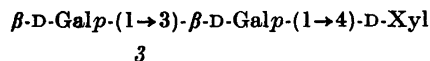
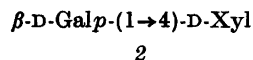


## Synthesis of *O*- $\beta$ -D-Galactopyranosyl-(1 $\rightarrow$ 4)-*O*- $\beta$ -D-xylopyranosyl-L-serine

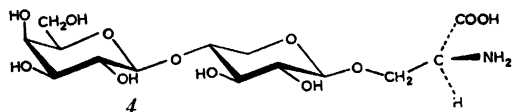
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The linkage region between the polysaccharide and protein chains in heparin and other animal proteoglycans has the structure 1.<sup>1</sup> Fragments of this linkage were needed for biosynthetic studies and we have previously synthesized *O*- $\beta$ -D-xylopyranosyl-L-serine<sup>2</sup> and the oligosaccharides 2 and 3.<sup>3</sup>



We now report the synthesis of *O*- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-*O*- $\beta$ -D-xylopyranosyl-L-serine (4).



4-*O*- $\beta$ -D-Galactopyranosyl-D-xylose (2) was prepared essentially as previously described,<sup>3</sup> but the use of silver triflate<sup>4</sup> and 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-galactopyranosyl bromide in the glycosidation step increased the total yield of pure 2 to 48 %, calculated from the galactosyl bromide.

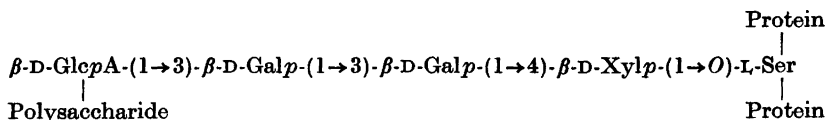
Disaccharide 2 was benzoylated and treated with hydrogen bromide in dichloromethane-acetic acid. The resulting glycosyl bromide was reacted with *N*-carbobenzyloxy-L-serine benzyl ester and silver triflate in nitromethane at  $-25^{\circ}\text{C}$ . The resulting glycoside was obtained in 72 % yield. Removal of protecting groups by hydrogenation over palladium followed by treatment with methanolic ammonia yielded the title compound (4). Some  $\beta$ -elimination, yielding the disaccharide 2, obviously occurred during the latter treatment, and the product was therefore purified by chromatography.

In the  $^{13}\text{C}$  NMR spectrum of 4 (Table 1), signals for all the fourteen carbon atoms could be identified by comparison with the corresponding spectra of methyl  $\beta$ -D-xylopyranoside,<sup>5</sup> methyl  $\beta$ -D-galactopyranoside,<sup>5</sup> serine, and *O*- $\beta$ -D-xylopyranosyl-L-serine.<sup>2</sup> In this spectrum, the signals assigned to the serine carbon atoms and C-1 of xylose, however, were doublets of unequal intensities (ratio  $\approx$  2:1), separated by 0.1–0.3 ppm. This is most probably due to partial racemization of the serine residue, known to occur during the basic conditions of the *O*-deacylation.<sup>6</sup>

**Experimental.** Melting points are corrected. Concentrations were performed at reduced pressure and a bath temperature below  $40^{\circ}\text{C}$ . Optical rotations were recorded at room temperature ( $22-24^{\circ}\text{C}$ ) with a Perkin-Elmer 241 instrument.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with a JEOL FX-100 NMR spectrometer. Analytical TLC was performed on silica gel F<sub>254</sub> plates. Sulfuric acid was used as spray reagent. Preparative separations were performed on silica gel columns (Merck Kieselgel 60, 0.040–0.063 mm). For paper chromatography, Whatman No. 4 paper was used. Silver nitrate and ninhydrin were used for detection. Spectrograde nitromethane (Matheson and Co.) was distilled under reduced pressure over phosphorus pentoxide immediately before use. Syrupy 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-galactopyranosyl bromide was prepared essentially as described by Fletcher<sup>7</sup> for the corresponding D-glucose derivative. It showed  $[\alpha]_{\text{D}}^{20} +192^{\circ}$  (c 0.5, chloroform).

All new substances gave  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra in agreement with the postulated structures. The purities of all compounds were checked by TLC and/or PC in different solvent systems.

**Benzyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl- $\beta$ -D-galactopyranosyl)-2,3-anhydro- $\beta$ -D-ribopyranoside (6).** A solution of 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-galactopyranosyl bromide (11.2 g) in nitromethane (40 ml) was added, during a period of 5 min, to a stirred solution of benzyl 2,3-anhydro- $\beta$ -D-ribopyranoside<sup>8</sup> (3.46 g), silver triflate (4.63 g) and 2,4,6-collidine (2.38 ml) in nitromethane (80 ml), cooled to  $-25^{\circ}\text{C}$ . The reaction mixture was warmed to room temperature, diluted with toluene (400 ml) and filtered. The solution was washed successively with 2 M sulfuric acid and aqueous sodium hydrogen carbonate, dried over sodium sulfate and concentrated. The resulting syrup was fractionated on a silica gel column (700 g) using toluene-ethyl acetate (85:15) as eluent.



1

Table 1.  $^{13}\text{C}$  NMR spectra of **4** and some reference substances in  $\text{D}_2\text{O}$  (Chemical shifts in ppm are relative to external tetramethylsilane).

Serine		Xylose				Galactose							
C-1	C-2	C-3	C-1	C-2	C-3	C-4	C-5	C-1	C-2	C-3	C-4	C-5	C-6
Methyl $\beta$ -D-xylopyranoside													
			105.1	74.0	76.9	70.4	66.3						
Methyl $\beta$ -D-galactopyranoside													
								104.9	71.8	73.9	69.8	76.2	62.1
L-Serine													
174.5	57.7	61.8											
O- $\beta$ -D-Xylopyranosyl-L-serine													
173.0	55.8	69.1	104.1	74.0	76.7	70.4	66.4						
Compound <b>4</b>													
173.2 <sup>a</sup>	55.9 <sup>a</sup>	69.3 <sup>a</sup>	104.2 <sup>a</sup>	73.8	74.9	77.7	64.2	103.1	71.9	73.8	69.9	76.6	62.4
173.0	55.8	69.0	103.9										

<sup>a</sup> Ratio between the two peaks  $\approx$  1:2.

The main fraction contained pure **6** (10.6 g) which crystallized from methanol, m.p. 152–153°C,  $[\alpha]_{\text{D}} + 89^\circ$  (c 0.5, chloroform). Anal.:  $\text{C}_{45}\text{H}_{46}\text{O}_{13}$ ; C, H.

*Benzyl 4-O-( $\beta$ -D-galactopyranosyl)-2,3-anhydro- $\beta$ -D-ribofuranoside (7)*. Compound **6** (10.0 g) was dissolved in boiling methanol (500 ml) and 0.1 M sodium methoxide (50 ml) in methanol was added. After 3 h, the solution was neutralized with methanol-washed Dowex 50 ( $\text{H}^+$ ), concentrated and triturated with hexane to give **7** as an amorphous powder (4.5 g),  $[\alpha]_{\text{D}} - 8^\circ$  (c 0.5,  $\text{H}_2\text{O}$ ).

*Benzyl 4-O-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-xylopyranoside (8)*. A solution of **7** (4.35 g) in 5% aqueous sodium hydroxide was kept at 100°C for 24 h, neutralized (Dowex 50,  $\text{H}^+$ ) and concentrated. The syrup obtained was crystallized from methanol to give material with m.p. 40–42°C (3.2 g),  $[\alpha]_{\text{D}} - 38^\circ$  (c 0.5,  $\text{H}_2\text{O}$ ).

Sugar analysis of **8** showed the presence of galactose and xylose in the molar ratio 1:1. No arabinose was observed in the crystalline product.

*4-O- $\beta$ -D-Galactopyranosyl-D-xylose (2)* was prepared by catalytic hydrogenation of **8** as previously described.<sup>3</sup> The product, which did not crystallize, showed  $[\alpha]_{\text{D}} + 18^\circ$  (c 0.5,  $\text{H}_2\text{O}$ ).

*4-O-( $\beta$ -D-Galactopyranosyl)- $\alpha$ -D-xylopyranosyl bromide hexabenzate (9)*. Disaccharide **2** (1.84 g) was benzoylated by treatment with benzoyl chloride in pyridine and worked up as usual. The syrupy product (5.97 g) showed two spots on TLC (toluene–ethyl acetate, 8:2) corresponding to the  $\alpha$  and  $\beta$  anomers.

A solution of the crude product in dry dichloromethane (15 ml) was cooled with ice, and acetic acid (12 ml), saturated with hydrogen bromide at room temperature, was added with stirring. After 90 min, when the reaction mixture contained a single carbohydrate component (TLC), the solution was diluted with dichloromethane (50 ml), washed first with ice-water and then with aqueous sodium hydrogen carbonate, dried over sodium sulfate and concentrated. The amorphous product (5.67 g) showed  $[\alpha]_{\text{D}} + 80^\circ$  (c 0.5, chloroform). In the  $^1\text{H}$  NMR spectrum of **9**, the signal for the anomeric proton of the xylopyranosyl residue appeared at  $\delta$  6.66,  $J_{1,2}$  4 Hz, demonstrating that it had  $\alpha$ -configuration.

*N-Benzylloxycarbonyl-3-O-[2,3-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)- $\beta$ -D-xylopyranosyl]-L-serine benzyl ester (10)*. Glycosyl bromide **9** (5.40 g) in nitromethane (25 ml) was added over a 60 s period to a stirred solution of *N*-benzyloxycarbonyl-L-serine benzyl ester (1.98 g) and silver triflate (1.54 g) in nitromethane (30 ml), cooled to  $-30^\circ\text{C}$ . After 3 min, pyridine (1 ml) was added to neutralize the liberated acid and the mixture was allowed to reach room temperature. Ethyl ether (150 ml) was added, salts removed by filtration and the solution washed first with 2 M sulfuric acid and then with aqueous sodium hydrogen carbonate. The solution was dried and concentrated to a syrup which was fractionated on a silica gel column (700 g) using toluene–ethyl acetate (8:2) as eluent. The main fraction contained pure **10** (5.07 g) and showed  $[\alpha]_{\text{D}} + 13^\circ$  (c 0.5, chloroform). The  $^{13}\text{C}$  NMR spectrum

of 10 showed, *inter alia*, signals for the anomeric carbon atoms of the xylose and galactose residues at 100.6 ppm and 102.5 ppm, respectively. The corresponding signals for *N*-benzyl-oxy-carbonyl-3-*O*-(2,3,4-tri-*O*-benzoyl- $\beta$ -D-xylo-pyranosyl)-L-serine benzyl ester<sup>4</sup> and methyl tetra-*O*-benzoyl- $\beta$ -D-galactopyranoside appear at 99.9 and 102.4 ppm, respectively. In the spectrum of 10, no other signals in the region of anomeric carbons were observed.

*O*- $\beta$ -D-Galactopyranosyl-(1 $\rightarrow$ 4)-*O*- $\beta$ -D-xylo-pyranosyl-L-serine (4). Compound 10 (4.91 g) was dissolved in ethyl acetate-ethanol-acetic acid-water (5:20:5:1, 150 ml) and hydrogenated at 0.5 MPa using 10% palladium on charcoal (2.0 g) as catalyst. The reaction was complete in 24 h. Filtration and concentration gave a syrup which was dissolved in methanol (250 ml). The solution was saturated with ammonia gas and left at +4°C for 4 days. Concentration gave a syrup, which was partitioned between water and chloroform. The water phase was washed several times with chloroform, concentrated to half the volume and lyophilized. The resulting syrup (2.6 g) showed two main silver nitrate-positive spots on paper chromatography (ethyl acetate-acetic acid-water, 3:1:1), one having the same  $R_F$  value as compound 2, the other corresponding to compound 4. The syrup was fractionated in 200 mg portions on a Sephadex LH-20 column (50  $\times$  2 cm) using ethanol-water (1:1) as eluent. The ninhydrin-positive fractions were collected and minor impurities removed by gel filtration on a Bio-Gel P-2 column (5  $\times$  100 cm) to give a total of 320 mg partially racemized 4, having  $[\alpha]_D^{25} = -27^\circ$  (c 0.5, H<sub>2</sub>O).

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## Tobacco Chemistry. 46. Syntheses of (12*R*,13*S*)- and (12*S*,13*R*)-8,12-Epoxy-14-labden-13-ol and (12*S*,13*R*)-8,13-Epoxy-14-labden-12-ol, Three Tobacco Diterpenoids

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In a previous communication we described the synthesis of four tobacco diterpenoids, the (12*R*,13*R*)- and (12*S*,13*S*)-8,12-epoxy-14-labden-13-ols (1, 2) and the (13*R*)- and (13*S*)-8,13-epoxy-14-labden-12-ones (3, 4) from (12*Z*)-abienol (5),<sup>1</sup> a compound which has been proposed as an appropriate precursor of the tobacco labdanoids.<sup>2</sup> We now report the preparation of three additional tobacco labdanoids by peracid oxidation of (12*E*)-abienol (10), the double bond isomer of starting material 5.

Treatment of (12*E*)-abienol (10) with *m*-chloroperbenzoic acid in chloroform afforded four products of low polarity (6-9), which according to GC-integration were present in the ratio 49:6:29:16, as well as two compounds of higher polarity, the (12*S*,13*S*,14 $\xi$ )- and (12*R*,13*R*,14 $\xi$ )-8,12-13,14-diepoxy-15-labdanols constituting some 20% of the total reaction mixture. The latter two compounds are formed by a novel type of mechanism, which will be discussed elsewhere.<sup>3</sup>

Of the less polar products, 6 and 7 proved to be identical to two of the stereoisomers of (12 $\xi$ ,13 $\xi$ )-8,12-epoxy-14-labden-13-ol, which have previously been encountered in Greek tobacco and then designated "Ia" and "Ic", respectively.<sup>4</sup> The configuration at C-12 in the tetrahydrofurans 6 and 7 was deduced from the <sup>13</sup>C NMR spectra (cf. Table 1). The C-12 and C-17 signals for compound 6 appeared at  $\delta$  81.7 and 21.4, respectively, which is close to the corresponding values, 81.6 and 21.3 ppm, for (12*R*,13*R*)-8,12-epoxy-14-labden-13-ol (1) and is consistent with a 12*E*-configuration in compound 6.<sup>1</sup> Since the C-12 and C-17 signals were present at  $\delta$  85.5 and 25.5 for compound 7 and at  $\delta$  85.3 and 25.4 for (12*S*,13*S*)-8,12-epoxy-14-labden-13-ol (2), it was concluded that compound 7 has the 12*S*-configuration. With these results at hand the chiralities at C-13 followed from a consideration of the reaction mechanism involved in the peracid oxidation of (12*E*)-abienol (10) (cf. Scheme 1). Thus, an S<sub>N</sub>2 type of oxide opening at the secondary C-12 in the intermediate epoxides 13 and 14, or their equivalents, by attack of the 8 $\alpha$ -hydroxyl group affords the (12*R*,13*S*)- and (12*S*,13*R*)-8,12-epoxy-14-labden-13-ols (6, 7), respectively.