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Sugar/Steroid/Sugar Conjugates: Sensitivity of Lipid Binding to Sugar Structure

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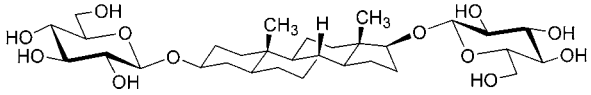
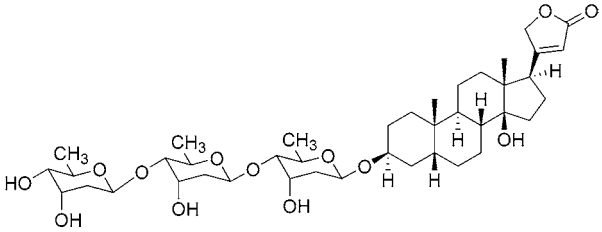
ABSTRACT



Three steroids, each bearing a sugar on rings A and D, have been synthesized. Their effect on the "melting" behavior of a lipid bilayer depends on whether the sugar is glucose, galactose, or mannose. Packing constraints dictate how the lipid bilayer responds to the sugars.

In the bark, fruit, and roots of many plants (as well as in some marine organisms) dwell the saponins, compounds with sugar units covalently bound to one or two sites of a steroid or triterpene framework (the "aglycone"). Saponins are the key ingredient in a host of traditional Chinese, African, and Indonesian medicines.[1,2] Commercial applications of saponins can be ascribed in large measure to their polar/apolar ("amphiphilic") nature. These include foaming agents, emulsifiers, and preservatives, as well as agents for combating cholesterol, microbes, fungi, viruses, tumors, and molluscs.[1,2] Digitoxin, a cardiac glycoside drawn below, is perhaps the most famous member of the clan.



1993 was a good year for saponin synthesis. Nishizawa reported the total synthesis of osladin, an intensely sweet saponin.[3] Danishefsky completed the synthesis of a complex saponin, desgalactotigonin.[4] And Schmidt prepared holotoxin A (a saponin having six hexose units) without resorting to lengthy protection−deprotection procedures.[5] In 1999, Yu and Hui published the synthesis of several saponins by a one-pot sequential glycosylation.[6] We cite the preceding work only as a representative sample of what has been a much more widespread synthetic effort.

Our interest focuses here on synthetic "bidesmosidic" saponins with sugars attached to both rings A and D of a steroid. The resulting sugar/"facial" hydrophobe/sugar combination presented intriguing possibilities with regard to colloidal properties in water. In particular, we wondered how such a molecule would interact with a lipid bilayer, considering that (1) sugar groups are not expected to tolerate a bilayer's hydrophobic interior, (2) such a saponin would be too short to span an entire membrane from one polar surface to the other, and (3) since steroids are rigid, the saponin molecule, which would presumably align parallel to the bilayer's lipid chains, cannot readily "loop" back to place both sugars at the same membrane surface. Uncertainties in membrane adsorption, caused by the hydrophobic and geometric restrictions, led us to synthesize double-glycosy-

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(1) Hostettman, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, England, 1995.

(2) Waller, G. R., Yamasaki, K., Eds. *Saponins Used in Traditional and Modern Medicine*; Plenum Press: New York, 1996. Waller, G. R., Yamasaki, K., Eds. *Saponins Used in Food and Agriculture*; Plenum Press: New York, 1996.

(3) Yamada, H.; Nishizawa, M. *Synlett* **1993**, 54.

(4) Randolph, J. T.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1993**, *115*, 8457.

(5) Han, X.-B.; Jiang, Z−H.; Schmidt, R. R. *Liebigs Ann. Chem*. **1993**, 853.

(6) Yu, B.; Yu, H.; Hui, Y.; Han, X. *Tetrahedron Lett*. **1999**, *40*, 859.

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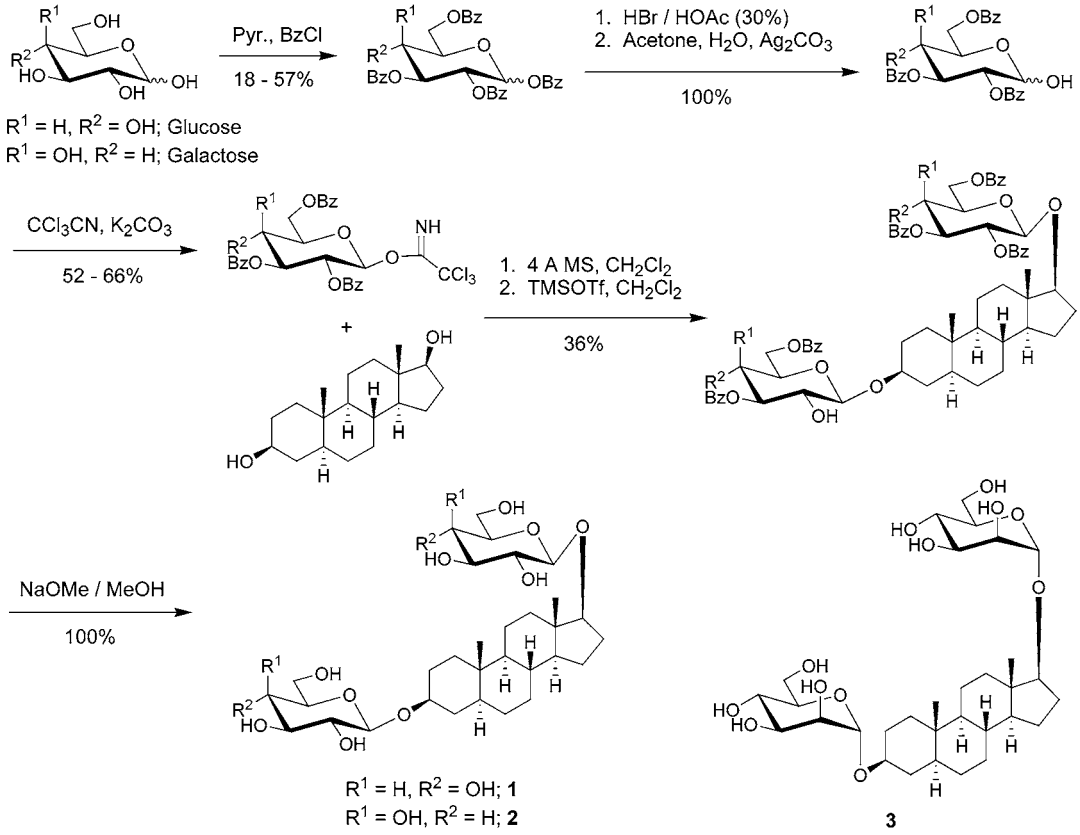
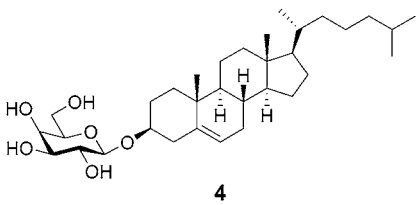
Scheme 1



lated saponins with three different sugars (glucose, galactose, and mannose). As will be shown, their membrane binding displayed an unexpected sensitivity to the sugar structure.

The synthesis of the glucose−glucose and galactose−galactose saponins (**1** and **2**, respectively) from 5-α-androstane-3-*β*,17-*β*-diol is given in Scheme l. Mannose−mannose saponin **3**, with its α-configuration at the glycosidic center, was made in an identical fashion. Yields reported in Scheme 1 have not been optimized. Structure verifications consisted of ^{1}H and ^{13}C NMR, FAB−HRMS, and EA (see Supporting Information for full details). None of the three compounds was soluble to the extent of 0.1 wt % in water at room temperature.

For comparison purposes, the monodesmosidic compound **4**, with a single galactose fixed to a cholesteryl aglycone, was prepared in a 75% overall yield using the general procedure given in Scheme 1.



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Many saponins interact with cell membranes, as manifested, for instance, in the capacity to induce hemolysis in red blood cells.[7] We have previously examined the effect of the monodesmoside digitonin on giant vesicles.[8] While digitonin's membrane behavior is well-established and understandable given its "conventional" amphiphilic structure, the interaction mode of our present novel bidesmosides, with their dual polar moieties, was not obvious a priori. To develop a preliminary picture of their comparative properties, we turned to differential scanning calorimetry (DSC), which measures the influence of incorporated components on the fluidity of membrane bilayers. Thin films of **1**, **2**, **3**, or **4** plus dipalmitoylphosphatidylcholine (DPPC), in a molar ratio of 1:10, were prepared by drying chloroform solutions in a glass vial under a stream of N_2 followed by vacuum drying for at least 16 h. Hydration of the mixed films, achieved by adding Milli-Q-purified water (5 mg/2 mL), was accomplished by manually shaking while warming with a heat gun to produce a homogeneous opaque suspension. This suspension was vortexed for 1 min, sonicated in a water bath for l h at 70−85 °C, and then subjected twice to a freeze−thaw cycle (−20 to 23 °C) to provide samples suitable for DSC. Typical runs, obtained with a Hart Scientific DSC instrument, were carried out with a 30 min hold at 20 °C and an ensuing scan up to 60 °C at a 5 °C/h heating rate to detect the endothermic peak resulting from the DPPC bilayer "melting" (i.e., a conversion from a "solid" gel phase into a more fluid

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(8) Menger, F. M.; Keiper, J. S. *Angew. Chem., Int. Ed.* **1998**, *37*, 3433.

liquid-crystalline phase). Duplicate scans on fresh samples indicated that the data are reproducible.

Pure DPPC bilayers display a sharp melting peak at a T_m = 41.3 °C. Incorporating 10 mol % cholesterol into the DPPC bilayer broadens the melting peak while lowering the T_m to about 39.5 °C. The DSC plots for DPPC containing 10 mol % **1**−**4** are given in Figure 1. Saponin **3** plus DPPC

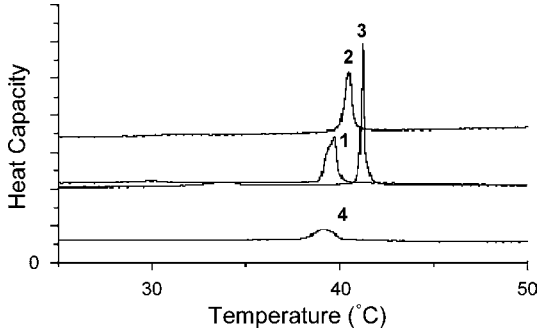

Figure 1. Endothermic plots for 10 mol % **1**−**4** in DPPC obtained by DSC at a scanning rate of 5 °C/h.

gives a sharp DSC peak virtually identical in position and shape to that of pure DPPC. In contrast, the T_m of the galactose−galactose **2** system is shifted downward to 40.5 °C, and the peak is noticeably broadened. The T_m for DPPC plus the glucose−glucose saponin **1** is further shifted to 39.6 °C with the peak being now substantially broadened. Since the bilayers are overwhelmingly composed of the carrier lipid, DPPC, the seemingly small variations in peaks caused by the saponins are in fact worthy of note. First, the shifts in T_m for **1** and **2** are indicative of membrane incorporation. Second, despite their general structural similarities, saponins **1**−**3** provided T_ms having nontrivial differences. How might these unexpected differences be explained in molecular terms?

Assuming the saponins **1**−**3** did incorporate into the vesicles, we can offer the following interpretation of the data. Two packing modes for the bidesmosidic saponins in a bilayer can be envisioned (Figure 2). It is possible that two saponins line up to span a normal lipid bilayer. Alternatively, a single saponin molecule might span a bilayer that, via interdigitation, locally contracts to accommodate the guest. Of the two models, we prefer the latter because it does not demand that polar sugars embed themselves within a hydrocarbon environment (as does the former model). The interdigitation model is in fact not a new idea; biophysical studies have been carried out on bilayers incorporating a component quite similar in concept to the present saponins, a bolaamphiphile (a double-headed surfactant with a hydrophobic length roughly half of the bilayer thickness).[9,10] It was proposed that the membrane narrows itself by interdigitating in the vicinity of the bolaamphiphile molecules.

(9) Lemmich, J.; Honger, T.; Mortensen, K.; Ipsen, J. H.; Bauer, R.; Mouritsen, O. G. *Eur. Biophys. J.* **1996**, *25*, 61.
(10) Duwe, H. P.; Kaes, J.; Sackman, E. *J. Phys. France* **1990**, *51*, 945.

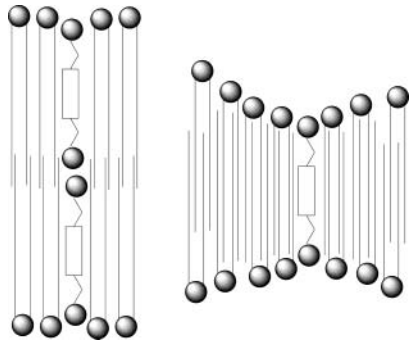

Figure 2. Schematic models for the adsorption of bidesmosidic saponin-like compounds into a bilayer. The shaded circles represent polar groups, and the rectangles represent the steroidal aglycone.

Keeping the interdigitation model in mind, we can explain the DSC differences among the three sugars with one additional assumption: a guest molecule within a membrane bilayer causes maximal perturbation to the motional freedom of the phospholipid chains (and thus to their melting behavior) when the guest and lipid chains have intimate contact. If this is true, then one sees from Figure 3 that

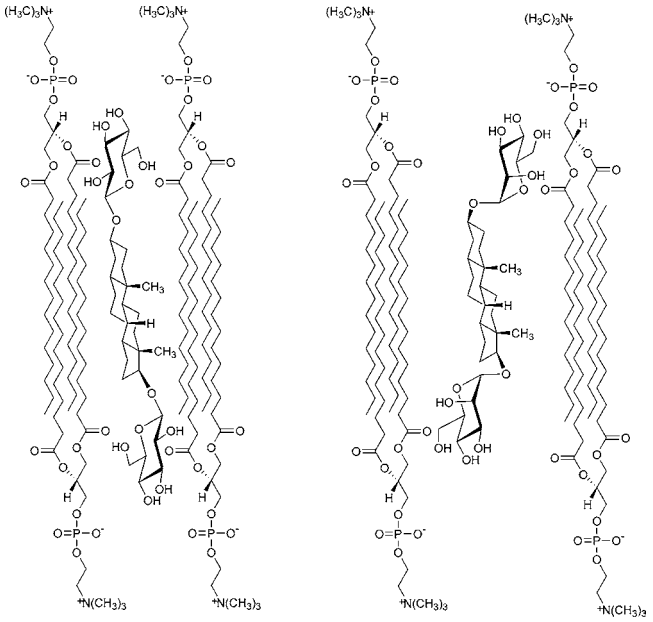

Figure 3. Structural representation of the glucose−glucose steroid **1** (left) and the mannose−mannose steroid **3** (right) interdigitated into a DPPC bilayer.

glucose−glucose **1** should have a greater T_m reduction than mannose−mannose **3** (with galactose−galactose **2** being intermediate in behavior) as is observed. Stated in another way, even if mannose−mannose **3** causes large defects due to its axial glycosidic linkage, the large interdefect regions of the phospholipid manifest a rather normal DSC.

We note that although it is tempting to account for the lack of a T_m shift with **3** by speculating that it did not enter the membrane bilayer, there was no visual evidence of the water-insoluble bolaamphiphile floating in the suspension which resembled that of the other samples analyzed. An alternate rationale for **3**'s T_m can be offered in terms of a "canceling out" effect, whereby the decrease in T_m afforded by perturbation of bilayer chain movement is countered by the increase in T_m associated with contraction of the bilayer.[11] Since **3** alone lacks a T_m shift, the canceling-out rationale would seem to be an ad hoc addition to our model.

The DSC of **4**, in which the melting plot almost disappears into the baseline, is consistent with the interdigitation model. With only a single sugar, this compound can slip its steroid moiety between the chains without inducing a bilayer contraction. The resulting tight packing has a dramatic and long-range effect on the DSC.

In summary, three bidesmosidic saponins were synthesized and evaluated for their influence on phospholipid membranes. The surprising dependence of the DSC data on the nature of the sugar may be understandable in terms of packing constraints arising from sugar stereochemistry. The preparation and analysis of additional analogues containing sugar moieties, including branched saccharides and xylose derivatives, may provide more insight into the nature of bidesmosidic saponin−membrane interaction.

Acknowledgment. This work was supported by the National Institutes of Health.

Supporting Information Available: Synthetic details and characterization data, including ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis for compounds **1**−**4**, as well as DSC sample preparation and procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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