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Three-Dimensional Structures of OSW-1 and Its Congener

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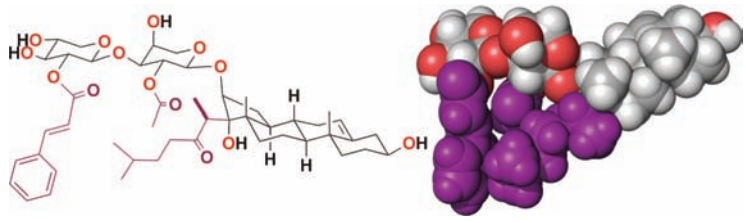
ABSTRACT



The 3D structures of an antitumor glycosylsterol OSW-1 and its closely related congener were investigated by NMR studies and an X-ray crystallographic analysis. The disaccharide moiety was found as a structural scaffold for the formation of a hydrophobic cluster by the biologically required functionalities.

OSW-1 (**1**) and one of the related congeners (**2**) are acylated glycosylsterols isolated from the bulbs of *Ornithogalum saundersiae* (Figure 1).[1] They are structurally unique among an abundant class of natural products called saponins,[2] regarding the position of the disaccharide attachment to the sterol aglycone (at C16β-OH of the D ring) and the presence of acyl side chains on each monosaccharide residue (L-arabinose and D-xylose residues).[1−3] Compound **1** has attracted considerable attention since its discovery because of its exceptionally potent and selective antitumor activities (IC_{50} = 0.25 nM, HL-60 cells) superior to those used in the clinical treatment of cancer such as doxorubicine or paclitaxel.[1b,4] Congener **2** differs from **1** only at the O2″-acyl side chain of the D-xylose residue and is equally potent in the cytotoxicity assay (IC_{50} = 0.20 nM, HL-60 cells).[1b] While the cytotoxicity of compound **1** against certain tumor cells has been related to its ability to induce apoptosis via

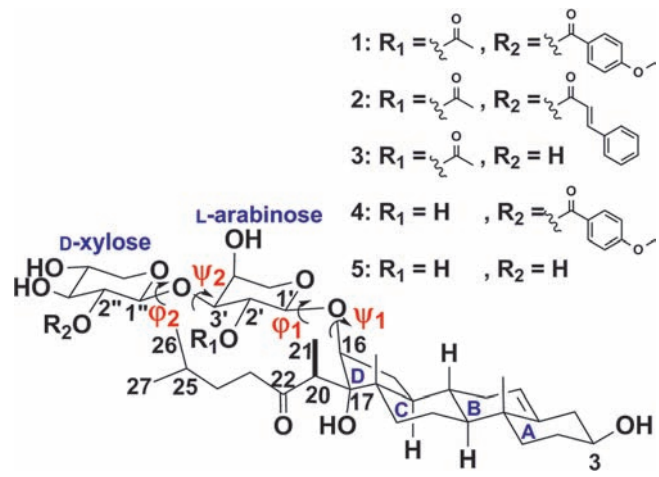


Figure 1. Antitumor glycosylsterols OSW-1 (**1**) and its congeners (**2**−**5**).

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caspase-8 activation,[4] their cellular target(s) or the molecular mechanism of their action is still not well understood.[5]

Synthetic and degradative studies have shown that both the sterol aglycon and the acylated disaccharide moiety are required to retain the extremely potent cytotoxicity against tumor cells exhibited by compounds **1** and **2**.[1,6,7] However, the relationships between the biologically required functionalities and the overall molecular structures of the glycosylsterols have not been investigated in detail. Characterization of the three-dimensional structures of the glycosylsterols should facilitate the elucidation of the structural basis for their biological activity and for the design of more effective analogues. Here we report the structural analysis of the glycosylsterols **1** and **2** by solution NMR studies and the first crystallographically resolved solid-state structure for **2**. We provide new insights into the role of the disaccharide moiety in the overall molecular structure of the glycosylsterols.

The sample material for the glycosylsterols **1** and **2** was prepared from the plant source.[1] Using a standard sample for compound **1**, we first established an HPLC method to rapidly analyze the presence of the desired natural product in the fractions during the purification. This eliminated the need for activity-guided fractionation, which is laborious and time-consuming for a simple preparative purpose. The microwave-assisted extraction method[8] was found to enable efficient extraction of the compound from the crude methanolic extract of the lyophilized plant material. Subsequently, we performed a three-step purification involving dichloromethane extraction, silica gel column chromatography, and ODS column chromatography, which provided both compounds **1** and **2**. Their biological activity was verified by cytotoxicity assay against HeLa cells (**1**: $IC_{50} = 0.5$ nM; **2**: $IC_{50} = 0.7$ nM, HeLa cells).

In the ^{1}H NMR spectra for compounds **1** and **2** in $CDCl_3$, CD_3CN, and CD_3OD (Table S1, Supporting Information), which have different polarity, only one set of peaks was observed with no dramatic chemical shift changes, indicating the preorganized nature of their overall structures.[9] The conformation of the disaccharide moiety and its relative orientation to the aglycon were analyzed on the basis of the conformation of monosaccharide residues and glycosidic linkages defined by a set of φ, ψ dihedral angles.[10] The coupling constants ($^3J_{H,H}$) and intraresidue NOEs for the monosaccharide residues of compounds **1**−**2** correspond to those of the standard chair conformation for of the pyranose rings ($^1C_4$ for the L-arabinose and $^4C_1$ for the D-xylose residues). The 2D experiments in CD_3OD showed interresidue NOEs in agreement with close to *syn* conformations for both of the glycosidic linkages. NOE crosspeaks were observed between H1′ and H16 (also H15 for compound **1**) and between H1″ and H3′, H4′ (Figure 2 and Figure S3, Supporting Information). The glycosidic linkages of oligosaccharides are known to favor a *syn* conformation (for β-D-sugars, $\varphi \approx 60°$, $\psi \approx 0°$),[10b] where the transglycosidic C−H bonds are oriented nearly parallel to each other due to the exo anomeric effect and the nonbonded steric interaction.[11] It thus suggests that the structural characteristics found in oligosaccharides are pronounced in the disaccharide moiety of the glycosylsterols **1** and **2**. As a consequence of having the *syn* conformers for both glycosidic linkages, two monosaccharide residues would be arranged in a linear fashion where the C2′-OH of the arabinose and the C2″-OH of the xylose residues emerge from the same face of their trunk (Figure 2). Consistent with this picture, additional weak NOEs are also present between H26/27 and H32/36, H33/35, and between H26/27 and H34 in compound **1**. It indicates the close proximity between the C2″-*p*-methoxybenzoyl (*p*-MBz) group and the C2′-acetyl group, the C26/27 methyl groups of the C20−C27 sterol side chain (Figure 2). Similarly for compound **2**, NOEs are detected between

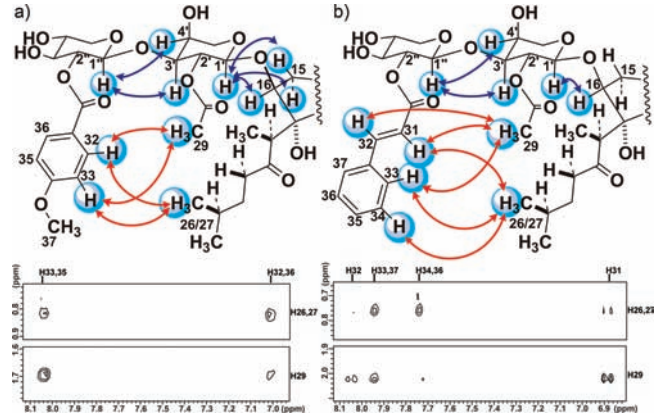

Figure 2. Selected NOESY correlations (500 MHz, 293 K, CD_3OD) for compounds **1** (a) and **2** (b). NOEs shown by blue arrows represent the *syn* conformation for the glycosidic linkages. The interresidue NOEs between the hydrophobic side chains are shown in red arrows (the corresponding crosspeaks in the NOESY spectra are shown in bottom panels).

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(9) Both compounds **1** and **2** are insoluble in water.

(10) The φ_i and ψ_i glycosidic torsion angles are defined as follows: H1−C1−OX−CX and C1−OX−XX−HX, respectively, where X denotes a linkage position (i.e., X = C16 or C3′) and *i* denotes a residue number. See: (a) Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: Amsterdam, 1998; Chapter 3. . (b) Dixon, A. M.; Venable, R.; Widmalm, G.; Bull, T. E.; Pastor, R. E. *Biopolymers* **2003**, *69*, 448.
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H26/27 and H32, H33/35, H34/36 and between H29 and H31, H32. It suggests that the average distances between the C2″-cinnamate and the C2′-acetyl group and the C26/27 methyl groups of the C20−C27 sterol side chain are also within 4 Å. Therefore, the data show that both compounds **1** and **2** having the same disaccharide backbone structure display a similar hydrophobic cluster formed by two acyl side chains of the disaccharide moiety and the sterol side chain. For compound **1**, the same NOE patterns at similar chemical shifts were observed among the three side chains (C2′-acetyl, C2″-*p*-MBz, and the C20−C27 groups) in both protic (CD_3OD) and aprotic solvent (CD_3CN). It indicates that the conformation of the disaccharide determines the relative orientation of the acyl side chains to the sterol side chain independent of the hydrogen-bonding capability of the solvents.

The presence of a stable hydrophobic cluster in solution was also suggested in an observation that the intrinsically less reactive *p*-MBz group of compound **1** can be hydrolyzed preferentially over the acetyl group to give compound **3** instead of compound **4** under conditions using ∼6 M NH_3 in CH_2Cl_2/MeOH = 1:9.[1a] A typical basic hydrolysis condition should give products with a selectivity order of acetyl group > *p*-MBz group.[12] It is in support of the structure where the C2′-acetyl group is sterically protected by the C2″-*p*-MBz group and the sterol side chain.

Despite the ubiquity of saponins in nature, the X-ray structural data for this class of compounds are still scarce largely because of their poor physicochemical properties, which make it difficult to prepare pure samples and crystallize.[2,13] Extensive screening of organic−aqueous mixed solvents with various mixing ratios and employment of a vapor-diffusion method finally led to fine needle-shaped colorless crystals from H_2O−MeCN (1:2) for both compounds **1** and **2**. Only crystals of compound **2** grew to a size suitable for synchrotron radiation diffraction experiments. Single crystals of compound **2** were solved in the orthorhombic, $P2_12_12_1$ space group (a = 5.988(5) Å, b = 27.539(5) Å, c = 28.749(5) Å).[14] The crystal packing shows that two modes of molecular interfaces alternate in a monolayer assembly along the *b* axis (Figure 3a). In one mode, an antiparallel dimer is formed via a hydrophobic interaction between the longest sides of the molecule (Figure 3b). In another, an antiparallel pair of molecules is held together via a hydrophilic sugar−sugar interaction (Figure 3c, left panel). The interface is stabilized by the hydrogen-bonding network, which involves the hydrogen-bonding pairs of the ring oxygen (O5″) of the xylose residue and O4″ (2.90 Å), O4′ and a water molecule (Ow2; 2.71 Å), O3″ and a water molecule (Ow1; 2.92 Å), and the two water molecules (2.74 Å). Along the *a* axis, pairs of the hydrated glycosterols are

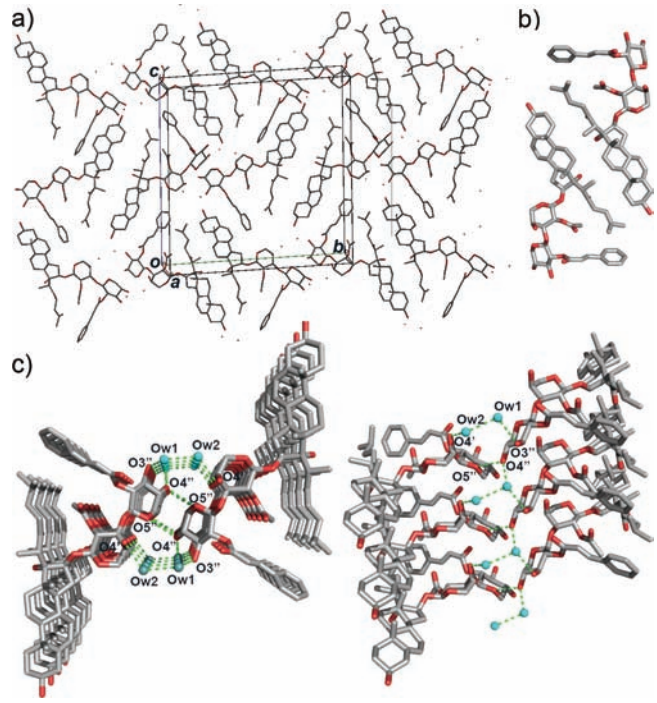

Figure 3. (a) Crystal packing with the unit cell viewed along the *a* axis. Compound **2** is shown in stick representation and water molecules as cyan balls. (b) Hydrophobic dimer. (c) Water-mediated sugar−sugar interaction shown in two perspectives.

stacked in parallel on their flat faces, leading to a zipper-like supramolecular structure (Figure 3c, right panel). The water molecule (Ow1) bound to each layer stabilizes the vertical assembly by hydrogen bonds (2.48 Å) to the C4″−OH group of the next layer. As a consequence, a pair of channels of water molecules is formed at the sugar−sugar interface. Thus, the presence of the acylated disaccharide moiety enables dual modes of supramolecular association for compound **2** in the solid state.

The monomeric structure of compound **2** in the solid state, which was obtained from the aqueous organic solution, is consistent with the NMR analysis for compounds **1** and **2** in organic solvents (Figure 4). Since the structural differences between compounds **1** and **2** are only at the C2″-aromatic ester side chains, it is likely that compound **1** displays similar solid-state characteristics to those of compound **2** in this work. The sterol aglycon displays ring conformations (A−D rings) similar to those found in the crystal structure of cholesterol.[15] The C20−C27 side chain is in fully extended conformations along the long axis of the sterol moiety, and its orientation appears to be stabilized by a hydrogen bond between the C17-OH and C22-carbonyl group pointed to the α face. Both of the pyranose rings are found in the expected chair conformation ($^1C_4$ for the L-arabinose and $^4C_1$ for the D-xylose residues). Because of the exoanomeric effect,[10] both φ angles adopt the gauche conformation ($\varphi_1 = -20.10°$, φ_2

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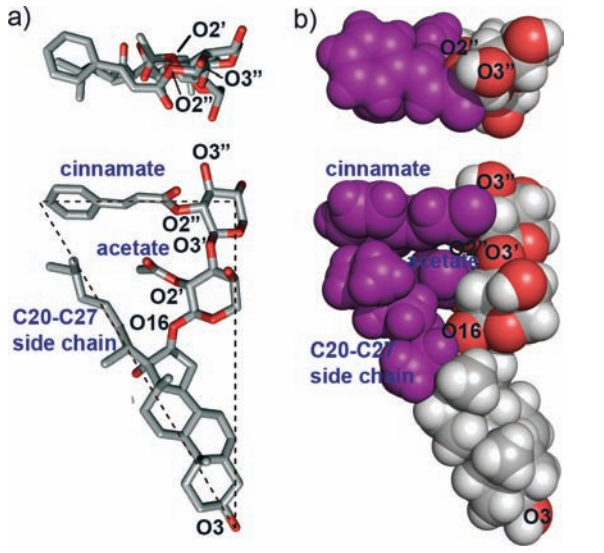

Figure 4. Molecular structure of compound **2** in stick drawing (a) and in CPK representation (b) shown in two perspectives. Dotted line in (a) shows the triangular molecular shape for **2**. Hydrophobic side chains are colored in magenta in (b).

= 4.37°).[16] The ψ angles are found in the range (ψ_1 = −68.77°, and ψ_2 = 47.84°) represented by a preferred conformation predicted in the conformational analysis based on nonbonded steric interactions around the glycosidic linkages.[10] The *syn* conformations for both glycosidic linkages lead the disaccharide moiety to form an overall extended conformation with a slight helical twist with its axis lying on the same plane of sterol with an angle of ∼27° with respect to the long axis of the sterol moiety (Figure 4a). As a result, two notable structural features emerge. First is the specific positioning of the acyl side chains, which results in the generation of a hydrophobic cluster in agreement with our NMR analysis for compounds **1** and **2** (Figure 4b). Both the C2′-acetate and C2″-cinnamate are pointed in the same direction toward the sterol side chain, perpendicular to the long axis of the disaccharide backbone. The C2″-cinnamate and C2′-acetate groups and the C20−C27 sterol side chain pack against each other, making van der Waals contacts. Second, the conjugation of the disaccharide moiety with its relative orientation to the sterol moiety gives rise to a molecular shape of a flat right triangle (Figure 4a bottom). Therefore, the presence of the disaccharide moiety in compound **2** enables the formation of a hydrophobic cluster, distinct molecular shape as well as dual modes of supramolecular structures in the solid state.

The hydrophobic cluster found in this work may have a functional importance based on the previous structure−activity studies for compound **1**. Inversion of the C16 stereochemistry,[6d] which alters the relative orientation of the entire disaccharide moiety, removal of the acyl side chains of the disaccharide moiety,[1,6a−c] or truncation of the sterol side chain[6c] leads to loss of activity by more than 2 orders of magnitude. Thus, the structural modifications, which would abolish the hydrophobic cluster of the glycosylsterols, resulted in inactive compounds. It is of interest to note that upon a closer inspection of the crystal structure for compound **2**, a resemblance can be found in the hydrophobic cluster shown in Figure 4b to a folded structure formed by benzyl group of Phe, methyl group of Ala (or methylene group of Gly), and isoamyl group of Leu residues in proteins. Hydrophobic clusters in proteins are often formed by the side chains of noncontinuous amino acid residues displayed on suitable secondary structures. Some of these found on protein surfaces function as recognition motifs for other proteins to bind.[17] The structural basis for the biological importance of the acylated disaccharide moieties in **1**−**2** may be attributed to their ability to preorganize a hydrophobic recognition motif. Chemists have been inspired by versatile carbohydrate structures and de novo design of bioactive molecules based on carbohydrate scaffolds is an ongoing area of research.[18] The structural motif in the glycosylsterols revealed in this work may represent a possible example for carbohydrate mimicry of a protein motif.[19]

In conclusion, the three-dimensional structures of a highly potent antitumor glycosylsterol OSW-1 (**1**) and its congener **2** were characterized by NMR analysis and X-ray structural analysis using synchrotron radiation, which revealed a flat triangular overall molecular shape. We provide evidence that the disaccharide moiety serves as a structural scaffold in the formation of a hydrophobic cluster motif by the biologically required functionalities as well as of the overall conformation. Our findings also present new opportunities for the design of more effective analogues for **1** and **2** and carbohydrate-based mimics of protein motifs.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) For simplicity, (φ,ψ) are described as in ref 11. The hydrogen atoms were placed geometrically based on the heavy atom positions in the crystal structure.

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