The Calyxolanes: New 1,3-Diphenylbutanoid Metabolites Isolated from the Caribbean Marine Sponge *Calyx podatypa*[1,2]

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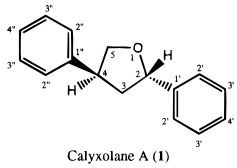
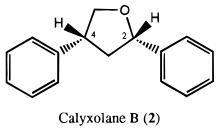
Calyxolanes A (**1**) and B (**2**) are rare 1,3-diphenylbutanoid compounds isolated from the marine sponge *Calyx podatypa* collected in Puerto Rico. Their structures, including relative stereochemistry, have been determined by spectroscopic methods. The unique 2,4-diphenyloxolane function in **1** and **2** was established by 2D $^1H-^1H$ and $^1H-^{13}C$ NMR correlation experiments and confirmed by mass spectral analysis. A suggestion is made as to their biogenetic origin.

The common Caribbean sponge *Calyx podatypa* de Laubenfels (class Demospongiae, order Haplosclerida, family Oceanapiidae) has been extensively characterized from the chemical point of view and is known to be a source of a variety of sterols,[4] proline-derived cyclic dipeptides (diketopiperazines),[5] and a series of cytotoxic antimicrobial alkaloids containing a 3-substituted pyridine ring system.[6] Our specimen of *C. podatypa* was collected near Mona Island off the west coast of Puerto Rico in 1992.

Minced, freeze-dried specimens (927 g) were extracted exhaustively with $CHCl_3$−MeOH (1:1) (5 × 1 L), and after filtration the crude extract was concentrated to yield 62 g of a dark green residue (*ca.* 6.6% of the dry wt). The crude extract was suspended in H_2O and then was partitioned against *n*-hexane, $CHCl_3$, and *n*-BuOH (4 × 400 mL each). The *n*-hexane solubles (*ca.* 14.1 g) were passed through a Bio-Beads SX-2 column using toluene as eluent, while the collection of fractions was guided by TLC and NMR. The slowest-moving fraction obtained was purified by successive column chromatography on Si gel and HPLC, yielding analytically pure calyxolane A (**1**) and calyxolane B (**2**).

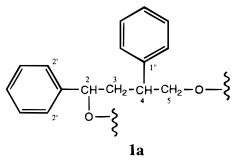


Calyxolane A (**1**)



Calyxolane B (**2**)

Calyxolane A (**1**) gave rise to a molecular ion peak at *m*/*z* 224.1193 upon HREIMS, which suggested a molecular formula of $C_{16}H_{16}O$ (Δ 0.8 mmu), corroborated by ^{13}C-NMR data that showed 16 carbons (see Table 1). The nine degrees of unsaturation required by the molecular formula could be ascribed to six double bonds [two CH_0 type: δ 143.6, 142.0; and 10 CH type: 128.6 (2 × C), 128.4 (2 × C), 127.3 (2 × C), 127.2, 126.6, 125.5 (2 × C)], leaving three rings present in the molecule. The IR (neat) spectrum showed no hydroxyl absorptions[7] but contained strong aromatic C−H stretching bands at 3055 and 3020 cm^{-1}. The latter, together with the UV absorption at λ_{max} 206 nm, multiple ^1H-NMR resonances between 7.26−7.38 ppm integrating for 10 protons, and 12 ^{13}C-NMR resonances in the 125−129 ppm region, indicated the presence of two phenyl residues in **1**.



1a

Figure 1. Structural fragment of calyxolane A (**1**).

^1H and ^{13}C NMR (Table 1) identified a total of four nonaromatic carbon atoms: a methylene group (^1H and ^{13}C resonances, respectively, δ 2.34 and 2.48, 42.7), a methine (δ 3.54, 44.4), and two oxygenated carbons, one of which is primary (δ 3.95 and 4.47, 75.1) and the other secondary (δ 5.24, 80.6). From the COSY spectrum it was clear that all the nonaromatic protons in **1** comprised an isolated spin system as revealed by consecutive correlations between H2-H3α/H3β, H3α/H3β-H4, and H4-H5α/H5β. An analysis of the $^1H-^1H$ COSY, $^1H-^{13}C$ COSY (HETCOR) and APT NMR spectra of **1** led to partial structure **1a** (Figure 1). Since there is only one oxygen atom in the molecular formula of **1**, it had to be attached to both C2 and C5. Enhancement of the ^{13}C-NMR signals at δ 128.6 (C2′) and δ 142.0 (C1″) during an INAPT experiment upon irradiation of H2 (δ 5.24) and H5α (δ 4.47), respectively, placed the benzene rings at C2 and C4 of the oxolane ring. The 2,4-disubstituted oxolane structure was also supported by an ion peak at *m*/*z* 194 [$M^+ - CH_2O$] in the HREIMS spectrum of **1** (Scheme 1), which ruled out a symmetrical 2,5-disubstituted structure. The coupling constants for H2-H3α/H3β, H3α/H3β-H4, and H4-H5α/H5β (Table 1) also indicated that the protons were attached to carbons in a five-membered ring. Detailed analysis of the HREIMS spectrum (Scheme 1) completely confirmed the entire carbon sequence depicted in structure **1**.

The *trans* relative stereochemistry shown between H2 and H4 rests in part upon the observed small difference in chemical shifts of the C3 diastereotopic protons and the absence of any significant NOE correlation between H2 and H4. Indeed, the aromatic anisotropic effect experienced by each of the H3 protons is expected to be roughly the same when a *trans* 1,3-relationship exists between the phenyl residues. This causes the H3 methylene protons to have very similar chemical shift values. The (2*S**,4*S**) relative stereochemistry shown is also consistent with the moderately strong optical

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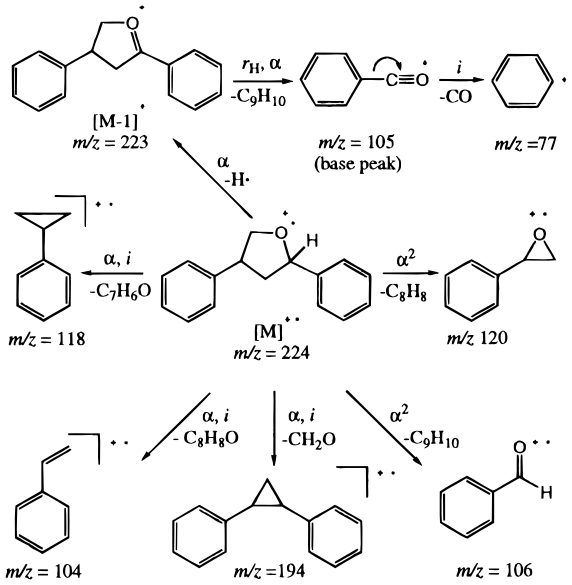
Table 1. ^{1}H-NMR and ^{13}C-NMR Spectral Data for Calyxolanes A (**1**) and B (**2**)[a]

	calyxolane A		calyxolane B	
position	δ_H, mult, intrg (*J* in Hz)	δ_C (mult)[b]	δ_H, mult, intrg (*J* in Hz)	δ_C (mult)[b]
2	5.24, dd, 1H (5.7, 7.8)	80.6 (d)	5.08, dd, 1H (5.7, 10.2)	81.8 (d)
3α	2.34, ddd, 1H (5.7, 8.4 12.6)	42.7 (t)	2.02, q, 1H (10.5)	43.7 (t)
3*β*	2.48, ddd,1H (7.8, 7.8, 12.6)		2.77, m, 1H (6.3)	
4	3.54, m, 1H (7.8)	44.4 (d)	3.65, m, 1H (8.1)	46.0 (d)
5α	4.47, t,1H (8.0)	75.1 (t)	4.03, t, 1H (8.4)	75.1 (t)
5*β*	3.95, t, 1H (8.1)		4.37, t, 1H (8.4)	
1′		143.6 (s)		142.6 (s)
2′	7.37, m, 2H	128.6 (d, 2 × C)	7.37, m, 2H	128.6 (d, 2 × C)
3′	7.32, m, 2H	127.3 (d, 2 × C)[c]	7.32, m, 2H	127.2 (d, 2 × C)[c]
4′	7.26, m, 1H	127.2 (d)[d]	7.26, m, 1H	127.4 (d)[d]
1″		142.0 (s)		141.7 (s)
2″	7.37, m, 2H	128.4 (d, 2 × C)	7.37, m, 2H	128.4 (d, 2 × C)
3″	7.38, m, 2H	125.5 (d, 2 × C)[c]	7.38, m, 2H	125.7 (d, 2 × C)[c]
4″	7.26, m, 1H	126.6 (d)[d]	7.26, m, 1H	126.6 (d)[d]

[a] ^{1}H- and ^{13}C-NMR spectra were recorded at 25 °C in $CDCl_3$ at 300 MHz and 75 MHz, respectively. [b] Number of attached protons were determined by APT experiments. [c–d] Signals within a column may be reversed.

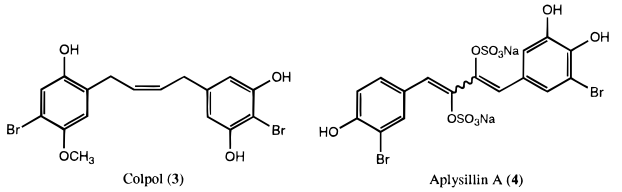
Scheme 1. Main fragmentation processes of the calyxolanes with suggested ion structures



rotation displayed by calyxolane A in $CHCl_3$ solution ($[\alpha]^{22}_D$ +34.6°) (see below).

Compound **2** (calyxolane B; $C_{16}H_{16}O$, as deduced from HREIMS, ^{1}H- and ^{13}C-NMR data) was easily identified as the *cis*-2,4-diphenyl isomer of **1** from comparison of their HREIMS, ^{1}H- and ^{13}C-NMR spectra. Its structure was deduced from ^{1}H-, ^{13}C-, ^{1}H−^{1}H COSY, and ^{1}H−^{13}C COSY NMR spectral data. This compound exhibited ^{1}H- and ^{13}C-NMR spectra nearly identical to those of **1** (see Table 1). Nevertheless, two major differences between **2** and **1** in the ^{1}H- and ^{13}C-NMR spectra were observed: the ^{1}H-NMR signals ascribed to H3α*β* in **2** showed considerable differences in chemical shifts, and C4 had shifted from *δ* 44.4 in **1** to *δ* 46.0 in **2**. Epimerization of **2** at C4 would account for these spectral differences. In confirmation of this assignment, a significant NOE correlation between protons H2 and H4 was observed. Interestingly, the fact that **2** does not exhibit any detectable optical rotation despite having an unsymmetrical structure suggests that calyxolane B must have opposite relative stereochemistry at both chiral centers [*i.e.*, (2*S**,4*R**)]. Alternatively, the lack of optical activity may indicate that calyxolane B is racemic.

Biogenetically, **1** and **2** could belong to the rare C_6−C_4−C_6 natural products, which are derived from a polyketide or a mixed polyketide−shikimate pathway.[8] To the best of our knowledge, colpol (**3**)[9] and aplysillin A (**4**)[10] are the only other C_6−C_4−C_6 compounds isolated from marine organisms, the Red Sea alga *Colpomenia sinuosa* and the Caribbean sponge *Aplysina fistularis fulva*, respectively. While the proposed biosynthesis [C_6−C_4−C_6] of **3** was not supported by any confirming data, it is likely that colpol and the calyxolanes are dimers of a biosynthetic equivalent of styrene [so-called C_6C_2-dimers].[8,11] They are, however, joined together in different manners (1,1 and 1,2, respectively) and their biosynthetic origins are therefore not identical. Moreover, the calyxolanes possess certain structural features so far unreported for compounds of this type. On the one hand, compounds **1** and **2** lack functionality at the benzene rings, and, on the other hand, the unprecedented 1,4-oxidation pattern of the butanoid chain paves the way for subsequent cyclization into a tetrahydrofuran ring. Unfortunately, the paucity of compounds **1** and **2** precluded our attempts to assess their biological activities.



Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 600 FT-IR spectrophotometer. ^{1}H-NMR, ^{13}C-NMR, ^{1}H−^{1}H COSY, HETCOR, and 2D NOESY spectra were recorded on a General Electric QE-300 or Bruker DPX-300 spectrometers. ^{1}H-NMR chemical shifts were recorded with respect to internal Me_4Si, and ^{13}C-NMR chemical shifts are reported in parts per million relative to $CDCl_3$ (77.0 ppm). Optical rotations were determined on a Perkin-Elmer Polarimeter model 243B. HREIMS were recorded on a VG-Fisons Autospec MS system at the Research and Materials Characterization Center of the University of Puerto Rico. A 12L Labconco freeze-dry system was used for lyophilization of the marine organism. Column chromatography was carried out on Analtech Si gel (35−

75 mesh), and TLC analyses were performed using Analtech glass-packed precoated Si gel plates. High performance liquid chromatography (HPLC) was performed on a Beckman Instruments System Gold chromatograph equipped with ultraviolet (model 167) and refractive index (model 156) detectors using a Si gel Partisil 10 semipreparative column (9.4 mm × 50 cm). All solvents used were either spectral grade or were distilled from glass prior to use.

Animal Material. *Calyx podatypa* is a dark reddish-brown to black, soft sponge of irregular mass and smooth-textured surface with scattered excurrent openings that form volcano-like projections. It inhabits reefs, especially small patch reefs in exposed areas. Maroon sponge zoanthids may be found growing on the surface. A voucher specimen (no. MI-030) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Collection and Extraction. Minced and freeze-dried specimens of *C. podatypa* (927 g) collected near Mona Island, Puerto Rico, were extracted exhaustively with $CHCl_3$−MeOH (1:1) (5 × 1 L). After filtration the crude extract was evaporated under vacuum to yield a residue (62 g) that was suspended in H_2O and then partitioned between *n*-hexane (4 × 400 mL), $CHCl_3$ (4 × 400 mL), and *n*-BuOH (4 × 400 mL). The hexane extract was concentrated *in vacuo* to yield 14.1 g of an oily residue which, after filtration under vacuum in toluene solution, was fractionated by size-exclusion chromatography on a Bio-Beads SX-2 column with toluene as eluent. The combined portions (TLC guided using $CHCl_3$ as solvent and iodine as detection reagent) were concentrated to obtain three main fractions. The last fraction was chromatographed successively over a Si gel column with $CHCl_3$ followed by HPLC using 98:2 hexane−EtOAc to afford 2.5 mg of calyxolane A (**1**) (0.004% of the crude extract) and 3.8 mg of calyxolane B (**2**) (0.006% of the crude extract).

Calyxolane A (1): colorless oil; $[\alpha]^{22}_D$ +34.6° (*c* 0.3, $CHCl_3$); IR (neat) 3055, 3020, 2950, 2914, 2844, 1675, 1442, 1252, 1097, 1013, 794, 752, 689 cm^{-1}; UV (CH_3OH) λ_{max} 206 (ϵ 16 000) nm; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; HREIMS *m/z* (rel int) [M^+] calcd for $C_{16}H_{16}O$ 224.1201, found 224.1193 (13), 223 (16), 210 (8), 194 (24), 193 (16), 179 (9), 133 (11), 120 (10), 118 (18), 117 (20), 115 (12), 106 (9), 105 (100), 104 (21), 91 (20), 77 (38).

Calyxolane B (2): colorless oil; $[\alpha]^{22}_D$ 0° (*c* 0.4, $CHCl_3$); IR (neat) 3049, 3027, 2958, 2920, 2844, 1637, 1440, 1250, 1091, 1015, 787, 688 cm^{-1}; UV (CH_3OH) λ_{max} 206 (ϵ 16 000) nm; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; HREIMS *m/z* (rel int) [M^+] calcd for $C_{16}H_{16}O$ 224.1201, found 224.1202 (54), 223 (25), 210 (8), 194 (66), 193 (65), 179 (47), 178 (25), 133 (36), 120 (28), 118 (39), 117 (74), 116 (39), 115 (53), 106 (16), 105 (100), 104 (33), 91 (10), 90 (65), 77 (71).

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References and Notes

(1) Taken in part from the Ph.D. Dissertation of O. M. Cóbar, University of Puerto Rico, 1996.
(2) Presented in part at the 19th Senior Technical Meeting of the American Chemical Society, Puerto Rico Section, Villa Parguera Guest House, Lajas, Puerto Rico, 3−4 November, 1995.
(3) Undergraduate student supported by the NIH-MARC U*STAR Program of the University of Puerto Rico.
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(7) EIMS of an aliquot of calyxolane A treated with BSTFA showed that **1** remains underivatized as no active functional groups are present on this molecule.
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