dioxane was subjected to hydrogenation at atmospheric pressure, using 200 mg of 10% palladium-on-charcoal catalyst. Reduction was allowed to proceed for 4 hr. The catalyst was removed by filtration and removal of solvent *in vacuo* left a solid residue. Two recrystallizations from 95% ethanol afforded 107 mg (53%) of a light yellow, crystalline solid: mp 263–265° dec, $[\alpha]^{25}D$ −6.8° (0.5%, EtOH), λ_{max}^{EtOH} 304 mμ (ε 13,060).

Anal. Calcd for $C_{29}H_{33}N_5O_{13}$: C, 52.80; H, 5.04; N, 10.62. Calcd for $C_{29}H_{33}N_5O_{13}\cdot 0.5H_2O$: C, 51.97; H, 5.11; N, 10.47. Found: C, 52.24; H, 5.36; N, 10.47.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-[*p*-(acetylglycylamino)benzamido]-2(1H)-pyrimidinone (VIe).—A solution of 118 mg (1.0 mmole) of acetylglycine, 0.140 ml of triethylamine, and 4 ml of dimethylformamide was cooled to −5°. After addition of 0.095 ml of ethyl chlorocarbonate, the mixture was stirred occasionally and kept between 0 and −5° for 20 min. A cold solution of 280 mg (0.5 mmole) of Va in 4 ml of dimethylformamide was added and the mixture was stirred at −5° for 15 min. The reaction flask was stoppered and stored at 0° for 18 hr, then left at room temperature for an additional 18 hr. The mixture was evaporated to dryness *in vacuo* and the residue was dissolved in chloroform. The chloroform solution was extracted with saturated sodium bicarbonate solution, cold 20% hydrochloric acid, and water. After drying over sodium sulfate, evaporation of chloroform left a solid residue. Recrystallization from 95% ethanol gave 125 mg (38% yield) of slightly yellow, crystalline solid: mp 265° dec, $[\alpha]^{25}D$ −6.2° (0.5%, EtOH), λ_{max}^{EtOH} 304 mμ (ε 12,000).

Anal. Calcd for $C_{29}H_{33}N_5O_{13}$: C, 52.80; H, 5.04; N, 10.62. Calcd for $C_{29}H_{33}N_5O_{13}\cdot 0.5H_2O$: C, 51.97; H, 5.11; N, 10.47. Found: C, 52.24; H, 5.20; N, 10.43; loss on drying, 1.35.

1-β-D-Glucopyranosylcytosine Hydrochloride (IX). From Ammonolysis of IVa.—A suspension of 820 mg (1.39 mmoles) of IVa in 30 ml of absolute ethanol was saturated with ammonia at 0°. The mixture was heated in a glass-lined bomb for 36 hr at 95–100°. The cloudy solution was concentrated to dryness *in vacuo*. The white residue was dissolved in 30 ml of hot 95% ethanol and treated with charcoal. The hot solution was acidified to pH 2 with concentrated hydrochloric acid and cooled. The precipitate was filtered and thoroughly washed with warm absolute ethanol. Recrystallization from 95% ethanol gave 360 mg (83%) of white, crystalline solid: mp 205° dec, $[\alpha]^{25}D$ +23.3° (2%, water), λ_{max}^{EtOH} 281 mμ (ε 10,930). For comparison, an authentic sample was prepared independently following known procedures.[21,22]

Acetylation of IX.—A mixture of 125 mg (0.4 mmole) of IX, 3 ml of dry pyridine, and 3 ml of acetic anhydride was warmed until complete solution occurred. After standing at room temperature for 20 hr, the solution was quenched into 20 ml of ice–water. The solid was collected by filtration and recrystallized from alcohol with charcoal treatment. After drying, 145 mg (72%) of fine needles was obtained, mp 218.5–219.5° (lit.[19] mp 218.5°).

1-(2′-Deoxy-2′-ureido)-β-D-glucopyranosylcytosine (X). A. From Ammonolysis of IVc.—A suspension of 325 mg (0.54 mmole) of IVc in 30 ml of absolute ethanol was saturated with ammonia at 0°. The mixture was heated in a glass-lined bomb for 48 hr at 115°. The mixture was evaporated to dryness *in vacuo*, dissolved in hot 90% ethanol, and treated with charcoal. A white precipitate formed on standing. After drying, 91.8 mg (54%) of white, crystalline solid was obtained: mp 279–280° dec, $[\alpha]^{25}D$ +71.2° (0.9%, water), $\lambda_{max}^{0.1\ N\ HCl}$ 277 mμ (ε 11,670).

B. From Ammonolysis of IVb.—Ammonolysis of 350 mg (0.51 mmole) of IVb was carried out as described for IVc. After recrystallization from 90% ethanol, 94.5 mg (59%) of white, crystalline solid was obtained: mp 279–280° dec, $[\alpha]^{25}D$ +70.9° (0.9%, water). The physical constants of X agreed with those previously reported.[20]

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(22) J. J. Fox and I. Goodman, *ibid.*, **73**, 3256 (1951).

Relationships between Some Uronic Acids and Their Decarboxylation Products[1]

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2-Furaldehyde, 5-formyl-2-furoic acid, and reductic acid (2,3-dihydroxy-2-cyclopenten-1-one), all products from the decarboxylation of hexuronic acids, were found to be essentially end products in the reaction. Structural relationships between the 2-furaldehyde and reductic acid and the parent uronic acids were obtained by preparation of D-galacturonic-2-C^{14}, -1-C^{14}, and D-glucuronic acid-1-C^{14} from which pertinent C^{14}-labeled decomposition products were isolated. The 2-furaldehyde arising from the 1-C^{14}-labeled uronic acids contained over 99% of the activity in the aldehyde group. D-Galacturonic acid-2-C^{14} was converted to reductic acid-C^{14} which was shown to be a mixture of the reductic acids-1-C^{14} and -2-C^{14} in the approximate ratio 9:1. Conversely, the reductic acid-C^{14} from D-galacturonic acid-1-C^{14} was found to be a mixture of reductic acids-1-C^{14} and -2-C^{14} in the ratios 1:9. Thus, reductic acid is formed from hexuronic acids *via* two different mechanisms. In addition to isotopic-labeling experiments, a series of carbohydrate derivatives was examined for their potential as a source of reductic acid.

The decarboxylation of uronic acids and glycuronans in hot aqueous acid to give near-stoichiometric quantities of carbon dioxide is a well-known reaction of considerable practical importance, forming the basis for various analytical procedures which determine uronic acids in the presence of other carbohydrate materials. Although it has been the subject of extensive studies by various investigators,[3–5] the reaction sequence is still a matter of some controversy, and in recent years several mechanisms have been suggested.[4–7] The basis of these proposals rests largely on kinetic studies which have compared decarboxylation rates of uronic acids with other carbohydrate and organic acids. Recently, however, Anderson and Garbutt[8] have verified that the carbon dioxide evolved in this reaction arises from C-6 of the uronic acid; they used D-glucuronic acid-6-C^{14} as a representative model.

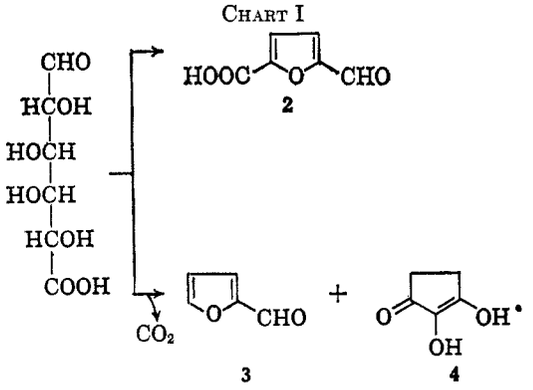
Although the above proposals are consistent with the observed reaction kinetics and products, they remain largely untested and unevaluated. This paper pre-

(1) Presented at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 11–16, 1966.
(2) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.
(3) E. W. Taylor, W. F. Fowler, Jr., P. A. McGee, and W. O. Kenyon, *J. Am. Chem. Soc.*, **69**, 342 (1947).
(4) D. M. W. Anderson and S. Garbutt, *J. Chem. Soc.*, 3204 (1963), and earlier papers in that series.
(5) E. Stutz and H. Deuel, *Helv. Chim. Acta*, **41**, 1722 (1958), and earlier papers.
(6) H. S. Isbell, *J. Res. Natl. Bur. Std.*, **33**, 45 (1944).
(7) V. P. Kiseleva, A. A. Konkin, and Z. A. Rogovin, *Zh. Prikl. Khim.*, **27**, 1133 (1954).
(8) D. M. W. Anderson and S. Garbutt, *Talanta*, **8**, 605 (1961).

sents data concerning the structural relationships between hexuronic acid and its decarboxylation products. Isotopically labeled D-galacturonic acid and D-glucuronolactone are used as representative hexuronic acids. In addition, the possible interconversion of the decarboxylation products was examined, and the decomposition products of some selected carbohydrate derivatives are compared to those of hexuronic acids.

In hot, aqueous acidic solutions, D-galacturonic acid liberates near-stoichiometric quantities of carbon dioxide and deposits considerable quantities of insoluble "humins." In addition, the reaction generates in isolable yield (Chart I) 5-formyl-2-furoic acid,[9] 2-furaldehyde, and reductic acid[10] (2,3-dihydroxy-2-cyclopenten-1-one). The possibility of interconversions of these three compounds was investigated at conditions under which they were shown to be formed from D-galacturonic acid. Reductic acid could not be detected in reacting solutions of either 2-furaldehyde or 5-formyl-2-furoic acid. 2-Furaldehyde degraded rapidly forming insoluble humins, but 5-formyl-2-furoic acid exhibited a relatively high stability although some decomposition occurred, as evidenced by the formation of 2-furaldehyde (demonstrated chromatographically) and accompanying black solids. Since yields of reductic acid as low as 2% could have been detected, it is unlikely that either 2-furaldehyde or 5-formyl-2-furoic acid are precursors for this compound. Although 2-furaldehyde has been reported as a source of reductic acid in small yield,[11] there was no evidence of its occurrence in the solutions encountered in our work. In agreement with Reichstein and Oppenaur,[10] reductic acid was found to be stable, and therefore cannot contribute to the formation of 2-furaldehyde.

CHART I



The decarboxylation rate of 5-formyl-2-furoic acid was compared with that of D-galacturonic acid under the conditions of the analytical procedure. The liberation of carbon dioxide was very slow, the rate being less than 2% of that of D-galacturonic acid; tests were not extended to determine if decarboxylation is quantitative. The low rate, however, rules out 5-formyl-2-furoic acid as an intermediate in the decarboxylation of D-galacturonic acid.

In addition to the above tests for interconvertibility, a series of carbohydrates was tested to determine their potential as sources of 5-formyl-2-furoic acid and reductic acid. The conversion was studied during a 4-hr reaction period by periodic chromatographic examination of a refluxing 12% hydrochloric acid solution containing the material. Under these conditions D-galacturonic acid readily produced 2-furaldehyde, reductic acid, and 5-formyl-2-furoic acid. The other compounds tested were 5-keto-D-gluconic acid, alginic acid, D-glucuronolactone, 2-keto-D-gluconic acid, L-arabinose, and D-xylose, but only 5-keto-D-gluconic acid gave detectable quantities of 5-formyl-2-furoic acid. The first four compounds all produced reductic acid, but only 5-keto-D-gluconic acid gave yields comparable to D-galacturonic. All the materials tested readily produced 2-furaldehyde.

It is interesting to note that no trace of pentose could be observed during the decomposition of the compounds which are sources of reductic acid. The appearance of pentoses during hexuronic acid decarboxylation has been previously investigated but generally discounted.[4,12,13] The possibility of pentoses as sources for reductic acid has generally been accepted on the evidence of Reichstein and Oppenaur[10] who isolated reductic acid in small yield (less than 0.5%) from D-xylose prepared from corncobs. The findings of the present work, using chromatographic detection methods, do not substantiate this fact although yields of less than 1.0% may have escaped detection. The absence of substantial amounts of reductic acid in reacting pentose solutions indicates that 2-furaldehyde formation in this system proceeds through a different intermediate from that by which reductic acid is formed in uronic acid decarboxylation. Common intermediates have been suggested in some proposed mechanisms.[5,6]

It is noteworthy that, aside from the hexuronic acids and the carbohydrate acids capable of decarboxylation reported herein, the only other substantial sources of reductic acid are Theander's methyl β-D-3-oxo-D-glucopyranoside[14] and the related and probably interconvertible-2-oxo isomer.

Structural relationships between the uronic acids and their decarboxylation products were studied with isotopic-labeled compounds. D-Galacturonic acid-1-C^{14} and -2-C^{14} were prepared from the correspondingly labeled aldoses. The method of Sell and Link[15] with minor modifications, was used to prepare the D-galacturonic acids. D-Glucuronolactone was obtained by oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose, prepared by Mehltretter's procedure.[16] Rapid oxidation was effected by using oxygen and a platinum catalyst as described by Marsh.[17]

2-Furaldehyde-C^{14} was prepared from D-galacturonic acid-1-C^{14} and D-glucuronolactone-1-C^{14}. This was oxidized to 2-furoic acid-C^{14}, which in turn was converted to 2-chloromercurifuran.[18] In each case, the mercurial derivative contained less than 1% of the radioactivity of the 2-furoic-C^{14} precursor, indicating that the aldehydic carbon of the 2-furaldehyde comes entirely from the aldehyde group of the parent uronic acid. Since the 2-furoic acid-C^{14} was found to have a

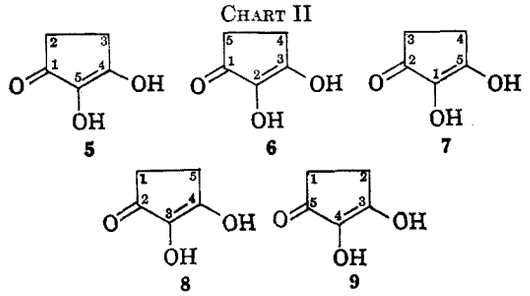
(9) E. Stutz and H. Deuel, *Helv. Chim. Acta*, **39**, 2126 (1956).
(10) T. Reichstein and R. Oppenaur, *ibid.*, **16**, 988 (1933); **17**, 390 (1934).
(11) Dutch Patent 61,296 (1948); *Chem. Abstr.*, **42**, 7788 (1948).
(12) H. Franken, *Biochem. Z.*, **250**, 53 (1932); **257**, 245 (1933).
(13) C. M. Conrad, *J. Am. Chem. Soc.*, **53**, 2282 (1931).
(14) O. Theander, *Acta Chem. Scand.*, **12**, 1897 (1958).
(15) H. M. Sell and K. P. Link, *J. Am. Chem. Soc.*, **60**, 1813 (1938).
(16) O. T. Schmidt, *Methods Carbohydrate Chem.*, **2**, 322 (1963).
(17) C. A. Marsh, *J. Chem. Soc.*, 1578 (1952).
(18) H. Gilman and G. F. Wright, *J. Am. Chem. Soc.*, **55**, 3302 (1933).

specific activity essentially equal to that of the uronic acid from which it was generated, it was concluded that there was no appreciable kinetic isotope effect involved in the formation of 2-furaldehyde. Such an effect might have been expected on the basis of the findings of Bonner and Roth,[19] who report a 23% discrepancy in the radioactive assay of D-xylose-1-C^{14} and the 2-furoic acid-C^{14} formed from it.

D-Galacturonic acid-1-C^{14} and -2-C^{14} were diluted with pectin and converted to reductic acid-C^{14} using conditions described by Reichstein and Oppenaur.[10] From reductic acid-C^{14}, succinic acid-C^{14} was prepared by potassium permanganate oxidation and its activity measured. That from the 1-C^{14}-labeled uronic acid had a specific activity of approximately 10% of the parent reductic acid-C^{14}, whereas that from the -2-C^{14}-labeled compound contained about 90% of the activity of the original reductic acid-C^{14}. The activity of the methylene carbon atoms of the succinic acid-C^{14} prepared from either D-galacturonic acid-1-C^{14} or -2-C^{14} was negligible.

Since reductic acid may undergo enolization, it has a plane of symmetry through C-2; the methylene carbons are equivalent as are the two oxygen-bearing carbons, C-1 and C-3. If it is assumed that the skeletal carbon chain is not cleaved during decarboxylation, five arrangements of the original chains are possible (Chart II: numbers refer to the original numbering in the hexuronic acid, C-6 being lost by decarboxylation).

CHART II



Since the methylene carbon atoms of the reductic acid-C^{14} formed from either D-galacturonic acid-1-C^{14} or -2-C^{14} exhibit negligible activity, arrangements **5**, **8**, and **9** do not occur. The measured isotope distribution indicates, however, that the remaining structures, **6** and **7**, occur in the reaction mixture in the approximate ratios of 1:9; thus it is concluded that reductic acid is formed from hexuronic acid by two mechanisms.

All proposed mechanisms assign the origin of the aldehyde group of 2-furaldehyde and C-2 of reductic acid to C-1 of the uronic acid. The radiochemical data showing that less than 1% of the activity of the 2-furaldehyde resides in the furan nucleus is in accord with this proposal. Conclusions regarding reductic acid formation are more complex. As would be expected, the finding that reductic acid is formed from hexuronic acid by two different mechanisms is inconsistent with any of the proposals. In the major pathway, which produces approximately 90% of the reductic acid, C-1 of the hexuronic acid appears as C-2 of the reductic acid. This finding is in accord with previous proposals. The finding of a minor reaction, which gives rise to about 10% of the reductic acid wherein C-1 of the reductic acid arises from C-1 of the hexuronic acid, shows that a second unpredicted and, as yet unexplained, mechanism is operating in the reaction.

(19) W. A. Bonner and M. R. Roth, *J. Am. Chem. Soc.*, **81**, 5454 (1959).

Experimental Section

Materials and Methods.—The sugars, hexuronic acids, and 2-furaldehyde used in this work were obtained commercially. Calcium 5- and 2-keto-D-gluconate samples were obtained through the courtesy of the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill. Specific activities of isotopically labeled compounds were determined by oxidation with the Van Slyke–Folch reagent,[20] collection of CO_2, and measurement of the $C^{14}O_2$ concentration in an ion chamber using a vibrating reed electrometer.

Preparation of Reductic Acid.[10]—A suspension of 100 g of pectin in 500 ml of 5% sulfuric acid was autoclaved at 150° for 1.5 hr. After cooling, the solution was filtered, neutralized with barium carbonate, and refiltered, and the filtrate was percolated through a column containing an excess of Dowex 50 (H). The solution was concentrated to a syrup to give crystalline reductic acid, which was isolated on a filter, washed with cold acetone, and sublimed (0.2 mm, 150°), mp 211–212°, lit.[10] mp 212–213°, λ_{max} 267 mμ (ϵ 13,300) (95% ethanol). Reductic acid could also be obtained in pure form by recrystallization from dimethylformamide.

Materials purified by either method ran as a single spot on silica gel HF thin layer plates using chloroform–acetic acid (9:1) as irrigant. Detection of purified materials was accomplished with aniline hydrogen phthalate spray which produces a characteristic green color. Short-wave ultraviolet radiation provides more sensitive detection of the compound.

Preparation of 5-Formyl-2-furoic Acid.—This compound was prepared essentially by the methods described by Votoček and Krošlák.[21] It was difficult, when using the conditions originally described (refluxing methanol saturated with hydrogen chloride), to consistently reproduce the reported yields. Hence, a solution of 250 mg of calcium 5-keto-D-gluconate in 5 ml of 5% methanolic hydrogen chloride was refluxed and aliquots were removed periodically for chromatography on silica gel thin layer plates using benzene–methanol (98:2) as irrigant and aniline hydrogen phthalate as spray reagent. Methyl 5-formyl-2-furoate appeared after 0.5 hr and reached a maximum concentration after 3.5 hr. Subsequently, 5.0 g of calcium 5-keto-D-gluconate was treated for 3 hr in 100 ml of 5% methanolic hydrogen chloride. The solution was then cooled, diluted with 100 ml of water, extracted three times with ether, and the dried (sodium sulfate) ether extracts were evaporated in a stream of dry air. The residue crystallized readily to give 600 mg (17.4%) of methyl 5-formyl-2-furoate, which was obtained pure after two recrystallizations from water, mp 91–92°. The acid was prepared from the ester using the procedure of Votoček and Krošlák[21] with the exception that the saponification solution was deionized with Dowex 50 (H) and the acid isolated by evaporation of the aqueous solution, mp 200–201°.

Tests for Interconversion of 2-Furaldehyde, 5-Formyl-2-furoic Acid, and Reductic Acid. Decarboxylation of 5-Formyl-2-furoic Acid.—Approximately 5% solutions of 2-furaldehyde (distilled) and 5-formyl-2-furoic acid in 12% hydrochloric acid were refluxed and aliquots were periodically withdrawn over an interval of 80 min. The aliquots were examined by thin layer chromatography (silica gel) using benzene–methanol (98:2) as irrigant and aniline hydrogen phthalate spray reagent. At the end of the reaction period, 2-furaldehyde had reacted to a considerable degree, as evidenced by the solids produced, with no observed traces of reductic acid. 5-Formyl-2-furoic acid decomposed more slowly, and a considerable amount of the original material crystallized upon cooling the solution after reaction. Chromatograms indicated that 2-furaldehyde was being released slowly.

The rate of carbon dioxide evolution from 5-formyl-2-furoic acid (97.91 mg) was measured in the usual way. After a 3-hr reaction (complete decarboxylation of D-galacturonic acid) 1.98 mg (6.5% of theoretical) of carbon dioxide was obtained.

(20) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940); D. D. Van Slyke, J. Plazin, and J. R. Weisiger, *ibid.*, **191**, 299 (1951).

(21) E. Votoček and A. Krošlák, *Collection Czech. Chem. Commun.*, **10**, 259 (1938); *Chem. Abstr.*, **32**, 8410 (1938).

A solution of 26 mg of reductic acid in 200 ml of 12% hydrochloric acid was brought to a boil and slowly distilled through a 10-in. Vigreux column. The distillate was collected and 2-furaldehyde content was measured spectrophotometrically at 276 mμ. After reaction for 2 hr, the total yield of 2-furaldehyde was less than 1%.

Selected Carbohydrates as Sources of Reductic Acid.—A 5% solution of D-galacturonic acid in 12% hydrochloric acid was refluxed, and 1-μg aliquots of the solution were chromatographically examined on silica gel HF thin layer plates (chloroform–acetic acid, 9:1). Both reductic acid and 5-formyl-2-furoic acid appeared within 1 hr and persisted until the end of the experiment (4 hr).

The following compounds were examined in the same manner: L-arabinose, D-xylose, alginic acid, D-glucuronolactone, and calcium 5-keto- and 2-keto-D-gluconate.

Preparation of D-Galacturonic Acids-C^{14} and Their Conversion to Reductic Acid-C^{14}.—D-Galactose-1-C^{14} (20 μcuries, about 11.4 mg) was mixed with 9.0 g of inert D-galactose and dried at 65° *in vacuo* for 10 hr. This material was converted to 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose-1-C^{14}, as described by Sell and Link,[15] by allowing the reaction to proceed for 48 hr rather than the prescribed 24 hr. A yield of 12.1 g (92.7%) was obtained. This material was converted to D-galacturonic acid as described, except that an ion-exchange resin (Dowex 50) was used to convert potassium di-*O*-isopropylidene-D-galacturonate to the acid. The final yield of pure D-galacturonic acid-1-C^{14} was 3.14 g (32.4%), 0.441 μcurie/mmole. For conversion to reductic acid, 1.503 g of the above uronic acid was mixed with 48.0 g of pectin and the conversion was performed as described above. The resulting reductic acid-C^{14} was recrystallized from dimethylformamide and then from ethanol to give 1.7 g of pure material, which was identical in its physical properties with the inert material prepared previously and had a specific activity of 0.0129 μcurie/mmole.

D-Galacturonic acid-2-C^{14} was synthesized in a similar fashion starting with 9.0 g of inert D-galactose and 7.1 mg of the 2-C^{14} isomer (3.06 μcuries/mg). The resulting D-galacturonic acid-2-C^{14} (2.0 g, 0.478 μcurie/mmole), after dilution with 48 g of pectin, was converted to reductic acid having a specific activity of 0.0197 μcurie/mmole.

Preparation of D-Glucuronolactone-1-C^{14}.—To 2.5 g of powdered, anhydrous D-glucose was added 1.9 mg (6.92 μcuries/mg) of D-glucose-1-C^{14}, and the mixture was converted to 1,2-*O*-isopropylidene-α-D-glucofuranose (yield, 1.4 g) using Mehltretter's procedure.[16] This material was oxidized to the uronic acid derivative using the procedure described by Marsh.[17] Complete oxidation required a 5-hr reaction time. The oxidation mixture was filtered, the filtrate treated with Dowex 50 (H), and the effluent concentrated to a syrup. The syrup was taken up in 6 ml of water and heated on a steam bath for 2 hr and then evaporated. The resulting syrup, which was predominately D-glucuronic acid, was diluted with 1.5 g of inert D-glucuronolactone; the mixture was taken into solution on a steam bath with the addition of a minimum amount of water. Crystalline D-glucuronolactone was obtained from this solution and was recrystallized from water–ethanol and dried to give 816 mg of material having a specific activity of 0.256 μcurie/mmole.

Conversion of the Hexuronic Acids-1-C^{14} to 2-Furoic Acid-α-C^{14}. Decarboxylation of 2-Furoic Acid-α-C^{14}.—D-Galacturonic acid (708.6 mg) was mixed with 289.4 mg of the above-prepared D-galacturonic acid-1-C^{14} and charged to a 1-l. flask containing 500 ml of 12% HCl. The solution was brought to reflux and slowly distilled, the rate of production of 2-furaldehyde in the distillate being followed spectrophotometrically. After a 7-hr reaction, when the yield of 2-furaldehyde was 27% of theoretical and 225 ml of distillate was collected, the reaction was stopped. To the distillate was added 2.25 g of NaOH and 1.6 g of silver oxide. The suspension (held at 25°) was stirred rapidly and air was passed through the solution. Spectra measurements indicated that all the 2-furaldehyde was converted to 2-furoic acid in less than 1 hr. After reaction for 1 hr, the suspension was filtered, passed through Dowex 50 (H), and evaporated to a white solid. The resulting 2-furoic acid (sublimed at 80° and 0.1 mm) had mp 132–133° and a specific activity of 0.135 μcurie/mmole. A portion of this material (67.6 mg) was diluted with 696.0 mg of inert 2-furoic acid and the mixture was decarboxylated using the procedure of Gilman and Wright.[18] After recrystallization, the resulting 2-chloromercurifuran had a specific activity of 9.07×10^{-5} μcurie/mmole and thus contained 0.75% of the total activity.

In an analogous experiment, 749.0 mg of the previously synthesized D-glucuronolactone-1-C^{14}, diluted with 272.0 mg of inert material, gave 2-furoic acid-α-C^{14} having a specific activity of 0.189 μcurie/mmole which on subsequent dilution and decarboxylation gave a 2-chloromercurifuran which contained less than 1% of the initial radioactivity.

Succinic Acid-C^{14} from Reductic Acids-C^{14}.—In a typical experiment, a solution of 800 mg of reductic acid-C^{14} (1.29×10^{-2} μcurie/mmole), obtained from D-galacturonic acid-1-C^{14}, in 50 ml of water was cooled on an ice bath, and 43 ml of saturated aqueous potassium permanganate was added dropwise with stirring. After 20 min of stirring, sufficient 30% hydrogen peroxide was added to discharge the purple color, and the resulting manganese dioxide was removed by filtration. The filtrate was passed through a column of Dowex 50 (H) and evaporated to give crude succinic acid-C^{14}. After recrystallization from water, 690 mg of material was obtained, mp and mmp 182°, having an infrared pattern superimposable with that of an authentic sample. The specific activity was 1.39×10^{-3} μcurie/mmole.

An analogous experiment using reductic acid-C^{14}, obtained from D-galacturonic acid-2-C^{14} and diluted with inert carrier reductic acid-C^{14} (4.87×10^{-3} μcurie/mmole), gave succinic acid-C^{14} having a specific activity of 4.44×10^{-3} μcurie/mmole.

Chemical Degradation of Succinic Acid-C^{14} Obtained from D-Galacturonic Acids-1-C^{14} and -2-C^{14}.—The succinic acid used in this experiment was a diluted sample having a specific activity of 2.28×10^{-3} μcurie/mmole and was obtained from D-galacturonic acid-2-C^{14}. It was degraded by a Curtius reaction essentially as described by Benson and Bassham.[22] The diethylurethan was obtained without isolating the diazide in order to avoid handling the dry, explosive diazide. The urethan was recrystallized from water to constant melting point (110°) and had a specific activity of 2.20×10^{-3} μcurie/mmole. Ethylene diamine dihydrochloride was prepared from the urethan and was purified by several recrystallizations from water–methanol, mp 203°. Assay of a 30.3-mg sample showed it to have a specific activity of 1.35×10^{-5} μcurie/mmole.

In the same manner, a succinic acid-C^{14} derived from D-galacturonic acid-1-C^{14} having a specific activity of 4.0×10^{-4} μcurie/mmole gave ethylenediamine dihydrochloride having a specific activity of 5.9×10^{-7} μcurie/mmole.

(22) A. A. Benson and J. A. Bassham, *J. Am. Chem. Soc.*, **70**, 3939 (1948).