Trisphaerolide A, a Novel Polyketide from the Dominican Sponge *Erylus trisphaerus*

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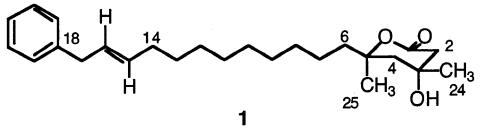
The structure of trisphaerolide A (**1**), a mildly cytotoxic metabolite isolated from extracts of the marine sponge *Erylus trisphaerus* collected in Dominica, has been elucidated by detailed analysis of spectroscopic data. Trisphaerolide A (**1**) has a putative polyketide biogenesis, incorporating a rare variant on the standard pathway, which involves the addition of methyl branches arising from C-2 carbons of acetate units to chain carbons that arise from C-1 of acetate units.

It has been proposed that a promising therapeutic strategy to selectively target cancer cells, which commonly have mutations in the p53 tumor suppressor gene, relative to normal cells, which have wild-type p53, would be to administer a DNA-damaging agent in combination with a G2 specific cell cycle checkpoint inhibitor.[1] Few G2 checkpoint inhibitors are known, and most of them are of limited clinical potential because they also interact with a broad range of other cellular processes. Therefore, it is of considerable importance to find new and specific G2 checkpoint inhibitors. Toward this end, a cell-based assay developed in one of our laboratories has been used to screen marine invertebrate and terrestrial plant extracts for their ability to inhibit the G2 cell cycle checkpoint.[2,3] Extracts of the Dominican marine sponge *Erylus trisphaerus* showed promising activity in the assay, and a bioassay-guided fractionation was initiated to isolate and identify the active component(s). Trisphaerolide A (**1**), a novel mildly cytotoxic polyketide, which was inactive in the G2 checkpoint inhibition assay, was isolated in the course of this work. Details of the isolation and structure elucidation of **1** are reported below.



1

The dark purple marine sponge *Erylus trisphaerus* was collected by hand using scuba at −5 to −10 m near Pointe Baptiste, Dominica. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. Extraction of thawed sponge specimens with MeOH, followed by fractionation of the concentrated MeOH extract via sequential application of Sephadex LH20 chromatography, centrifugal silica gel chromatography, and semipreparative HPLC, gave trisphaerolide A (**1**) as a white solid. The HREIMS of trisphaerolide A (**1**) contained a molecular ion at *m*/*z* 386.2818 consistent with a molecular formula of $C_{25}H_{38}O_3$ (calcd 386.2821). The ^{1}H, ^{13}C, DEPT, COSY, HSQC, and HMBC NMR data obtained from **1** readily identified a carboxyl group (^{13}C δ 169.9, s), a phenyl group (^{1}H δ 7.26, m, H-19/H-23; 7.17, m, H-21; 7.16, m, H-20/H-22; ^{13}C δ 141.2, C-18; 128.5, C-20/C-22; 128.3, C-19/C-23; 125.8, C-21), a 1,2-disubstituted olefin (^{1}H δ 5.53, dt, J = 15.3, 6.3 Hz, H-15; 5.50, dt, J = 15.3, 6.2 Hz, H-16; ^{13}C δ 132.1, C-16; 128.7, C-15), two oxygenated quaternary carbons (^{13}C δ 84.0, C-5; 69.5, C-3), two methylene groups with differentially shielded geminal protons (^{1}H δ 2.62, dd, J = 17.1, 2.4 Hz, H-2eq; 2.39, d, J = 17.1 Hz, H-2ax; 1.89, dd, J = 14.4, 2.4 Hz, H-4eq; 1.78, d, J = 14.4 Hz, H-4ax; ^{13}C δ 44.4, C-4; 43.8, C-2), a benzylic methylene group (^{1}H δ 3.31, d, J = 6.2 Hz, H-17; ^{13}C δ 39.1, C-17), an allylic methylene group (^{1}H δ 1.99, td, J = 7.0, 6.3 Hz, H-14; ^{13}C δ 32.5, C-14), a further eight methylene groups (^{13}C δ 44.6, C-6, 29.8/29.5/29.5(2C)/29.4, C-8/C-9/C-10/C-11/C-12; 29.1, C-13; 23.4, C-7), and two methyls (^{1}H δ 1.52, s, H-25; 1.36, s, H-24; ^{13}C δ 31.2, C-24; 28.0, C-25). These functionalities accounted for only six of the seven units of unsaturation required by the molecular formula, suggesting the presence of a second ring in the structure of **1**.

HMBC correlations observed between the methylene ^{1}H resonance at δ 3.31 (H-17/H-17′) and ^{13}C resonances assigned to the phenyl group (δ 141.2, C-18; 128.3, C-19/C-23) and the olefin (δ 132.1, C-16; 128.7, C-15) allowed the placement of a methylene group between the phenyl and olefinic moieties. The C-15 olefinic resonance at δ 128.7 showed an additional HMBC correlation to a proton resonance at δ 1.99 (H-14/H-14′), assigned to a pair of allylic methylene protons. COSY correlations were observed between the allylic methylene resonance at δ 1.99 (H-14/H-14′) and both the H-15 olefinic resonance (δ 5.53; dt, J = 15.3, 6.3 Hz) and the benzylic methylene resonance (^{1}H δ 3.31; H-17/H-17′), confirming the presence of the 4-phenyl-2-butenyl fragment (C-14 to C-23) in **1**.

HMBC data also identified the structure of a second major fragment of trisphaerolide A (**1**). The ^{13}C resonances of the oxygenated quaternary carbons (δ 69.5, C-3; 84.0, C-5) were each correlated to a different singlet methyl resonance (^{1}H δ 1.36, H-24; 1.52, H-25), consistent with the downfield shift of the methyl signals in the ^{1}H spectrum. Additional correlations were observed between the methyl resonance at δ 1.36 (H-24) and both of the methylene resonances at δ 43.8 (C-2) and 44.4 (C-4), situating the oxygenated methyl-bearing carbon (C-3; δ 69.5) between the two methylenes (C-2 and C-4). The carboxyl carbon (δ 169.9; C-1) showed HMBC correlations to both of the geminal methylene protons (^{1}H δ 2.62 and

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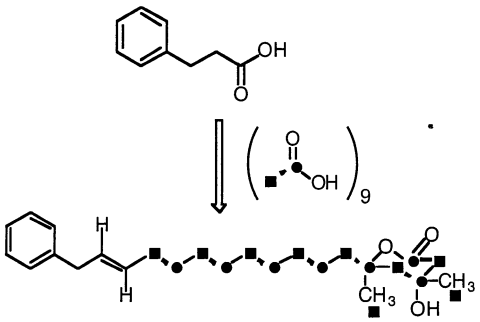

Figure 1. Proposed polyketide biogenesis for trisphaerolide A (**1**).

2.39) on C-2, establishing the point of attachment of C-1. HMBC correlations were observed between the methyl resonance at δ 28.0 (C-25) and both H-4 and H-4′ (δ 1.89, 1.78) resonances, linking C-4 and C-5 (δ 84.0). The protons and carbons of the C-25 methyl group (^{1}H δ 1.52; ^{13}C δ 28.0) and a methylene group (C-6: ^{1}H δ 1.58; ^{13}C δ 44.6) showed mutual HMBC correlations, allowing the carbon chain to be extended further to C-6.

To complete the planar structure of **1**, the second ring required by the molecular formula was assigned to a δ-lactone functionality between C-1 and C-5 in order to accommodate the relative chemical shifts of both C-5 and its attached methyl group in comparison to the equivalent chemical shifts at C-3, which must, as a consequence, carry a hydroxyl group. Since the only unassigned carbons remaining were aliphatic methylene groups, the elucidation of the carbon skeleton could be completed by joining the 4-phenyl-2-butenyl and δ-lactone partial structures by inserting a chain of seven methylene carbons between C-6 and C-14.

The stereochemistry of the $\Delta^{15,16}$ olefin could be assigned as *E* by consideration of the coupling constant between H-15 and H-16 (15.3 Hz) as well as the downfield shift of the allylic methylene carbon (C-14, ^{13}C δ 32.5). The existence of long-range coupling between H-2eq (^{1}H δ 2.62, dd, J = 17.1, 2.4 Hz) and H-4eq (^{1}H δ 1.89, dd, J = 14.4, 2.4 Hz), confirmed by the presence of COSY correlations between them, suggested that they were in a W-configuration around a chair conformation of the six-membered δ-lactone ring, and thus equatorial. This assignment allowed the relative stereochemistry around the lactone ring to be determined by a series of 1D NOESY experiments. In particular, irradiation of H-4ax (δ 1.78) caused enhancement of the signals of H-2ax (δ 2.39), H-6 (δ 1.58), and H-24 (δ 1.36), requiring both C-24 and C-6 to be equatorial. Enhancement of the signal at δ 1.89 (H-4eq) upon irradiation of H-25 (δ 1.52) is consistent with the axial orientation of the C-25 methyl group, as shown.

Trisphaerolide A (**1**) was inactive in the G2 checkpoint assay but did show modest in vitro cytotoxicity (IC_{50} 30 μg/mL) against MCF7 human breast cancer cells. Figure 1 shows a proposed biogenesis for **1** that suggests it is a polyketide derived from a phenylpropanoic acid starter unit that is extended in the normal manner with seven acetate units to give the linear carbon skeleton of the phenyl substituent, which terminates in the δ-lactone. In this proposal, the carbons on the chain (C-3 and C-5) that bear the branching methyl groups are derived from C-1 of acetate residues. This represents a rather rare variation on the polyketide pathway[4] that is known to occur in metabolites of marine bacteria[5] and marine dinoflagellates.[6] Feeding studies[4−6] have shown that the branching methyls in this polyketide variant come from C-2 carbons of acetate units, as illustrated in Figure 1. The fact that this variation on the polyketide pathway is known from marine microorganism metabolism raises the possibility that trisphaerolide A (**1**) is not produced by sponge cells but is a metabolite of a symbiotic or dietary microorganism.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO J-1010 polarimeter equipped with a halogen lamp (589 nm) and a 10 mm microcell. ^{1}H, ^{13}C, COSY-gr, HSQC-gr, and HMBC-gr spectra (optimized for $^{2,3}J$ = 8 Hz) for **1** were recorded on a Bruker AMX500 NMR spectrometer, while the 1D NOESY spectra were measured on a Bruker AV400 NMR spectrometer. Chemical shifts were referenced to solvent peaks (δ_H 7.24 ppm, δ_C 77.0 ppm for $CDCl_3$). Low-resolution ES mass spectra were recorded on a Bruker Esquire LC mass spectrometer. The HREI mass spectrum was recorded on an AEI MS-50 mass spectrometer. Centrifugal chromatography was performed using a Chromatotron (Harrison Research). HPLC separations were achieved using a Waters 600 pump and a Waters 486 tunable absorbance detector. Solvents were all HPLC grade (Fisher), and those used for HPLC were filtered prior to use.

Animal Material. A type sample of *Erylus trisphaerus* (De Laubenfels) (Demospongiae, order Astrophorida, family Geodiidae) has been deposited in the Zoologisch Museum, University of Amsterdam, under sample #ZMA POR. 17068.

Extraction and Isolation. The dark purple sponge, *Erylus trisphaerus*, was collected by scuba diving at 5−10 m off Pointe Baptiste, Dominica (N 15°35′20″, W 61°20′30″) in 2000. Frozen sponge (70 g) was repeatedly extracted with methanol, assisted by sonication. The combined methanolic extracts were passed through a lipophilic gel column (Diaion HP 20, 75 mL) preequilibrated with methanol. The eluent was diluted with water (100 mL), run through the column again, and for a third time after dilution of the eluent to 3 L with water. The column was washed with water (150 mL) and the adsorbed material eluted sequentially with 20% acetone/water, 55% acetone/water, and acetone (150 mL each). Each fraction was concentrated under vacuum. Inspection of the crude fraction eluting with acetone showed **1** as a UV active spot on TLC at R_f 0.45 (eluent: 5% methanol in 1% acetic acid/chloroform). This fraction was first separated by gel permeation chromatography (Sephadex LH20, eluent: methanol) followed by silica gel centrifugal chromatography (eluent: 1, 3, and 5% methanol in 1% acetic acid/chloroform). Trisphaerolide A (**1**) was obtained as a pure compound by normal-phase HPLC (eluent: 20−40% ethyl acetate in hexane) in 0.01% yield, based on the dried extracted weight of the sponge.

Trisphaerolide A (1): white solid; $[\alpha]^{27}_D$ −5.3° (*c* 0.32, methanol); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (2H, m, H-19/H-23), 7.17 (1H, m, H-21), 7.16 (2H, m, H-20/H-22), 5.53 (1H, dt, J = 15.3, 6.3 Hz, H-15), 5.50 (1H, dt, J = 15.3, 6.2 Hz, H-16), 3.31 (2H, d, J = 6.2 Hz, H-17), 2.62 (1H, dd, J = 17.1, 2.4 Hz, H-2eq), 2.39 (1H, d, J = 17.1 Hz, H-2ax), 1.99 (2H, td, J = 7.0, 6.3 Hz, H-14), 1.89, (1H, dd, J = 14.4, 2.4 Hz, H-4eq), 1.78 (1H, d, J = 14.4 Hz, H-4ax), 1.58 (2H, m, H-6), 1.52 (3H, s, H-25), 1.36 (3H, s, H-24), 1.34 (2H, m, H-13), 1.25 (12H, m, H-7/H-8/H-9/H-10/H-11/H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9 (s, C-1), 141.2 (s, C-18), 132.1 (d, C-16), 128.7 (d, C-15), 128.5 (d, C-20/C-22), 128.3 (d, C-19/C-23), 125.8 (d, C-21), 84.0 (s, C-5), 69.5 (s, C-3), 44.6 (t, C-6), 44.4 (t, C-4), 43.8 (t, C-2), 39.1 (t, C-17), 32.5 (t, C-14), 31.2 (q, C-24), 29.8/29.5/29.5(2C)/29.4 (t, C-8/C-9/C-10/C-11/C-12), 29.1 (C-13), 28.0 (q, C-25), 23.4 (t, C-7); EIMS *m*/*z* 386 $[M]^+$ (2), 368 (30), 284 (52), 271 (6), 143 (96), 131 (28), 130 (43), 129 (30), 125 (76), 117 (62), 104 (64), 103 (94), 101 (98), 91 (100), 85 (46), 69 (15), 55 (37); HREIMS *m*/*z* 386.2818 (calcd for $C_{25}H_{38}O_3$, 386.2821).

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