

was prepared according to the method of Norris and Rigby.<sup>7</sup> *s*-Butyl isobutyrate was made from the proper alcohol and acid using sulfuric acid as catalyst. The boiling points of the esters were *t*-butyl acetate 109–110°, isopropyl acetate 86–87°, *n*-butyl acetate 123.5–124°, *s*-butyl isobutyrate 141–142°, all at 734 mm., and benzyl acetate 105–105.5° (18 mm.).

The reaction of *t*-butyl acetate in which 169 g. of HF was used gave 3 g. of acetophenone, identified by means of its semicarbazone. The melting point was 198–199°, and when mixed with a known sample gave a melting point of 197–199°. Isopropyl acetate gave 4 g. of acetophenone, m. p. and mixed m. p. of the semicarbazone was 197–199°. A higher boiling ketonic fraction weighing 5 g. was also obtained. Its oxime melted at 68–69.5°. Widman<sup>8</sup> reports the melting point of *p*-isopropyl acetophenone oxime as 70–71°. When treated with semicarbazide in the usual manner a crystalline solid, m. p. 197°, was obtained. When mixed with aceto-

(7) Norris and Rigby, *THIS JOURNAL*, **54**, 2088 (1932).

(8) Widman, *Ber.*, **21**, 2226 (1888).

phenone semicarbazone the m. p. was 169–175°.

Some higher boiling fractions were obtained in other experiments, but in quantities too small to permit positive identification.

To prove that the alkylbenzene obtained from *n*-butyl acetate and *s*-butyl isobutyrate was in both cases *s*-butylbenzene a diacetamino derivative was prepared. That of the *n*-butyl acetate melted at 188–189° and when mixed with the same derivative of the latter compound did not depress in melting point.

### Summary

Hydrogen fluoride has been found to be an effective agent to alkylate benzene by means of esters. Reactions were carried out in a copper bomb at 80 to 100°. *t*-Butylbenzene, isopropylbenzene, and diphenylmethane were made from benzene and the acetates. *n*-Butyl acetate and *s*-butyl isobutyrate both gave secondary butylbenzene. Ketones also were formed in the reactions. Acetophenone was identified from those reactions in which acetates were used.

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## The Isolation of Keturonic Acids as Crystalline Alkaloidal Salts<sup>1</sup>

By J. P. HART AND M. R. EVERETT

In studies of the bromine oxidation of dilute solutions of more than 50 carbohydrates, Everett and Sheppard<sup>2</sup> have demonstrated the accumulation of keturonic acids. They found the yields of these substances to be related to the *cis-trans* structures of the original carbohydrates. Structural studies of the keturonic acids required their isolation in pure form. We are now reporting the isolation of alkaloidal salts of several keturonic acids, and the structures indicated by the properties of these salts. In order to avoid confusion the authors have named the keturonic acids as ketose derivatives.<sup>3</sup> Individual acids are differentiated by numbering the carboxyl carbon, the

terminal carbon nearest the potential carbonyl being called one.<sup>4</sup>

Calcium *l*-sorbo-6-uronate has been isolated previously from *d*-glucose and *d*-gluconic lactone solutions oxidized by bromine,<sup>5,6</sup> and has been shown to be identical in properties with the calcium salts of Boutroux<sup>7</sup> and Kiliani.<sup>8</sup>

(4) The principal group of keturonic acids described in this paper are named as follows:

Keturonic acid	Parent aldohexoses
<i>d</i> -Sorbo-6-uronic	<i>l</i> -Glucose, <i>d</i> -Idose
<i>l</i> -Sorbo-6-uronic	<i>d</i> -Glucose, <i>l</i> -Idose
<i>d</i> -Fructo-6-uronic	<i>l</i> -Gulose, <i>d</i> -Mannose
<i>l</i> -Fructo-6-uronic	<i>d</i> -Gulose, <i>l</i> -Mannose
<i>d</i> -Tagato-6-uronic	<i>d</i> -Altrose, <i>l</i> -Galactose
<i>l</i> -Tagato-6-uronic	<i>l</i> -Altrose, <i>d</i> -Galactose
<i>d</i> -Psico-6-uronic	<i>l</i> -Allose, <i>d</i> -Talose
<i>l</i> -Psico-6-uronic	<i>d</i> -Allose, <i>l</i> -Talose

(1) Aided by a grant from the Research Appropriation of the University of Oklahoma Medical School.

(2) M. R. Everett and Fay Sheppard, University of Oklahoma Medical School Monograph, "Oxidation of Carbohydrates in Acid Solution," 1936.

(3) J. P. Hart and M. R. Everett, "A Suggested Nomenclature for Keturonic Acids," *Abs. of Papers*, 96th meeting of Am. Chem. Soc.

(5) E. W. Cook and R. T. Major, *THIS JOURNAL*, **57**, 773 (1935).

(6) J. P. Hart, Fay Sheppard, M. R. Everett, *J. Biol. Chem.*, **123**, lii (1938).

(7) L. Boutroux, *Compt. rend.*, **127**, 1225 (1907).

(8) H. Kiliani, *Ber.*, **55**, 2817 (1923).

TABLE I

Brucine-	M. p., °C. <sup>a</sup>	Ultimate analysis, %				Formula	Source
		Found		Calcd.			
		C	H	C	H		
<i>l</i> -Sorbo-6-uronate <sup>b,c</sup>	174-175 (dec.)	57.45	6.31	57.42	6.33	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub> ·H <sub>2</sub> O	Ca- <i>l</i> -sorbo-6-uronate
<i>l</i> -Sorbo-6-uronate <sup>b</sup>	174-175 (dec.)	57.42	6.38	57.42	6.33	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub> ·H <sub>2</sub> O	Oxidized <i>d</i> -glucose
<i>d</i> -Fructo-6-uronate <sup>d</sup>	192-192.5 (dec.)	59.44	6.22	59.19	6.17	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub>	Oxidized <i>d</i> -mannose
<i>l</i> -Fructo-6-uronate <sup>d</sup>	165-166	57.42	6.46	57.42	6.33	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub> ·H <sub>2</sub> O	Oxidized <i>d</i> -gulonic lactone
<i>l</i> -Fructo-6-uronate	165-166	57.63	6.33	57.42	6.33	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub> ·H <sub>2</sub> O	Oxidized <i>l</i> -rhamnose
<i>l</i> -Tagato-6-uronate	189-189.5 (dec.)	59.12	6.18	59.19	6.17	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub>	Oxidized <i>d</i> -galactose
<i>d</i> -Xyloketuronate	168-169	58.39	6.36	58.32	6.30	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>7</sub> ·H <sub>2</sub> O	Oxidized <i>d</i> -xylose
1-Desoxy- <i>l</i> -fructo-6-uronate	128-129	58.75	6.53	58.97	6.49	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> ·C <sub>6</sub> H <sub>10</sub> O <sub>6</sub> ·H <sub>2</sub> O	Oxidized <i>l</i> -rhamnose

<sup>a</sup> All melting points uncorrected with short stem thermometers. <sup>b</sup> M. p. two samples mixed 174-175° (dec.). <sup>c</sup> A dihydrate, m. p. 182° (dec.), obtained by recrystallization from 80% alcohol. <sup>d</sup> M. p. of two samples mixed 165-166°.

The authors have prepared the crystalline brucine salt of *l*-sorbo-6-uronic acid by decomposition of the pure calcium salt. They have found it to be identical in all of its properties with the brucine salt prepared from *d*-glucose solutions oxidized by bromine for forty-two days. Ultimate analyses of the reducing brucine salts isolated from similarly oxidized solutions of *d*-mannose, *d*-gulonic lactone, and *d*-galactose show that these compounds were all salts of hexuronic acids (Table I). The stability of these acids in bromine water, compared with the rapid oxidation of alduronic acids by bromine,<sup>9</sup> indicates that they have ketose structures. The naphthorescinol, orcinol, and Molisch tests for the principal reducing brucine salts isolated from oxidized solutions of the four aldohexoses (Table II) are characteristic and identical. This implies by analogy structures stereoisomeric with *l*-sorbo-6-uronic acid, but proof of structure is to be the subject of further investigations. Melting points, optical rotations, and Sumner reducing equivalents show each compound to be pure and unique (see Tables I and III). These findings verify the conclusions of Everett and Sheppard<sup>2,10</sup> based on quantitative studies of oxidized hexose solutions. The position of the carbonyl in the keturonic acid isolated from the oxidized solutions of *d*-xylose is undeter-

TABLE II

COLOR REACTIONS OF THE BRUCINE SALTS OF KETURONIC ACIDS<sup>10</sup>

Brucine-	Naphthorescinol	Bials	Molisch
<i>l</i> -Sorbo-6-uronate	Blue	Light green	Red
<i>d</i> -Fructo-6-uronate	Blue	Light green	Red
<i>l</i> -Fructo-6-uronate	Blue	Light green	Red
<i>l</i> -Tagato-6-uronate	Blue	Light green	Red
<i>d</i> -Xyloketuronate	Pink	Negative	Brown
1-Desoxy- <i>l</i> -fructo-6-uronate	Red brown	Negative	Negative

(9) M. R. Everett, B. Edwards and Fay Sheppard, *J. Biol. Chem.*, **104**, 11 (1934).

(10) M. R. Everett and Fay Sheppard, *Proc. Soc. Exptl. Biol. Med.*, **34**, 7 (1936).

TABLE III

ROTATIONS AND REDUCTIONS OF THE BRUCINE SALTS OF KETURONIC ACIDS

Brucine-	[α] <sup>25D</sup> <sup>a</sup>	Salt	<i>d</i> -Glucose equivalent by Sumner's method <sup>b</sup> Calcd. for free acid
<i>l</i> -Sorbo-6-uronate	-24°	0.25	0.80
<i>d</i> -Fructo-6-uronate	-15.5°	.31	.98
<i>l</i> -Fructo-6-uronate	-24.5°	.32	.95
<i>l</i> -Tagato-6-uronate	-17°	.28	.85
<i>d</i> -Xyloketuronate	-9°	.22	.75
1-Desoxy- <i>l</i> -fructo-6-uronate	-32°	.37	1.21

<sup>a</sup> For 1% aqueous solution. <sup>b</sup> For 2 mg. per ml. concentrations of the salts.

mined. Ultimate analysis and the stability of this acid in the presence of bromine establish it as a ketopenturonic acid.

Oxidation of *l*-rhamnose for twenty-one days produces two keturonic acids, 1-desoxy-*l*-fructo-6-uronic acid and *l*-fructo-6-uronic acid, which have been isolated and separated by fractional crystallization of their brucine salts. The lactone of the desoxyketuronic acid has been isolated by Votoček and Malachta.<sup>11</sup> An identical brucine salt of *l*-fructo-6-uronic acid has been isolated from oxidized solutions of *d*-gulonic lactone. This acid evidently arises from oxidation of the methyl group of *l*-rhamnose, *l*-rhammonic acid or 1-desoxy-*l*-fructo-6-uronic acid to a primary alcoholic group, an unusual reaction, previously unrecognized.

## Experimental

**Oxidation of Carbohydrate Solutions.**—One per cent. carbohydrate solutions were oxidized with bromine for forty-two days, using the procedure of Everett and Sheppard.<sup>2,9</sup> Four hundred ml. of a 1% aqueous solution of each carbohydrate was placed in a glass-stoppered bottle. Fifteen ml. of bromine was then added and the mixture kept in the dark at approximately 25°. The solution was shaken at two to three day intervals, and more bromine was added whenever the liquid phase disappeared during the oxidation. After forty-two days the solution was aerated with washed air.

(11) E. Votoček and S. Malachta, *An. soc. espan. fis. quím.*, **27**, 494 (1929).

**General Method for Isolation of Mixed Barium Salts.**—The aerated oxidized carbohydrate solution was shaken with an excess of lead carbonate, filtered, allowed to remain in the refrigerator at 10° for twenty-four hours, and filtered again. The remaining bromide ion was removed by shaking with moist freshly prepared silver carbonate, centrifuging, decanting and immediately saturating the solution with hydrogen sulfide. The precipitated lead and silver sulfides were filtered off, and the filtrate aerated to remove excess hydrogen sulfide. This treatment removed most of the dibasic sugar acids present in the oxidized solutions.<sup>2,6</sup> Analysis by the Sumner method showed little loss of reducing material. The solution was then treated with an excess of barium carbonate at 40 to 45° for two hours. The filtrate was concentrated *in vacuo* to one-twelfth volume, and the barium salts were precipitated by addition of 8 volumes of alcohol. The salts were allowed to settle, filtered with suction, and washed twice with alcohol and twice with ether. They were dried in a desiccator and the barium content determined gravimetrically. Yields of barium salts obtained from 4 g. of original carbohydrates were as follows: *d*-glucose, 3.9 g.; *d*-mannose, 3.0 g.; *d*-gulonic lactone, 3.1 g.; *d*-galactose, 2.0 g.; and *d*-xylose, 4.1 g.

**General Method of Preparing Solutions of Brucine Salts.**—Weighed portions of the mixed barium salts were dissolved in two to three times their weight of water, and the barium was removed quantitatively by addition of the calculated quantities of *N* sulfuric acid. Enough alcohol was then added to produce a 70% solution. The mixture was placed in the refrigerator for fifteen minutes, then centrifuged free of barium sulfate and the calculated quantity of 10% alcoholic brucine solution was added.

**Brucine *l*-Sorbo-6-uronate.**—A solution of brucine salts was prepared from 2.5 g. of the mixed barium salts obtained from oxidized *d*-glucose. Crystallization began in fifteen minutes and apparently was complete after eighteen hours in the refrigerator. The brucine salt was filtered off, washed three times with alcohol and once with ether, and dried in a desiccator. The 2.2 g. yield was recrystallized from a small quantity of hot water, from which it separated as the monohydrate on cooling. It also was obtained as the dihydrate by dissolving in the least amount of hot water, adding 5 volumes of alcohol and allowing to crystallize in the refrigerator.

The same salt was prepared by decomposing a suspension of calcium *l*-sorbo-6-uronate in dioxane with the calculated quantity of sulfuric acid. After removal of calcium sulfate the dioxane was removed *in vacuo*, and the sirupy acid dissolved in a little water. After addition of an equivalent quantity of alcoholic brucine solution, the salt was crystallized as above.

**Brucine *d*-Fructo-6-uronate.**—A solution of this salt was prepared from 1 g. of the mixed barium salts from oxidized *d*-mannose. Crystallization started during slow concentration of the solution *in vacuo*. The mixture was then placed in a desiccator in the refrigerator, and the small fraction of crystalline material that separated in two hours was discarded. The mother liquors gave a second crop (0.3 g.) of crystals of the desired salt within twenty-four hours. This material was recrystallized by dissolving in a little hot water and adding 5 volumes of methyl alcohol.

**Brucine *l*-Fructo-6-uronate.**—A solution of this salt was prepared from 2 g. of the mixed barium salts from oxidized *d*-gulonic lactone. The solution was concentrated *in vacuo* until crystals began to form, and was then placed in a desiccator in the refrigerator. After twelve hours 0.58 g. of the salt was obtained. It was recrystallized by dissolving in hot water and adding 4 volumes of alcohol.

**Brucine *l*-Tagato-6-uronate.**—A solution of the salt was prepared from 4 g. of the mixed barium salts from oxidized *d*-galactose. In this preparation methyl alcohol was substituted for ethyl alcohol. The solution was concentrated to 30 ml. *in vacuo* and placed in a desiccator in the refrigerator. After twelve hours a small fraction of non-reducing salt was filtered off. A large fraction of a second non-reducing salt was separated in three days. After one week a third fraction of crystals appeared. It contained the reducing salt, which was purified by dissolving in a small quantity of hot water, adding 8 volumes of alcohol and allowing to crystallize in the refrigerator. The yield of recrystallized salt was 0.52 g.

**Brucine *d*-Xyloketuronate.**—A solution of brucine salts was prepared from 1 g. of the mixed barium salts from oxidized *d*-xylose. The solution was concentrated to one-half volume *in vacuo* and then placed in a desiccator in the refrigerator. With occasional scratching, 0.2 g. of crystalline material separated in twelve hours. The salt was recrystallized by dissolving in a small quantity of hot water and adding 5 volumes of alcohol.

**Brucine 1-Desoxy-*l*-fructo-6-uronate.**—The mixed barium salts from *l*-rhamnose solution, that had been oxidized twenty-one days, were obtained as a viscous gum. The product from 4 g. of *l*-rhamnose was dissolved in 50 ml. of water, and an aliquot was analyzed for barium. The barium was removed with an equivalent amount of sulfuric acid, and the solution was concentrated to 20 ml. The calculated quantity of brucine was dissolved in 30 ml. of alcohol and added to the solution. The latter slowly was concentrated *in vacuo* until crystals appeared. The solution was then placed in a desiccator in the refrigerator for twenty-four hours. The yield was 1.1 g. of the brucine salt of *l*-fructo-6-uronic acid. After another twenty-four hours the mother liquor deposited 4.1 g. of brucine 1-desoxy-*l*-fructo-6-uronate. This salt was recrystallized by dissolving in a small volume of hot water, adding 6 volumes of alcohol, and placing in the refrigerator for three days.

## Summary

1. Six keturonic acids have been isolated as crystalline brucine salts.
2. Brucine *d*-fructo-6-uronate, *l*-fructo-6-uronate, *l*-tagato-6-uronate, and *d*-xyloketuronate are the first pure preparations of these acids described.
3. Isolation of *l*-fructo-6-uronic acid from oxidized *l*-rhamnose solution demonstrates that the methyl group is susceptible to controlled oxidation.