Verbalactone, a New Macrocyclic Dimer Lactone from the Roots of *Verbascum undulatum* with Antibacterial Activity

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A novel macrocyclic dimer lactone, named verbalactone, was isolated from the roots of *Verbascum undulatum* and exhibited interesting antibacterial activity. It is the first time that 1,7-dioxacyclododecane is reported as the ring system of a natural product. The structure and the absolute stereochemistry of the new compound were determined by spectral methods and chemical correlation.

The genus *Verbascum*, belonging to the family Scrophulariaceae, comprises more than 300 species. *Verbascum undulatum* Lam. is a biennial plant widely spread in the Balkan peninsula. In previous papers[1,2] we described the isolation of eight iridoid glycosides, derivatives of 6-*O*-α-L-rhamnopyranosyl aucubin, and four phenylethanoid glycosides (verbascoside, martynoside, arenarioside, and 6-*O*-acetyl-martynoside) from the aerial parts of *V. undulatum*.

Investigation of the roots of the plant led to the isolation and identification by means of spectral (1D and 2D NMR, MS) and chemical data of a new macrocyclic dimer lactone, 4*R*,6*R*,10*R*,12*R*,4,10-dihydroxy-2,8-dioxo-6,12-dipentyl-1,7-dioxacyclododecane, named verbalactone (**1**), accompanied by five known iridoid glycosides, harpagoside,[3] laterioside,[4] harpagide,[3] ajugol,[5] and aucubin,[6] and three phenylethanoid glycosides, verbascoside,[7] martynoside,[1] and 2-(3-hydroxy-4-methoxyphenyl)ethanol-1-*O*-α-L-rhamnopyranosyl(1→3)-[β-D-xylopyranosyl(1→6)]-(4-ferruloyl)-β-D-glucopyranoside.[8]

Compound **1**, $[\alpha]_D$ +7.3° (*c* 0.9, $CHCl_3$), was obtained as a colorless oil, and its molecular formula was determined by HRMS as $C_{20}H_{36}O_6$, [HRFABMS *m/z* $[M + H]^+$ 373.2602, calcd 373.2590]. In the EIMS the molecular ion was not observed, and the prominent ion was at *m/z* 187. From the IR spectrum it was clear that verbalactone contained a hydroxyl group (3520 cm^{-1}) and a carbonyl group (1711 cm^{-1}). The hydroxyl group was confirmed by the ^1H NMR spectrum (broad singlet at 3.73 ppm exchanged with D_2O). Interpretation of the above-mentioned spectrum by the COSY spectrum indicated the presence of six protons (1.96−4.94 ppm) participating in a ring and 11 protons (0.85−1.55 ppm) participating in a linear aliphatic chain with one terminal methyl group. The ^{13}C NMR spectrum displayed one carbonyl (172.8 ppm) corresponding to a lactone group and nine sp^3 carbons: one methyl, six methylenes, and two oxygenated methines. In the HMBC spectrum (Figure 1), the oxygenated carbon at 64.67 ppm had a weak ^2J correlation with the proton of the hydroxyl group, revealing that this carbon was bearing the hydroxyl and consequently the other oxygenated carbon (72.49 ppm) was participating in the lactone system. The protons of these two oxygenated methines were correlated with the carbonyl carbon. The carbon of the first methine, bearing the hydroxyl, was correlated with the proton of the second methine (4.94 ppm), which was additionally correlated with C-19 (25.50 ppm) of the aliphatic chain. Moreover, cross-peak correlations were observed between the methylene protons of position 5 (1.96, 2.05 ppm) and the methylene carbon of position 3 (39.32 ppm) on one hand and the methylene carbon 18 (31.64 ppm) of the aliphatic chain on the other hand. In all, compound **1** had a NMR profile very similar to the monomer lactone of 3,5-dihydroxydecanoic acid (**2a**−**d**).[9]

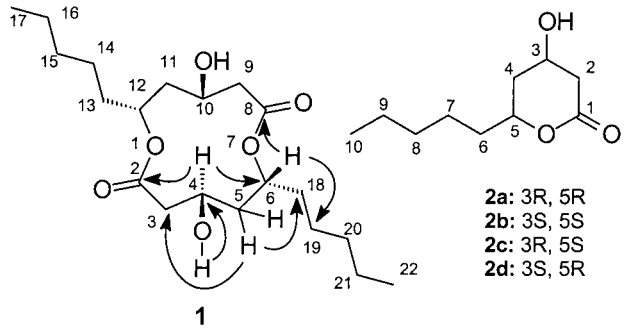

Figure 1. Structures of compounds **1** and **2a**−**d** and selected HMBC correlations for **1**.

The 3*R*,5*R* monomer lactone (**2a**) has never been found in higher plants, only in specific microorganisms. This isomer has been the object of several synthetic approaches.[10−13] The other three isomers **2b**−**d** are not natural products and have recently been synthesized.[9]

The fact that compound **1** has double the molecular weight of lactone **2** and displays small but important differences in its ^1H NMR and ^{13}C NMR spectra compared with those of all the possible stereoisomeres **2a**−**d** suggested that our compound was a symmetric dimer of the lactone **2**. This symmetric structure could explain the observation in the ^{13}C NMR and ^1H NMR spectra of only 10 carbons and 18 protons, respectively. This structure could also explain the difference in the IR spectrum in which the carbonyl of the dimer lactone **1** (1711 cm^{-1}) has a significantly lower energy of vibration, in comparison with **2** (1740 cm^{-1}), due to the larger size of the ring.

The dimer lactone was optically active, and the absolute stereochemistry was defined by chemical correlation. After alkaline hydrolysis and acid lactonization, verbalactone (**1**) yielded a compound identical ($[\alpha]_D$, NMR, MS, IR) with the

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Table 1. Antibacterial Activity (MIC μg/mL) of **1**[a]

Escherichia coli	250
Staphylococccus aureus	62.5
Pseudomonas aeruginosa	500
Klebsiella pneumoniae	250
Salmonella enteritidis	125
Staphylococccus epidermidis	62.5
Enterobacter aerogenes	>500
Lactobacillus casei	125

[a] Ampicillin MIC values for reference strains: *S. aureus*: 0.125 μg/mL. *E. coli*: 8.0 μg/mL.

monomer (3*R*,5*R*) lactone **2a** as the only product, confirming the absolute stereochemistry of **1** as 4*R*,6*R*,10*R*,12*R*. It is noteworthy that the 1,7-dioxacyclododecane is reported here for the first time as the ring system of a natural product.

The new compound was tested against three Gram-positive and five Gram-negative bacteria and exhibited interesting antibacterial properties (Table 1). The most sensitive Gram-positive microorganisms were found to be *Staphylococcus aureus* and *Staphylococcus epidermidis* with MIC = 62.5 μg/mL. The most sensitive Gram-negative microorganism was *Salmonella enteritidis*, with MIC = 125 μg/mL. In general, the MIC values of **1** were comparable with those of the noncyclic trimer of 3,5-dihydroxydecanoic acid, isolated from the marine microorganism *Exophiala pisciphila*.[14]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The IR spectrum was obtained on a Perkin-Elmer Paragon 500 instrument. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 and 200 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in ppm downfield from TMS. The ^{1}H−^{1}H and the ^{1}H−^{13}C NMR experiments were performed using standard Bruker microprograms. EIMS spectra were determined on a HP-6890 and HRMS on a AEI MS-90 spectrometer. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing Si gel 60 Merck (20−40 μm) or RP-18 Si gel 60 Merck (20−40 μm). Preparative TLC was performed on plates coated with Si gel 60 F_{254} Merck, 0.25 mm.

Plant Material. Plant material was collected at Nea Peramos (Attiki region, Greece) in May 1999. A voucher specimen (PROK008) is deposited in the herbarium of the laboratory of Pharmacognosy, University of Athens, Greece.

Extraction and Isolation. Air-dried, pulverized roots of *V. undulatum* (1 kg) were extracted with CH_2Cl_2 (3 × 2 L) and then with MeOH (3 × 2 L). After evaporation of the solvent from the dichloromethane extract, a portion of the residue (1.5 g) was chromatographed with MPLC (cyclohexane, EtOAc gradient) to afford compound **1** (331 mg). A portion of the methanolic extract (1.0 g) was also chromatographed with MPLC (H_2O, MeOH gradient) to afford five known iridoid glycosides, harpagoside (30 mg), laterioside (13 mg), harpagide (7 mg), ajugol (9 mg), and aucubin (14 mg), and three phenylethanoid glycosides, verbascoside (50 mg), martynoside (86 mg), and 2-(3-hydroxy-4-methoxyphenyl)ethanol-1-*O*-α-L-rhamnopyranosyl(1→3)-[*β*-D-xylopyranosyl(1→6)]-(4-ferruloyl)-*β*-D-glucopyranoside (14 mg).

Verbalactone (1): $[\alpha]^{25}_D$ +7.3° (*c* 0.9, $CHCl_3$); IR ν_{max} 3520, 1711, 1269, 1173 cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS, 400 MHz, δ ppm, *J* in Hz) 0.85 (6H, t, *J* = 7.0 Hz, H-17, 22), 1.22−1.31 (12H, m, H-14, 15, 16, 19, 20, 21), 1.49, 1.55 (4H, m, H-13, 18), 1.96 (2H, td, *J* = 15.2, 4.2 Hz, H-5a, 11a), 2.05 (2H, ddd, *J* = 15.2, 10.2, 3.1 Hz, H-5b, 11b), 2.68 (4H, d, *J* = 3.6 Hz, H-3, 9), 3.73 (2H, br, 3-OH, 10-OH), 4.06 (2H, ddd, *J* = 4.2, 3.6, 3.1 Hz, H-4, 10), 4.94 (2H, ddd, *J* = 10.2, 4.7, 4.6 Hz, H-6, 12); ^{13}C NMR ($CDCl_3$/TMS, 50 MHz, δ ppm) 172.85 (C-2, 8), 72.49 (C-6, 12), 64.67 (C-4, 10), 39.32 (C-3, 9), 38.11 (C-5, 11), 31.64 (C-13, 18), 31.36 (C-15, 20), 25.50 (C-14, 19), 22.43 (C-16, 21), 13.93 (C-17, 22); EIMS (70 eV) *m*/*z* 187 (78), 169 (60), 151-(21), 127 (100), 109 (55); HRFABMS *m*/*z* 373.2602 $[M + H]^+$ (Δ 1.2 mmu).

Hydrolysis of 1. To a solution of **1** (10 mg) in MeOH (2 mL) was added Na_2CO_3 (50 mg), and the reaction mixture was refluxed for 1 h. The mixture was neutralized with HCl (1 N) and filtered, and the filtrate evaporated. The residue was dissolved in acetonitrile (2 mL), HCl (1.5 mL, 0.1 N) was added, and the mixture was heated at 80 °C for 1 h, after which it was extracted with CH_2Cl_2−H_2O, and the organic phase was collected and evaporated. Compound **2a** was purified by preparative TLC (CH_2Cl_2−MeOH, 99:1).

Antibacterial Activity. The antibacterial activity was determined by the agar dilution method.[15] The strains of microorganisms employed were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and the following clinical isolates: *Staphylococcus epidermidis*, *Lactobacillus casei*, *Enterobacter aerogenes*, *Salmonella entritidis*, and *Klebsiella pneumoniae*. The microorganisms were maintained on tryptone soya agar (TSA, Oxoid) plates. The inoculum was prepared by culturing each organism in brain heart infusion broth (Oxoid) at 37 °C to a turbidity equivalent to McFarland 0.5 standard (1.5 × 10^8 CFU/mL) and subsequently diluting the organism suspension 1:32. Ten microliters of each diluted inoculum (10^4−10^5 CFU) was applied onto Mueller Hinton II Agar-MHA (BBL) plates containing doubling dilutions of **1** (range of final concentrations: 15.6−500 μg/mL). The MIC was taken as the lowest concentration that inhibited visible growth after incubation at 37 °C for 24 h. Ampicillin was used as reference standard. Plates containing only MHA and MHA and ethanol without **1** served as controls.

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