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Tethered Lipids from *Thapsia garganica*

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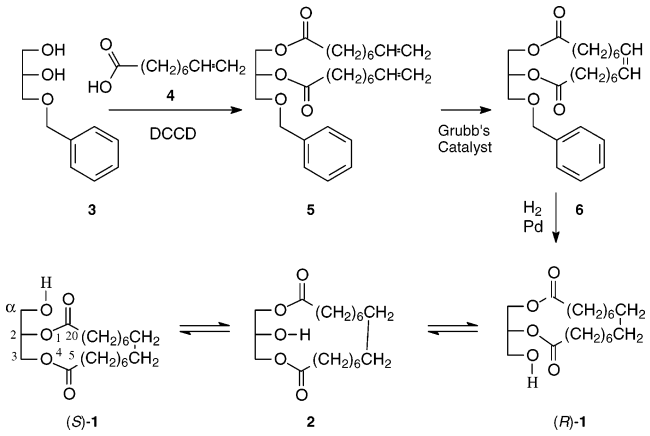
Abstract: The two macrocyclic lipids 2-hydroxymethyl-1,4-dioxacycloicosane-5,20-dione (**1**) and 3-hydroxy-1,5-dioxacyclohenicosane-6,21-dione (**2**) have been isolated from the fruits of *Thapsia garganica*. Tethered lipids are unprecedented in the plant kingdom.

Tethered lipids are lipids in which two of the oxygens of glycerol are included in a macrocyclic ring. The compounds have pronounced effects on the membranes of cells affording modulation of the activities of enzymes such as protein kinases and phospholipases. These properties have encouraged syntheses and testing of a number of such lipids.[1−3] Tethered lipids have never been found in nature except for archaebacteria, e.g., *Methanospirillium hangatei*.[4] The presence of terpenoid glycerol ethers in the membrane is assumed to enable the bacteria to grow in hostile environments. No other living organism has previously been reported to form tethered lipids. The present report, however, documents the presence of this group of compounds in the fruits of the Mediterranean plant *Thapsia garganica* L. (Apiaceae).

Our attempt to develop thapsigargin into a drug for treatment of prostate cancer[5,6] has made *T. garganica* a focus for our research for some years.[7] Encouraging developments in this project prompted us to isolate thapsigargin in 50 g amounts. In-depth examination of the NMR spectra of the obtained thapsigargin revealed the presence of unexpected signals. Rechromatography afforded two impurities, compounds **1** and **2**. The presence of the AB part of an ABX patterns at 3.74 ppm, two double doublets at 4.38 and 4.26 ppm, and a complex signal at 5.13 ppm, together with signals characteristic for α-protons in esters and signals that could be assigned to ester carbonyl carbons, strongly suggested compound **1** to be a 1-*O*,2-*O*-diacylglycerol. The absence of a signal originating in a terminal methyl group could only be explained by assuming the compound to be a tethered lipid. A molecular formula of $C_{19}H_{34}O_5$ proved the compound to be a diester of hexadecanedioic acid (**1**). Compound **2** possessed the same molecular formula, but the NMR spectrum revealed the presence of only one AB part of an ABX spectrum. Structure **2** explains this finding.

Scheme 1



Compound **1** possessed a small positive specific rotation. Synthesis of **1** (Scheme 1) established the absolute configuration at C-2 as *R*. An esterification of (*R*)-1-*O*-benzylglycerol (**3**) with 8-nonenoic acid (**4**) facilitated by dicyclohexylcarbodiimide (DCCD) afforded the diester **5**, which by treament with Grubb's first-generation catalyst was converted into the macrocyclic intermediate **6**. Palladium on charcoal catalyzed hydrogenation and hydrogenolysis yielded *S*-**1** with a specific rotation of −3.3°. The overall yield of the three steps was 48%. The negative rotation of

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the *S*-form proved that **1** isolated from *T. garganica* is the *R*-form. The considerably smaller numerical value of the specific rotation is explained by facile acyl migration between the oxygens on glycerol (Scheme 1). Leaving a solution of **1** in toluene with some silica gel added further support to this hypothesis. After stirring overnight, a considerable amount of **2** was observed by NMR. A silica gel-catalyzed migration of the acyl groups between O-1, O-2, and O-3 apparently occurs, eventually establishing equilibrium between (*R*)-**1**, **2**, and (*S*)-**1**. During the repeated chromatographic procedures for isolating **3** no doubt similar acyl migrations have occurred, affording some racemization of **1** and formation of **2**. The optical activity of **1**, although limited, reveals that *R*-**1** must be genuine. The possibility of **2** being an artifact formed during the purification procedure cannot be excluded.

Experimental Section

Plant Material. Fruits of *Thapsia garganica* L. were collected on the island of Ibiza, Spain, in July 2002. A voucher specimen is deposited at The Herbarium at the University of Copenhagen DFHSBC1.

Extraction and Isolation. The fruits (1 kg) were blended with 3 L of ethanol, and the mixture was left overnight. The mixture was filtered and the filtrate concentrated in vacuo. The residue was dissolved in toluene (1 L) and the solution washed three times with 330 mL of water. The organic phase was concentrated to give approximately 60 g of an oily dark residue. The residue was chromatographed over silica gel 60 (800 g, Merck 07734, 0.063−0.20 mm) and eluted with dichloromethane−ethyl acetate (20:1) with 0.5% of acetic acid, to which increasing amounts of ethyl acetate were added. The fractions containing thapsigargin were combined and concentrated to give 15 g of a viscid oil. A part of the residue (10 g) was rechromatographed over LiChroprep RP-18 (500 g, Merck 13900, 40−63 μm) and eluted with methanol−water (7:3) with 1% of acetic acid, to which increasing amounts of methanol were added, to give thapsigargin (4.0 g). The ^{1}H NMR spectrum contained some unexpected signals. Rechromatography over silica gel 60 (75 g) eluted with toluene−ethyl acetate (9:1) with 1% of acetic acid afforded compound **1** (200 mg, 300 ppm of plant material) and compound **2** (150 mg, 225 ppm of plant material).

2-Hydroxymethyl-1,4-dioxacycloicosane-5,20-dione (1): viscid oil; $[\alpha]^{25}_D$ +1.0° (*c* 0.097, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.13 (1H, ddd, J = 3.0, 5.1, 5.7, 6.5 Hz, H-2), 4.38 (1H, dd, J = 3.0, 12.3 Hz, H-3), 4.26 (1H, dd, J = 6.5, 12.3 Hz, H-3′), 3.75 (1H, dd, J = 5.7, 12.0 Hz, H-α), 3.72 (1H, dd, J = 5.1, 12.0 Hz, H-α′), 2.34 and 2.32 (4H, two overlapping t, J = 7.2 Hz, H-6 and H-19), 1.64 (4H, m, H-7 and H-18), 1.30 (20H, m, H-8−H-17); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.0 and 173.7 (C, C-5 and C-20), 72.4 (CH, C-2), 62.9 (CH_2, C-3), 61.5 (CH_2, C-α), 34.6 and 34.4 (CH_2, C-6 and C-19), 28.5 and 28.4 (CH_2, C-7 and C-18), 28.2, 27.9, 27.7, 27.6, 27.5, 27.4, 27.1, 27.0, 25.0, and 24.8 (CH_2, C-8−C-17); HRFABMS *m*/*z* 343.2514 $[M + H]^+$ (calcd for $C_{19}H_{35}O_5^+$, 343.2484); 365.2328 $[M + Na]^+$ (calcd for $C_{19}H_{34}NaO_5^+$, 365.2304).

3-Hydroxy-1,5-dioxacyclohenicosane-6,21-dione (2): viscid oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.15 (2H, dd, J = 12.0, 3.0 Hz, H-2 and H-4), 4.14 (2H, dd, J = 12.0, 3.0 Hz, H-2′ and H-4′), 4.04 (1H, quintet, J = 3.0, H-3), 2.33 (4 H, t, J = 7.2 Hz, H-7 and H-20), 1.62 (4H, m, H-8 and H-19), 2.27 (20H, m, H-9−H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.1 (C, C-6 and C-21), 68.5 (CH, C-3), 65.2 (CH_2, C-2 and C-4), 34.3 (CH_2, C-7 and C-20), 28.5, 28.4, 28.1, 25.0 (CH_2, C-8−C-19); HRFABMS *m*/*z* 343.2506 $[M + H]^+$ (calcd for $C_{19}H_{35}O_5^+$, 343.2484); 365.2319 $[M + Na]^+$ (calcd for $C_{19}H_{34}NaO_5^+$, 365.2304).

Synthesis of Compound (*S*)-1. To a solution of (*R*)-3-benzyloxy-1,2-propandiol (**3**, 200 mg, 1.1 mmol), **4** (400 mg, 2.5 mmol), and 4-(dimethylamino)pyridine (1.20 g, 9.8 mmol) in dry CH_2Cl_2 (60 mL) was added at 0 °C a solution of DCCD (2.02 g, 9.8 mmol) in dry CH_2Cl_2 (4 mL). The solution was filtered after stirring at room temperature for 4−5 h, and the precipitate rinsed with CH_2Cl_2. The solvent was evaporated in vacuo. Compound (*S*)-**5** (350 mg, 74%) was isolated by column chromatography over silica gel eluted with toluene−ethyl acetate (40:1). To a solution of (*S*)-**5** (229 mg, 0.5 mmol) in CH_2Cl_2 (55 mL) was added over 1 h a solution of Grubb's first-generation catalyst (50 mg) in CH_2Cl_2 (27 mL) at room temperature. After stirring for an additional 18 h at room temperature, the solvent was removed in vacuo. Compound (*S*)-**6** (160 mg, 68%) was obtained as a viscid oil by column chromatography of the residue over silica gel eluted with toluene−ethyl acetate (50:1), $[\alpha]^{25}_D$ +17.7° (*c* 0.10 $CHCl_3$). A 50 mL flask containing a solution of (*S*)-**6** (194 mg, 0.45 mmol) in ethanol−acetic acid (10:1 v/v, 14 mL) and 100 mg of palladium on charcoal (10%) was sealed with a rubber stopper. A syringe through the stopper connected 200 mL of hydrogen in a balloon to the contents of the flask. After stirring for 2 h the mixture was diluted with CH_2Cl_2 (30 mL) and filtered through Celite. The filtrate was concentrated in vacuo to give (*S*)-**1** (194 mg) as a viscid oil, $[\alpha]^{25}_D$ −3.3° (*c* 0.91, $CHCl_3$). The NMR spectra could be superimposed on those of (*R*)-**1** isolated from the plant material.

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Supporting Information Available: Tabulated NMR spectra of compounds **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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