673. *The Separation of Lactones from Reducing Sugars by Anion-exchange Resins.*

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The carbonate form of the strong-base resins De-Acidite FF micro-bead with 2% of cross linking has been shown to be suitable for the separation of lactones from reducing sugars.

DURING an investigation into the alkaline degradation of carbohydrates, the need arose for a method by which sugar acids could be separated from reducing sugars. An ion-exchange procedure was an obvious choice, but the situation was complicated by a number of factors. The acids in question existed in solution in equilibrium with their lactones, and preliminary experiments showed that, although the free acid was quite readily taken up on a column of an anion-exchange resin in the free-base form, sorption of the lactone was incomplete. This difficulty has already been reported by Berntsson and Samuelson [1] for sorption of gluconic acid on the weak-base resin Dowex-3, the lactone being completely taken up when stirred with an excess (unspecified) of the resin for 24 hours.

The choice of an anion-exchange resin is at once limited by the presence of alkali-labile reducing sugar. Thus, while for efficient sorption of the lactone, a strong-base resin would be preferred, the presence of a reducing sugar rules this out. Phillips and Pollard [2] showed that glucose and other sugars were strongly sorbed and rapidly degraded by the strong-base resin Amberlite IRA-400. However, Bryant and Overell [3] had noted that reducing sugars were not sorbed to an appreciable extent by Amberlite IRA-400 in the carbonate form, and that this could be used in a column for separation of acids such as citric, malic, and succinic from reducing sugars.

A further factor to be considered was the effect of the physical state of the resin on the sorption of a given lactone. Commercial anion-exchange resins are normally supplied in a particle size varying from *ca.* 0·25 to 1·0 mm. and, for these resins which are based on polystyrene, with a *ca.* 10% cross-linking of the polystyrene matrix. The rate and extent of sorption of large organic ions should be increased by reducing the particle size of the resin, and also by reducing the cross-linking to a nominal 2%, which is the lowest practical value.

It has now been found that the carbonate form of the strong-base resin De-Acidite FF micro-beads with 2% of cross linking gives satisfactory sorption of a number of lactones when it is stirred with their aqueous solutions for 24 hours and when the amount of lactone does not exceed *ca.* 10—15% of the theoretical exchange capacity of the resin. Further, recovery of the sorbed material was very high when aqueous ammonium carbonate was used as the eluant (see Table 1). These results contrast sharply with the result obtained on sorption of α-D-gluco*iso*saccharinolactone on the same resin in a *column*, the extent of

[1] Berntsson and Samuelson, *Acta Chem. Scand.*, 1955, **9**, 277.
[2] Phillips and Pollard, *Nature*, 1953, **171**, 41.
[3] Bryant and Overell, *ibid.*, 1951, **167**. 361.

sorption of the lactone being then only 48%, compared with 92% for the batch method with similar quantities.

Experiments with glucose, fructose, and xylose established that this carbonate resin had no measurable affinity for reducing sugars, and was without effect on such sugars in solution.

As a final demonstration of its suitability, a synthetic mixture of α-D-gluco*iso*saccharinolactone with a large excess of glucose was completely separated, and a 91% recovery of lactone achieved.

The two weak-base resins Amberlite IR-4B(OH) and De-Acidite G in the free-base form were also investigated. It is clear from the results in Table 1 that these resins do not take up the lactones satisfactorily, and that the sorbed material is not readily eluted. Further, both resins sorbed glucose from aqueous solution, as recorded by Reynolds [4] for Amberlite IR-4B(OH).

TABLE 1. *Sorption of lactones on anion-exchange resins.*

Resin (5 g.)	Lactone	Acid + lactone (milliequiv.)	Lactone (%)	Sorption of acid + lactone (%)	Elution of sorbed acid (%)
Amberlite IR-4B(OH)	D-Glucono-δ-lactone	5·1	*	98	82
	α-D-Gluco*iso*saccharino-lactone	2·65	71	89	54
De-Acidite G, free base (−16 + 50 mesh)	D-Glucono-δ-lactone	5·1	*	84	*
	α-D-Gluco*iso*saccharino-lactone	2·35	70	69	*
De-Acidite G, free base (micro-bead, 2% cross-linked)	D-Glucono-δ-lactone	5·1	*	97	35
	α-D-Gluco*iso*saccharino-lactone	2·4	68	74	53
De-Acidite FF, carbonate form (−16 + 50 mesh)	D-Glucono-δ-lactone	5·1	*	100	90
	α-D-Gluco*iso*saccharino-lactone	2·4	79	76	*
De-Acidite FF, carbonate form (micro-bead, 2% cross-linked)	D-Glucono-δ-lactone	5·1	*	99	97
	α-D-Gluco*iso*saccharino-lactone	5·3	71	56	*
	,, ,,	2·65	71	92	95
	,, ,,	1·58	79	100	97
	α-D-Glucosaccharino-lactone	2·45	83	95	98
	β-D-Gluco*meta*saccharino-lactone	2·5	66	95	99
	D-Saccharolactone	1·9	23	99	95
	Lactobionolactone	2·28	5	97	90

* Not determined.

It is of interest to examine the difference in behaviour of the various lactones investigated, as revealed by the extent of sorption of each lactone on different anion-exchangers. The extent of sorption of D-glucono-δ-lactone was practically independent of the resin employed. This is evidently due to the rapid hydrolysis of this lactone to the free acid in aqueous solution, the hydrolysis being followed by a relatively slow formation of the γ-lactone (see Table 2). These observations agree with earlier measurements [5] of the optical

TABLE 2. *Hydrolysis of* D-*glucono-δ-lactone.*

Time (hr.)	0	0·25	0·5	1·0	2·0	4	7	23·5	48
Lactone (%)	100·0	70·0	56·4	42·8	24·7	12·2	5·4	8·1	15·0

rotation of aqueous solutions of D-glucono-δ-lactone. Thus one is obviously concerned here with direct sorption of the free acid by the resin, hydrolysis of the δ-lactone to the free acid being accelerated by removal of the latter from the system by sorption as soon as it is formed. Lactobionolactone almost certainly behaves in a similar manner.

In contrast, the three D-glucosaccharinolactones were stable in solution. Thus, when

[4] Reynolds, *Nature*, 1955, **175**, 46.
[5] Isbell and Frush, *J. Res. Nat. Bur. Stand.*, 1933, **11**, 649.

the free acid was completely removed from an acid–lactone mixture by passage through a column of De-Acidite FF carbonate, no free acid was detected in the resulting lactone solution, even after 5 days at room temperature. It is clear that for these three lactones sorption by anion-exchange resins should take place less readily, and this conclusion is in accord with the experimental results. Whether the D-glucosaccharinolactones are sorbed directly by the resin, or the resin catalyses a prior hydrolysis to the free acids, remains to be established.

Experimental

The following solvents and sprays were used for chromatography with Whatman No. 1 paper at 25°. Solvents: A, ethyl acetate–acetic acid–water (10 : 1·3 : 1); B, butan-1-ol–pyridine–water (6 : 4 : 3). Sprays: a, silver nitrate–sodium hydroxide; [6] b, B.D.H. 4·5 indicator; [7] c, *p*-anisidine hydrochloride; [8] d, hydroxylamine–ferric chloride.[9]

Preparation of Lactones.—D-Glucono-δ-lactone was a commercial sample whose purity was checked by titration. α-D-Gluco-, α-D-gluco*iso*- and β-D-gluco*meta*-saccharinolactone, and D-saccharolactone were prepared by treating the calcium salt of the appropriate acid with an excess of washed Amberlite IR-120(H) resin to remove calcium, and finally heating the aqueous solution at *ca.* 70° for 1 hr. in the presence of the resin to bring about lactonisation. Lactobionolactone was obtained from the calcium salt as indicated, but the heating was omitted to avoid hydrolysis of the glycosidic link. The amount of acid + lactone present in each solution was determined by the addition of a two-fold excess of standard alkali, storage for 30 min. at 20° to decompose lactones, and back-titration with acid. Where appropriate, the amounts of free acid and lactone in solution were determined by direct titration with alkali to bromothymol-blue.

Preparation of Ion-exchange Resins.—Amberlite IR-4B(OH) was supplied in the free-base form and was freed from small amounts of chloride by washing in a column with a 2% solution of sodium carbonate. De-Acidite G (−16 + 50 mesh) was supplied in the chloride form, and was converted into the free-base form by washing with 3% ammonia solution. After the resin (80 g.) in a column had been treated with ammonia (3 l.) during 48 hr., appreciable amounts of chloride ion were still being removed. De-Acidite G (micro-beads, 2% cross-linked) was freed from fine material and treated as the large beads.

De-Acidite FF (−16 + 50 mesh) was converted into the carbonate form by treatment with a large excess of N-aqueous ammonium carbonate. The prepared resin still contained a small amount of chloride ion.

De-Acidite FF (micro-beads, 2% cross-linked) was freed from fines and converted into the carbonate form as above. The prepared material was free from chloride ion. Amberlite IR-120(H) was washed with distilled water until the effluent was free from chloride ions.

" AnalaR " or " Micro-analytical reagents (M.A.R.) " reagents were used in this and subsequent work.

Sorption of Lactones on Anion-exchange Resins.—An aqueous solution (50 ml.) (100 ml. for D-gluconolactone) of the acid–lactone mixture in amount as indicated in Table 1 was stirred with the air-dried resin (5 g.) for 24 hr. at room temperature. The resin was then transferred to a column, and the lactone remaining in the filtrate determined by titration as indicated previously. Sorbed material was eluted from the resin during 1 hr. with N-ammonium carbonate (100 ml.), except for the experiments in which Amberlite IR-4B(OH) was used, when the eluant was 2% aqueous sodium carbonate (250 ml.). The eluate was run directly on to a stirred suspension of Amberlite IR-120(H) (60 g.) to decompose excess of ammonium carbonate and the ammonium salt of the eluted acid. Removal of traces of ammonium ions was completed by passage of the acid solution through a column of the same resin (5 g.). With all the resins except De-Acidite FF carbonate (micro-beads, 2% cross-linked) the final acid solution was found to contain chloride ions eluted from the resin. These ions were eliminated by neutralisation of the solution with silver carbonate, and removal of the precipitated silver chloride by filtration. The free acid was then recovered by running the solution of the silver salt through a column of Amberlite IR-120(H) (5 g.), and the acid in the effluent determined as described. Results are recorded in Table 1.

[6] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[7] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[8] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[9] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

Sorption of α-D-*Glucoisosaccharinolactone on De-Acidite FF Carbonate (Micro-beads, 2% Cross-linked) in a Column.*—A solution of α-D-glucoisosaccharinolactone (2·4 milliequiv., 78% of lactone) in water (50 ml.) was run through a narrow (0·8 cm.) column of De-Acidite FF carbonate (micro-beads, 2% cross-linked) during 2·5 hr. The time taken for this operation was about five times longer than that usually accepted as adequate.[6] Titration of an aliquot part of the effluent and washings as described showed that only 1·15 milliequiv. (48%) of the original acid + lactone had been sorbed. Direct titration with alkali of a second portion established the absence of free acid, and from the two determinations it follows that the extent of sorption of the *lactone* was only 33%.

Sorption of Reducing Sugars on Anion-exchange Resins.—Amberlite IR-4B(OH). A solution of glucose (5 g.) in water (500 ml.) was run through a column of Amberlite IR-4B(OH) (5 g.). After the column had been washed with distilled water (6 l.) during 18 hr. sorbed glucose was eluted with a 2% sodium carbonate solution (180 ml.), and sodium ions were removed from the eluate with Amberlite IR-120(H) in batch and column as above. After elimination of chloride ions with silver carbonate as described, the solution was concentrated, under reduced pressure at room temperature, for paper chromatography. By using solvent A and sprays a and b, the presence of a considerable amount of glucose, but no acidic or neutral degradation products, was established.

De-Acidite G (−16 + 50 *mesh*). A solution of glucose (1 g.) in water (50 ml.) was stirred with De-Acidite G (5 g.) for 24 hr. After transference of the resin to a column, a portion of the solution was evaporated to small bulk and retained for chromatographic examination. The resin was then eluted with N-ammonium carbonate (100 ml.), and ammonium and chloride ions were removed as before. A portion of the eluate was then concentrated for chromatography.

Paper chromatography of the non-sorbed material and the eluate in solvent B, and application of sprays a and c, resulted in the detection of glucose only in the non-sorbed material, and a significant amount of glucose in the eluate. Further chromatography of the eluate in solvent A, and the use of sprays b and d, failed to detect any acids or lactones.

De-Acidite FF *carbonate (micro-beads, 2% cross-linked).* The behaviour of glucose, fructose, and xylose was investigated. The sugar (1 g.) was stirred with De-Acidite FF carbonate (micro-beads, 2% cross-linked) (5 g.) for 24 hr., and the resin separated from the non-sorbed sugar. Sorbed sugar was then recovered by elution as indicated, except for a simplification due to the absence of chloride ion in the eluate. In all three cases, paper chromatography of the non-sorbed material in solvent B, and the use of sprays a and c, detected nothing other than the original sugar. The eluates from the three experiments were chromatographed in solvents A and B, with sprays b and a, respectively. Neither the original sugar nor any degradation product was detected in the eluates. Testing with Tollens's reagent did reveal a trace of original sugar in the eluates from the fructose and xylose experiments, but comparison with standards showed that the amount of sugar present in the total eluate in both cases was certainly <1 mg.

Separation of Glucose and α-D-*Glucoisosaccharinolactone using De-Acidite* FF *Carbonate (Micro-beads, 2% Cross-linked).*—A mixture of glucose (2 g.) and α-D-glucoisosaccharinolactone (3·15 milliequiv., 79% lactone) in water (100 ml.) was stirred with De-Acidite FF carbonate (micro-beads, 2% cross-linked) (10 g.) for 24 hr. at room temperature. The resin was then transferred to a column and washed with water, and the combined filtrate and washings (1 l.) were concentrated to exactly 250 ml. under reduced pressure at room temperature. Non-sorbed lactone was determined by titration, and a correction applied for the small amount of acid formed from the glucose present. Sorption of the lactone was found to be virtually complete (99%). Chromatography of the non-sorbed material in solvent B, with sprays a and c, revealed the presence only of glucose.

The sorbed material was eluted from the resin during 2 hr. with N-ammonium carbonate (200 ml.), and the excess of eluant removed by evaporation to a thin syrup under reduced pressure. Passage of the diluted syrup (50 ml.) through a column of IR-120(H) (10 g.) resulted in the break-up of the ammonium salt to give a solution of the free acid. This modification in the treatment of the eluate economises in the use of IR-120(H) and thus eliminates a possible source of contamination of the solution with chloride ion. However, it can obviously be applied only when the eluate is known to be free from alkali-labile material. By titration of an aliquot part of the acid solution, the total acid present was found to be 2·84 milliequiv. A second elution of the resin with a similar amount of ammonium carbonate removed only a further 0·02

milliequiv. of acid. From these figures, the efficiency of elution of the sorbed acid was found to be 92%, and the recovery of the original acid 91%.

Paper chromatography of the eluate with solvent B and sprays a and c failed to reveal any glucose. With solvent A and sprays b and d, only α-D-gluco*iso*saccharinic acid and its lactone were detected.

Hydrolysis of Lactones.—D-*Glucono-δ-lactone.* This lactone (0·890 g., 5 milliequiv.) was dissolved in water (100 ml.) at 20°. At suitable intervals, aliquot parts of the solution were removed, and the free acid formed by hydrolysis was determined by a rapid direct titration with alkali. Results are given in Table 2.

D-*Glucosaccharinolactones.* Solutions of α-D-gluco-, α-D-gluco*iso*-, and β-D-gluco*meta*-saccharinolactone (*ca.* 2 milliequiv.) were obtained from the calcium salts of the parent acid as described. The solution of each lactone (50 ml.) was then run through a column of De-Acidite FF carbonate (−16 + 50 mesh) (5 g.) during 0·5 hr. This resulted in the sorption of all of the free acid and a small amount of the lactone, leaving a solution of the pure lactone. Titration of aliquot parts of each lactone solution failed to detect any free acid, even after the solution had been kept for 5 days at room temperature.

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[10] See, *e.g.*, Samuelson, " Ion Exchangers in Analytical Chemistry," Wiley, New York, 1953, p. 95.