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Polygonophenone, the First MEM-Substituted Natural Product, from *Polygonum maritimum*

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Abstract: An unprecedented natural acetophenone, polygonophenone (**1**), and a new resorcinol, polygonocinol (**2**), were isolated from the dichloromethane and methanol extracts of *Polygonum maritimum* and identified as 2-hydroxy-4-[(2-methoxyethoxy)methoxy] acetophenone and 2-methyl-5-nonadecylresorcinol, respectively. In addition, 11 known compounds were identified, namely, the sesquiterpenoid (+)-8-hydroxycalamene, four ferulic acid esters (tetracosyl, hexacosyl, octacosyl, and triacontyl ferulate), the arylpropane broussonin B, the flavonoids quercetin, quercitrin, and (+)-catechin, the hydroquinone glucoside isotachioside, and β-sitosterol. The structures of the new compounds were elucidated on the basis of their NMR and MS data. It is noteworthy that polygonophenone (**1**) is the first naturally methoxyethoxymethyl (MEM)-substituted natural product, and its isolation gives support for the use of MEM protection in biomimetic synthetic schemes.

More than 30 years after the introduction of methoxyethoxymethyl (MEM) as a protecting group in organic synthesis by Corey et al.,[1] numerous examples of its use have been described. Although the use of the MEM protecting group is considered as a classic synthetic strategy, the identification of a naturally MEM-substituted compound isolated from *Polygonum maritimum* L., as described herein, is a striking example of additional plant chemodiversity.

The plant *P. maritimum* belongs to the family Polygonaceae, which is comprised of 45 genera.[2] Members of the genus *Polygonum* are found all over the world, but mainly occur in regions with a temperate climate. The genus *Polygonum* is separated into about 200 species, and 36 of these grow in Europe.[3] Many of them show medicinal properties, while others are cultivated and a few are ornamental plants. *P. maritimum* is a perennial herb or small shrub 20−50 cm in height that can be found in sandy coasts in Europe, America, South Africa, and the Mediterranean region.

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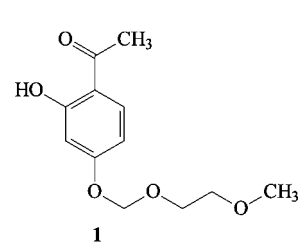
Several studies on the genus *Polygonum* have shown the presence of several flavonoids and other phenolics.[4−6] A very interesting property of *Polygonum* species is their capability to accumulate heavy metals from the soil.[7]

On the basis of phytochemical interest in the genus *Polygonum*, we investigated the chemical constituents of *P. maritimum* from Greece. This investigation led to the isolation and structure elucidation of two unknown natural products, a new acetophenone, polygonophenone (**1**), and a new resorcinol, polygonocinol (**2**). In addition, a known sesquiterpenoid, four ferulic acid ester derivatives, an arylpropane, three flavonoids, a hydroquinone glucoside, and a phytosterol were identified.



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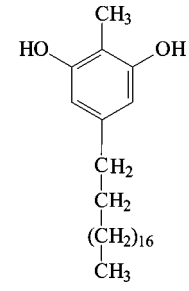

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Polygonophenone (**1**) was isolated as a white, amorphous compound, and its molecular formula was determined by HRMS as $C_{12}H_{16}O_5$. The IR spectrum of **1** showed the presence of a conjugated ketone (1637 cm^{-1}) and a hydroxyl forming a hydrogen bond (3312 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of a typical ABX system of three aromatic protons, a characteristically deshielded methyl singlet (2.56 ppm), an aliphatic methoxy group (3.38 ppm), a deshielded singlet (5.30 ppm) corresponding to a doubly oxygenated methylene, and a pair of monoxygenated methylenes (3.55, 3.81 ppm). The latter two methylenes were cross-coupled in the COSY spectrum. The ^{13}C NMR spectrum confirmed the presence of a conjugated ketone (202.4 ppm) and also showed the presence of two oxygenated aromatic carbons, a quaternary carbon, and three other protonated aromatic carbons constituting a trisubstituted aromatic ring. The ^{13}C NMR spectrum, in combination with the DEPT spectrum, confirmed the unusual observation of one doubly oxygenated methylene, two monoxygenated methylenes, and an aliphatic methoxy group, constituting a polyoxygenated side chain.

In the HMBC spectrum, the methyl protons at 2.56 ppm were

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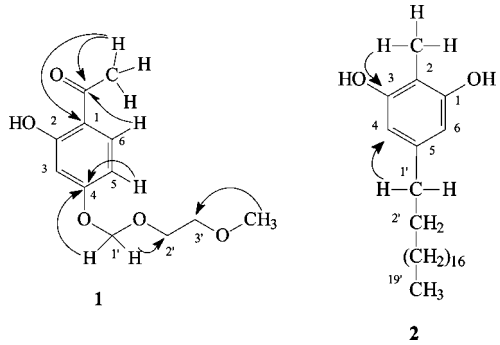

Figure 1. Structures of compounds **1** and **2** and selected HMBC correlations.

correlated with the ketone carbonyl and the nonoxygenated quaternary aromatic carbon, revealing **1** to be an acetophenone. The ketone carbonyl was also correlated only with the aromatic proton at 7.65 ppm, establishing the placement of the ABX system as depicted in Figure 1. The attachment position of the polyoxygenated side chain was confirmed by the HMBC correlations of the doubly oxygenated methylene protons at 5.30 ppm with the oxygenated aromatic carbon at 164.5 ppm on one hand and the monoxygenated methylene at 67.9 ppm on the other hand. The structure of the side chain was established by the ^3J correlation of the terminal methoxy protons with the second monoxygenated methylene at 71.2 ppm.

The placement of the side chain at position 4 rather than position 2 was based on the ^2J correlation of H-5 at 6.56 ppm with the carbon at 164.5 ppm, and not with the carbon 163.3 ppm, and was confirmed by the presence of a hydrogen bond forming hydroxyl, which could be possible only if the hydroxyl was placed at position 2. Consequently, the structure of **1** could be assigned as 2-hydroxy-4-[(2-methoxyethoxy)methoxy]acetophenone. Compound **1** has been previously reported as a synthetic intermediate.[8] To the best of our knowledge, the above-described polyxygenated side chain has never been reported from a natural source, and for this reason **1** may be considered as the first naturally MEM-substituted compound. The isolation of **1** gives support for the use of MEM protection in biomimetic synthetic schemes. The biosynthesis of the MEM side chain remains unknown. To exclude the possibility of an artifactual origin of **1**, the extraction was repeated using different solvents and plant material collected from two different positions and at two different seasons. In all cases, the presence of **1** was confirmed after isolation and NMR spectroscopy, supporting the natural origin of the MEM group.

Polygonocinol (**2**) was isolated as a white, amorphous compound, and its molecular formula was determined by HRMS as $C_{26}H_{46}O_2$. The EIMS showed a quasimolecular ion at *m*/*z* 390, a fragment at *m*/*z* 375 corresponding to the loss of a terminal methyl group, and 17 consecutive fragments differing by 14 amu. This fragmentation pattern suggested a molecule containing a long aliphatic chain. The structure of **2** was determined unambiguously by NMR studies. The ^{1}H NMR spectrum showed a broad singlet at 4.61 ppm that exchanged with D_2O, corresponding to a hydroxyl group. The HMQC spectrum showed that a two-proton singlet at 6.27 ppm corresponded to a two-carbon peak, observed at 107.8 ppm. The two aforementioned carbons, in combination with four deshielded quaternary carbons at δ 107.3 (C-2), 142.1 (C-5), and 154.5 (C-1,3) constituted a tetrasubstituted aromatic ring, with two symmetric oxygenated carbons.

Additionally, the ^{1}H NMR spectrum of **2** showed a three-proton singlet at 2.12 ppm. In the HMQC spectrum, this singlet was correlated with a carbon at 7.7 ppm. From the DEPT spectrum, this carbon corresponded to an aromatic methyl group. Also, the ^{1}H NMR spectrum showed a triplet (J = 8 Hz) at 2.45 ppm, integrating for two protons, a broad singlet at 1.58 ppm, integrating for two protons, a broad singlet at 1.25 ppm, integrating for 32 protons, and a triplet (J = 7 Hz) at 0.85 ppm, integrating for three protons. The COSY spectrum showed that all these protons were cross-coupled, confirming the presence of a long aliphatic chain, as suggested by the EIMS. The alkyl chain was unbranched, because in the DEPT spectrum only one terminal methyl group (14.1 ppm) was observed. The most deshielded alkyl chain protons were observed at 2.45 ppm, indicating that the alkyl chain was directly connected to the aromatic ring. From these data, it was evident that **2** contains an aromatic ring, symmetrically substituted with two hydroxyl groups, a methyl group, and an unbranched aliphatic chain with 19 carbons. The arrangement of these groups on the aromatic ring was established by the HMBC spectrum.

In the HMBC spectrum (Figure 1), the hydrogens of the aromatic methyl group gave a strong ^3J correlation only with the aromatic oxygenated carbons, and consequently, the methyl group should be placed in an *ortho* position between the two hydroxy groups. The H-1′ protons of the aliphatic chain were correlated in the HMBC spectrum only with the protonated aromatic carbons, C-4 and C-6, indicating that the aliphatic chain should be placed at C-5. The final structure of polygonocinol (**2**) was confirmed by the NOESY spectrum, in which the methyl hydrogens were correlated with the hydroxyl proton and not with any of the aromatic hydrogens. These hydrogens were correlated with the hydrogens of the aliphatic chain.

It must be noted that *P. maritimum* represents a new source of alkylresorcinols. Natural products of this category have been found only in 11 families of higher plants. Closely related alkylresorcinols with different side chains[9] or substitution patterns[10] have been reported.

In addition to the two new compounds, 13 other known products were identified: the sesquiterpenoid (+)-*cis*-8-hydroxycalamene,[11] four ferulic acid esters (ferulic acid tetracosyl ester, ferulic acid hexacosyl ester, ferulic acid octacosyl ester, and ferulic acid triacontyl ester),[12] and the arylpropane broussonin B.[13] In addition, the plant was found to be rich in the flavonoids quercetin,[14] quercitrin,[15] and (+)-catechin.[16] Finally, the hydroquinone glucoside isotachioside[17] and β-sitosterol[18] were identified. It should be noted that (+)-*cis*-8-hydroxycalamene, the four ferulic acid esters, broussonin B, and isotachioside were isolated for the first time from a plant of the Polygonaceae family.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu-160A spectrophotometer. The IR spectra were obtained on a Perkin-Elmer Paragon 500 instrument. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in ppm downfield to TMS. The 2D NMR experiments were performed using standard Bruker microprograms. HREIMS were obtained on a AEI MS-902 mass spectrometer.

Plant Material. The plant *Polygonum maritimum* was collected from the Heronisi coast, in Agios Andreas village, in Arcadia County of Peloponnesus, Greece, in August 2004, and identified by one of the authors (E.K.). A voucher specimen (no. KL 176) is deposited in the herbarium of the Department of Pharmacognosy, University of Athens. A second sample (no. KL 176b) was collected from the same region in April 2007 and a third sample (no. KL 176c) from Crete in September 2007.

Extraction and Isolation. The air-dried powder (1.2 kg) of the whole plant of *P. maritimum* was extracted with CH_2Cl_2 (3 × 5 L) and then with CH_3OH (3 × 5 L). The weight of the dried residue of the dichloromethane extract was 17.5 g, and the weight of the methanol extract was 110 g. The total dichloromethane extract was fractionated by column chromatography (CC) over Si gel 60 (Merck, 40−63 μm), using a cyclohexane, CH_2Cl_2, and CH_3OH gradient to afford 125 fractions altogether. Fractions 19−32 (1.6 g) were rechromatographed by CC [Si gel 60 Merck (20−40 μm), cyclohexane, CH_2Cl_2, and CH_3OH gradient] to afford (+)-8-hydroxycalamene (5 mg) and β-

sitosterol (8 mg). Fractions 49−72 (2.5 g) were rechromatographed by CC [Si gel 60 Merck (20−40 μm), cyclohexane, CH_2Cl_2, and CH_3OH gradient] to afford tetracosyl ferulate, hexacosyl ferulate, octacosyl ferulate, triacontyl ferulate (10 mg), and palmitic acid (12 mg). Moreover, fractions 61−200 of the previous column (0.49 g) were rechromatographed by CC [Si gel 60 Merck (20−40 μm), cyclohexane, CH_2Cl_2, and CH_3OH gradient] to afford polygonocinol (**2**) (6 mg). A part of the methanol extract (40 g) was fractionated by column chromatography over Si gel 60 (Merck, 40−63 μm), using a CH_2Cl_2 and CH_3OH gradient, to afford a total of 90 fractions. Fractions 29−39 (1.9 g) were rechromatographed by CC [Si gel 60 Merck (20−40 μm), CH_2Cl_2−CH_3OH gradient] to afford polygonophenone (**1**) (10 mg) and broussonin B (12 mg). Fractions 53−60 (3.4 g) were rechromatographed by CC [Si gel 60 Merck (20−40 μm), CH_2Cl_2−CH_3OH gradient] to afford quercetin (9 mg), quercitrin (7 mg), and (+)-catechin (5 mg). In addition, fractions 211−280 (0.4 g) of the previous column were rechromatographed by MPLC [RP-18 Si gel 60 Merck (20−40 μm), H_2O−CH_3OH gradient] to afford isotachioside (5 mg).

The second and the third samples of *P. maritimum* were extracted directly with EtOH, and the extracts obtained were submitted to chromatographic fractionation, as described above, leading to the reisolation of **1**.

Polygonophenone (1): UV ($CHCl_3$) λ_{max} 272 (4.76), 315 (1.98) nm; IR ($CHCl_3$) λ_{max} 3312, 2919, 1637, 1454, 1367, 1251, 990 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.56 (3H, s, $COCH_3$), 3.38 (3H, s, OCH_3), 3.55 (2H, m, H-3′), 3.81 (2H, m, H-2′), 5.30 (2H, s, H-1′), 6.56 (1H, dd, J = 8.8, 2.4 Hz, H-5), 6.60 (1H, d, J = 2.4 Hz, H-3), 7.65 (1H, d, J = 8.8 Hz, H-6), 12.63 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 25.9 ($COCH_3$), 58.6 (OCH_3), 67.9 (C-2′), 71.2 (C-3′), 92.7 (C-1′), 103.4 (C-3), 107.8 (C-5), 114.4 (C-1), 132.1 (C-6), 163.3 (C-2), 164.5 (C-4), 202.4 (CO); HREIMS *m*/*z* 240.0995 (calcd for $C_{12}H_{16}O_5$, 240.0998).

Polygonocinol (2): UV ($CHCl_3$) λ_{max} 241 (3.46), 271 (2.97), 2.80 (sh) nm; IR ($CHCl_3$) ν_{max} 3400, 3000, 1618, 1591, 1250, cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, t, J = 7.0 Hz, H-19′), 1.25 (32H, br s, H-3′-18′), 1.58 (2H, br s, H-2′), 2.12 (3H, s, CH_3-2), 2.45 (2H, t, J = 8.0 Hz, H-1′), 6.27 (2H, s, H-4,6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 7.7 (CH_3−2), 14.1 (C-19′), 22.7, 29.7, 31.9 (C-2′-18′), 36.1 (C-1′), 107.3 (C-2), 107.8 (C-4,6), 142.1 (C-5), 154.5 (C-1,3); HREIMS *m*/*z* 390.3497 (calcd for $C_{26}H_{46}O_2$, 390.3498).

Supporting Information Available: Spectroscopic data are available free of charge via the Internet at http://pubs.acs.org.

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