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Amides of Glucuronic, Galacturonic, and Mannuronic Acids

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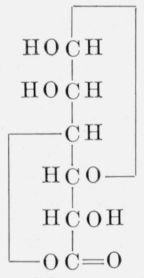
Heretofore the amides of glucuronic, galacturonic, and mannuronic acid were not known, and methods were not available for their preparation. It has now been found possible to obtain the amides of uronic acids from the corresponding 1-amino-uronamides by selective hydrolysis of the glycosylamino group. An aqueous solution of the 1-amino-uronamide is treated with an acid or a suitable cation exchange resin that replaces the glycosylamino group by a hydroxyl, forming the free amide. The new compounds, like the sugars, have a free reducing group, and hence they are capable of existing in the pyranose, furanose, and open-chain forms and of displaying mutarotation. D-Glucuronamide, D-mannuronamide, and D-galacturonamide were separated in the alpha pyranose form. The beta pyranose modification of D-galacturonamide was also obtained. The amides are stable crystalline substances, that should prove to be useful representatives of a new class of carbohydrate derivatives.

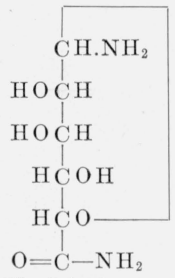
I. Introduction

The development of methods for the production of uronic acids [1] [1] and the recognition of their possible therapautic uses [2] make desirable the investigation of the amino derivatives of these substances. In a previous paper from this laboratory [3] it was shown that mannuronic lactone (I) reacts with 2 moles of ammonia, and that the crystalline product, 1-aminomannuronamide (II) contains a glycosylamino group and an amide group.[2] It has now been found that similar products can be obtained from the esters or lactones of glucuronic and galacturonic acids and that all three 1-amino-uronamides can be selectively hydrolyzed to give new crystalline amides (III, IV, V, VI) of the corresponding uronic acids. Although the amides of a number of substituted uronic acids are known [4], previous attempts to prepare the unsubstituted amides have been unsuccessful, presumably because of contamination with the 1-amino-uronamides. This difficulty has now been overcome by first isolating the amino-uronamide, and thereafter removing the glycosylamino group.

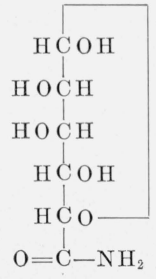
In all three 1-amino-uronamides, the glycosylamino group is so labile that it is hydrolysed



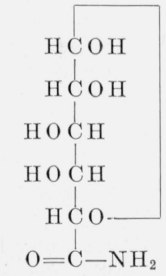
I. D-Mannuronic lactone.



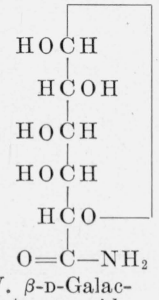
II. 1-Aminomannuronamide.



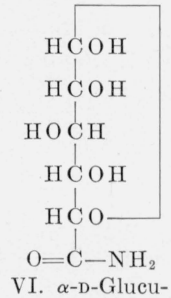
III. α-D-Mannuronamide.



IV. α-D-Galacturonamide



V. β-D-Galacturonamide.



VI. α-D-Glucuronamide.

[1] Figures in brackets indicate the literature references at the end of this paper.

[2] The uronic acids also react with alkyl and aryl amines to form substituted 1-amino-uronamides. A number of these compounds and some of their characteristic reactions will be described in a future publication.

slowly by dilute acid at room temperature. Figures 1, 2, and 3 show the pH titration curves obtained when 0.1 *N* HCl was added portion-wise to 75 ml of a solution containing 0.275 g of the 1-amino-uronamide. Sufficient time was allowed after each addition of acid for the pH of the solution to attain equilibrium. The 1-aminomannuronamide used was substantially pure, but the analogous derivatives of galacturonic and glucuronic acid were amorphous or partially crystalline, and, as indicated by the titration curves, had a purity of 87 and 68 percent, respectively. The nearly horizontal portion on the left of the curve is caused by the cleavage of ammonia from the glycosylamino group; the leveling off of the curve at the right of the figure is caused by hydrolysis of the amide nitrogen. On evaporation, solutions obtained after the addition of acid to pH 4 gave the pure crystalline amide corresponding to the 1-amino-uronamide used.

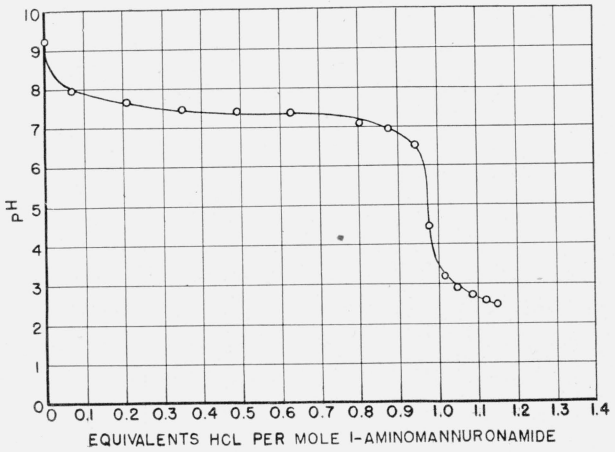


FIGURE 1. pH *titration curve for 1-aminomannuronamide.*

0.1 *N* HCl added portionwise to 75 ml of a solution containing 0.275 g of 1-aminomannuronamide.

Conversion of the 1-amino-uronamide to the corresponding amide was accomplished in several ways. The amino compound was treated with an equivalent quantity of dilute acid, and the resulting ammonium salt of the acid employed was removed by means of ion-exchange resins. When sulfuric acid was used for the hydrolysis, the ion-exchange resins were unnecessary, and the sulfate ion was removed by an equivalent quantity of barium hydroxide. It was also found that ion exchange resins of the carboxylic acid type were particularly suitable for hydrolysis of the 1-amino-

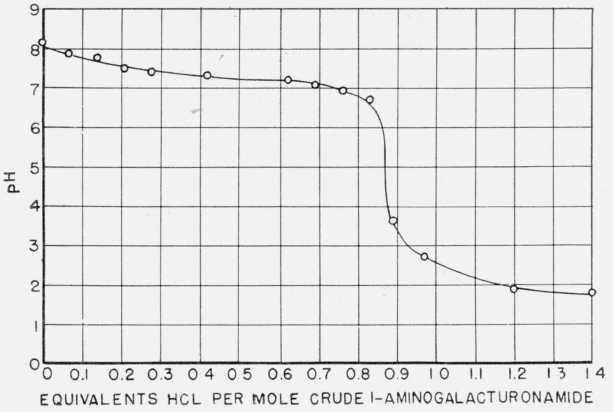


FIGURE 2. pH *titration curve for 1-aminogalacturonamide.*

0.1 *N* HCl added portionwise to 75 ml of a solution containing 0.275 g of crude 1-aminogalacturonamide.

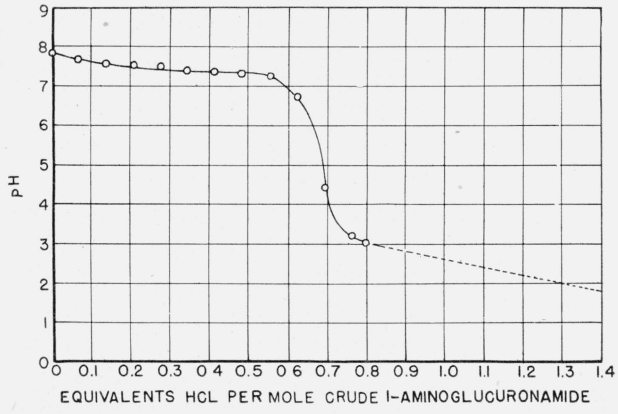


FIGURE 3. pH *titration curve for 1-aminoglucuronamide.*

0.1 *N* HCl added portionwise to 75 ml of a solution containing 0.275 g of crude 1-aminoglucuronamide.

uronamides, and that when these resins were used, previous acid treatment was unnecessary.

The new amides are substituted sugars capable of existence in the open chain, and the several ring modifications characteristic of the aldoses. Two crystalline modifications of galacturonamide have been isolated. One of these is an alpha pyranose analogous to α-D-galactopyranose; the other is a hydrate having the beta pyranose structure. Mannuronamide crystallizes as an anhydrous alpha pyranose, and glucuronamide as an alpha pyranose hydrate.

It was previously reported [3] that 1-aminomannuronamide could be acetylated and that the crystalline acetate, on deacetylation, yielded crystalline 1-*N*-acetylaminomannuronamide. In analogous manner, 1-*N*-acetylaminogalacturon-

amide has now been obtained; a comparative study of the properties of these two substances will be reported in a later publication.

II. Experimental Details

1. D-Mannuronamide

D-Mannuronamide was prepared from the 1-amino-D-mannuronamide previously reported, by removal of the glycosylamino group with a cation exchange resin of the carboxylic acid type, as follows: Twenty grams of 1-aminomannuronamide was dissolved in 500 ml of water, and the solution was passed slowly through a column of cation exchange resin.[3] The effluent was treated with a decolorizing carbon, filtered, and concentrated to a thick sirup, to which methanol was added from time to time until the total volume was 75 ml. After 24 hr, the truncated prisms that had formed were collected on a funnel, washed with aqueous methanol, and dried in a vacuum desiccator; they weighed 11.5 g. A second crop weighing 4.8 g was obtained by concentrating the mother liquor and again adding methanol. Mannuronamide was recrystallized in high yield by dissolving the material in water at room temperature, concentrating the solution to a sirup under reduced pressure, and cautiously adding methanol as crystallization proceeded. After one recrystallization, mannuronamide melted sharply at a point between 145° and 150° C, which depended on the rate of heating. In 4-percent aqueous solution, $[\alpha]_D^{20}=+2.4$ (1.4 min); $-10.4°$ (30 min). The properties were unchanged by further recrystallization. Analysis: Calculated for $C_6H_{11}O_6N$: C, 37.31; H, 5.74; N, 7.25. Found: C, 37.5; H, 5.9; N, 7.3.

Mannuronamide has also been prepared equally satisfactorily by the methods described for the preparation of galacturonamide and glucuronamide.

2. 1-Amino-D-galacturonamide

Fifty grams of α-D-galacturonic acid was converted to the methyl ester by dissolving it in 1,500 ml of a 0.02 *N* solution of HCl in absolute methanol and maintaining the solution at 0° C for 65 hr [5]. The methyl galacturonate was not isolated, but a stream of dry ammonia gas was bubbled through the solution until it was substantially saturated, and it was then allowed to stand for several days in the refrigerator. The partially crystalline material that separated was collected on a filter, washed with methanol, and dried in air. It weighed 24 g. The crude material gave the following analysis: Calculated for $C_6H_{12}O_5N_2$: C, 37.50; H, 6.29; N, 14.58. Found: C, 36.2; H, 6.5; N, 13.3.

1-Aminogalacturonamide is difficult to purify; it separates from solution as double-refracting clumps of indeterminate structure that sometimes contain amorphous material. Titration of a solution of the crude 1-amino-galacturonamide with dilute acid gave the curve shown in figure 2. The inflection indicates a purity of approximately 87 percent, and shows that the material is largely a glycosyl amine analogous to 1-aminomannuronamide (see fig. 1). Three minutes after dissolution in water the crude 1-aminogalacturonamide in 2-percent solution had a specific rotation of $-21.4°$. On standing there was a gradual drift in rotation, and after 102 hr $[\alpha]_D^{20}=-24.4°$. The substance was used without further purification for the preparation of crystalline galacturonamide.

3. D-Galacturonamide

To 20 g of crude 1-amino-D-galacturonamide dissolved in 500 ml of water, 145 ml of 0.7 *N* H_2SO_4 was added gradually over the course of 2 hr. The resulting solution was then treated with an exactly equivalent quantity of a solution of barium hydroxide in order to remove sulfate ion. The mixture was filtered, and the filtrate concentrated under reduced pressure to a thick sirup, which crystallized after standing for 2 days in a vacuum desiccator over calcium chloride. The crystals were separated, washed with aqueous methanol and air-dried; they weighed 15.0 g. A small second crop was separated from the mother liquor.

In a separate experiment the ammonium and sulfate ions were removed by successive passage of the acid-treated solution through columns of cation and anion exchange resins,[4] and the amide was obtained from the effluent in approximately the same yield as that reported above.

Galacturonamide crystallizes in both the alpha and beta modifications. The former is anhydrous, the latter a monohydrate. To prepare the beta modification, 10 g of the crude galacturonamide

[3] Amberlite IR-50, Resinous Products & Chemicals Co., Philadelphia, Pa.

[4] Zeo-Rex H and De-Acidite, Permutit Co., New York, N. Y.

was dissolved with slight warming in 10 ml of water, and the solution was treated with a decolorizing carbon, filtered, and allowed to stand for 2 hr at room temperature. Ethanol was then added almost to the point of turbidity (approximately 60 ml). In the course of several hours, long, slender prisms separated abundantly. The crystals were collected on a filter, washed with aqueous ethanol, and air-dried; they weighed 7.7 g. Additional material was reclaimed from the mother liquor. β-Galacturonamide hydrate was twice recrystallized by the method described. Analysis: Calculated for $C_6H_{11}O_6N.H_2O$: C, 34.12; H, 6.21; N, 6.63. Found: C, 34.2; H, 6.5; N, 6.9.

β-Galacturonamide monohydrate is stable in air, but when heated in a vacuum oven at 60° C, it slowly loses water. In a melting point tube heated at about 6 deg a minute, the substance melts at 113° to 116° C. It then resolidifies and melts with decomposition at 172° to 178° C. In 4-percent aqueous solution $[\alpha]_D^{20}=-21.4°$ (3 min); +14.6° (22 hr).

By crystallization in the following manner anhydrous α-galacturonamide was obtained: Ten grams of pure β-galacturonamide monohydrate was dissolved in 20 ml of warm water, and the solution was filtered with the aid of a decolorizing carbon and concentrated in vacuum to a thick sirup ($n_D^{20}=1.507$). Thirty milliliters of hot methanol was added, and the mixture was kept in a stoppered flask at a temperature of 60° C for a period of 4 hr. α-Galacturonamide crystallized in good yield in slender flat plates, which were separated, washed with methanol, and air-dried. The yield was 8 g. Analysis: Calculated for $C_6H_{11}O_6N$: C, 37.31; H, 5.74; N, 7.25. Found: C, 37.3; H, 5.9; N, 7.2.

α-Galacturonamide melts with decomposition at 172 to 178° C, is soluble in water, slightly soluble in methanol and ethanol, and insoluble in ether. In 4-percent aqueous solution $[\alpha]_D^{20}=+80°$ (3 min); +15.9° (22 hr).

4. 1-*N*-Acetylamino-D-galacturonamide

In the procedure described below, 1-amino-D-galacturonamide was acetylated and the O-acetyl groups of the sirupy acetate were removed by ammoniacal methanol to yield 1-*N*-acetylaminogalacturonamide. Five grams of finely powdered 1-aminogalacturonamide was added to an ice-cold mixture containing 20 ml of acetic anhydride and 50 ml of pyridine. The suspension was shaken at 0° C for 20 min and at room temperature for 18 hr.[5] Ten volumes of petroleum ether were then added in order to precipitate the acetate, which separated as a sirup.[6] The sirupy phase was extracted successively with several portions of petroleum ether and finally was dissolved in methanol. The methanolic solution was saturated at 0° C with ammonia and concentrated under reduced pressure to a sirup that was then extracted three times with isopropyl ether. When the residue was dissolved in a few milliliters of ethanol and allowed to stand, large rectangular prisms separated from the solution in the course of 2 days. The yield of crude product was 3 g.

To recrystallize, *N*-acetylaminogalacturonamide was dissolved in six parts of hot water. The solution was filtered and cooled to room temperature, and isopropanol was added almost to the point of turbidity. The hydrated crystals, large rectangular prisms, when air-dried, melted at 103° to 107° C, $[\alpha]_D^{20}=-48.1°$ (water, c=2). The constants were unchanged after further crystallization. Analysis: Calculated for $(C_8H_{14}O_6N_2)_2.5H_2O$: C, 34.41; H, 6.86; N, 10.03; H_2O, 16.13. Found: C, 34.6; H, 6.9; N, 10.0; H_2O, 15.8.

5. 1-Amino-D-glucuronamide

Six grams of pure glucurone was suspended in 120 ml of absolute methanol at 0° C, and a stream of dry ammonia gas was passed into the solution until it was substantially saturated. The material was stored for several days in the refrigerator, and during this time a flocculent amorphous precipitate formed. When separated, and dried over calcium chloride in a vacuum desiccator, it weighed 3.5 g. In 2-percent aqueous solution the crude material showed a gradual change in specific rotation over the course of many days. $[\alpha]_D^{20}=-14.0°$ (5 min); −7.9° (28 hr); −3.9° (5 days).

Figure 3 shows the change in pH when a solution of the crude 1-aminoglucuronamide was titrated slowly at room temperature with 0.1 *N* acid. The inflection is less sharp than that of

[5] The acetylation mixture assumed a striking deep-blue color, which disappeared slowly after the addition of petroleum ether.

[6] Extraction with chloroform was found to be impracticable because of the relatively high solubility of the crude acetate in water. In one preparation the acetylation mixture was poured into ice water, and after several chloroform extractions, most of the acetate was still present in the aqueous portion.

the titration curve of 1-aminomannuronamide but indicates the presence of a glycosyl amine with a purity of approximately 68 percent. The crude 1-aminoglucuronamide was used without purification for the preparation of crystalline glucuronamide.

6. D-Glucuronamide

Crude 1-amino-D-glucuronamide (3.1 g) was dissolved in 50 ml of water, and 128 ml of 0.0993 *N* HCl was added over the course of 1 hr.; the pH of the resulting solution was 3.0. The material was then passed through columns of cation and anion exchange resins [7] to remove ammonium chloride, and concentrated under reduced pressure to a thin sirup, which crystallized on addition of methanol. The crystals weighed 2.35 g.

To recrystallize, one part of the glucuronamide was dissolved in three parts of water with slight warming. The solution was filtered with the aid of a decolorizing carbon, and isopropyl alcohol was added almost to the point of turbidity. α-Glucuronamide monohydrate crystallized in high yield in the form of slender prisms, which were separated after 24 hr and dried in a vacuum desiccator over calcium chloride. Heated slowly, they melted at 113° to 117° C, resolidified, and finally decomposed above 150° C. $[\alpha]_D^{20}=+78°$ (3 min); +31.6° (22 hr) (water, c=2). Analysis: Calculated for $C_6H_{11}O_6N.H_2O$; C, 34.12; H, 6.21; N, 6.63. Found: C, 34.2; H, 6.2; N, 6.7.

III. Summary

Methods are reported for the preparation of the amides of mannuronic, galacturonic, and glucuronic acids. It has been found that the corresponding 1-amino-uronamides prepared by a method previously reported are selectively hydrolysed at room temperature by dilute acid, which replaces the glycosylamino group by hydroxyl. After removal of the ammonium salt of the acid employed, the free uronamides were crystallized. These substances, like the sugars, have a free reducing group and hence are capable of existing in the pyranose, furanose, and open-chain forms, and of displaying mutarotation.

D-Mannuronamide $C_6H_{11}O_6N$, truncated prisms, melting point 145° to 150° C with decomposition, has a specific rotation, $[\alpha]_D^{20}$, of +2.4° (1.4 min) changing in 30 min to −10.4° (water, c=4). It thus appears to be an alpha pyranose modification.

D-Galacturonamide crystallizes in two modifications: an anhydrous alpha pyranose form, $C_6H_{11}O_6N$, slender flat plates, melting point 172° to 178° C with decomposition, has a specific rotation, $[\alpha]_D^{20}$, of +80° (3 min), and +15.9° (22 hr) (water c=4); a beta pyranose hydrate $C_6H_{11}O_6N.H_2O$, long slender prisms, melting point 113° to 116° C with decomposition, has a specific rotation, $[\alpha]_D^{20}$, of −21.4° (3 min), and +14.6° (22 hr) (water, c=4).

By low-temperature acetylation of 1-aminogalacturonamide followed by deacetylation with ammonia, a crystallinehydrate of 1-*N*-acetylaminogalacturonamide was obtained, $(C_8H_{14}O_6N_2)_2.5H_2O$, which melted at 103° to 107° C, and had a specific rotation, $[\alpha]_D^{20}$, of −48.1° (water, c=2).

D-Glucuronamide was obtained by selective hydrolysis of crude 1-aminoglucuronamide. The crystals, long slender prisms, were found to be an alpha pyranose hydrate $C_6H_{11}O_6N.H_2O$, melting point 113° to 117° C. $[\alpha]_D^{20}=+78°$ (3 min); +31.6° (22 hr) (water, c=2).

The authors are indebted to Nancy B. Holt for assistance in various phases of the work, and to Rolf A. Paulson for the analyses reported in this paper.

IV. References

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[4] S. Luckett and F. Smith, J. Chem. Soc. **1940,** 1506; H. G. Sammons, J. Shelswell, and R. T. Williams, Biochem. J. **35,** 557 (1941); S. P. James and F. Smith, J. Chem. Soc. **1945,** 739.

[5] E. F. Jansen and R. Jang, J. Am. Chem. Soc. **68,** 1457 (1946).

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[7] Amberlite IR-100-H and IR-4-B, Resinous Products and Chemicals Co., Philadelphia, Pa.; other commercially available ion exchange resins are equally satisfactory.