

Supplementary material to Aquino et al. "Heparins from porcine and bovine intestinal mucosa: are they similar drugs?"

(Thromb Haemost 2010; 103.4)

2D NMR spectra of heparins from bovine and porcine intestinal mucosa

Correlation-peaks in the 2D $^1\text{H}/^1\text{H}$ COSY and TOCSY spectra allowed us to trace the preponderant spin systems of bovine and porcine heparins. The COSY spectrum of porcine heparin showed two major correlation signals due to scalar-couplings between ^1H -anomeric protons and H2 of the α -glucosamine and α -iduronic acid units (A1 vs. A2 and I1 vs. I2, respectively, in Fig. 2A). It was also possible to identify clearly the correlation signal between H-5 and H-4 of α -iduronic acid residues (I5 vs. I4). In contrast, bovine heparin showed seven correlation signals in the 4.78 - 5.42 ppm region of the spectrum. Three of them are between ^1H -anomeric protons and H2 of the α -glucosamine residues (Fig. 1D). Four correlation signals were observed for the α -iduronic acid, two of them between I1 and I2 and another two between U5 and U4.

Following identification of the cross peaks in the COSY spectra, the TOCSY experiments (Fig. 2, B and E) allowed to assign unequivocally the spin systems to obtain most of the ^1H chemical shifts, as indicated in Table 1. NOESY spectra showed similar results (not shown). Based on the ^1H -chemical shifts, the peaks of correlation in the $^1\text{H}/^{13}\text{C}$ HSQC spectra (Fig. 2, C and F) were assigned to obtain the values of ^{13}C chemical shifts, as shown in Table I.

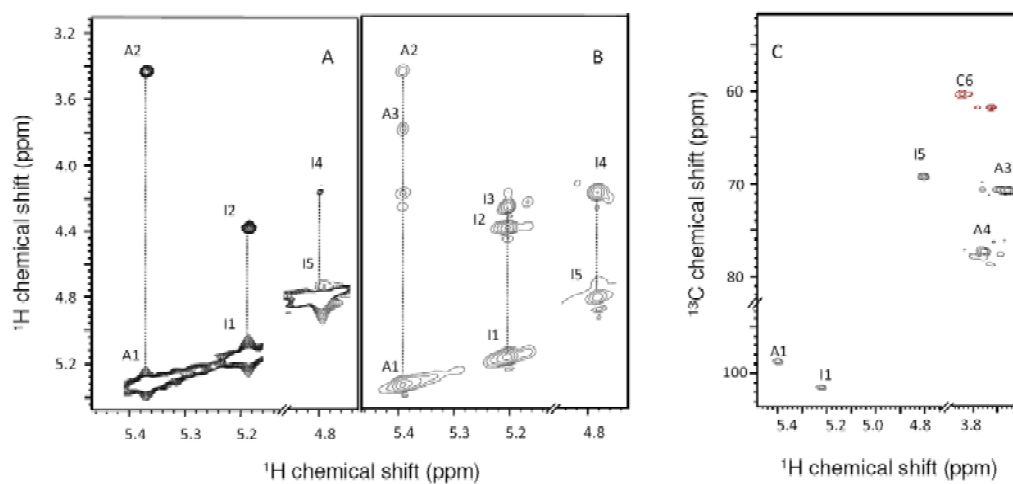


Figure 2 (A,B and C)

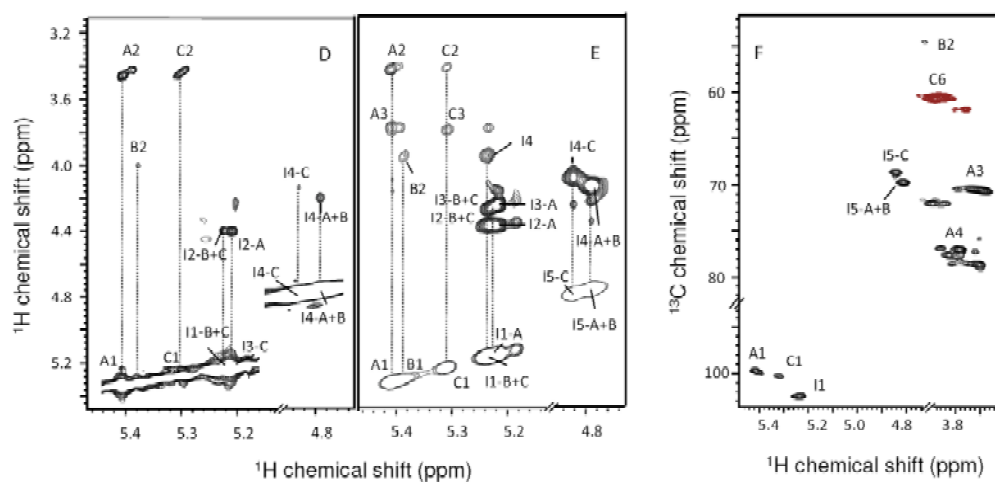


Figure S1 (D,E and F)

Figure S1: Strips from the $^1\text{H}/^1\text{H}$ COSY (A and D), $^1\text{H}/^1\text{H}$ TOCSY (B and E) and $^1\text{H}/^{13}\text{C}$ HSQC (C and F) spectra of porcine (A, B and C) and bovine heparins (D, E and F) at 800 MHz. About 20 mg of each sample were dissolved in 0.5 mL of 99.9% D_2O , and the NMR spectra were recorded at 25°C . ^1H Chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm. The residual water was suppressed by presaturation. See the legend of Fig. 1 for the nomenclature of the residues. The signal indicated as C6 in Panels C and F (red) is from non-sulfated C6/H6 of α -glucosamine units. In Panel F the signal indicated as B2 corresponds to C2/H2 of *N*-acetylated α -glucosamine.

Analyses of mixtures of heparins from bovine and porcine intestinal mucosa

Figure S2: The 4.75 - 4.94 (A), 5.18 - 5.27 (B) and 5.26 - 5.47 (C) ppm regions of the 1D ¹H NMR spectra at 800 MHz of mixtures containing increasing amounts of heparin from bovine intestine added to a fixed amount of porcine heparin. Varying amounts of heparin from bovine intestine (2 to 50 mg) were added successively to 20 mg of porcine heparin dissolved in 0.5 mL of 99.9% D₂O. After each addition an NMR spectrum was recorded. The signals that are characteristic of bovine heparin increase over the course of the titration curve (see asterisks in the panels). See the legend of Fig. 1 for the nomenclature of the signals.

Figure S3: Peak areas of the signals assigned to H1 of the α -glucosamine units (A) and to H1 (B) and H5 (C) of the α -iduronic acid residues over the course of titration curves, adding increasing amounts of bovine heparin to a fixed amount of porcine heparin. The signal areas were derived from the ¹H NMR spectra shown in Fig. S1. See the legend of Fig. 1 for the nomenclature of the signals indicated in the panel. Note that the spectral areas of B1, C1, I1-B+C and I5-C increase and those of A1, I1-A and I5-A decrease in accordance with the amount of bovine heparin added to the mixtures.

Figure S4: 4.75 - 4.94, 5.18 - 5.27 and 5.26 - 5.47 ppm regions of the 1D ¹H NMR spectra at 800 MHz of three batches containing mixtures of heparins from bovine and porcine intestine. The amounts of bovine heparin in these batches were estimated based on the curves shown in Fig. S2 and are indicated to the right of the panels.

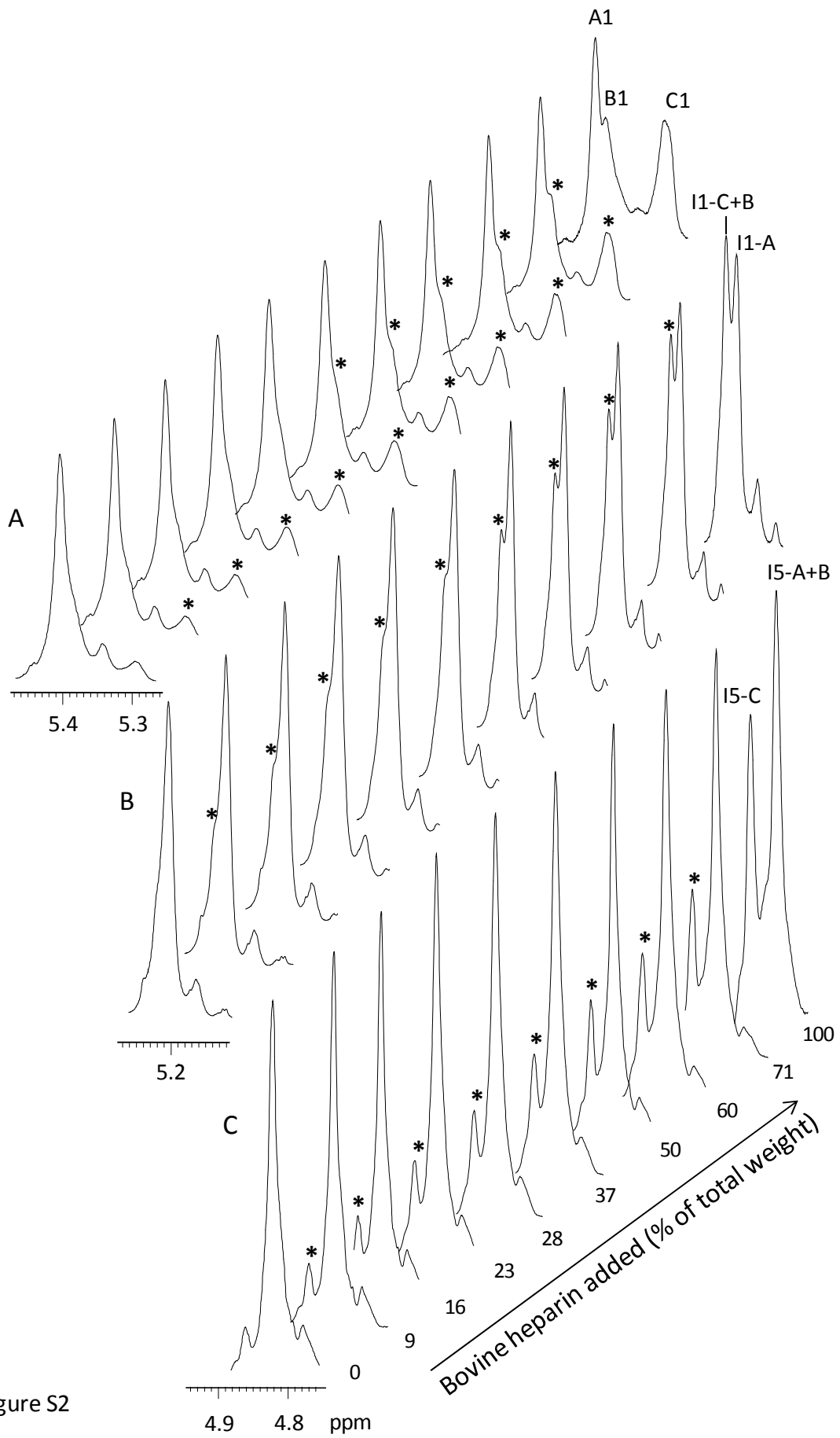


Figure S2

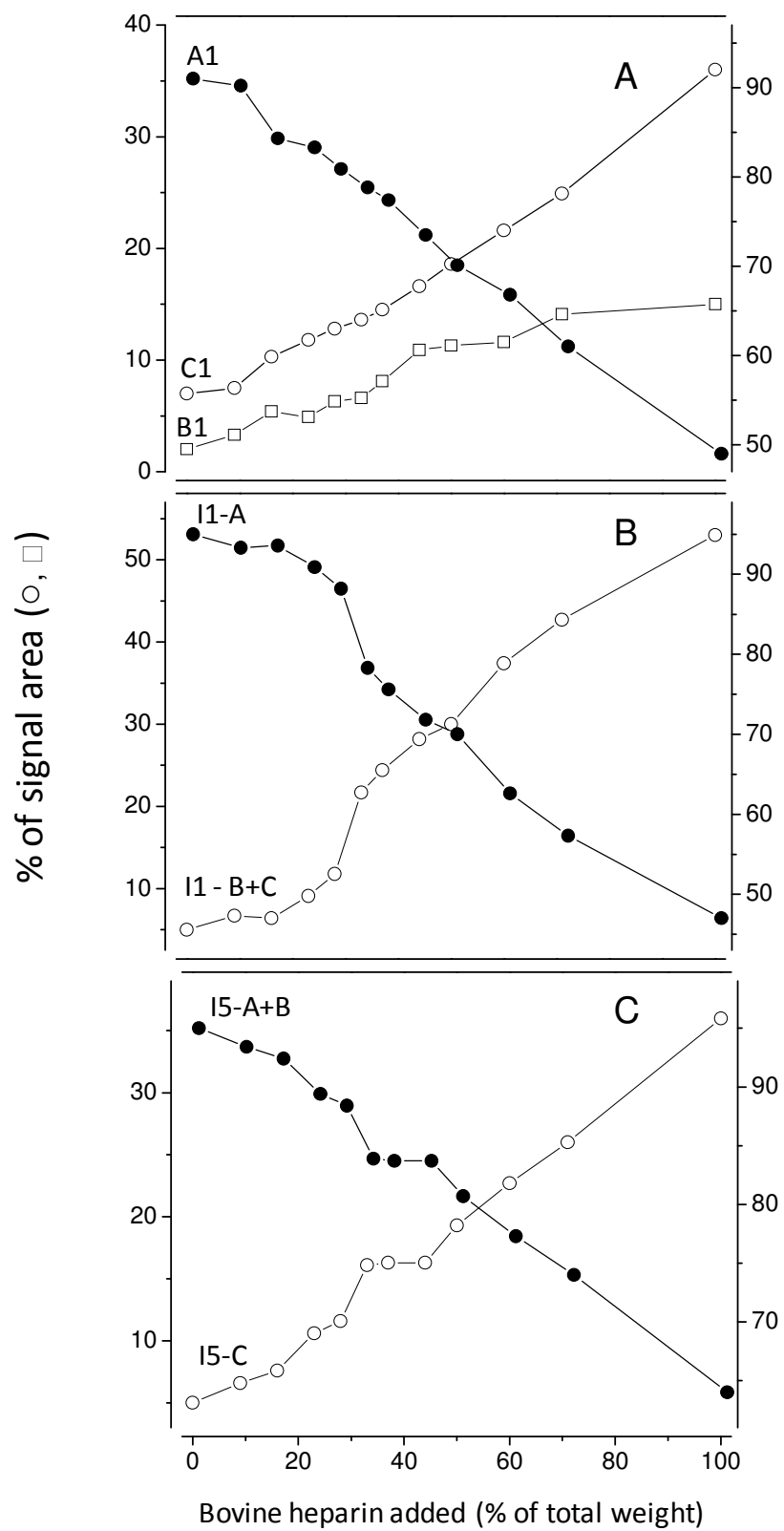


Figure S3

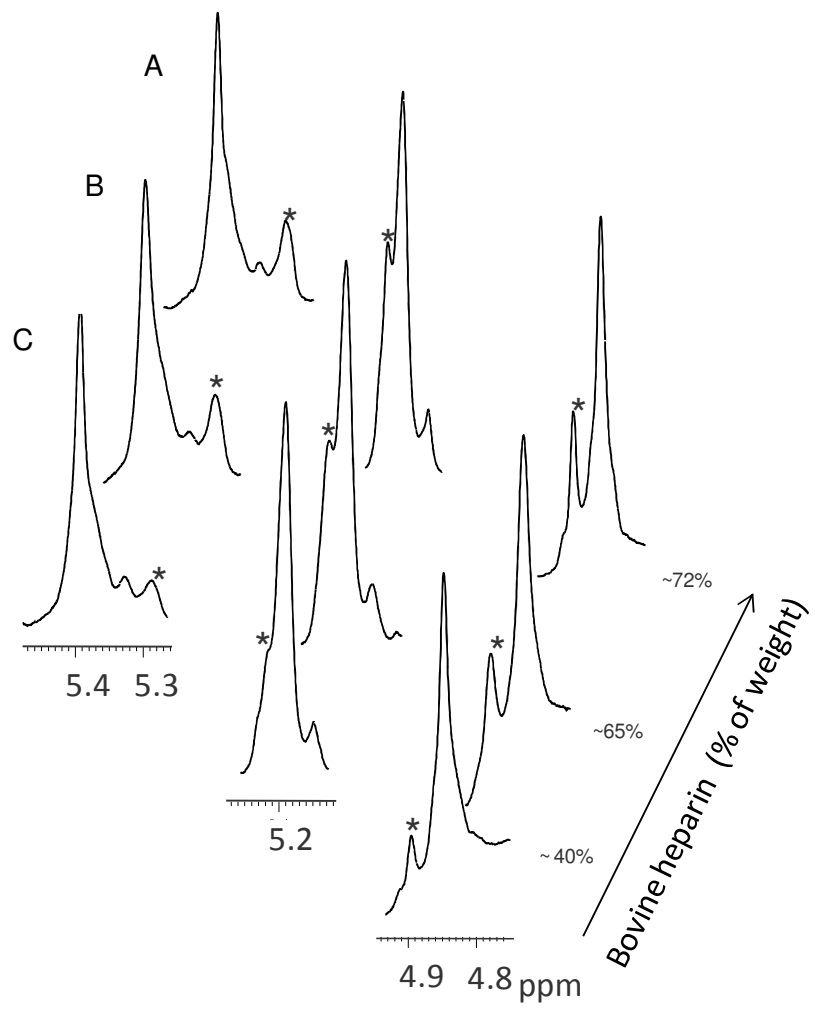


Figure S4

Table S1. Anticoagulant activity of batches containing mixtures of heparins from bovine and porcine intestine

Source of heparin (% of the total) ^a		
Porcine	Bovine	Anticoagulant activity (as IUmg ⁻¹)
~ 85	~ 15	150
~ 40	~ 60	129
~ 30	~ 70	104
100	-	197 ± 15 ^b
-	100	104 ± 22 ^b

^aSee Fig. S3.

^bData from the assays shown in Fig. 5A.