Hudson [10], horse oxyhemoglobin was prepared according to Ferry *et al.* [11] and beef cytochrome c according to Neilands [12]. Crystalline beef metmyoglobin was a gift from Mr. Å. Åkeson. Egg albumine, lysozyme and bovine serum albumine were crystalline commercial preparations. Phosphate buffers were prepared from the sodium salts and when other anions were studied the corresponding sodium salts were also used in order to avoid a cation effect on the adsorption phenomena. The adsorption experiments were carried out at + 4°C in 10 ml centrifuge tubes which were agitated on a simple manual rotator during the incubation. The adsorbent was then centrifuged off and protein remaining in the supernatant solution was determined from the adsorption at 280 mμ (or 405 mμ in case of the heme proteins) corrected for a blank, obtained by omitting the protein from the test system. Only a rather narrow pH-range (5.6−7.8) was studied as calcium phosphate is unstable outside this range. All experiments were carried out in the presence of phosphate buffer, as it was found impossible to free the gel from free phosphate by repeated washing on the centrifuge. For an explanation of this effect se Ref. [13,14]

Preliminary experiments were carried out with a reaction time of 30 min, but as it was later observed that the adsorption of proteins on the gel was very rapid, a shorter reaction time (5 min) was used in the following experiments.

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Halogen-Metal Interconversion with Dibromobithienyls

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Bithienyl dicarboxylic acids are useful intermediates for a study of optically active bithienyls [1] and of thiophene analogues to fluorene. Most bithienyls have been prepared by the Ullmann reaction [2-4], which usually gives low yields, especially in the absence of activating substituents such as $-NO_2$ or $-CO_2CH_3$. The coupling of Grignard reagents with $CuCl_2$ was used in a few cases by Steinkopf *et al.*[5,6] for the preparation of bithienyls. In the thiophene series, however, organolithium compounds are available through halogen-metal interconversion, in cases where Grignard reagents are formed only with difficulty [7,8]. Thus the present author and Karlsson [9] were able to demonstrate that good yields of bithienyls can be obtained on coupling 2- and 3-thienyllithium with $CuCl_2$.

It has now been found that 4,4′-dibromo-3,3′-bithienyl (I) and 3,3′-dibromo-2,2′-bithienyl (II) are obtained in 50−60 % yield by treating 4-bromo-3-thienyllithium and 3-bromo-2-thienyllithium, respectively, with $CuCl_2$ at −70°. The NMR-spectra of the products showed that no rearrangement had occurred during the coupling. II was obtained earlier in lower yield by Steinkopf *et al.*[6] by the coupling of 3-bromo-2-thiophenemagnesium bromide, obtained through the entrainment Grignard reaction of 2,3-dibromothiophene, with $CuCl_2$.

It was shown earlier [10] that thienyllithium derivatives in which the Li is not in the most acidic position, undergo a complex series of rearrangements when they are obtained by halogen-metal interconversion. They are, however, sufficiently stable at −70° to be of great use for the preparation of substituted thiophenes. It has now been found that the Li derivatives obtained from dibromobithienyls are stable at −70°. Treatment of I with excess *n*-butyllithium at −70° followed by carbonation gave 4,4′-dicarboxy-3,3′-bithienyl, which melts with decomposition at 275−280°. Its structure is demonstrated by the occurrence in its NMR-spectrum of a coupling constant of 3.4 c/s between the two groups of equivalent ring-hydrogens. This is of the magnitude characteristic of J_{25} in 3,4-disubstituted thiophenes [11]. The properties (m.p., IR- and NMR-spectra) of this acid differ from those of 2,2- and 5,5′-dicarboxy-3,3′-bithienyl, which have been obtained through metalation of 3,3′-bithienyl with excess *n*-butyllithium [12].

Similarly reaction of II with excess *n*-butyllithium at −70° followed by carbonation, gave 3,3′-dicarboxy-2,2′-bithienyl, which melted with decomposition at 289−291°. This acid was prepared earlier in low yield by Nord *et al.*[2] by the Ullmann coupling of the less easily obtained methyl 2-bromo-3-thiophenecarboxylate. They state the m.p. to be 175−185°, but this is probably due to a printing error as the m.p. of the dimethyl ester is the same as that obtained in the present investigation.

Experimental. 4,4′-Dibromo-3,3′-bithienyl. 74 g (0.55 mole) of anhydrous $CuCl_2$ were added at −70° to a solution of 4-bromo-3-thienyllithium prepared in the usual way [13] at −70° from 570 ml of 0.92 N *n*-butyllithium and 107 g (0.44 mole) of 3,4-dibromothiophene [14]. After standing at −70° for 2 h the mixture was allowed to attain room temperature overnight, and was then treated under cooling with 4 N hydrochloric acid. The ether phase was extracted several times with 4 N hydrochloric acid in order to remove cuprous chloride and was then extracted with water. It was subsequently dried, and ether and butyl bromide were removed *in vacuo*. The residue was recrystallized from ligroin (Norit) and yielded 37 g (52 %) of pure 4,4′-dibromo-3,3′-bithienyl, m.p. 127−129°, crystallizing in large flakes. (Found: C 29.76; H 1.32; Br 49.16. Calc. for $C_8H_4Br_2S_2$: C 29.64; H 1.24, Br 49.35.) The NMR-spectrum in dimethyl sulphoxide (23 wt %) consists of two strongly coupled doublets with a splitting of 3.4 c/s. The shifts of the two groups of equivalent ring hydrogens are 5.22 ppm and 5.11 ppm relative to the solvent peak.

3,3′-Dibromo-2,2′-bithienyl. This was prepared as described above from 138 g (0.57 mole) of 2,3-dibromothiophene [15] in 100 ml of ether, 630 ml of 1.0 N *n*-butyllithium and 100 g (0.74 mole) of anhydrous $CuCl_2$. Yield 56 g (61 %) of 3,3′-dibromo-2,2′-bithienyl, which crystallized from ligroin in beautiful almost cubic crystals, m.p. 102−104° (Found: C 29.62; H 1.24; Br 49.35. Calc. for $C_8H_4Br_2S_2$: C 29.64; H 1.24; Br 49.35.) (Literature values [6]: m.p. 96−97°). The NMR-spectrum in dimethyl sulphoxide (28 wt %) consists of two doublets with a splitting of 5.4 c/s. The shifts of the two groups of equivalent ring-hydrogens are 5.23 ppm and 4.66 ppm relative to the solvent peak.

4,4′-Dicarboxy-3,3′-bithienyl. A solution of 16.2 g (0.050 mole) of 4,4′-dibromo-3,3′-bithienyl in 100 ml of anhydrous tetrahydrofuran was added over a period of 5 min to 130 ml of 1.20 N *n*-butyllithium cooled to −70°. After a few minutes the mixture was poured onto solid carbon dioxide covered with ether. The reaction mixture was hydrolyzed with water, and the ether phase was extracted with sodium carbonate solution. On acidification with dilute hydrochloric acid the crude acid precipitated out (10.1 g, 80 %). The product was washed twice with 250 ml portions of ether and then recrystallized from glacial acetic acid, yielding 7.1 g of pure 4,4′-dicarboxy-3,3′-bithienyl in small prisms, m.p. 275−280° (decomp.). (Found: C 46.80; H 2.70; S 25.23. Calc. for $C_{10}H_6O_4S_2$: C 47.23; H 2.38; S 25.21). The resonances of the ring protons in its NMR-spectrum in dimethyl sulphoxide (15 wt %) consist of two doublets with a splitting of 3.4 c/s. The shifts of the two groups of equivalent ring-hydrogens are 5.64 ppm and 4.84 ppm relative to the solvent peak.

4,4′-Dicarbomethoxy-3,3′-bithienyl was prepared through methylation with diazomethane, m.p. 147−148°.

3,3′-Dicarboxy-2,2′-bithienyl. This was prepared as described above from 10.0 (0.031 mole) of 3,3′-dibromo-2,2′-bithienyl and 76 ml of 0.92 N *n*-butyllithium. Yield 5.5 g (70 %) of crude acid. Purification as above gave 3.5 g of pure acid, m.p. 289−291° (decomp.) (Found C 46.67; H 2.50; S 25.01. Calc. for $C_{10}H_6O_4S_2$: C 47.23; H 2.35; S 25.21.) In the NMR-spectrum in dimethyl sulphoxide solution (34 wt %) the resonances of all four ring-hydrogens coincide at 4.97 ppm relative to the solvent peak.

3,3′-Dicarbomethoxy-2,2′-bithienyl was prepared through methylation with diazomethane, m.p. 150−151°. (literature value [2]: m.p. 145−147°.)

The NMR-spectra were obtained at 40 Mc/s with a Varian Associates model V-4 300 B high resolution NMR-spectrometer and a flux-stabilized 12 in. electromagnet obtained from the same company. The magnet sweep was calibrated using the modulation side-band technique.

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Separation of Uronic Acids by Paper Electrophoresis

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The purpose of this work was to develop a rapid and convenient method for qualitative and quantitative analysis of mixtures of uronic acids. Paper chromatography of uronic acids in the solvents most commonly used for chromatography of sugars does not give satisfactory results. In basic solvents such as pyridine-ethyl acetate-water, the uronic acids do not move, and in acidic solvents such as acetic acid-ethyl acetate-water, the mobilities of the different uronic acids are nearly identical. The most satisfactory solvents are mixtures of both basic and acidic components, such as the solvent introduced by Fischer and Dörfel [1]: Pyridine-ethyl acetate-acetic acid-water, 5:5:1:3. However, the separation is not very satisfactory, it is not possible to distinguish between glucuronic and guluronic acid, it is time-consuming and for quantitative work it is necessary beforehand to transform all lactones to uronic acids. Paper electrophoresis of uronic acids has been used by Jayme and Kringstad [2] and Hoffman, Linker and Meyer [3] but no details of the mobilities of the different uronic acids were given. The possibility of using paperelectrophoresis for separation of uronic acids was therefore further investigated.

Materials and methods. The glucuronic and galacturonic acids used were commercial preparations. The guluronic and mannuronic acids were prepared from hydrolysates of alginic acid by chromatographic separation of the lactones [4] and transforming the lactones by addition of alkali to uronic acid salts. The determination of the pK values has been described earlier [5]. The electrophoresis was carried out in an LKB paper electrophoresis apparatus on Schleicher & Schüll 2043b paper strips. The

Table 1. pK_s values and mobilities in acidic medium.

	pK_s	M_m
Glucuronic acid	3.20	1.10
Mannuronic acid	3.38	1.00
Galacturonic acid	3.42	0.95
Guluronic acid	3.65	0.88