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Dodecyl sorbitan ethers as antimicrobials against Gram-positive bacteria

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ABSTRACT

A range of amphiphilic sorbitan ethers has been synthesized in two steps from sorbitan following an acetalization/hydrogenolysis sequence. These sorbitan ethers and the acetal intermediates have been evaluated as antimicrobials against Gram-negative and Gram-positive bacteria. No antimicrobial activity was observed for Gram-negative bacteria. However, the compounds bearing a linear dodecyl chain exhibit antimicrobial activity (MIC as low as 8 μg/mL) against Gram-positive bacteria such as *Listeria monocytogenes*, *Enterococcus faecalis* and *Staphylococcus aureus*. Encouraged by these preliminary results, dodecyl sorbitan was tested against a range of resistant strains and was found to be active against vancomycin-, methicillin- and daptomycin-resistant strains (MIC = 32–64 μg/mL).

Antimicrobials are essential compounds to prevent and cure bacterial infections.[1] In this respect, they are widely used in medicine but also in other fields such as in cosmetics, food,[2] textile[3] and packaging.[4] However, the misuse and overuse of antimicrobials have led to the emergence of antimicrobial resistance (AMR). AMR presents a serious problem for public health as it threatens the prevention and the treatment of bacterial infections and compromises the success of major surgery and cancer chemotherapy.[5] More worrying, humanity is now facing infections by superbugs that are resistant to all known antimicrobial chemotherapy.[6] In this context, the development of new effective antimicrobials is highly desirable.

Sorbitan esters, known under the trade name Span®, are surface active compounds with very low impacts on health (*i.e.* no acute toxicity) and environment (*i.e.* readily biodegradable).[7] For example, sorbitan monolaurate (Span 20, E493), one of the most representative compound of this family, is widely used in food,[8] pharmaceutical and cosmetic industries.[9] Similarly to other carbohydrate fatty esters such as sucrose monolaurate,[10] lauroylmaltose and maltotriose,[11] and methyl 6-*O*-lauroyl-α-D-glucopyranoside,[12] sorbitan monolaurate exhibits interesting antimicrobial activity against Gram-positive bacteria. However, its activity could be seriously altered by the hydrolysis of the ester bond by cellular esterases, producing the corresponding fatty acid and the inactive polyol. For example, it has been shown that monolaurin can be quickly hydrolyzed ($t_{1/2}$ of about 5 min) in the presence of *Staphylococcus aureus* cells.[13] In order to increase the life-time of antimicrobials, the *ester* linkage could be replaced by an *ether* group thus preventing such hydrolysis. For examples, 1-*O*-dodecylglycerol exhibits an enhanced activity against *Enterococcus faecium* than monolaurin[14] and methyl 6-*O*-dodecanyl-α-D-glucopyranoside has an improved activity against *S. aureus* and *Listeria* spp. compared to its ester counterpart.[12] However, such approach has never been reported on sorbitan derivatives, probably due to the difficulty to prepare the corresponding ethers. In this context, we have previously prepared a range of sorbitan ethers[15] as robust analogues of span derivatives and we now report their antimicrobial activity against Gram-positive bacteria.[16]

A range of sorbitan ethers has been synthesized in two steps from sorbitan – a dehydration product of sorbitol – following an acetalization/hydrogenolysis sequence (Scheme 1).[15] First, acid-catalysed acetalization of non-protected sorbitan **1** with linear aldehydes gave a range of sorbitan acetals **2**–**6** with 29–81% isolated yields.[17] Noteworthy, these compounds exist as a mixture

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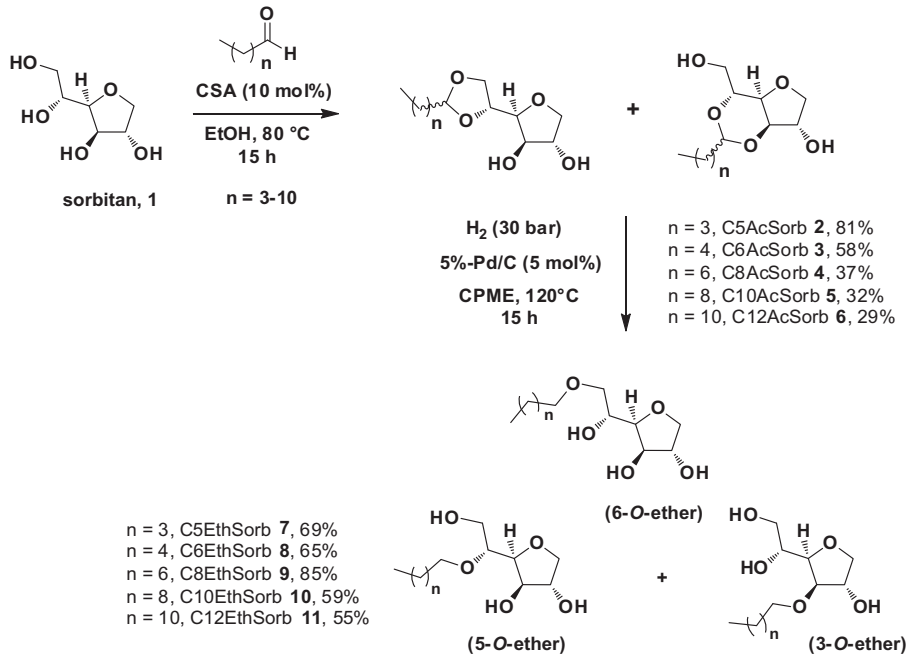

Scheme 1. Preparation of sorbitan ethers from sorbitan through acetalization/hydrogenolysis sequence.

Table 1
Antimicrobial activities of sorbitan acetals **2**–**6** and sorbitan ethers **7**–**11** against Gram positive bacteria.

Entry	Compounds	MIC values (μg/mL)[a]		
		L. monocytogenes (CIP 103575)	*E. faecalis* (ATCC® 29212™)	*S. aureus* (ATCC® 29213™)
1	C5AcSorb **2**	> 512	> 512	> 512
2	C5EthSorb **7**	> 512	> 512	> 512
3	C6AcSorb **3**	> 512	> 512	> 512
4	C6EthSorb **8**	> 512	> 512	> 512
5	C8AcSorb **4**	> 512	> 512	> 512
6	C8EthSorb **9**	> 512	> 512	> 512
7	C10AcSorb **5**	> 512	> 512	> 512
8	C10EthSorb **10**	> 512	> 512	> 512
9	C12AcSorb **6**	8	8	32
10	C12EthSorb **11**	32	32	32
11	dodecanal	> 512	> 512	> 512

[a] MIC values were determined by serial dilution (2 by 2) of each compound from 512 μg/mL to 0.25 μg/mL.

of five-membered and six-membered isomers. Subsequent reductive cleavage of the mixture with hydrogen furnished the corresponding sorbitan ethers **7**–**11** with 55–85% isolated yields.[17] These species were obtained as an inseparable mixture of regioisomers with the alkyl chain grafted either at the position 3, 5 or 6. Noteworthy, this lack of selectivity is not detrimental for such low-added value compounds (Span 20 is actually a mixture of products).

The antimicrobial activity of sorbitan acetals (abbreviated CxAcSorb, with x = number of carbons of the alkyl chain, Ac = acetal, Sorb = sorbitan) and sorbitan ethers (abbreviated CxEthSorb, with x = number of carbons of the alkyl chain, Eth = ether, Sorb = sorbitan) were first evaluated against Gram-negative bacteria, namely, *Escherichia coli* (ATCC® 8739™) and *Pseudomonas aeruginosa* (ATCC® 27853™) by microdilution as previously described.[18] Unfortunately, no antimicrobial activity was observed for these bacteria (MIC >512 μg/mL). As a result, their antimicrobial activities were next evaluated against Gram-positive bacteria such as *Listeria monocytogenes* (CIP 103575), *Enterococcus faecalis* (ATCC® 29212™) and *S. aureus* (ATCC® 29213™). The results are gathered in Table 1.

No antimicrobial activity has been detected for sorbitan acetals **2**–**5** or ethers **7**–**10** bearing an alkyl chain with ten or less carbons (Table 1, entries 1–8). On the contrary, sorbitan acetal C12AcSorb **6** exhibits a significant antimicrobial activity against *S. aureus* (MIC = 32 μg/mL) and was found slightly more active against *L. monocytogenes* and *E. faecalis* (MIC = 8 μg/mL) (Table 1, entry 9). In that case, hydrolysis studies have been performed in order to determine whether the antimicrobial activity arises from the acetal derivative itself or from the hydrolyzed product. These studies were conducted at 40 °C at pH = 7 (physiological pH) but also at pH = 5 and 1 in order to get further information on the stability of such compounds (Fig. 1). At pH = 1, about 70% of the acetal was hydrolyzed after the first hour and the compound was totally degraded after 20 h. In sharp contrast, at pH = 7 or 5, only a slight degradation (about 4–6%) was observed after 1 h at 40 °C and about 92–94% of the compound still remains after 20 hours at this temperature. These results indicate that the rate of hydrolysis of acetal is slow under these conditions.

Moreover, the antimicrobial activity of dodecanal – the remaining amphiphilic product after hydrolysis – was also evaluated and no significant antimicrobial activity was observed (Table 1, entry

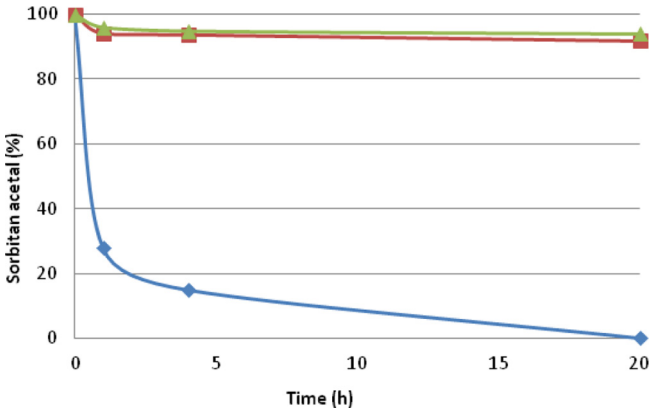

Fig. 1. Hydrolysis studies of sorbitan acetal at 40 °C at pH = 7 (▲), pH = 5 (■) or pH = 1 (◆).

11). From these experiments, it is clear that the antimicrobial activity of acetal C12AcSorb **6** is due to the compound itself. The corresponding ether, C12EthSorb **11**, was next considered. Satisfyingly, this compound was found to be active against all strains with a MIC of 32 μg/mL (Table 1, entry 10). Finally, these results show that only sorbitan derivatives bearing a dodecyl chain exhibit interesting activities.

Encouraged by these preliminary results, a wider range of bacterial strains has been studied. Nevertheless, considering the hydrolysis potential of acetals, only C12EthSorb **11** was considered for further evaluation. *L. monocytogenes* strains including a reference (CIP 103575) and three clinical isolated strains were first investigated (Table 2). Interestingly, C12EthSorb **11** was found active for all clinical strains with a MIC of 16 μg/mL (Table 2, entry 1). However, this compound is by far less active than amoxicillin. Indeed, the MIC of this classical β-lactam antibiotic is about 1–2 μg/mL for all strains studied (Table 2, entry 2).

The antimicrobial activity of compound C12EthSorb **11** was next studied against *E. faecalis* (ATCC® 29212™, 015206179901 and 015205261801) and *E. faecium* strains (CIP 103510, Van A 0151850763 and 015205731401) (Table 3). The compound was found to be active against all strains with MIC of 8–16 μg/mL. More importantly, it is also active against a vancomycin-resistant strain at 16 μg/mL (Van A – 0151850763) that is 16 times more active than amoxicillin.

A wide range of *S. aureus* strains was finally considered to establish the scope of the antimicrobial activity of C12EthSorb **11** and oxacillin was used as reference. The compounds were tested against two reference, six methicillin-resistant and three daptomycin-resistant *S. aureus* strains (Table 4). Satisfyingly, C12EthSorb **11** gave interesting antimicrobial activities (32–64 μg/mL) for most strains. Contrary to oxacillin that is very active against ATCC 25923 and ATCC 29213 strains and less effective against resistant bacteria, C12EthSorb **11** displays similar activities on resistant strains.

In conclusion, we have demonstrated that sorbitan ethers could have interesting antimicrobial activities against Gram-positive bacteria providing an alkyl chain length of 12 carbons. Dodecylsorbitan ethers (mixture of 3 isomers) were found to be active (MIC as low as 8 μg/mL) against *L. monocytogenes*, *E. faecalis, E. faecium* and *S. aureus*. Moreover, this compound presents a moderate antimicrobial activity against some antibiotic-resistant strains (MIC = 32–64 μg/mL). Considering the levels of antimicrobial activity reached, this compound is more likely to be used as preservative than as therapeutic agent.

Table 2
Antimicrobial activity of C12EthSorb **11** against *L. monocytogenes* strains.[a.]

Entry	Compounds	MIC values (μg/mL)[a]			
		CIP 103575	LM1[b]	LM2[b]	LM3[b]
1	C12EthSorb **11**	32	16	16	16
2	amoxicillin	1	1	2	1

[a] MIC values were determined by serial dilution (2 by 2) of each compound from 512 μg/mL to 0.25 μg/mL.
[b] Clinical isolated strains: LM1: 015189074801; LM2: 015170199001; LM3: 015181840701.

Table 3
Antimicrobial activity of C12EthSorb **11** against *E. faecalis* and *E. faecium* strains.

Entry	Compounds	MIC values (μg/mL)[a]					
		E. faecalis			*E. faecium*		
		ATCC 29212	015206179901	015205261801	CIP 103510	0151850763	015205731401
1	C12EthSorb **11**	8	16	8	16	16	8
2	amoxicillin	0.5	0.5	0.5	32	256	128

[a] MIC values were determined by serial dilution (2 by 2) of each compound from 512 μg/mL to 0.25 μg/mL.

Table 4
Antimicrobial activity of C12EthSorb **11** against *S. aureus (references), methicillin- and daptomycin-resistant S. aureus.*

Entry	Compounds	MIC values (μg/mL)[a]										
		S. Aureus		methicillin-resistant *S. Aureus*						daptomycin-resistant *S. aureus*		
		ATCC 25923	ATCC 29213	LAC USA 300	MU3	HT 2004–0012	LY 1999–0053	HT 2002–0417	HT 2006–1004	ST 2015–0188	ST 2014–1288	ST 2015–0989
1	C12EthSorb **11**	32	32	32	64	32	32	32	32	64	64	256
2	oxacillin	32	0.5	64	256	32	64	64	128	64	64	128

[a] MIC values were determined by serial dilution (2 by 2) of each compound from 512 μg/mL to 0.25 μg/mL.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.09.015.

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