Furanoterpene fatty acid esters from the Australian marine sponge *Coscinoderma mathewsi*

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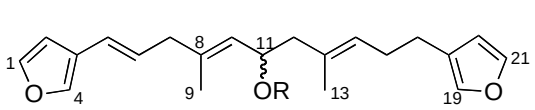
Dedicated to Professor Torbjorn Norin

Abstract
Chemical investigation of the sponge *Coscinoderma mathewsi* collected near Mooloolaba, S.E. Queensland, has led to the isolation of the furanoterpene esters **1a** – **1c** together with the known furanoterpenes **2** – **4**. NMR spectroscopic assignments are provided for the esters, while GC/MS analysis of FAME derivatives **5a** – **5c** confirmed the identity of the fatty acid components.

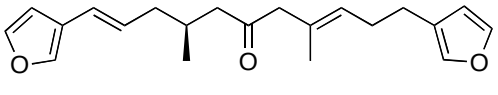
Keywords: Sesterterpenoid, fatty acid, FAMES, NMR, sponges, *Coscinoderma*

Introduction

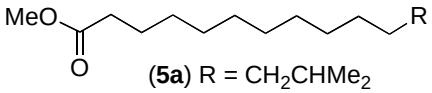
Lipid-containing metabolites have been found in both marine microorganisms and in marine invertebrates such as sponges.[1,2] Fatty acid amide examples include the malyngamide metabolites found in the extracts of cyanobacteria such as *Lyngbya* spp,[3,4] while the fatty acid ester derivatives of variabilin,[5] bengazoles,[6,7] bengamides[7,8] and sterols[9] are typical examples from marine sponges. An ongoing screening program directed at identifying furanoterpenes in sponges collected in S.E. Queensland waters indicated the dictyoceratid sponge *Coscinoderma mathewsi* as showing evidence of both β-substituted furanoterpene and lipid metabolites. A detailed chemical study of this sponge has now led to the isolation of the furan fatty acid esters (**1a**) – (**1c**), together with the known terpenes (**2**) – (**4**). This paper reports structural studies on the isolated metabolites, together with a FAME analysis of derivatives (**5a**) – (**5c**) that identifies the lipid side chains (Figure 1).



(**1a**) R = $CO(CH_2)_{11}CHMe_2$
(**1b**) R = $CO(CH_2)_{10}CHMeCH_2Me$
(**1c**) R = $CO(CH_2)_{13}Me$
(**2**) R = H; 11*R*
(**3**) R = Ac



(**4**)



(**5a**) R = CH_2CHMe_2
(**5b**) R = $CHMeCH_2Me$
(**5c**) R = $(CH_2)_3Me$

Figure 1. Structures of furanoterpenes isolated from the marine sponge *Coscinoderma mathewsi* and of some FAME derivatives.

Result and Discussion

Extraction of the sponge sample with DCM/MeOH 1:1 gave a dark-brown extract that was fractionated by silica-gel flash chromatography (hexane/DCM => DCM => MeOH), followed by reverse phase HPLC using a CH_3CN:H_2O gradient to give (+)-tetradehydrofurospongin-1 (**2**), its acetyl derivative (**3**),[10] and (-)-untenospongin C (**4**),[11] all identified by comparison with literature data. During the investigation of these compounds, a less polar column fraction attracted attention since the ^{1}H NMR spectrum resembled that of the acetyl derivative of tetradehydrofurospongin-1, showing a multiplet signal at δ_H 5.63, similar in appearance to the H-11 signal for the acetyl derivative of tetradehydrofurospongin-1. However there was no corresponding acetyl signal present at δ_H 1.97. Instead there were diagnostic terminal methyl signals in the region δ_H 0.84 – 0.87 and a broad signal for a methylene envelope at approx δ_H 1.20 that suggested fatty acid ester substitution at the C-11 position of the terpene skeleton. The fraction was further purified through RP-HPLC using a solvent gradient from 80% CH_3CN/H_2O to 100% CH_3CN and gave eight fractions SF1-SF8, each of which was examined by proton NMR spectroscopy. Each of the fractions contained the H-11 signal diagnostic for an ester substituent however there were differences for the upfield methyl and methylene signals that hinted at the presence of different fatty acids in the eight fractions. Further evidence for the presence of a fatty ester side chain was the carbonyl signal between 172.5 to 173.0 ppm observed in the ^{13}C NMR spectrum in all eight fractions. Typically the three bond coupling from a oxymethine proton to an adjacent ester functionality (*H*-C-O-*C*O-R) is about 2.7 Hz.[12] When a geHMBC experiment optimised for a long range $J_{C,H}$ of 4 Hz was run on fraction F5, a correlation from the proton at δ_H 5.62 (H-11) to the ester carbonyl at 173.0 ppm (C-1') was detected. Other correlations to the ester carbonyl were also observed from the fatty acid ester signals at δ_H 2.21 (H-2') and δ_H 1.56 (thus identified as H-3').

The fatty acid substituents present in fraction SF5 were predominantly branched chain fatty acids by ^{1}H NMR since, in addition to the signals for the parent furanoterpene skeleton that were present, the ^{1}H NMR of the major component showed methyl signals at δ_H 0.84 (6H, d, H-14' &

H-15') that were diagnostic for a terminal *iso*- branched chain. 1D proton decoupling and TOCSY experiments were conducted on this sample to identify additional ^{1}H NMR signals. Irradiation of the H-14'/H-15' signal identified a signal at δ_H 1.49 as H-13', while irradiation of H-2' at δ 2.21 (2H, t) led to the assignment of H-3' at δ_H 1.56 and also to some of the overlapping methylene signals (δ_H 1.22 - 1.32) which extended to the *iso*-branched terminus. These data were consistent with structure **1a**, however it was apparent that the sample also contained smaller amounts of other furan fatty acid esters. A full diagnosis of the fatty acid side chains in these other components was not possible by ^{1}H NMR for two reasons: firstly, there was extensive signal overlap for the upfield methylene protons; and secondly, since the methylene region could not be integrated accurately, it was not possible to determine the chain length of the fatty acids. A GC/MS analysis of this fraction revealed components containing *m/z* 550 and *m/z* 578 which corresponded to derivatives of tetradehydrofurospongin-1 containing C15 and C17 fatty acyl side chains respectively. A prominent ion at *m/z* 355 apparent in each spectrum could possibly be related to a McLafferty-type rearrangement (producing *m/z* 368) and loss of a methyl group.

To confirm the component fatty acids, fraction SF5 was treated with 1.5M HCl in methanol, and the resulting mixture of fatty acid methyl esters (FAME) analyzed by GC/MS. The major component (**5a**) eluted at 22.2 min and gave a parent ion *m*/*z* 256 with fragmentation at *m*/*z* 225 [M^+-31]; *m*/*z* 201 [M^+-55]; *m*/*z* 200 [M^+-56]; *m*/*z* 191 [M^+-65] and *m*/*z* 74 [McLafferty] which were all diagnostic for a 13-methyltetradecanoate fatty acid ester (*iso*-15:0)[13] side chain. A second component (**5b**) eluted at 22.3 min also with *m*/*z* 256 gave fragment peaks at *m*/*z* 227 [M^+-29]; *m*/*z* 196 [M^+-60]; *m*/*z* 195 [M^+-61]; *m*/*z* 177 [M^+-79] and *m*/*z* 74 which were all diagnostic for 12-methyltetradecanoate fatty acid ester (*anteiso*-15:0)[13] side chain (**1b**). Another component (**5c**) that eluted at 22.6 min was identified as the methyl ester of pentadecanoic acid (*n*-C15:0). Thus the three major components present from fraction SF5 were furanoterpenes with an *iso*-15:0 (**1a**), *anteiso*-15:0 (**1b**) and linear 15:0 (**1c**) side chains. Their FAME derivatives **5a** – **5c** were present in the ratio 21.4: 6.8: 1.0 from integration of the GC/MS trace. Other components present in trace amounts in the FAME sample gave molecular ions at *m/z* 242, 270, 284 and 298 respectively, suggesting the presence of saturated C14, C16, C17 and C18 fatty acids.

Metabolite (**2**) has been isolated in both enantiomeric forms. The (+)-isomer was first isolated, from a sponge of the genus *Spongia*, and named tetradehydrofurospongin-1,[14] while the (-)-isomer, isolated later from *Hippospongia* sp. was named untenospongin B.[11] Our sample of metabolite (**2**) had a small, positive specific rotation, similar to the reported literature value for the stereoisomer with 11*R* stereochemistry.[10a,11] Owing to the first report naming the (+)-isomer as tetradehydrofurospongin-1, this name is used in preference to untenospongin B. In contrast, the ester mixture (**1a**) – (**1c**) and the acetyl derivative (**3**), that were isolated both showed a small negative value for their specific rotations. Notably, the acetyl derivative **3** showed an $[\alpha]_D$ -5.0 (c 0.03, $CHCl_3$) in contrast to the literature reported value of +28.9 (*c* 1.5, $CHCl_3$) for a synthetic sample of the compound that was known to have 11*R* stereochemistry.[10a] Although these data

may imply 11*S* stereochemistry for the acylated compounds, the small rotations obtained and the small amount of material available prevent unequivocal assignment of absolute stereochemistry. In particular, there was insufficient material to confirm the absolute configuration at C-11 in compounds **1a** – **1c** by chemical correlation to acetyl derivative **3**. The C-12' configuration of the *anteiso*-15:0 acid was not evaluated. The configuration of (-)-untenospongin C has been deduced as 8*S* by correlation with the known dihydrofurospongin-2.[11]

The furanosesterterpene chemistry reported for this sample of *Coscinoderma mathewsi* contrasts with earlier reports of diterpenes,[15] sesterterpenes,[16] sulphated sesterterpenes,[17] and alkaloids[18] from this sponge genus. Our collection of this sponge from Mooloolaba represents the southernmost known occurrence of the species along the East coast of Australia, and so extends its geographic range. The specimen collected may represent a slight morphological variant compared to specimens from tropical waters, a fact that may explain the difference in metabolite profile.

Experimental Section

General Procedures. Optical rotations were obtained using a JASCO-P1010 polarimeter. One and two dimensional NMR spectra were acquired using Bruker DRX-500 or Bruker AMX-400 instruments. NMR spectra were obtained in deuterochloroform at room temperature. Samples were internally referenced to $CHCl_3$ at either δ_H 7.25 and δ_C 77.0. High and low resolution mass measurements were obtained from a Finnigan MAT 900 XL-Trap electrospray (ESI) mass spectrometer with a Finnigan API III electrospray source. Gas chromatography/ mass spectrometry (GC/MS) spectra were recorded on a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer, carrying a Zebron ZB-5 capillary column (30 mL x 0.32 mm ID x 0.25 μm df, 5% phenyl polysiloxane) with a Shimadzu AOC-20i auto injector. Reverse Phase high performance liquid chromatography (RP HPLC) was performed with an Agilent 1100 series instrument with Water μBondapak semipreparative columns (10μm, 7.8 x 300mm). Columns were connected to a variable wavelength UV detector and detection at 254 nm. Flow rates were at 1.5 mL/ min.

Biological material

Specimens of *Coscinoderma mathewsi* were collected from the Inner Gneerings, a group of shoals off Mooloolaba (Australia), using SCUBA at a depth of 10-15 m on 16 January 2006. Samples were taken back to the laboratory where they were stored at -20^{0}C until extraction. The sponge was a dull grey colour on the exterior with a beige interior. A voucher specimen (QM G324322) is lodged at the Queensland Museum. Photographs of the sponge material are available from the authors.

Extraction and Isolation of Metabolites

The specimen of *Coscinoderma mathewsi* (wet weight 189 g) was cut into small pieces and extracted exhaustively with DCM/MeOH (1:1) (3 x 300 mL). The crude extract was concentrated under vacuum to a dark brown oil (678 mg), and subjected to Si flash chromatography using a solvent gradient (hexanes => DCM => MeOH). The CH_2Cl_2/EtOAc (3:2) fraction was further purified by RP HPLC using CH_3CN/H_2O (7:3) to afford tetradehydrofurospongin-1 (**2**) (2.3 mg) eluting at 20.4 min. Next the CH_2Cl_2/EtOAc (4:1) fraction was purified by RP HPLC using CH_3CN/H_2O (8:2) and afforded untenospongin C (**4**) (0.5 mg, 17.9 min) and the acetyl derivative of tetradehydrofurospongin-1 (**3**) (2.0 mg, 21.4 min). The 100% CH_2Cl_2 fraction was purified by RP HPLC using a solvent gradient of CH_3CN/H_2O (9:1) to 100% CH_3CN over 10 min, followed by 100% CH_3CN for 30 min and 100% MeOH for another 30 min. This afforded the eight fractions SF1 to SF8.

Tetradehydrofurospongin-1 (2).[10] Colourless oil; $[\alpha]_D$ +11.4 (c 3.10, $CHCl_3$), lit.[10a] +21.8 (c 0.78, $CHCl_3$); LREIMS *m*/*z* 349 $[M+Na]^+$; ^1H and ^{13}C NMR – identical with literature data (ref 10a).

Tetradehydrofurospongin-1 acetyl derivative (3).[10] Colourless oil; $[\alpha]_D$ -5.0 (c 0.03, $CHCl_3$), lit.[10a] +28.9 (c 1.5, $CHCl_3$); LREIMS *m*/*z* 349 $[M+Na-Ac]^+$; GC-MS 368 (<1), 149, 95, 81, 69 (100%); ^1H and ^{13}C NMR – identical with literature data (ref 10b).

Untenospongin C (4):[11] colourless oil; $[\alpha]_D$ -4.6 (c 0.18, $CHCl_3$), lit.[11] -9.3 (c 1.0, $CHCl_3$); LREIMS *m*/*z* 365 $[M+K]^+$; ^1H and ^{13}C NMR - identical with literature data (ref 11).

Tetradehydrofurospongin-1 fatty acid ester derivatives (1a, 1b, 1c). $[\alpha]_D$ of mixture -12.5 (c 0.06, $CHCl_3$); GC-MS *m*/*z* 550 $[M]^+$ (<1), 429 (5), 355 (30), 281 (15), 221 (15), 207 (10), 147 (15), 44 (40), 40 (60); ^1H NMR of component **1a** ($CDCl_3$) δ 7.34 (1H, br s, H-4), 7.31 (1H, br s, H-1), 7.30 (1H, br s, H-21), 7.16 (1H, br s, H-19), 6.48 (1H, br s, H-2), 6.23 (1H, br s, H-20), 6.22 (1H, d, *J* = 15 Hz, H-5), 5.84 (1H, dt, *J* = 15, 7 Hz, H-6), 5.62 (1H, m, H-11), 5.18 (1H, d, *J* = 7 Hz, H-15), 5.15 (1H, d, *J* = 7 Hz, H-10), 2.80 (2H, br s, H-7), 2.40 (2H, t, *J* = 7 Hz, H-17), 2.31 (1H, m, H-12a), 2.21 (2H, t, *J* = 7 Hz, H-2'), 2.20 (2H, q, *J* = 7 Hz, H-16), 2.13 (1H, m, H-12b), 1.70 (3H, s, H-9), 1.61 (3H, s, H-14), 1.56 (2H, m, H-3'), 1.49 (1H, m, H-13'), 1.32 – 1.22 (16H, m, CH_2), 1.13 (2H, m, H-12'), 0.84 (6H, d, *J* = 6 Hz, H-14', H-15'); ^{13}C NMR of component **1a** ($CDCl_3$) δ 173.0 (C-1'), 143.2 (C-1), 142.7 (C-21), 139.3 (C-4), 138.7 (C-8), 138.5 (C-19), 131.2 (C-13), 127.2 (C-15), 127.1 (C-6), 124.6 (C-18), 124.2 (C-10), 124.0 (C-3), 121.2 (C-5), 110.8 (C-20), 107.3 (C-2), 69.5 (C-11), 44.7 (C-12), 42.3 (C-7), 39.1 (C-12'), 34.2 (C-2'), 29.7 - 27.4 (8C), 28.2 (C-16), 28.0 (C-13'), 25.0 (C-3'), 24.3 (C-17), 22.7 (C-14' & C-15'), 16.8 (C-9), 16.1 (C-14).

Preparation of fatty acid methyl ester derivatives (FAMEs)

Furanoterpene fatty acid ester SF5 was treated with 1.5M of HCl in MeOH (1 mL) in a screw-capped vial and refluxed for 1 hour at 65°C. The residual liquid was removed by a stream of N_2 (g) before addition of 0.5 mL toluene that was also then removed under a stream of N_2 (g). The crude transmethylated fatty ester was dissolved in 1 mL of hexane and passed through a short

silica column (0.5g silica) using hexane (10 mL) as a solvent. The eluted FAME was evaporated to dryness prior to GC-MS analysis which revealed (**5a**) *iso*-15:0, Rt 22.2 min), (**5b**) (*anteiso*-15:0, Rt 22.3 min) and (**5c**) (*n*15:0, Rt 22.6 min) in the ratio 21.4: 6.8: 1.0.

Methyl 13-methyltetradecanoate (*iso*-15:0) (5a). GC-MS *m/z* 256 $[M]^+$ (2), 225 (1), 213 (7), 199 (3), 185 (2), 171 (1), 157 (4), 143 (9), 129 (4), 111 (1), 101 (6), 87 (42), 74 (100), 69 (20), 57 (20), 55 (25), 43 (66), 41 (59).

Methyl 12-methyltetradecanoate (*anteiso*-15:0) (5b). GC-MS *m/z* 256 $[M]^+$ (1), 225 (2), 213 (4), 199 (14), 185 (1), 177 (1), 157 (2), 143 (9), 129 (4), 115 (4), 101 (4), 87 (46), 74 (100), 57 (35), 43 (61), 41 (69).

Methyl pentadecanoate (*n*-15:0) (5c). GC-MS *m/z* 256 $[M]^+$ (4), 225 (2), 213 (3), 199 (2), 185 (2), 171 (2), 157 (3), 143 (12), 129 (6), 115 (2), 97 (7), 87 (45), 76 917), 74 (100), 69 (15), 57 (18), 55 (27), 43 (40).

Supplementary Information Available

Figures S1 – S2. ^{1}H NMR for the fraction containing compounds **1a** – **1c** and GC-MS data for compounds **5a** – **5c**. Figure S3. A surface photograph of a specimen of *Coscinoderma mathewsi*.

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