 3.07 (3H, s, 13-OCH_3), 2.55 (1H, m, H-8β), 2.45 (1H, m, H-20β), 2.25 (1H, m, H-20α), 2.10 (1H, m, H-8α), 1.65 (1H, m, H-22β), 1.56 (2H, m, H-12β, 22α), 1.50 (1H, m, H-10β), 1.30 (1H, m, H-10α), 1.10 (1H, m, H-12α), 1.09 (3H, d, J = 6.0 Hz, H-24); ^{13}C NMR (125 MHz, DMSO-d_6) 165.7 (C_q-1), 145.5 (CH-3), 141.1 (CH-5), 137.0 (CH-7), 135.4 (CH-18), 133.3 (CH-14), 133.0 (CH-15), 131.7 (CH-16), 131.1 (CH-6), 130.2 (CH-17), 127.8 (CH-19), 127.5 (CH-4), 120.0 (CH-2), 78.9 (CH-13), 69.8 (CH-21), 65.9 (CH-9), 64.0 (CH-11), 62.7 (CH-23), 55.1 (13-OCH_3), 48.0 (CH_2-10), 44.6 (CH_2-12), 43.4 (CH_2-8), 42.1 (CH_2-22), 36.6 (CH_2-20), 24.4 (CH_3-24); ESIMS (+) m/z 455 [M + Na]$^+$; ESIMS (−) m/z 431 [M − H]$^-$; HRFABMS (+) m/z 455.2418 [M + Na]$^+$ (calcd 455.2410 for $C_{25}H_{36}O_6Na$).

Wortmannilactone C (3): amorphous powder; $[\alpha]_D^{20} = -9.2$ (c = 0.85, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.78), 261 (4.88), 271 nm (4.91). IR (KBr) ν_{max} 3520, 1711 cm^{-1}; ^{1}H (500 MHz, DMSO-d_6) 7.06 (1H, dd, J = 15.0, 11.0 Hz, H-3), 6.55 (1H, dd, J = 15.0, 11.0 Hz, H-5), 6.13 (1H, dd, J = 15.0, 11.0 Hz, H-4), 6.05 (1H, dd, J = 15.0, 11.0 Hz, H-6), 5.99 (2H, m, H-15, 16), 5.92 (1H, dd, J = 15.0, 11.0 Hz, H-14), 5.88 (1H, m, H-7), 5.78 (1H, dd, J = 15.0, 11.0 Hz, H-17), 5.62(1H, d, J = 15.0 Hz, H-2), 5.47 (1H, m, H-18), 5.43 (1H, m, H-13), 5.14 (1H, m, H-21), 4.68 (1H, d, J = 4.0 Hz, 19-OH), 4.61 (1H, d, J = 4.0 Hz, 9-OH), 4.50 (1H, d, J = 4.5 Hz, 11-OH), 4.38 (1H, d, J = 5.0 Hz, 23-OH), 3.88 (1H, m, H-19), 3.73 (1H, m, H-9), 3.54 (1H, m, H-23), 3.49 (1H, m, H-11), 2.33 (1H, m, H-8β), 2.21 (2H, m, H-8α, 12β), 2.07 (1H, m, H-12α), 1.85 (1H, m, H-20β), 1.75 (1H, m, H-20α), 1.54 (3H, m, H-10β, 22), 1.21 (1H, m, H-10α), 1.03 (3H, d, J = 6.0 Hz, H-24); ^{13}C NMR (125 MHz, DMSO-d_6) 166.0 (C_q-1), 144.7 (CH-3), 140.4 (CH-5), 137.4 (CH-18), 135.3 (CH-7), 132.9 (CH-6), 132.7 (CH-14), 132.3 (CH-15), 130.3 (CH-16), 130.2 (CH-13), 129.9 (CH-17), 128.3 (CH-4), 120.3 (CH-2), 70.9 (CH-19), 69.6(CH-21), 67.3 (CH-11), 66.5 (CH-9), 62.7 (CH-23), 44.8 (CH_2-22), 43.4 (CH_2-20), 40.5 (CH_2-12), 40.4 (CH_2-10), 39.5 (CH_2-8), 24.1 (CH_3-24); ESIMS (+) m/z 441 [M + Na]$^+$, ESIMS (−) m/z 417 [M − H]$^-$; HRFABMS (+) m/z 419.2442 [M + H]$^+$ (calcd 419.2439 for $C_{24}H_{35}O_6$).

Wortmannilactone D (4): amorphous powder; $[\alpha]_D^{20} = -3.2$ (c = 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.79), 261 (4.89), 271 nm (4.93); IR (KBr) ν_{max} 3519, 1713 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) 7.04 (1H, dd, J = 15.0, 11.0 Hz, H-3), 6.55 (1H, dd, J = 15.0, 11.0 Hz, H-5), 6.12 (1H, dd, J = 15.0, 11.0 Hz, H-4), 6.05 (1H, m, H-6), 5.95 (2H, m, H-14, 15), 5.84 (3H, m, H-7, 16, 17), 5.61(1H, d, J = 15.0 Hz, H-2), 5.45 (1H, m, H-13), 5.31 (1H, dd, J = 15.0, 9.0 Hz, H-18), 5.14 (1H, m, H-21), 4.62 (1H, d, J = 4.5 Hz, 9-OH), 4.51 (1H, d, J = 5.0 Hz, 11-OH), 4.40 (1H, d, J = 5.0 Hz, 23-OH), 3.72 (1H, m, H-9), 3.56 (1H, m, H-23), 3.50 (1H, m, H-11), 3.47 (1H, m, H-19), 3.06 (3H, s, 19-OCH_3), 2.32 (1H, m, H-8β), 2.21 (1H, m, H-8α), 2.19 (1H, m, H-12β), 2.08 (1H, m, H-12α), 1.95 (1H, m, H-20β), 1.85 (1H, m, H-20α), 1.54 (3H, m, H-10β, 22), 1.1 (1H, m, H-10α), 1.03 (3H, d, J = 6.0 Hz, H-24); ^{13}C NMR (125 MHz, DMSO-d_6) 165.9 (C_q-1), 144.9 (CH-3), 140.6 (CH-5), 135.5 (CH-7), 133.7 (CH-14), 133.3 (CH-16), 133.0 (CH-18), 132.9 (CH-6), 132.1 (CH-15), 130.9 (CH-13), 129.6 (CH-17), 128.3 (CH-4), 120.2 (CH-2), 80.8 (CH-19), 69.8(CH-21), 67.1 (CH-11), 66.4 (CH-9), 62.7 (CH-23), 55.5 (19-OCH_3), 44.7 (CH_2-22), 41.4 (CH_2-20), 40.2 (CH_2-10), 39.8 (CH_2-12), 39.5 (CH_2-8), 24.2 (CH_3-24); ESIMS (+) m/z 455 [M + Na]$^+$; ESIMS (−) m/z 431 [M − H]$^-$; HRFABMS (+) m/z 455.2420 [M + Na]$^+$ (calcd 455.2410 for $C_{25}H_{36}O_6Na$).

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