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Positional isomers of thioxylobiose, their synthesis and inducing ability for D-xylan-degrading enzymes in the yeast *Cryptococcus albidus*[*,†]

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ABSTRACT

Isomeric *S*-linked 2-thioxylobiose **10**, 3-thioxylobiose **17**, and 4-thioxylobiose **19** were conveniently prepared by SN2 displacement of suitable triflylglycoses with the sodium salt of 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranose, either in *N,N*-dimethylformamide, or in oxolan in the presence of a sodium complexing agent. Allyl 3,5-*O*-isopropylidene-2-*O*-trifluoromethanesulfonyl-β-D-lyxofuranoside was a convenient electrophilic precursor for **10**, which was smoothly obtained after a short sequence of deprotection involving conversion to the 1-propenyl glycoside. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose and 1,2,3-tri-*O*-benzoyl-4-*O*-trifluoromethylsulfonyl-β-L-arabinopyranose were the respective precursors for **17** and **19**. 4-Thioxylobiose has a highly stimulatory effect on the synthesis of enzymes of the xylanolytic system in the yeast *Cryptococcus albidus* when applied to the cells in the presence of the natural disaccharide inducer (1→4)-β-D-xylobiose.

INTRODUCTION

The xylanolytic system of the yeast *Cryptococcus albidus* has been shown to comprise three enzymes which differ in their function and cellular localization, namely, an extracellular *endo*-(1→4)-β-D-xylanase (EC 3.2.1.8), a plasma membrane localized β-D-xyloside permease involved in the transport of D-xylose oligosaccharides, and an intracellular β-D-xylosidase (EC 3.2.1.27) which hydrolyses xylobiose or xylotriose to D-xylose[3].

The xylanolytic system is inducible by (1→4)-β-D-xylobiose or (1→4)-β-D-xylotriose, oligosaccharides derived from the polysaccharide. Methyl β-D-xylopyranoside is also a very efficient inducer. The system can also be induced by positional isomers of (1→4)-β-D-xylobiose, namely, (1→2)-β-D-xylobiose and (1→3)-β-D-xylobiose; however, the behavior of these compounds suggested that they may not function as direct

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inducers, but rather as precursors of other inducing species[3]. The enzyme activities appear much earlier in the presence of (1→4)-β-D-xylobiose than in the presence of positional isomers. It has been, in fact, further established that (1→2)-β-D-xylobiose and (1→3)-β-D-xylobiose can be transformed into (1→4)-β-D-xylobiose inside the induced cells by a pathway involving hydrolytic and glycosyl transfer reactions catalyzed by β-D-xylosidase and D-xylanase[4]. Although there are examples of glycanase induction by positional isomers of oligosaccharides derived from the corresponding natural polysaccharide, *e.g.*, induction of cellulase by sophorose[5,6], the unexpected response of the cells of *C. albidus* to positional isomers of xylobiose prompted us to reinvestigate the nature of the natural inducer of the xylanolytic system using thio analogs of xylobioses that would be resistant to the enzyme transformation.

1-Thioglycosides[7-9] and analogs of oligosaccharides having a sulfur atom in the interglycosidic linkage[10] have been shown to be excellent "gratuitous" inducers of glycosidases, and their first application as inducers of cellulase and xylanase also proved to be promising[11,12]. Such thio analogs of the three positional isomers of xylobiose have now been prepared. This includes an improved access to 4-thioxylobiose[12]. The compounds were examined for their resistance to xylanolytic enzymes, and for their induction ability, in the anticipation of finding a new efficient xylanase inducer and explaining the behavior of linkage isomers of D-xylobiose.

RESULTS AND DISCUSSION

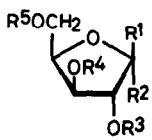
Synthesis of thioxylobioses **10**, **17**, *and* **19**. — Thiooligosaccharides which bear a sulfur in place of an oxygen atom at the interglycosidic linkage may be conveniently prepared by SN2 reactions of activated thioglycoses with secondary trifluoromethanesulfonates[10]. This procedure has been used for the preparation of octa-*O*-acetyl-β-2-thiosophorose[13,14]. However, despite claims to the contrary[13], all attempts to deprotect the 2-thiodisaccharide using basic or acidic reagents failed[14]. An alternative synthesis involving hydrolysis of methyl β-2-thiosophoroside was no more successful[14], pointing out a peculiar reactivity of 2-thiodisaccharides which has been attributed to the participating ability of sulfur in reactions at a vicinal anomeric position[10]. 2-Thiosophorose, however, has been recently prepared by use of a more readily cleavable anomeric protecting group, such as the 1-propenyl substituent[15]. This approach has now been extended to the preparation of 2-thioxylobiose.

Glycosidation of D-xylose with allyl alcohol, in the presence of hydrogen chloride under kinetic conditions, gave in good yield a 1:1 mixture of anomeric allyl D-xylofuranosides (**1**) which were subsequently protected at C-3–C-5 by acetonation yielding, after chromatographic separation, the α-D (**2**) and β-D anomer (**3**). The anomeric configuration of **2** and **3** was assigned on the basis of the chemical displacement, in the n.m.r. spectrum, for C-1 which was found to resonate at a field 7.3 p.p.m. higher for the β-D anomer (**3**) as compared to the α-D anomer (**2**), in agreement with data reported in the literature for pairs of anomers in the furanose series[16].

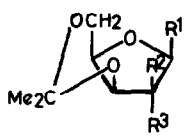
As expected from steric considerations, inversion of configuration at C-2 in **3** by

pyridinium dichromate oxidation, followed by sodium borohydride reduction of the resulting uloside **4**, was completely stereoselective, resulting in a 76% yield of the D-*lyxo* isomer **5**. Electrophilic activation of **5** was obtained by conventional esterification with trifluoromethanesulfonic anhydride in dichloromethane, in the presence of pyridine. Displacement of the resulting triflate group of **6** with the sodium salt of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose[17] was carried out in *N,N*-dimethylformamide solution and was completed within 4 h at room temperature, affording the protected 2-thioxylobioside **7** in 68% yield. Confirmation of the position and anomeric configuration of the thiodisaccharide linkage came from the ^{13}C- and ^{1}H-n.m.r. spectra which showed a strong shielding for C-2 (δ 53.8) and H-2 (δ 3.57) in **7** with a large *trans*-diaxial coupling constant of 9.8 Hz for H-1′,2′.

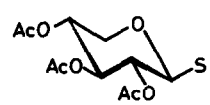
Rearrangement of the allyl group in **7**, under neutral conditions, with tris-(triphenylphosphine)rhodium(I) chloride[18] in aqueous ethanol–toluene, provided the

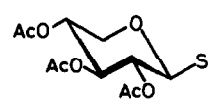


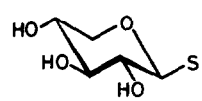
1 R^1,R^2 = H, OCH_2CHCH_2; $R^3 = R^4 = R^5$ = H
2 R^1 = H, $R^2 = OCH_2CHCH_2$; R^3 = H ; $R^4,R^5 = Me_2C$
3 $R^1 = OCH_2CHCH_2$; R^2 = H; R^3 = H; $R^4,R^5 = Me_2C$



4 $R^1 = OCH_2CHCH_2$; R^2R^3 = O
5 $R^1 = OCH_2CHCH_2$; R^2 = OH; R^3 = H
6 $R^1 = OCH_2CHCH_2$; $R^2 = OSO_2CF_3$; R^3 = H

7 $R^1 = OCH_2CHCH_2$, R^2 = H, R^3 = 

8 $R^1 = OCHCHCH_3$, R_2 = H, R^3 = 

9 $R^1 = OCH_2CHCH_3$, R^2 = H, R^3 = 

10 R = H
11 R = Ac

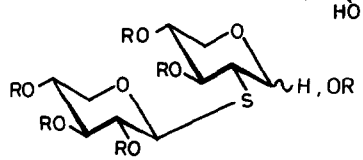


TABLE I

^{1}H-N.m.r. data (400 MHz, D_2O) for 2-thioxylobiose (**10**), 3-thioxylobiose (**17**), and 4-thioxylobiose (**19**)

Compound	*Anomer*	*Chemical shift* (δ)											
		H-1	*H-2*	*H-3*	*H-4*	*H-5*a	*H-5*e	*H-1'*	*H-2'*	*H-3'*	*H-4'*	*H-5'*a	*H-5'*e
10	α	5.48d	3.11dd	3.83dd	3.76–3.66m	[a]	[a]	4.80d	3.36t	3.56–3.49m	3.76–3.66m	3.40m	4.09m
	β	4.81d	2.83dd	3.61dd				4.75d	3.40t				
17	α	5.27d	3.77	3.17t	[a]	[a]	[a]	4.64d	3.40t	3.55t	3.80–3.70	3.43	4.11d
	β	4.67d	3.46dd	2.94t	3.76	3.49dd	4.15dd	4.78d					
19	α	5.53d	3.66dd	3.75t	3.13td	3.97t	3.89dd	4.64d	3.41t	3.72t	3.52m	[a]	4.08dd
	β	4.66d	3.35t	[a]		[a]	4.14dd	4.63d					

		Coupling constants (Hz)											
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5e}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'e}$	$J_{5a,5e}$
10	α	3.4	10.9	9.0	[a]	[a]	[a]	9.8	9.3	[a]	[a]	[a]	[a]
	β	8.1	10.5	8.8	[a]	[a]	[a]	9.7	9.4	[a]	[a]	[a]	[a]
17	α	3.4	[a]	10.3	[a]	5.2	11.3	9.7	9.2	8.9	[a]	5.4	[a]
	β	7.8	10.5	10.5	[a]								
19	α	3.6	9.4	9.8	[a]	5.0	11.7	9.6	9.6	9.5	[a]	4.8	11.4
	β	7.9	8.5	[a]	[a]		12.2						

[a] Not recorded

1-propenyl 2-thioxylobioside **8** in good yield. This step was followed by sequential Zemplén deacetylation of **8** to **9** and simultaneous smooth hydrolysis at pH ~ 2 of the enol ether and isopropylidene protecting groups in **9**, to afford 2-thioxylobiose (**10**) as an amorphous powder which was finally purified by l.c. The overall yield from the allyl derivative **7** was 70%. The structure of the 2-thiodisaccharide was confirmed by its f.a.b. mass spectrum which showed, in the presence of sodium iodide, a main ion for the cationized molecular species. The position and anomeric configuration of the thioglycosidic linkage were clearly inferred from the ^{13}C- and ^{1}H-n.m.r. spectra (Tables I and II) which showed high field shielding for C-2 and H-2 signals, as compared to the corresponding atoms in (1→2)-β-D-xylobiose[19]; a large coupling constant for $J_{1,2}$, in agreement with a β-D-anomeric configuration at the 1-thioxylopyranosyl moiety and the expected coupling constant for $^{4}C_{1}$ (D) conformations in both xylopyranosyl rings in the 2-thiodisaccharide **10**. Similar structural confirmations were drawn from the ^{1}H-n.m.r. spectrum of the hexaacetate **11** (Table III), which was obtained by pyridine–acetic anhydride acetylation of a 1:1 α,β-anomeric mixture of **10**.

A convenient precursor for the synthesis of 3-thioxylobiose (**17**) is 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethanesulfonyl-α-D-allofuranose[20]. Reaction of the sodium salt of 2,3,5-tri-*O*-acetyl-1-thio-β-D-xylopyranose[17], in dry oxolan, with this readily available triflate, in the presence of 1,7,10-trioxa-4,13-diazacyclopentadecane[13] (Kriptofix 21) for 2 h at room temperature, resulted in the formation of the crystalline 3-thiodisaccharide **12**. Its structure was inferred from the ^{1}H- and ^{13}C-n.m.r. data, which showed high-field signals for C-3 and H-3 of the D-glucofuranosyl unit, in agreement with the thioglycosyl substitution at this site, and a large *trans*-diaxial coupling constant for $J_{1',2'}$ confirming the β-D-anomeric configuration of the D-xylopyranosyl unit.

Selective hydrolysis of the 5,6-*O*-isopropylidene group in **12** to **13** was readily achieved in 50% aqueous acetic acid, allowing chain shortening at C-5 to the corresponding D-xylose derivative by use of a glycol cleavage reagent. Oxydation of the diol **13** was performed, in the dark at 0°, with one mole of sodium metaperiodate. Under these conditions, the molecule was very rapidly cleaved at the C-5–C-6 bond without any overoxidation of the thiol group[21]. Subsequent sodium borohydride reduction of the C-5 aldehydo thiodisaccharide gave the protected 3-thioxylobiose derivative **14** in good overall yield from **12**. The hexa-*O*-acetyl-3-thioxylobioside **15** was then obtained by sequential hydrolysis of the residual 1,2-*O*-isopropylidene protecting group in **14**, in 60% aqueous acetic acid, followed by pyridine–acetic anhydride peracetylation of the resulting partially acylated product. Incidentally, the pure α-D anomer **16** was obtained in crystalline form in an attempted direct crystallisation of the anomeric mixture **15**. Its ^{1}H-n.m.r. spectrum, which showed the expected high-field shielding for H-3 and the signals for both xylopyranosyl units in the $^{4}C_{1}$ (D) conformation, is in agreement with the expected structure **16**. Zemplén *O*-deacetylation of **15** gave 3-thioxylobiose **17**, further characterized by its ^{1}H- and ^{13}C-n.m.r. data (Tables I and II).

4-Thioxylobiose (**19**) had previously been prepared[12] by SN2 displacement of the triflate group in 1,2,3-tri-*O*-benzoyl-4-*O*-trifluoromethylsulfonyl-β-L-arabinopyrano-

TABLE II. ^{13}C-N.m.r. data (50 MHz, D_2O) for 2-thioxylobiose (**10**), 3-thioxylobiose (**17**), and 4-thioxylobiose (**19**)

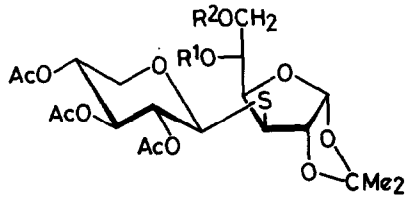
Compound	*Anomer*	*Chemical shift* (δ)									
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*
10	α	94.0	50.5	73.0[a]	70.6	61.3	86.9	72.8[a]	77.2	69.2	68.8
	β	96.4	53.2	[b]	[b]	[b]	85.8	[b]	[b]	[b]	[b]
17	α	91.5	70.7	50.9		62.1					
					68.8		85.5	73.3	77.2	69.2	67.6
	β	97.6	72.6	54.1		67.1					
19	α	93.3				62.4					
			[b]	[b]	46.0		85.0	73.6	77.8	69.5	66.8
	β	97.3				66.8					

[a] Assignments may be reversed. [b] Not assigned.

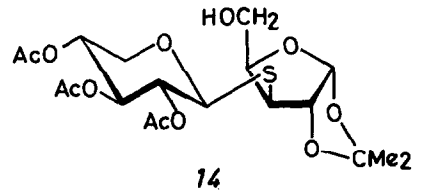
TABLE III. ^{1}H-N.m.r. data (400 MHz, $CDCl_3$) for peracetylated α-D anomer of 2-thioxylobiose (**11**), 3-thioxylobiose (**16**), and 4-thioxylobiose (**20**)

Compound	*Chemical shift*[a] (δ)											
	H-1	*H-2*	*H-3*	*H-4*	*H-5*a	*H-5*e	*H-1'*	*H-2'*	*H-3'*	*H-4'*	*H-5'*a	*H-5*e
11	6.18d	3.11dd	5.35dd	4.98td	3.65t	3.86t	4.70d	4.81t	5.09t	4.90dt	3.36dd	4.19dd
16	6.15d	4.95dd	3.27t	4.91dt	3.66dd	3.88dd	4.89d	4.63t	5.09t	4.86dt	3.43dd	4.23dd
20	6.25d	5.01dd	5.26t	3.13dt	3.83t	3.91dd	4.69d	4.86t	5.12t	4.87td	[b]	4.22dd
	Coupling constant (Hz)											
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5e}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4'5'a}$	$J_{4'5'e}$	$J_{5a,5b}$
11	3.5	11.3	9.4	10.8	5.8	11.1	8.3	8.3	8.3	8.5	4.9	11.8
16	3.4	11.0	10.8	10.2	5.5	11.2	7.1	7.1	7.2	7.4	4.4	12.0
20	3.7	[b]	[b]	11.3	5.8	12.0	7.7	7.9	7.8	8.1	4.7	[b]

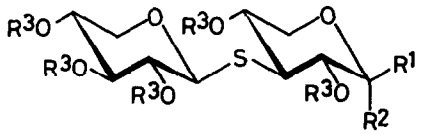
[a] Resulting from 2D ^{1}H-COSY assignations. [b] Not assigned.



12 $R^1,R^2 = Me_2C$
13 $R^1,R^2 = H$



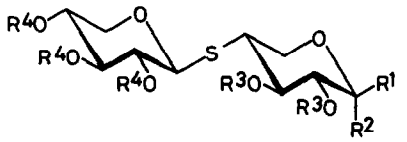
14



15 $R^1,R^2 = H,OAc$; $R^3 = Ac$
16 $R^1 = H$, $R^2 = OAc$, $R^3 = Ac$
17 $R^1,R^2 = H,OH$; $R^3 = H$

se[22] with the sodium salt of 1-thio-β-D-xylopyranose in *N',N''N'''*-hexamethylphosphoramide. Although the overall yield is acceptable, this method involves a high-boiling toxic solvent and a reacetylation step, and is therefore not suitable for the preparation in quantity of this thiodisaccharide. The activation procedure used for the preparation of **17** appeared consequently more suitable. Indeed, reaction of the 4-*O*-arabinosyl triflate[22] with the sodium salt of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose in oxolan in the presence of Kriptofix 21 for 3 h at room temperature gave in one step the known acylated 4-thiodisaccharide **18** in 92% yield, from which 4-thioxylobiose **19** could be obtained by conventional *O*-deacylation. Comparison of the n.m.r. data for **19** (Tables I and II), as well as of the fully acetylated derivative **20** (Table III) with the series of 2- and 3-thiodisaccharides confirmed the previously proposed structures[12].

Resistance of thioxylobioses **10**, **17**, *and* **19** *to xylanolytic enzymes of* C. albidus. — Solutions of (1→4)-β-D-xylobiose and thioxylobioses (20mM) were incubated separately with *C. albidus* cells, induced for D-xylan-degrading enzymes with methyl β-D-xylopyranoside, and subsequently permeabilized with toluene. At time intervals, aliquots were analyzed by t.l.c. for D-xylose formation. (1→4)-β-D-Xylobiose disappeared from the mixture within 20 h and, as it has been reported earlier[4], the products were D-xylose, and transitionally also (1→4)-β-D-xylotriose as a result of glycosyl-transfer reactions. The final product was only D-xylose. No evidence for a similar hydrolysis of all three



18 $R^1 = H$, $R^2 = OBz$, $R^3 = Bz$, $R^4 = Ac$
19 $R^1, R^2 = H, OH$; $R^3 = R^4 = H$
20 $R^1, R^2 = H, OAc$; $R^3 = R^4 = Ac$

thioxylobioses was obtained, which indicated that thioxylobioses **10**, **17**, and **19** are resistant to the action of β-D-xylosidase present in the permeabilized cells used as an enzyme source.

Induction ability of thioxylobioses **10**, **17**, *and* **19**, *and methyl 1-thio-β-D-xylopyranoside.* — D-Glucose-grown cells of *C. albidus* were incubated with thioxylobioses and methyl 1-thio-β-D-xylopyranoside at a 2mM concentration, and their ability to induce the xylanolytic system was compared with that of xylobiose and methyl β-D-xylopyranoside. The levels of extracellular β-D-xylanase and intracellular β-D-xylosidase are presented in Table IV. The level of β-D-xylanase is shown after a 24-h incubation. The values correspond to the maximum level of the enzyme because β-D-xylanase is stable in the induction medium. The values of intracellular β-D-xylosidase activity correspond to the time of the culmination of its specific activity in the cells. β-D-Xylosidase, similarly to β-D-xyloside permease, undergoes a significant decay after disappearance of the inducer from the cell-culture medium.

TABLE IV

Induction of extracellular β-D-xylanase and intracellular β-D-xylosidase in *Cryptococcus albidus*

Compound[a]	*β-D-Xylanase after 24 h (U/mL)*	*β-D-Xylosidase*	
		m Units in cells present in 1 mL	*Maximum value at (h)*
(1→4)-β-D-Xylobiose	0.34	53	6
Methyl 1-thio-β-D-xylopyranoside	0.01	2	8
2-Thioxylobiose (**10**)	0.01	3	10
3-Thioxylobiose (**17**)	0.03	4	10
4-Thioxylobiose (**19**)	0.08	12	22
(1→4)-β-D-Xylobiose + 4-thioxylobiose (**19**)	0.85	77	10
(1→4)-β-D-Xylobiose + 3-thioxylobiose (**17**)	0.35	50	6
(1→4)-β-D-Xylobiose + 2-thioxylobiose (**10**)	0.37	55	6
(1→4)-β-D-Xylobiose + methyl 1-thio-β-D-xylopyranoside	0.40	60	8

[a] Concentrations of each compound was 2mM.

The data in Table IV show that, at a 2mM concentration, all three thioxylobioses and methyl 1-thio-β-D-xylopyranoside are very poor inducers. A significant increase of β-D-xylanase over the control enzyme level was observed after a long-time incubation with 4-thioxylobiose. The cells did not respond to the presence of 2-thioxylobiose, 3-thioxylobiose, and methyl 1-thio-β-D-xylopyranoside.

All thio analogs were also tested in a combination with xylobiose. The reason for these experiments was a possibility that the inability of thio analogs to induce the xylanolytic system could be due to their inability to be taken up by the noninduced cells. The data in Table IV indicate that this may be true for 4-thioxylobiose, because its presence stimulated considerably the induction effect of (1→4)-β-D-xylobiose itself. The level of activities of the two tested enzymes in the presence of both disaccharides exceeded the sum of activities induced by the compounds separately. This interesting effect of 4-thioxylobiose was investigated in more detail. Figure 1 shows the time course of appearance of xylanolytic enzymes in the cells incubated with (1→4)-β-D-xylobiose, 4-thioxylobiose, and with both of them. The induction efficiency of 4-thioxylobiose is very low in comparison with that of (1→4)-β-D-xylobiose. The increase of the specific activity of β-D-xyloside permease in the presence of 4-thioxylobiose, shortly after transfer of the cells into the induction medium, should not be regarded as a specific effect of 4-thioxylobiose since a similar increase of β-D-xyloside permease activity was observed in the cells transferred to a medium containing no inducer[3]. The most pronounced effect of 4-thioxylobiose on xylobiose induction is the stabilization of the level of the cell-bound enzymes, β-D-xyloside permease and β-D-xylosidase, which undergo rapid inactivation after disappearance of xylobiose from the induction medium.

In attempts to explain the unexpected behavior of 4-thioxylobiose, the dependence of the level of induced extracellular β-D-xylanase on the concentration of (1→4)-

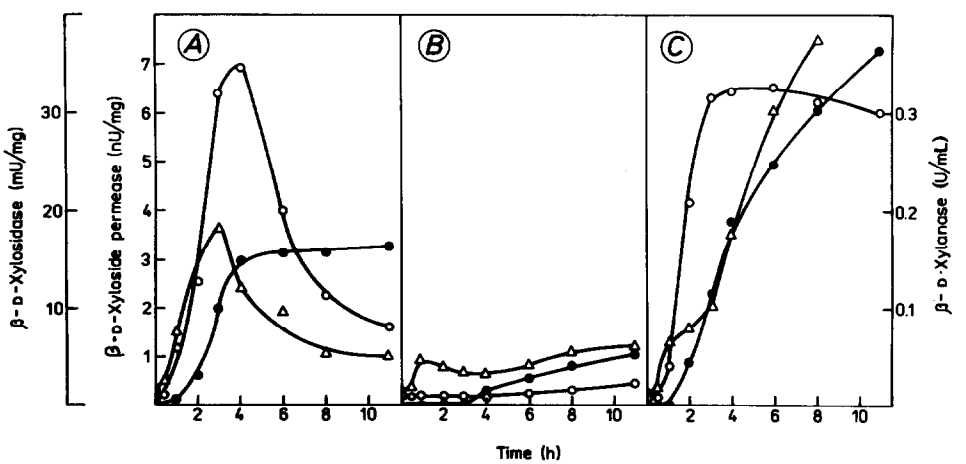


Fig. 1. Time course of the appearance of β-D-xylanase (●), β-D-xylosidase (○), and β-D-xyloside permease (△) during incubation of the cells with: (A) (1→4)-β-D-xylobiose; (B) 4-thioxylobiose (19); and (C) (1→4)-β-D-xylobiose plus 4-thioxylobiose (19).

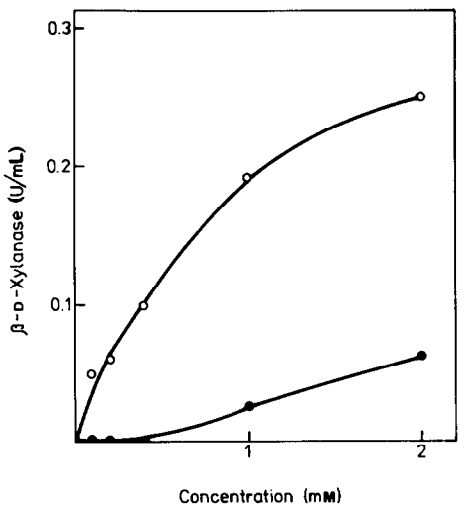


Fig. 2. Activity of extracellular β-D-xylanase after a 22-h incubation of the cells with (1→4)-β-D-xylobiose (o) and 4-thioxylobiose (**19**) (•) as a function of their concentration.

β-D-xylobiose and 4-thioxylobiose was established. The results (Fig. 2) indicate that the thio analog is not recognized by the cells, particularly at low concentrations. This might be a consequence of a low affinity of the xylobiose transport system for 4-thioxylobiose, which operates in noninduced cells and which may be represented by a low level of β-D-xyloside permease synthesized constitutively. Once the cells are induced, the thio analog becomes transported more efficiently. Another reason for the stimulating effect of 4-thioxylobiose on xylobiose-induced synthesis of the xylanolytic system in *C. albidus* could be an inhibition of the uptake or intracellular degradation of xylobiose, resulting in a longer persistence of (1→4)-β-D-xylobiose outside or inside the cells. The first possibility, namely the inhibition of the xylobiose uptake, could be rejected on the basis of the results reported in Table V. 4-Thioxylobiose (2mM) did practically not influence

TABLE V

Level of radioactivity in the medium during induction of the xylanolytic system of *C. albidus* by (1→4)-β-D-[U-^{14}C]xylobiose (2mM) in the absence and in the presence of 2 mM 4-thioxylobiose (**19**)

Time of incubation (*h*)	*Original radioactivity of (1→4)-β-D-[U-^{14}C]xylobiose in the medium* (*%*)	
	4-Thioxylobiose absent	*4-Thioxylobiose present*
0.5	99	100
1	91	89
2	25	20
3	6	7
4	4	5

the rate of disappearance of (1→4)-β-D-[U-^{14}C]xylobiose from the medium. The experiments to monitor the effect of 4-thioxylobiose on the persistance of intracellular (1→4)-β-D-xylobiose from the cells indicated that there is no significant accumulation of xylobiose inside the cells. The fraction of extracted radioactivity was always very low in (1→4)-β-D-xylobiose. The aforementioned observations suggested that 4-thioxylobiose interacts with cell macromolecules involved in the regulation of the formation of the xylanolytic system of the yeast *C. albidus*. However, under circumstances in which the compound does not enter the cells, it may readily behave as biologically inactive.

It will be of interest to examine, in the future, the behavior of the available thioxylobioses in procaryotic microorganisms, in which xylanolytic systems may not be phylogenetically related to *C. albidus*. Challenging is also the study of other biochemical potential of thioxylobioses, *e.g.*, as enzyme inhibitors.

EXPERIMENTAL

General methods for chemical syntheses. — Melting points were determined in capillary tubes, with a Büchi 535 apparatus and are corrected. Optical rotations were measured with a Jobin–Yvon (Paris) "Digital Micropolarimeter" at mutarotational equilibrium. N.m.r. spectra (^{1}H, 400 MHz; ^{13}C, 50 MHz) were recorded with Bruker AM 400 and AC 200 spectrometers, respectively. The references were the central line of the $CDCl_3$-triplet (δ 76.9 for ^{13}C) for solutions in $CDCl_3$; signals at δ 29.2 (^{13}C) and 2.17 (^{1}H) for solutions in ($^2H_6$)acetone and external tetramethylsilane for solutions in D_2O. For additional informations, 2D ^{1}H-COSY and 2D ^{1}H–^{13}C CORR spectra were obtained. Positive f.a.b.-mass spectra (Xe, accelerating potential 8 kV) were recorded with a ZAB-SEQ (VG) spectrometer. Reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by u.v. light or charring with H_2SO_4. Solutions were concentrated with a Büchi rotatory evaporator at $<45°$, except when otherwise stated. Separations were performed by flash chromatography on Silica Gel 60 (230–400 mesh, Merck) with a Büchi 680 equipment fitted with a Knauer refractometric detector 188.00. L.c. (4×10^3 kPa) of unprotected disaccharides was carried out with a Perkin–Elmer 250 chromatograph, fitted with an LC 250 isocratic pump, a LC 30 refractometric detector, and a 1020 S integrator, on a Lichrosorb NH_2 (7 μm) column (250 × 10 mm). Microanalyses were performed by the Microanalytical Laboratory of the CNRS, in Vernaison.

Substrates. — 4-*O*-β-D-Xylopyranosyl-D-xylose [(1→4)-β-D-xylobiose] and the U-^{14}C-labeled disaccharide (spec. radioactivity, 94 GBq/mol) were prepared from phenyl β-D-xylopyranoside or phenyl D-[U-^{14}C]xylopyranoside, respectively, by incubation with purified β-D-xylanase from *C. albidus*[23]. Methyl β-D-xylopyranoside and the U-^{14}C-labeled xylopyranoside (spec. radioactivity, 3.3 TBq/mol) were prepared by chemical synthesis[24]. Methyl 1-thio-β-D-xylopyranoside was a generous gift from Professor C. K. DeBruyne (Laboratorium voor Biochemie, Rijksuniversiteit Ghent, Belgium).

Growth of the microorganism and the induction experiments. — The cells of the yeast *Cryptococcus albidus* CCY 17-4-1 were grown, harvested, and incubated with the

substrate and substrate analogs as previously described[3]. Concentrations of substrates and analogs were 2mM, unless otherwise stated.

Enzyme assays. — The activity of extracellular xylanase was determined in cell-free medium, on beechwood xylan as substrate[3]. One unit of xylanase and β-D-xylosidase is defined as the amount of enzyme that liberates 1 μmol of D-xylose equivalent and 1 μmol of 4-nitrophenol in 1 min from 1 mg of the corresponding substrate, in 0.5 mL of 50mM acetate buffer (pH 5.4) at 30°. β-D-Xyloside permease was assayed on intact cells by use of methyl β-D-[U-^{14}C]xylopyranoside as substrate[24]. One unit of the transport system corresponds to 1 μmol of the substrate taken up by the cells in 1 min under the described conditions[24]. Hydrolysis of (1→4)-β-D-xylobiose and thioxylobioses was monitored by t.l.c. (17:8:10 ethyl acetate–acetic acid–water, Kavalier microcrystalline cellulose, aniline phthalate detection[25]) of supernatants from suspensions of toluene-permeabilized cells induced with methyl β-D-xylopyranoside (8 mg dry-weight/mL) in acetate buffer solution (pH 5.4, 50 mM) containing the respective compounds (20mM). Proteins were determined by the Lowry method[26].

Allyl α,β-D-xylofuranoside (**1**). — D-Xylose (11 g, 73.3 mmol) was added to allyl alcohol (250 mL) containing HCl (1.25 g), and the suspension, which resulted in a clear solution after 1 h, was stirred for a further 5 h. The acid was neutralized by shaking with powdered $NaHCO_3$, and the resulting precipitate was removed by centrifugation. Concentration of the clear solution under reduced pressure at 40° gave the anomeric mixture **1** (10.28 g, 74%), syrup; ^{13}C-n.m.r. (50 MHz, D_2O): δ 135.1, 134.8 (=CH), 119.7, 119.3 (=CH_2), 108.0 (C-1β), 101.5 (C-1α), 83.6 (C-4β), 81.4 (C-2β), 79.5 (C-4α), 78.0 (C-2α), 76.3 (C-3α and β), 70.5, 70.3 (CH_2O), 62.2 (C-5β), and 61.7 (C-5α).

Anal. Calc. for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 49.47; H, 7.84.

Allyl 3,5-O-isopropylidene-α- (**2**) *and -β-D-xylofuranoside* (**3**). — The anomeric mixture of allyl xylosides **1** (5.45 g, 28 mmol), in anhydrous acetone containing anhydrous $CuSO_4$ (6.85 g) and H_2SO_4 (2M, 68.5 μL), was shaken for 20 h at room temperature. The filtered solution was neutralized by addition of NH_4OH (109 μL), filtered again, and concentrated to a syrup which was dissolved in dichloromethane and washed with water. The dried organic solution was concentrated to a slightly yellow syrup (5.39 g, 23.4 mmol, 83%), which showed two spots in t.l.c. (1:1 ethylacetate–hexane). Column chromatography of an aliquot (5 g, 1:2 ethylacetate–hexane) yielded successively the α anomer **2** (2.19g, 44%) and the β anomer **3** (2.26 g, 45%), both as syrups. For **2**: $[\alpha]_D^{20}$ + 202° (*c* 3.9, chloroform); ^{13}C-n.m.r. [($^2H_6$)acetone, 50 MHz]: δ 135.0 (=CH), 116.3 (=CH_2), 102.0 (C-1), 97.6 (Me_2C), 77.5 (C-4), 76.0 (C-2), 71.6 (C-3), 69.3 (CH_2O), 60.4 (C-5), 28.0, and 19.7 [(CH_3)$_2$C]. For **3**: $[\alpha]_D^{20}$ − 179° (*c* 1.3, chloroform); ^{13}C-n.m.r. [($^2H_6$)acetone]: δ 135.6 (=CH), 115.4 (=CH_2), 109.3 (C-1), 97.8 (Me_2C), 80.8 (C-4), 75.7 (C-2), 74.9 (C-3), 67.7 (CH_2O), 60.8 (C-5), 27.0, 20.6 [(CH_3)$_2$C].

Anal. Calc. for $C_{11}H_{18}O_5$: C, 57.39; H, 7.83. Found for **2**: C, 56.56; H, 7.60. For **3**: C, 56.76; H, 7.36.

Allyl 3,5-O-isopropylidene-β-D-threo-pentofuranos-2-uloside (**4**).— To a solution of **3** (1.37 g, 6 mmol) in dichloromethane (300 mL) at 0° were added pyridinium dichromate (3.32 g, 8.8 mmol), freshly activated molecular sieve powder (5 g), and acetic

acid (100%, 0.59 mL), and the suspension was stirred at room temperature. After 15 h, t.l.c. (2:1 ethylacetate–hexane) showed an optimal conversion of **3** into a slower migrating component. The mixture was poured onto the top of a short silica gel column which was washed with ethyl acetate. Concentration of the eluate gave **4** as a syrup (0.76 g, 56%), which was not further characterized and was used as such for the preparation of **5**.

*Allyl 3,5-*O*-isopropylidene-β-*D*-lyxofuranoside* (**5**). — To a stirred solution of **4** (1.2 g, 5.26 mmol) in aqueous 1:1 (v/v) ethanol (33 mL) at 0° was added powdered BH_4Na (260 mg, 7.6 mmol) in small portions and the reaction was further stirred for 2 h at 20°. Neutralization with aqueous acetic acid (10%) and concentration of the resulting solution resulted in a sirupy residue which was extracted with dichloromethane and then concentrated to a colorless syrup (**5**; 0.92 g, 76%), homogeneous in t.l.c. (1:1 ethyl acetate–hexane); ^{13}C-n.m.r. [50 MHz, ($^2H_6$) acetone]: δ 135.5 (=CH), 115.3 (=CH_2), 101.1 (C-1), 98.1 (Me_2C), 73.9 (C-4), 73.5 (C-3), 68.2 (C-2), 67.4 (CH_2O), 61.2 (C-5), 26.7, and 20.9 [(CH_3)$_2$C].

*Allyl 3,5-*O*-isopropylidene-2-*O*-trifluoromethanesulfonyl-β-*D*-lyxofuranoside* (**6**). — Trifluoromethanesulfonic anhydride (1.13 mL, 4 mmol) was added dropwise to a solution of the allyl lyxoside **5** (0.84 g, 3.65 mmol) in dichloromethane (24 mL) containing pyridine (1.5 mL, 19 mmol). After being stirred at 25° for 2 h, the mixture was poured into ice–water (50 mL) containing an excess of $NaHCO_3$ and shaken vigourously. The layers were separated, and the aqueous layer was extracted with dichloromethane (30 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed by distillation, under reduced pressure, to yield **6** as an oily residue (1.26 g, 95%), homogenous in t.l.c. (1:1 ethyl acetate–hexane), which was used without further characterization in the following step.

*Allyl 3,5-*O*-isopropylidene-2-*S*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-2-thio-β-*D*-xylofuranoside* (**7**). — Sodium hydride (105 mg, 4.4 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose (0.97 g, 3.3 mmol) in dry oxolan (30 mL) at room temperature. The suspension was stirred in an inert atmosphere until hydrogen formation had ceased. The solution was then concentrated under reduced pressure and the residue was dissolved in *N,N*-dimethylformamide (15 mL). To this stirred solution was then added dropwise allyl 3,5-*O*-isopropylidene-2-*O*-trifluoromethylsulfonyl-β-D-lyxofuranoside (**6**) (1.2 g, 3.3 mmol) in *N,N*-dimethylformamide (15 mL). After being stirred for 1 h at room temperature, the mixture was concentrated at 50° under reduced pressure and the residue, dissolved in dichloromethane, was washed with water (50 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (1:1 ethyl acetate–hexane) of the residue on silica gel column (1.6 g) gave **7** (1.13 g, 68%), glass, $[\alpha]_D^{20}$ −64° (*c* 1.2, chloroform); ^{1}H-n.m.r. [($^2H_6$)acetone, 400 MHz]: δ 5.35 (t, H-3′), 5.19 (d, H-1), 5.09 (d, H-1′), 5.04 (t, H-2′), 5.03 (dt, H-4′), 4.46 (d, H-3), 4.30 (dd, H-5′*e*), 4.22 (dd, H-4), 4.08 (dd, H-5a), 3.88 (dd, H-5b), 3.68 (dd, H-5′*a*), and 3.57 (s, H-2); $J_{1,2}$ 1.6, $J_{2,3}$ < 1.5, $J_{3,4}$ 4.2, $J_{4,5a}$ 4.8, $J_{4,5b}$ 5.2, $J_{5a,b}$ 12.0, $J_{1',2'}$ 9.8, $J_{2',3'}$ 8.4, $J_{3',4'}$ 8.5, $J_{4',5'a}$ 9.3, $J_{4',5'e}$ 5.1, and $J_{5'a,e}$ 11.5 Hz; ^{13}C-n.m.r. [($^2H_6$)acetone, 50 MHz]: δ 135.1 (=CH), 115.6 (=CH_2), 108.4 (C-1), 98.5 (Me_2C), 83.4 (C-1′), 75.8 (C-3), 74.6 (C-4), 72.1 (C-3′), 70.2 (C-2′), 68.7 (C-4′), 68.2

(CH_2O), 65.3 (C-5'), 60.6 (C-5), 53.8 (C-2), 26.8, 20.9 [(CH_3)$_2$C], and 19.9 (CH_3CO); f.a.b.m.s. (4-nitrobenzyl alcohol, NaI): *m/z* 1031 (0.1, [2 M + Na]$^+$), 527 (10, [M + Na]$^+$), and 259 (30, acylated xylopyranosyl cation).

Anal. Calc. for $C_{22}H_{32}O_{11}S$: C, 52.38; H, 6.35; S, 6.35. Found: C, 52.79; H, 6.53; S, 6.32.

1-Propenyl 3,5-O-isopropylidene-2-S-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-thio-β-D-xylofuranoside (**8**). — A solution of **7** (800 mg, 1.6 mmol), chlorotriphenylphosphinerhodium (420 mg, 0.45 mmol), and diazabicyclo[2.2.2]octane (96 mg, 0.85 mmol) in 7:3:1 ethanol–toluene–water (154 mL) was stirred at 90° for 6 h. The solution was then concentrated under reduced pressure and the residue was dissolved in ether (30 mL). After filtration and washing with water, the solution was dried (Na_2SO_4), concentrated to 10 mL, and filtered through a small silica gel column in order to remove the residual catalyst. Concentration of the clear solution under reduced pressure gave **8** as syrup (630 mg, 78%), $[\alpha]_D^{20}$ −70° (*c* 1, chloroform).

Anal. Calc. for $C_{22}H_{32}O_{11}S$: C, 52.38; H, 6.35; S, 6.35. Found: C, 51.91; H, 6.56; S, 6.10.

1-Propenyl 3,5-O-isopropylidene-2-S-β-D-xylopyranosyl-2-thio-β-D-xylofuranoside (**9**). — To a solution of the acylated thiodisaccharide **8** (530 mg, 1.05 mmol) in methanol (30 mL) was added methanolic sodium methoxide (M, 138 μL), and the mixture was stirred for 2 h at room temperature. The solution was then de-ionized by passing it through a mixed bed of Amberlite ion-exchange resins IRC-50 (H^+) and IRA-910 (OH^-). Evaporation, dissolution of the glassy residue in water, and freeze-drying gave **9** as an amorphous powder (370 mg, 88%), which was used in the following step without further characterization.

2-S-β-D-Xylopyranosyl-2-thio-D-xylose, (2-thioxylobiose, **10**). — A solution of **9** (370 mg, 0.92 mmol) in 0.015M HCl (16 mL) was heated at 65° for 6 h. The acid was then neutralized by pouring it through a small column of Amberlite IRA-93 anion-exchange resin. Freeze-drying of the solution gave **11** as an amorphous powder which showed a main component in l.c. with k' 1.4 (41:9 acetonitrile–water). Preparative l.c. and freeze-drying gave **10** as a white powder (162 mg, 60%), $[\alpha]_D^{20}$ −33° (*c* 0.84, water); f.a.b.m.s. (glycerol, NaI): *m/z* 629 (3, [2 M + Na]$^+$), and 321 (72, [M + Na]$^+$).

Anal. Calc. for $C_{10}H_{20}O_8S{\cdot}0.5H_2O$: C, 39.12; H, 6.19; S, 10.44. Found: C, 39.19; H, 6.20; S, 9.38.

1,3,4-Tri-O-acetyl-2-S-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-thio-α,β-D-xylopyranose (**11**). — Crude **10** (10 mg, 33 mmol) was acetylated with 1:1 (v/v) acetic anhydride in pyridine to give, after conventional processing, **11** (16 mg, 88%), as a syrup having (^{1}H.n.m.r., integrals for H-1,2) a 2:1 proportion of α,β anomers; f.a.b.m.s. (4-nitrobenzyl alcohol, NaI): *m/z* 573 (10, [M + Na]$^+$) and 259 (56, acylated xylopyranosyl cation).

1,2:5,6-Di-O-isopropylidene-3-S-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-3-thio-α-D-glucofuranose (**12**). — Sodium hydride (209 mg, 7.25 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose[17] (1.93 g, 6.6 mmol) in dry oxolan (50 mL) at 0°. The suspension was stirred in an inert atmosphere until hydrogen evolution had

ceased. Kriptofix 21 (251 mg, 1.2 mmol) was then added, followed by dropwise addition of a solution of 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose[20] (2.48 g, 6.6 mmol) in dry oxolan (20 mL) at 20°. After being stirred for 2 h at room temperature, the mixture showed a main spot on t.l.c. (1:2 ethyl acetate–hexane). It was filtered and the filtrate was concentrated to a residue which was dissolved in dichloromethane and washed with water. The dried solution (Na_2SO_4) was evaporated to a foam, which crystallized from its dichloromethane solution by addition of light petroleum ether, yielding **12** (1.9 g, 54%), m.p. 172.5°, $[\alpha]_D^{20}$ −86° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$, 400 MHz): δ 5.85 (d, H-1), 5.15 (t, H-3′), 4.95 (t, H-2′), 4.95 (dt, H-4′), 4.76 (d, H-2), 4.74 (d, H-1′), 4.27 (dd, H-5), 4.21 (dd, H-4), 4.10 (dd, H-5′*e*), 4.09 (dd, H-6b), 3.97 (ddd, H-6a), 3.55 (d, H-3), 3.37 (dd, H-5′*a*), 2.05–2.02 (9 H, $3CH_3CO$), and 1.50–1.30 [12 H, 2 $(CH_3)_2C$]; $J_{1,2}$ 3.5, $J_{2,3}$ 0, $J_{3,4}$ 3.8, $J_{4,5}$ 8.5 $J_{5,6a}$ 5.9, $J_{5,6b}$ 4.5, $J_{6a,b}$ 8.7, $J_{1',2'}$ = $J_{2',3'}$ = $J_{3',4'}$ = 8.0, $J_{4',5'a}$ 8.5, $J_{4',5'e}$ 3.6, and $J_{5'a,e}$ 11.7 Hz; ^{13}C-n.m.r. ($CDCl_3$, 50 MHz): δ 169.0 (CH_3CO), 112.0, 109.4 [2 $(CH_3)_2C$], 104.9 (C-1), 86.1 (C-2), 82.5 (C-1′), 79.9 (C-4), 73.8 (C-5), 71.7 (C-3′), 69.9 (C-2′), 68.3 (C-4′), 67.4 (C-6), 65.0 (C-5′), 50.0 (C-3), 26.0 [$(CH_3)_2C$], and 20.6 (CH_3CO); f.a.b.m.s. (4-nitrobenzyl alcohol): *m/z* 535 (2, $[M + H]^+$) and 259 (34, acylated xylopyranosyl cation).

Anal. Calc. for $C_{23}H_{34}O_{12}S$; C, 51.69; H, 6.37; S, 5.99. Found: C, 51.84; H, 6.37; S, 6.27.

*1,2-*O*-Isopropylidene-3-*S*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-3-thio-α-*D*-glucofuranose* (**13**). — A solution of **12** (1.5 g, 2.81 mmol) in 50% aqueous acetic acid (75 mL) was heated at 60° for 1 h, then cooled to room temperature and poured into saturated aqueous $NaHCO_3$. Extraction with dichloromethane and concentration of the extract gave **13** as a foam (1.10 g, 2.23 mmol, 79%), homogeneous in t.l.c. (1:1 ethyl acetate–hexane), which crystallized with difficulty from a mixture of ether–light petroleum ether (150 mg), m.p. 89°, $[\alpha]_D^{20}$ −86° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$, 400 MHz): δ 5.80 (d, H-1), 5.19 (t, H-3′), 4.96 (t, H-2′), 4.94 (m, H-4′), 4.77 (d, H-2), 4.64 (d, H-1′), 4.25 (dd, H-4), 4.24 (dd, H-5′*e*), 3.91 (ddd, H-5); 3.82 (dd, H-6b), 3.66 (dd, H-6a), 3.54 (d, H-3), 3.39 (dd, H-5′*a*), 2.20–2.00 (9 H, 3 CH_3CO), and 1.50–1.30 [6 H, 2$(CH_3)_2C$]; $J_{1,2}$ 3.5, $J_{2,3}$ 0, $J_{3,4}$ 3.8, $J_{4,5}$ 9.0, $J_{5,6a}$ 8.3, $J_{5,6b}$ 3.4, $J_{6a,b}$ 11.6, $J_{1',2'}$ 8.9, $J_{2',3'}$ 8.8, $J_{3',4'}$ 8.6, $J_{4',5'a}$ 9.5, $J_{4',5''e}$ 3.5, and $J_{5'a,e}$ 11.6 Hz; ^{13}C-n.m.r. ($CDCl_3$, 50 MHz): δ 169 (CH_3CO), 112.0 [$(CH_3)_2C$], 104.6 (C-1), 86.2 (C-2), 83.3 (C-1′), 78.5 (C-4), 71.9 (C-3′), 69.9 (C-5), 69.8 (C-2′), 68.1 (C-4′), 65.7 (C-6), 64.0 (C-5′), 50.7 (C-3), 26.0 [$(CH_3)_2C$], and 20.4 (CH_3CO); f.a.b.m.s. (glycerol, NaI): *m/z* 517 (7, $[M + Na]^+$), 495 (0.2, $[M + H]^+$), 259 (66, acylated xylopyranosyl cation), and 235 (100, glucofuranosyl cation).

Anal. Calc. for $C_{20}H_{30}O_{12}S$: C, 48.58; H, 6.07; S, 6.48. Found: C, 48.80; H, 6.06; S, 6.18.

*1,2-O-Isopropylidene-3-*S*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-3-thio-α-*D*-xylofuranose* (**14**). — Aqueous sodium metaperiodate (520 mg, 2.24 mmol) was added to a stirred solution of the diol **14** (1.1 g, 2.23 mmol) in 1:1 ethanol–water (20 mL) at 0°. After 30 min, the reaction was terminated by addition of a saturated solution of $NaHCO_3$, and the solution was extracted with dichloromethane. The extract was dried (Na_2SO_4) and concentrated to a syrupy residue (1 g, 2.2 mmol) which was dissolved in 2-propanol (40

mL). To this stirred solution, $NaBH_4$ (120 mg, 3.2 mmol) was added at 0°. After 30 min, the base was neutralized by careful addition of aqueous acetic acid (10%) and the solution was concentrated to 20 mL. To this was added water (20 mL) and the solution was again concentrated to 20 mL, and then extracted with dichloromethane. Concentration of the dried (Na_2SO_4) organic extract gave **14** as a foam (0.9 g, 87%), which crystallized with difficulty from dichloromethane–light petroleum ether (0.4 g, 39%), m.p. 196°, $[\alpha]_D^{20}$ −90° (*c* 0.75, chloroform); ^{1}H-n.m.r. ($CDCl_3$, 400 MHz): δ 5.82 (d, H-1), 5.17 (t, H-3′), 4.96 (t, H-2′), 4.94 (td, H-4′), 4.77 (d, H-2), 4.61 (d, H-1′), 4.48 (td, H-4), 4.22 (dd, H-5′*e*), 3.80 (dd, H-5a), 3.75 (dd, H-5b), 3.50 (d, H-3), 3.36 (dd, H-5′*a*), 2.10–2.00 (9 H, 3 CH_3CO), 1.50, and 1.30 [6 H, 2$(CH_3)_2C$]; $J_{1,2}$ 3.5, $J_{2,3}$ 0, $J_{3,4}$ 4.1, $J_{4,5a}$ 6.2, $J_{4,5b}$ 6.3, $J_{5a,b}$ 11.9, $J_{1',2'}$ 8.6, $J_{2',3'} = J_{3',4'}$ 8.5, $J_{4',5'a}$ 9.0, $J_{4',5'e}$ 5.0, and $J_{5a,e}$ 11.5 Hz; ^{13}C-n.m.r. ($CDCl_3$, 50 MHz): δ 169 (CH_3CO), 112.0 [$(CH_3)_2C$], 104.5 (C-1), 86.9 (C-2), 83.1 (C-1′), 79.0 (C-4), 71.9 (C-3′), 69.7 (C-2′), 68.3 (C-4′), 65.8 (C-5), 62.5 (C-5′), 49.8 (C-3), 26.0 [$(CH_3)_2C$], and 20.5 (CH_3CO); f.a.b.m.s. (glycerol, NaI): *m/z* 487 (56, $[M + Na]^+$).

Anal. Calc. for $C_{19}H_{28}O_{11}S$: C, 49.24; H, 6.05; S, 6.91. Found: C, 48.91; H, 5.83; S, 6.96.

*1,2,4-Tri-*O*-acetyl-3-*S*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-3-thio-α,β-*D*-xylopyranose (hexa-*O*-acetyl-3-thioxylobiose*, **15**). — A solution of **14** (200 mg, 0.43 mmol) in aqueous acetic acid (60%, 15 mL) was heated at 55° for 48 h. It was then freeze-dried to a foam (150 mg, 82%) which was acetylated in 3:4 acetic anhydride–pyridine (7 mL) for 15 h. The extract was washed with aqueous $KHSO_4$ (10%) and with water, and dried (Na_2SO_4). Concentration gave **15** as a foam (180 mg, 92%, $\alpha:\beta \approx$ 1:1 by ^{13}C-n.m.r. C-1 and C-3), from which the α anomer **16** (80 mg, 16%) crystallized by dissolution in methanol, m.p. 182.5°, $[\alpha]_D^{20}$ −17° (*c* 0.35, chloroform); f.a.b.m.s. (4-nitrobenzyl alcohol, NaI): *m/z* 573 (2, $[M + Na]^+$) and 259 (36, acylated xylopyranosyl cation).

Anal. Calc. for $C_{22}H_{30}O_{14}S$: C, 48.00; H, 5.45; S, 5.82. Found: C, 47.75; H, 5.50; S, 6.40.

*3-*S*-β-*D*-Xylopyranosyl-3-thio-*D*-xylose, (3-thioxylobiose*, **17**). — To the anomeric mixture of **15** (60 mg, 0.11 mmol) in methanol (10 mL) was added methanolic sodium methoxide (0.25M, 0.15 mL). After 2 h stirring, the base was neutralized by shaking with Amberlite IRN 77 (H^+) cation-exchange resin and, after filtration, concentration yielded **17** (30 mg, 92%) as a glassy residue, which was freeze-dried to a white powder from its aqueous solution, k′ 1 (41:9 acetonitrile–water), $[\alpha]_D^{20}$ −6.9° (*c* 1.75, methanol), $[\alpha]_D^{20}$ −77° (*c* 0.34, water); f.a.b.m.s. (glycerol, NaI): *m/z* 618 (3, $[2 M + Na]^+$), 321 (100, $[M + Na]^+$), and 299 (14, $[M + H]^+$).

Anal. Calc. for $C_{10}H_{18}O_8S \cdot H_2O$: C, 37.97; H, 6.33; S, 10.13. Found: C, 38.03; H, 6.15; S, 10.02.

*1,2,3-Tri-*O*-benzoyl-4-*S*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-4-thio-α-*D*-xylopyranose* (**18**). — Sodium hydride (88 mg, 3.1 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose[17] (810 mg, 2.8 mmol) in dry oxolan (50 mL), and the suspension was stirred in an inert atmosphere until hydrogen evolution had ceased. Kryptofix 21 (105 g, 0.21 mmol) was added, followed dropwise by a solution of 1,2,3-tri-*O*-benzoyl-4-*O*-trifluoromethylsulfonyl-β-L-arabinopyranose[22] (1.65 g, 2.8

mmol) in dry oxolan (20 mL) at 20°. After stirring for 3 h at room temperature, a main component was detected (t.l.c., 1:2 ethyl acetate–hexane). The suspension was filtered, the filtrate concentrated, and the residue in dichloromethane was washed with water until neutral. The dried (Na_2SO_4) solution was concentrated to yield **18** as a foam (1.9 g, 92%), $[\alpha]_D^{20}$ +94° (*c* 0.36, chloroform); ^{1}H-n.m.r. ($CDCl_3$, 400 MHz): δ 8.15–7.25 (15 H, PhCO), 6.69 (d, H-1), 5.90 (t, H-3), 5.52 (dd, H-2), 5.10 (t, H-3′), 4.88 (t, H-2′), 4.82 (dt, H-4′), 4.75 (d, H-1′), 4.12 (dd, H-5*e*), 4.07 (t, H-5*a*), 4.02 (dd, H-5′*e*), 3.48 (dt, H-4), 3.26 (dd, H-5′*a*), and 2.10–1.70 (9 H, $3CH_3$ CO); $J_{1,2}$ 3.6, $J_{2,3}$ 9.8, $J_{3,4}$ 10.3, $J_{4,5a}$ 11.1, $J_{4,5e}$ 6.1, $J_{5a,e}$ 11.8, $J_{1',2'}$ 8.1, $J_{2',3'}$ 8, $J_{3'4'}$ 8, $J_{4',5'a}$ 8.3, $J_{4',5'e}$ 4.8, and $J_{5'a,5e}$ 11.8 Hz; ^{13}C-n.m.r. ($CDCl_3$, 50 MHz), δ 134–128 (C_6H_5CO), 90.6 (C-1), 82.4 (C-1′), 71.6 (C-3,3′), 69.3 (C-2′), 68.6 (C-2), 68.2 (C-4′), 64.7 (C-5), 64.5 (C-5′), 44.3 (C-4), and 20.6 (CH_3CO); lit.[12] $[\alpha_D]$ +65° (*c* 0.74, chloroform).

Anal. Calc. for $C_{37}H_{36}O_{14}S$: C, 60.30; H, 4.90: S, 4.35. Found: C, 60.42; H, 4.99; S, 4.52.

4-S-β-D-Xylopyranosyl-4-thio-D-xylose (*4-thioxylobiose*, **19**). — Compound **18** (650 mg, 0.89 mmol) was stirred with M methanolic sodium methoxide (3.8 mL) for 1 h at room temperature. The base was neutralized by shaking with Amberlite IRN 77 (H^+) cation-exchange resin, the suspension filtered, and the filtrate concentrated to a residue which was freeze-dried from its aqueous solution to yield **19**, glass, k′ 1.22 (41:9 acetonitrile–water), $[\alpha]_D^{20}$ −9.5° (*c* 4.2, methanol), $[\alpha]_D^{20}$ −56° (*c* 0.4, water); f.a.b.m.s. (glycerol, NaI): *m/z* 321 (24, $[M + Na]^+$); lit.[12] $[\alpha]_D$ −22.9° (*c* 2.6, methanol).

Anal. Calc. for $C_{10}H_{18}O_8S$: C, 40.26; H, 6.04; S, 10.74. Found: C, 40.04; H, 6.25; S, 10.42.

1,2,3-Tri-O-acetyl-4-S-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4-thio-D-xylopyranose (*hexa-O-acetyl-4-thioxylobiose*, **20**). — A solution of **19** (80 mg, 0.27 mmol), in 1:1 pyridine–acetic anhydride (8 mL), was kept at room temperature for 3 h, then poured into a saturated solution of $NaHCO_3$ and extracted with dichloromethane. The extract was washed with aqueous $KHSO_3$ (10%), then water, dried (Na_2SO_4), and concentrated to yield **20** as a foam (120 mg, 80%), $[\alpha]_D^{20}$ −40° (*c* 0.33, chloroform), which was shown (^{1}H-n.m.r., integrals for H-1, H-4) to contain a 1:1 proportion of α,β anomers at C-1); f.a.b.m.s. (4-nitrobenzyl alcohol, NaI): *m/z* 573 (10 $[M + Na]^+$) and 259 (100, acylated xylopyranosyl cation).

Anal. Calc. for $C_{22}H_{30}O_{14}S$: C, 48.00; H, 5.45; S, 5.82. Found: C, 47.66; H, 5.57; S, 6.49.

REFERENCES

1 A. Gadelle, J. Defaye, and C. Pedersen, *Carbohydr. Res.*, 200 (1990) 497–498.
2 J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 212 (1991) 51–58.
3 P. Biely and E. Petráková, *J. Bacteriol.*, 160 (1984) 408–412.
4 P. Biely and E. Petráková, *FEBS Lett.*, 178 (1984) 323–326.
5 M. Mandels, F. W. Parrish, and E. T. Reese, *J. Bacteriol.*, 83 (1962) 400–408.
6 T. Nisizawa, H. Suzuki, M. Nakayama, and K. Nisizawa, *J. Biochem. (Tokyo)*, 70 (1971) 375–385.
7 H. V. Rickenberg, G. N. Cohen, G. Buttin, and J. Monod, *Ann. Inst. Pasteur*, 91 (1956) 829–857.

8 G. N. Cohen and J. Monod, *Bacteriol. Rev.*, 21 (1957) 169–194.
9 H. Okada and H. O. Halvorson, *Biochim. Biophys. Acta*, 82 (1964) 538–546.
10 J. Defaye and J. Gelas, in Atta-ur-Rahman (Ed.), *Studies in Natural Product Chemistry*, Vol. 8, Elsevier, Amsterdam, 1991 pp. 315–357.
11 D. Rho, M. Desrochers, L. Jurasek, H. Driguez, and J. Defaye, *J. Bacteriol.*, 149 (1982) 47–53.
12 J. Defaye, H. Driguez, M. John, J. Schmidt, and E. Ohleyer, *Carbohydr. Res.*, 139 (1985) 123–132.
13 K. Hamacher, *Carbohydr. Res.*, 128 (1984) 291–295.
14 C. Orgeret, Thèse de Doctorat en Chimie, Université de Grenoble, 1984.
15 J. Defaye and J.M. Guillot, *Abstr. Eur. Symp. Carbohydr., 5th*, 1989, A-48.
16 R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, *Can. J. Chem.*, 53 (1975) 1424–1433.
17 J. Stanek, M. Šindlerova, and M. Černý, *Collect. Czech. Chem. Commun.*, 30 (1965) 197–302.
18 E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 38 (1973) 3224.
19 E. Petráková and P. Kovác, *Chem. Zvesti*, 35 (1981) 551–566.
20 L. D. Hall and D. C. Miller, *Carbohydr. Res.*, 47 (1976) 299–305.
21 M. L. Wolfrom and Z. Yosizawa, *J. Am. Chem. Soc.*, 81 (1959) 3474–3476.
22 J. F. Batey, C. Bullock, E. O'Brien, and J. M. Williams, *Carbohydr. Res.*, 43 (1975) 43–50.
23 Z. Krátký, P. Biely, and M. Vršanská, *Carbohydr. Res.*, 93 (1981) 300–303.
24 Z. Krátký and P. Biely, *Eur. J. Biochem.*, 112 (1980) 367–373.
25 S. M. Partridge, *Nature (London)*, 164 (1949) 443.
26 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.