Cacofurans A and B, New Furanoditerpenes from a Marine Sponge

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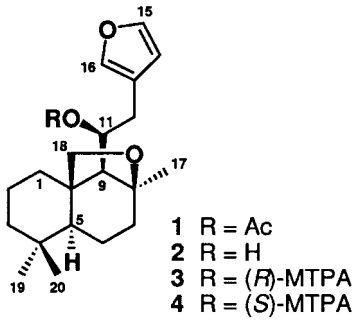
Two new labdane-class diterpenes, cacofurans A (**1**) and B (**2**), have been isolated from a sponge *Cacospongia* sp. Their structures were determined by analyzing spectroscopic data, by chemical transformations, and by X-ray diffraction. Cacofurans **1** and **2** inhibited the development of fertilized sea urchin eggs at concentrations of 0.5 and 5 μg/mL and showed an actin-disrupting effect on the NBT-II cell line at 10 μg/mL.

A large number of labdane diterpenes have been reported from natural sources, particularly terrestrial plants. Some are endowed with significant biological properties such as inhibition of platelet aggregation[1] and cell differentiation-inducing activity.[2] However, relatively few labdanes have been reported from marine sources.[3] Two examples are certain aldehydes found in the mollusk *Pleurobranch meckelii*, which probably act as defense chemicals,[4] and a cytotoxin isolated from the tunicate *Lissoclinum voeltzkowi*.[5] As part of our search for novel bioactive metabolites from coral reef organisms,[6] we discovered that an extract of a sponge of the genus *Cacospongia* exhibited moderate cytotoxicity. Consequently, we decided to characterize the metabolites contained therein. In this report we describe their isolation and the elucidation of their structures.

A specimen (wet wt, 140 g) of a tube-shaped sponge *Cacospongia* sp. (family Thorectidae)[7] was collected at Yonaguni Island, Okinawa. Extraction with acetone and removal of solvent furnished material that was then extracted with EtOAc. Chromatographic separation of the extract over silica gel followed by HPLC gave two new diterpenes, cacofuran A (**1**) and B (**2**), as major constituents.



Cacofuran A (**1**) showed a molecular ion at *m*/*z* 360 on EIMS, and a molecular formula of $C_{22}H_{32}O_4$ was determined by HREIMS (Δmmu −0.1). Of the seven degrees of unsaturation, three were assigned to a β-substituted furan (δ 7.13 brs, 7.09 brs, and 6.22 brs; δ 143.5 d, 140.0 d, 122.1 s, 111.2 d), one to an acetoxyl group (δ 169.7 s, 21.1 q; δ 1.71 s; 1735, 1240 cm^{-1}), and the remaining three to rings. Downfield-shifted methylene protons [δ 2.86 dd, 2.93 dd (H-12ab)] revealed coupling to an oxymethine proton at δ 5.61 br dd (H-11), having an HMBC correlation with the acetyl carbonyl group. These methylene protons also showed HMBC cross-peaks with furan carbon atoms (δ 140.0, 122.1, and 111.2 d). Another downfield-shifted methylene group [δ 3.49 d (H-18a), 4.24 d (H-18b); δ 74.3 t] and a remaining oxygen atom must be part of a five-membered cyclic ether, which was confirmed by the HMBC correlations [δ 4.24/δ 49.4 s (C-10), 59.6 d (C-9), 82.0 (C-8); δ 3.49 brd/δ 49.4; δ 1.48 s (Me-17)/δ 82.0, 59.6]. The *trans*-decalin portion of the molecule, a common structural unit in terpenoids, was elucidated by analyzing the 2D NMR spectra (COSY, HMQC, and HMBC) and by comparing the data with those of similar terpenoids.[8]

Cacofuran B (**2**) exhibited a molecular ion at *m*/*z* 318 and molecular formula $C_{20}H_{30}O_3$ (Δmmu −0.7). Since its ^1H and ^{13}C NMR data were similar to those of **1**, it was assumed to be the desacetyl derivative. Confirmation was obtained by treating **2** with acetic anhydride and pyridine, which gave a compound showing an identical R_f value on TLC and identical ^1H NMR and MS data with those of **1**. As in the case for **1**, the ^1H and ^{13}C NMR data of **2** were assigned by 2D NMR analysis (COSY, HMQC, HMBC) and by comparing the data with those for **1**.

The relative stereochemistry of the tricyclic portion was deduced from the positive NOEs observed between the following signals: H-9/H-5, H-5/Me-19, and Me-20/H-18b. The syn-diaxial arrangement of the protons at the C-5 and C-9 positions confirms the trans-fusion of the A/B rings. Similarly, the NOE reveals that the angular methylene substituent lies diaxially alongside the Me-20 group. The absolute stereochemistry at C-11 of **2** was solved by applying Mosher's method.[9] Treatment of **2** with either (+)-*S*-methoxytrifluoromethylphenyl acetyl chloride (MTPACl) or (−)-*R*-MTPACl and pyridine gave the (+)-*R*- or (−)-*S*-MTPA esters **3** or **4**. As the $\Delta\delta$ values (δ**4** − δ**3**) were negative for the side chain [−0.063 (H-12a), −0.066 (H-12b), −0.032 (H-14), −0.102 (H-15), and −0.109 (H-16)] and positive for the tricyclic portion [+0.133 (H-18a), +0.093 (H-18b), +0.073 (H-19), and +0.034 (H-20)], it was concluded that the C-11 center has the *S* configuration. The C-11 stereocenter, thus determined, permits the elucidation of the configuration of the attached tricyclic portion. On adding D_2O to a solution of **2** in C_6D_6, the signal at δ 2.50 (d, J = 6.1 Hz) due to the C-11 hydroxyl proton disappeared. At the same time, the multiplicity of the resonance of the adjoining oxymethine proton (δ 4.11, ddd) changed to a doublet of doublets. As the latter signal showed no coupling with the C-9 proton, it can be concluded that the dihedral angle between H-9 and H-11 is about 90°. Fur-

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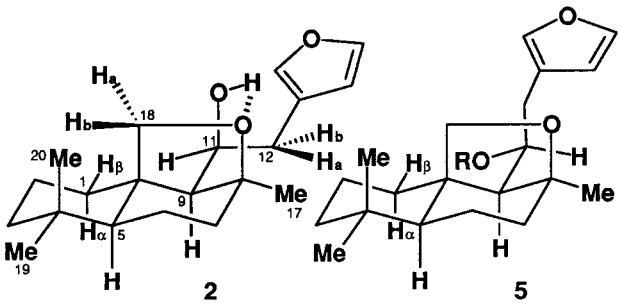

Figure 1. Stereochemical correlation in **2** and **5**.

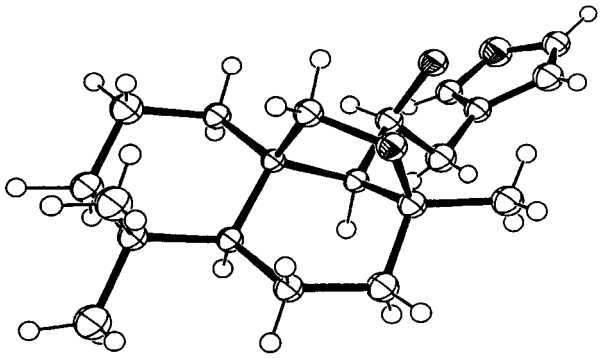

Figure 2. Computer-generated ORTEP drawing of cacofuran B (**2**).

thermore, the hydroxyl absorption (3550 cm^{-1}) did not change on dilution in CCl_4 solution. The above results indicate that the C-11 hydroxyl proton is hydrogen-bonded to the ether oxygen atom. Moreover, rotation about the C-9/C-11 bond is frozen. Positive NOEs were observed between Me-17/H12ab, Me-17/OH-11, H-11/H-18a, H-11/H-1β, and H-18a/H-1β, enabling the absolute configuration of the tricyclic portion to be assigned as 5*S*, 8*S*, 9*S*, 10*R*, and 11*S* (Figure 1). It is therefore seen that the side chain of **2** prefers to adopt a conformation eclipsing the angular methyl group. However, the esters **3** and **4**, as well as **1**, where hydrogen bonding is absent, could conceivably populate the alternative conformation in which the side chain eclipses the ether bridge (**5**).

Since compound **2** gave colorless crystals, its relative stereochemistry was determined by X-ray diffraction (Figure 2). The distance between the ether and hydroxyl oxygen atoms was found to be 2.766 Å, confirming the presence of the hydrogen bonding. The torsion angle between H-9 and H-11 was found to be 76°. Clearly, the structure agrees with the conclusions drawn from the spectroscopic evidence.

Tests on the P388 and K562 cell lines showed that both **1** and **2** are moderate cytotoxins, having IC_{50} values greater than 1 μg/mL. TMR-phalloidin images showed the drug caused a major disruption to the actin cytoskeleton of NBT-II cells and roughly 50% of the cells survived the drug treatment. Cell−cell contacts at the Adherens junctions were disrupted, and surviving cells contained bleb-like structures and numerous small intracellular vesicles. In an inhibitory test against the development of sea urchin eggs,[10] **1** and **2** were effective at levels of IC_{50} 0.5 and 5 μg/mL, respectively. On the other hand, neither **1** nor **2** displayed any antimicrobial activity against bacteria such as *E. coli*, *B. cereus*, and MRSA.

Some 40 articles have been published on sponges belonging to the genus *Cacospongia*. In most cases, the metabolites reported were found to be sesterterpenes.[11] The present result is noteworthy in that it describes the first isolation of a bridged tricyclic diterpene.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Jasco DIP-370 polarimeter. IR spectra were recorded on a Hitachi 260-10 spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a JEOL A-500 or on an EX-270 NMR spectrometer. Mass spectra were measured on a Hitachi M-2500 instrument.

Animal Material. The sponge (140 g, wet wt) was collected in an underwater cave (−20 m) by hand, using scuba, at Yonaguni Island, Okinawa, on September, 1992. The specimen was kept frozen after collection. A voucher specimen (G301468) was deposited at the Queensland Museum, South Brisbane, Australia. A photo is available as Supporting Information.

Extraction and Isolation. The frozen sample was brought back to the laboratory and extracted with acetone three times. After concentration, the EtOAc-soluble portion was taken up to give 0.71 g (0.51% of the fresh animal) of extract. A portion (0.50 g) was first separated over a Si gel column (CH_2Cl_2) to give a diterpene-containing fraction (294 mg), which on further separation over a Si gel column (hexanes−EtOAc) and finally by reversed-phase HPLC (Cosmosil $5C_{18}$, MeOH−H_2O, 10:1) gave **1** (31.2 mg, 6.2% of the extract) and **2** (143.2 mg, 28.6%).

Cacofuran A (1): glass; $[\alpha]_D$ −30° (*c* 0.40, CH_2Cl_2); IR (CCl_4) 2940, 1735, 1240, 1030 cm^{-1}; ^1H NMR (C_6D_6) δ 0.74 (3H, s, Me-19), 0.81 (3H, s, Me-20), 0.98 (1H, dt, *J* = 13.5, 3.0 Hz, H-3α), 1.03 (1H, m, H-5), 1.10 (1H, qt, *J* = 13.5, 2.7 Hz, H-2β), 1.25 (1H, brd, *J* = 13.5 Hz, H-3β), 1.34 (1H, m, H-7β), 1.38 (1H, m, H-2α), 1.43 (1H, m, H-1α), 1.48 (3H, s, Me-17), 1.50 (1H, m, H-6β), 1.63 (1H, brs, H-9), 1.65 (1H, m, H-7α), 1.71 (3H, s, OCO*CH*$_3$), 1.72 (1H, m, H-6α), 1.97 (1H, brd, *J* = 13.5 Hz, H-1β), 2.86 (1H, dd, *J* = 15.0, 8.4 Hz, H-12a), 2.93 (1H, dd, *J* = 15.0, 6.0 Hz, H-12b), 3.49 (1H, d, *J* = 8.1 Hz, H-18a), 4.24 (1H, d, *J* = 8.1 Hz, H-18b), 5.61 (1H, brdd, *J* = 6.0, 8.4 Hz, H-11), 6.22 (1H, brs, H-14), 7.09 (1H, brs, H-15), 7.13 (1H, brs, H-16); ^{13}C NMR (C_6D_6) δ 20.8 t (C-2), 21.1 q (OCO*CH*$_3$), 21.6 t (C-6), 21.8 q (C-20), 23.6 q (C-17), 28.7 t (C-12), 32.9 q (C-19), 33.3 t (C-1), 33.9 s (C-4), 42.1 t (C-3), 42.2 t (C-7), 49.4 s (C-10), 52.6 d (C-5), 59.6 d (C-9), 73.4 d (C-11), 74.3 t (C-18), 82.0 s (C-8), 111.2 d (C-14), 122.1 s (C-13), 140.0 d (C-16), 143.5 d (C-15), 169.7 s (O*C*OCH$_3$); HREIMS *m/z* 360.2297 (calcd for $C_{22}H_{32}O_4$, 360.2298); EIMS *m/z* 360 (M^+, 21), 300 (33), 279 (42), 237 (100).

Cacofuran B (2): needles, mp 141−145 °C (hexanes−EtOAc); $[\alpha]_D$ −15°(*c* 2.0, CH_2Cl_2); IR (CCl_4) 3550, 2950, 1030 cm^{-1}; ^1H NMR (C_6D_6) δ 0.73 (3H, s, Me-19), 0.75 (3H, s, Me-20), 0.95 (1H, ddd, *J* = 2.7, 12.8, 13.4 Hz, H-3β), 1.01 (1H, dd, *J* = 4.3, 12.8 Hz, H-5), 1.07 (1H, tq, *J* = 2.7, 13.4 Hz, H-2β), 1.22 (1H, m, H-3α), 1.26 (1H, m, H-9), 1.28 (1H, m, H-7β), 1.33 (1H, m, H-1β), 1.37 (1H, m, H-2α), 1.44 (3H, s, Me-17), 1.51 (1H, m, H-6β), 1.61 (1H, m, H-1α), 1.71 (1H, m, H-6α), 1.88 (1H, brd, *J* = 13.1 Hz, H-7α), 2.50 (1H, d, *J* = 6.1 Hz, OH-11), 2.61 (1H, brdd, *J* = 14.3, 5.7 Hz, H-12b), 2.86 (1H, dd, *J* = 14.3, 7.3 Hz, H-12a), 3.68 (1H, d, *J* = 8.4 Hz, H-18a), 4.11 (1H, ddd, *J* = 5.7, 6.1, 7.3 Hz, H-11), 4.16 (1H, d, *J* = 8.4 Hz, H-18b), 6.20 (1H, brs, H-14), 7.15 (1H, brs, H-15), 7.17 (1H, brs, H-16); ^{13}C NMR (C_6D_6) δ 20.6 t (C-2), 21.6 t (C-6), 21.6 q (C-20), 24.5 q (C-17), 32.8 t (C-12), 32.8 q (C-19), 33.1 t (C-1), 33.8 s (C-4), 42.0 t (C-3), 42.0 t (C-7), 49.0 s (C-10), 52.0 d (C-5), 61.0 d (C-9), 71.5 d (C-11), 74.4 t (C-18), 82.6 s (C-8), 111.5 d (C-14), 123.3 s (C-13), 140.0 d (C-15), 143.2 d (C-16); HREIMS *m/z* 318.2186 (calcd for $C_{20}H_{30}O_3$, 318.2193); EIMS *m/z* 318 (M^+, 1), 300 (2), 237 (100 rel %).

Acetylation of 2. A solution of **2** (3.7 mg) in dry pyridine (0.1 mL) and Ac_2O (0.1 mL) was stirred under N_2 for 1 h at room temperature, then at 50 °C for 6 h. The mixture was concentrated in vacuo, and the residue purified on preparative TLC to give **1** (2.2 mg, 52%), identical in all respects with authentic material.

(+)-*R*-MTPA Ester 3. A mixture of of compound **2** (5.0 mg), dry pyridine (50 μL), and (+)-*S*-MTPA chloride (15 μL) was kept standing at 60 °C for 2 h. The residue obtained after evaporating the pyridine was separated by preparative TLC (hexanes−EtOAc, 4:1) to give (+)-*R*-MTPA ester **3** (5.54 mg, 75%): plates; mp 131−135 °C (MeOH); $[\alpha]_D$ +11.3° (*c* 0.981,

$CHCl_3$); IR (CCl_4) 2950, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.812 (3H, s, Me-19), 0.822 (3H, s, Me-20), 1.408 (3H, s, Me-17), 2.991 (1H, dd, J = 15.2, 9.6 Hz, H-12b), 3.077 (1H, dd, J = 15.2, 4.6 Hz, H-12a), 3.217 (1H, brd, J = 8.6 Hz, H-18a), 4.032 (1H, d, J = 8.6 Hz, H-18b), 5.589 (1H, m, H-11), 6.335 (1H, brs, H-14), 7.276 (1H, brs, H-15), 7.370 (1H, brs, H-16); ^{13}C NMR ($CDCl_3$) δ 20.1, 21.1, 21.8, 22.8, 26.9, 32.0, 33.0, 33.8, 41.3, 41.6, 49.0, 52.2, 55.8, 59.1, 74.0, 76.9, 81.8, 110.5, 121.3, 127.1, 128.4, 129.7, 140.0, 143.5, 166.5; EIMS m/z 534 (M^+, 76), 453 (28), 300 (68), 189 (100 rel %).

(−)-*S*-MTPA Ester 4. It was prepared in a manner similar to that described above. **4**: gum; $[\alpha]_D$ −28.5° (c 0.554, $CHCl_3$); IR (CCl_4) ν_{max} 2950, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.856 (3H, s, Me-20), 0.885 (3H, s, Me-19), 1.403 (3H, s, Me-17), 2.925 (1H, dd, J = 15.0, 9.2 Hz, H-12b), 3.014 (1H, dd, J = 15.0, 5.3 Hz, H-12a), 3.350 (1H, d, J = 8.6 Hz, H-18a), 4.125 (d, J = 8.6 Hz, H-18b), 5.622 (1H, m, H-11), 6.303 (1H, brs, H-14), 7.174 (1H, brs, H-15), 7.261 (1H, brs, H-16); ^{13}C NMR ($CDCl_3$) δ 20.4, 21.2, 21.7, 22.9, 32.5, 33.0, 33.9, 41.7, 41.8, 52.6, 55.3, 59.5, 74.1, 76.8, 81.9, 110.7, 116.4, 120.9, 127.8, 128.6, 129.7, 131.7, 140.1, 143.3, 164.1; EIMS m/z 534 (M^+, 40), 453 (28), 300 (31), 189 (100 rel %).

X-ray Diffraction of 2.[12] Suitable colorless crystals of **2** were obtained by recrystallization (MeOH). The crystal (0.2 × 0.2 × 0.7 mm) belongs to the orthorhombic system, space group $P2_12_12_1$, with a = 10.3286(1) Å, b = 23.0770(4) Å, c = 7.2476(1) Å, V = 1727.49(4) $Å^3$, Z = 4, D_{calcd} = 1.224 g/cm^3, λ(Mo Kα) = 0.71069 Å. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to 2θ of 55°. A total of 2315 reflections were collected. The structure was solved by direct methods (SIR 92) and refined by a full matrix least squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.050, R_w = 0.056 for 1810 observed reflections [$I > 10.00\sigma(I)$] and 208 variable parameters.

Cytotoxicity Testing. The assay with P388 and K562 cell lines was carried out by Dr. T. Natori, Kirin Brewry Co. Ltd. Cacofuran was tested using an image-based high content imaging workstation. The rat bladder epithelial carcinoma cell line (NBT-II) was treated with cacofurans A and B at 10 μg/mL. Phase contrast images of control and drug-treated cells were recorded after 16 h using a microscope described by Choidas et al.[13] The effects of the drugs on the actin cytoskeleton were analyzed by staining permeabilized cells with TMR-phalloidin according to Choidas et al.[13]

Sea Urchin Egg Test. Sea urchin eggs and sperms were collected from mature specimens of *Tripneustes gratilla*. Soon after fertilization, the eggs were dropped onto the test media which were previously prepared from a graded concentration of cacofurans dissolved in EtOH and filtered seawater. After allowing the test media to stand, the embryos were observed with a light microscope at certain intervals.

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Supporting Information Available: Sponge photograph. This material is available free of charge via the Internet at http://pubs.acs.org.

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