Meroterpenoid MAPKAP (MK2) Inhibitors Isolated from the Indonesian Marine Sponge *Acanthodendrilla* sp.

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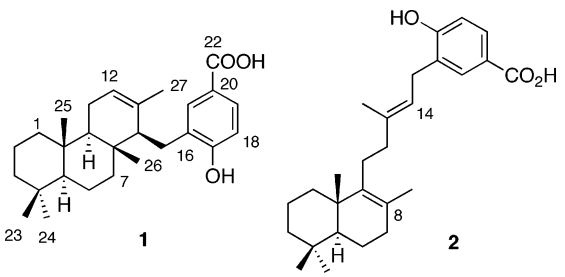
(+)-Makassaric acid (**1**) and (+)-subersic acid (**2**), new meroterpenoid inhibitors of the protein kinase MK2, have been isolated from the marine sponge *Acanthodendrilla* sp. collected in Indonesia. The structures of (+)-makassaric acid (**1**) and (+)-subersic acid (**2**) were determined by spectroscopic analysis.

MAPKAP kinase 2 (MK2) has been shown to be critical in the regulation of TNF-α production.[1] TNF-α is a pleiotropic cytokine involved in various inflammatory processes, and therefore, MK2 kinase inhibitors represent potential therapeutic agents to treat inflammatory diseases such as rheumatoid arthritis, where TNF-α plays a key role. A screen of marine sponge crude extracts for MK2 inhibitors revealed that the MeOH extract from the Indonesian sponge *Acanthodendrilla* sp. had significant activity. Fractionation of the *Acanthodendrilla* sp. extract led to the isolation of (+)-makassaric acid (**1**) and (+)-subersic acid (**2**), two new meroterpenoid MK2 inhibitors, whose structures are described below.

Acanthodendrilla sp. (order Dendroceratida, family Dictyodendrillidae) was harvested by hand using scuba on reefs off Palau Badi near Makassar, Indonesia. Freshly collected sponge was repeatedly extracted with EtOH. The EtOH extracts were combined, concentrated in vacuo, and then partitioned between H_2O and EtOAc. Repeated fractionation of the EtOAc-soluble materials via Sephadex LH-20 and flash silica gel column chromatography, followed by reversed-phase HPLC, gave pure samples of the new meroterpenoids (+)-makassaric acid (**1**) and (+)-subersic acid (**2**).



Subersic acid isolated from *Acanthodendrilla* sp. in the present study had NMR and MS data matching that reported in the literature for (−)-subersic acid.[2] The optical rotation of this sample was similar in magnitude to the literature value for (−)-subersic acid, but the sign of rotation was opposite ($[\alpha]^{25}_D$ +39°) of that reported ($[\alpha]^{25}_D$ −46°).[2] Therefore, (+)-subersic acid (**2**) isolated from *Acanthodendrilla* sp. is the enantiomer of the (−)-subersic acid isolated from *Jaspis splendens*, which was assigned the 5*R*,10*R* absolute stereochemistry.

(+)-Makassaric acid (**1**) was obtained as an optically active pale yellow oil that gave a $[M - H]^-$ ion in the HRESIMS at *m/z* 409.2748, appropriate for a molecular formula of $C_{27}H_{38}O_3$, requiring nine sites of unsaturation. The ^{13}C/HMQC NMR data obtained for **1** contained resonances that could be assigned to 27 carbon atoms, consistent with the HRMS data. Since the HMQC data showed that only 36 of the hydrogen atoms were attached to carbon (5 × CH_3, 7 × CH_2, 7 × CH, 8 × C), there had to be two exchangeable hydrogens attached to oxygen. Preliminary inspection of the ^{1}H and ^{13}C NMR spectra (Table 1) indicated that the structure of **1** had both benzenoid and terpenoid fragments. Resonances that could be assigned to a trisubstitued benzene ring (δ 114.6 (C-18), 121.2 (C-20), 128.3 (C-19), 129.4 (C-16), 130.6 (C-21), 159.0 (C-17)), a trisubstituted olefin (δ 121.8 (C-12) and 134.6 (C-13)), and a carboxylic acid or ester (δ 167.5 (C-22)) were observed in the ^{13}C NMR spectrum, accounting for six of the nine required sites of unsaturation. Therefore, makassaric acid had to contain three additional rings.

COSY data readily established a 1, 2, 4 substitution pattern on the aromatic ring (δ 6.82, bd, J = 8.1 Hz (H-18); 7.58, bd, J = 8.1 Hz (H-19); 7.74, bs (H-21)), and HMBC correlations situated methylene, hydroxyl, and carboxylic acid functionalities at C-16 (δ 25.1 (C-15) correlated to 7.74 (H-21)), C-17 (δ 159.0 (C-17) correlated to 2.47 (H-15), 2.62 (H-15), 6.82 (H-18), 7.58 (H-19), 7.74 (H-21)), and C-20 (δ 167.5 (C-22) correlated to 7.58 (H-19), 7.74 (H-21)), respectively (see Table 1). An olefinic methyl (δ 1.35 (bs)/21.8 (C-27)) and four aliphatic methyls (δ 0.79 (s)/21.5 (C-23); 0.81 (s)/14.5 (C-26); 0.85 (s)/33.2 (C-24); 0.87 (s)/15.3 (C-25)) could be readily identified from the ^{1}H and ^{13}C NMR data. The presence of five methyl appendages and 20 carbons not accounted for by the benzoic acid fragment indicated that the terpenoid portion of (+)-makassaric acid (**1**) consisted of a tricyclic diterpene, which incorporated a trisubstituted olefin and four aliphatic methyls. These required structural features are similar to the structural components of the co-occurring (+)-subersic acid (**2**),[2] except that an additional ring in **1** had to replace one of the olefins in **2**. Cyclization between the olefinic carbons C-8 and C-14 in **2** and the concomitant formation of a $\Delta^{12,13}$ olefin results

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Table 1. NMR Data for (+)-Makassaric Acid (**1**) Recorded in DMSO-d_6

atom #	δ_H	δ_C	HMBC[a]
1	0.75 m	39.1	H-2, H-3_{eq}, H-25
1′	1.57 m		
2	1.33−1.35	18.1	H-1′
3_{ax}	1.11 m	41.3	H-1′, H-23, H-24
3_{eq}	1.33		
4		32.8	H-2, H-3_{eq}, H-23, H-24
5	0.86	55.4	H-1′, H-3_{eq}, H-7_{eq}, H-23, H-24
6	1.51 m	18.4	H-7_{ax}, H-7_{eq}
7_{ax}	1.19 m	40.4	H-6, H-26
7_{eq}	1.98 dm, J = 12.6 Hz		
8		36.5	H-6, H-7_{eq}, H-11, H-14, H-15, H-26
9	1.21	54.6	H-7_{eq}, H-12, H-25, H-26
10		36.8	H-1′, H-25
11	1.86 m	22.2	H-9
12	5.31 m	121.8	H-11, H-27
13		134.6	H-11, H-14, H-15, H-15′, H-27
14	2.32 m	54.0	H-12, H-15, H-15′, H-26, H-27
15	2.47	25.1	H-21
15′	2.62 dd, J = 15.0, 9.6 Hz		
16		129.4	H-14, H-15, H-15′, H-18
17		159.0	H-15, H-15′, H-18, H-19, H-21
18	6.82 d, J = 8.1 Hz	114.6	
19	7.58 d, J = 8.1 Hz	128.3	H-21
20		121.2	H-18
21	7.74 bs	130.6	H-15, H-15′, H-19
22		167.5	H-19, H-21
23	0.79 s	21.5	H-24
24	0.85 s	33.2	H-23
25	0.87 s	15.3	H-9
26	0.81 s	14.5	H-7_{ax}, H-9
27	1.35 bs	21.8	H-12

[a] Chemical shifts of proton resonances correlated to the carbon resonance listed in the δ ^{13}C column. Experiments optimized for both 2 and 8 Hz.

in an additional cyclohexene ring that satisfies all the structural requirements of **1**.

The NMR data obtained for (+)-makassaric acid (**1**) confirmed this structural assignment. COSY correlations established the spin system from H-9 (δ 1.21) to the methylene protons at C-15 (δ 2.47 and 2.62). HMBC correlations from the H-15 protons (δ 2.47 and 2.62) to the three aromatic carbons C-16 (δ 129.4), C-17 (159.0), and C-21 (130.6) and the aliphatic carbons at δ 36.5 (C-8), 54.0 (C-14), and 134.6 (C-13) linked the C-9/C-15 fragment to the trisubstitued benzene ring through a single bond between C-15 and C-16. The presence of ring C was further supported by HMBC correlations between C-14 (δ 54.0) and H-12 (δ 5.31), H-27 (1.35), and H-26 (0.81); between C-8 (δ 36.5) and H-6 (δ 1.51), H-7_{eq} (1.98), H-11 (1.86), and H-14 (2.32); and between C-9 (δ 54.6) and H-26 (δ 0.81), H-7_{eq} (1.98), H-12 (δ 5.31), and H-25 (0.87). 2D NMR data also routinely confirmed the structure of the A and B rings in makassaric acid (**1**) as shown (see Table 1).

The Me-23, Me-24, Me-25, and Me-26 resonances in the ^{1}H NMR spectrum of (+)-makassaric acid (**1**) were poorly resolved, preventing a straightforward analysis of the relative stereochemistry of the ABC ring system from ROESY and 1D NOESY data. However, the chemical shifts of the ring carbons C-1 to C-6 and the methyl carbons C-23 and C-24 observed for **1** (δ 39.1 (C-1), 18.1 (C-2), 41.3 (C-3), 32.8 (C-4), 55.4 (C-5), 18.4 (C-6), 21.5 (C-23), 33.2 (C-24), 15.3 (C-25)) are close to those observed for (+)-subersic acid (**2**) (recorded in DMSO-d_6: δ 38.5 (C-1), 18.5 (C-2), 41.3 (C-3), 32.9 (C-4), 51.4 (C-5), 18.6 (C-6), 21.5 (C-23), 33.1 (C-24), 15.8 (C-25)), indicating that the relative stereochemistry for the AB ring junction of **1** is trans as in **2**.[2] 1D NOESY correlations observed between H-14 (δ 2.32) and both H-7_{ax} (1.19) and H-9 (1.21) and between H-25 (δ 0.87) and both H-6_{ax} (1.51) and H-11_{ax} (1.86) are only possible if the B/C ring junction is also trans, H-14 is axial, and the B and C rings both adopt chair conformations. This is supported by the observation of a weak 1D NOESY correlation between the axial ring junction proton H-5 (δ 0.86) and H-7_{ax} (1.19). Assuming that (+)-makassaric acid belongs to the same enantiomeric series as the co-occurring (+)-subersic acid (**2**), the absolute stereochemistry of **1** is 5*S*, 8*S*, 9*R*, 10*S*, 14*R*.

(+)-Makassaric acid (**1**) and (+)-subersic acid (**2**) inhibited MK2 with IC_{50}'s of 20 and 9.6 μM, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 polarimeter with sodium light (589 nm). UV spectra were recorded with a Waters 2487 dual λ absorbance detector. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 spectrometer. ^{1}H chemical shifts are referenced to the residual DMSO-d_6 signal (δ 2.49 ppm), and ^{13}C chemical shifts are referenced to the DMSO-d_6 solvent peak (δ 43.95 ppm). Low- and high-resolution ESI-QIT-MS were recorded on a Bruker-Hewlett-Packard 1100 Esquire-LC mass spectrometer. Merck type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography. A Waters 2 g Sep-Pak silica cartridge was used for normal-phase flash chromatography. Reversed-phase HPLC purifications were performed on a Waters 600E system controller liquid chromatograph attached to a Waters 996 photodiode array detector. All solvents used for HPLC were Fisher HPLC grade.

Materials and Methods for the MK2 Kinase Assay. MAPKAPK2 kinase activity was assessed using human recombinant MK2 containing residues 41 through 353 in an ELISA-based assay. The kinase reaction was performed on 96-well streptavidin-coated plates using a biotinylated 13-mer peptide derived from LSP1 in 20 mM Hepes pH 7.4, 10 mM $MgCl_2$, 3 mM DTT, and 1 μM ATP. The reaction was stopped after 30 min incubation at room temperature and washed in PBS 0.05% Tween 20. Polyclonal anti phospho-LSP1 antibodies were then added to the plate along with goat anti-rabbit labeled with europium in 20 mM MOPS, 150 mM NaCl, 0.025% Tween 20, 0.02% gelatin, and 1% BSA for 1 h at room temperature. The plate was then washed in PBS 0.05% Tween 20, and enhancement solution from Perkin-Elmer was added before counting on a Victor 2 reader from Perkin-Elmer.

Sponge Material. Specimens of *Acanthodendrilla* sp. (close to *A. australis*, Berquist 1995) were collected by hand using scuba at a depth of 10−15 m near Palau Badi, Makassar, Sulawesi, Indonesia, in October 1996. A voucher sample has been deposited at the Zoological Museum of Amsterdam (POR ZMA 17430).

Isolation of (+)-Makassaric Acid (1) and (+)-Subersic Acid (2). Freshly collected sponge (79 g) was cut into small pieces and extracted repeatedly with EtOH (3 × 300 mL). The combined EtOH extracts were concentrated in vacuo and the resultant oil was shipped to Vancouver, where it was partitioned between EtOAc (3 × 15 mL) and H_2O (45 mL). The combined EtOAc layers were evaporated to dryness, and the resulting oil was chromatographed on Sephadex LH-20 in 4:1 MeOH/CH_2Cl_2 to give late eluting MAPKAP kinase-2 active fractions. These active fractions were combined and further fractionated using silica gel flash chromatography employing a step gradient from 19:1 hexanes/EtOAc to EtOAc. A fraction eluting with 3:1 hexanes/EtOAc was active. Pure samples of (+)-makassaric acid (**1**) (2.3 mg) and (+)-subersic acid (**2**) (4.3 mg) were obtained from this mixture via C_{18} reversed-phase

HPLC using a CSC-Inertsil 150A/ODS2, 5 μm, 25 × 0.94 cm column, with 7:3 MeCN/H_2O as eluent.

(+)-Makassaric acid (1): pale yellow oil; $[\alpha]^{25}_D$ +7.3° (*c* 5.4, MeOH); UV (MeOH) λ_{max} 217 (ϵ 9711), 254 (ϵ 8715) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; negative ion HRESIMS $[M - H]^-$ *m/z* 409.2748 (calcd for $C_{27}H_{37}O_3$, 409.2743).

(+)-Subersic acid (2): $[\alpha]^{25}_D$ +39.3° (*c* 3.26, MeOH); ^{1}H and ^{13}C NMR data recorded in $CDCl_3$ were identical to that reported in the literature for (−)-subersic acid;[2] HRESMS negative ion $[M - H]^-$ *m/z* 409.2740 (calcd for $C_{25}H_{37}O_3$, 409.2743).

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References and Notes

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