

ART. LI.—*Contributions from the Sheffield Laboratory of Yale College.* No. XXXVIII.—*On the Oxidation product of Glycogen with Bromine, Silver Oxide and Water;* by R. H. CHITTENDEN, Ph.B., Assistant in Physiological Chemistry.

WHILE submitting an aqueous solution of glycogen to the action of bromine in an open vessel with the aid of heat, it was observed that the strong opacity of the fluid gradually disappeared, and that, after the removal of the free bromine by partial evaporation, a perfectly clear fluid remained which contained considerable combined bromine.

This reaction, indicating union between the glycogen and bromine, pointed to the possibility of the formation of an acid from the glycogen by oxidation, in a manner analogous to the formation of "dextronsäure" from dextrin, and "lactonsäure" from lactose, as described by Habermann,\* Barth and Hlasiwetz.† The following experiments were undertaken to form, if possible, a corresponding acid from glycogen. The glycogen employed was prepared from the muscular tissue of *Pecten irradians*,‡ and was as pure as could be obtained. The process of oxidation was as follows: fifty grams of glycogen dried at 100° C., were dissolved in 300 c.c. of distilled water, and this solution transferred to a champagne flask fitted with a caoutchouc stopper, in which was a small stout glass tube drawn out to a point.§ Forty grams of bromine were then added and the stopper wired in. The flask was then heated in a water bath until the red vapors of bromine had entirely disappeared, which required about two hours' boiling. A heavy light yellow or white precipitate formed at first, which completely disappeared by the time the bromine had all been taken up. At the end of this first treatment the fluid was perfectly clear and of a pale yellow color. After cooling, the gases were allowed to escape, by breaking the end of the tube in the cork, and, being collected, were found to consist mainly of carbonic acid and bromoform. The stopper was then removed and 40 grams more bromine

\* Annalen der Ch. u. Pharm., clxii, 297. † Ibid., cxxii, 96.

‡ This Journal, III, vol. x, p. 26.

§ Annalen der Ch. u. Pharm., clxx, 315.

added. The flask was then closed and heated as before until the bromine had all been taken up, after which, on cooling, the gases were liberated and 40 grams more bromine added, and the flask treated as before. After this, 10 grams of bromine were added three times, so that 150 grams of bromine were employed in the oxidation of 50 grams of dried glycogen.

The fluid was then transferred to an evaporating dish and heated on a water-bath until somewhat concentrated. When cool the fluid was diluted with an equal volume of water, then mixed with freshly precipitated and thoroughly washed silver oxide until all bromine was removed from the fluid. After the silver bromide had completely settled, the fluid was filtered off and the silver contained in it precipitated by hydrogen sulphide. The silver sulphide was removed by filtration, and the filtrate upon partial evaporation left a yellowish-red fluid with strong acid taste and reaction. This was an impure solution of an acid which decomposed carbonates with avidity. In this manner 150 grams of dried glycogen were oxidized, in parts of 50, giving in all sufficient acid for the following experiments. Two methods of purification were employed. The first consisted in treating this impure solution of the acid with chemically pure animal charcoal, and precipitating the filtrate with an excess of alcohol, to remove inorganic salts derived from the glycogen, which the latter always contains in small quantity. This alcoholic filtrate is evaporated on the water-bath, when a moderately pure solution of the acid results.

The second and better method, however, is to treat the impure acid with pure calcium carbonate, on the water-bath, when a soluble calcium salt is obtained which is filtered off, and, after concentration, crystallizes out on standing several days. After washing the crystals with a little cold water, they are dissolved in a large quantity of hot water, and precipitated while still hot by basic lead acetate. This precipitate of a lead salt of the acid is washed with hot water, then emulsified with water and decomposed by hydrogen sulphide. The lead sulphide is removed by filtration, the fluid evaporated, and then mixed with an excess of dilute alcohol. The precipitate, if any forms, is filtered off, and the filtrate on evaporation leaves the pure acid as a thick colorless syrup, which, after standing several months, shows as yet no signs of crystallization. A solution of the acid in water has an acid reaction on litmus; a strong acid taste; is not precipitated by alcohol, and dissolves freshly precipitated hydrated copper oxide to an azure-blue fluid, which remains blue when heated, but after long boiling shows strong reducing action.

*Calcium Salt.*—On treating an aqueous solution of the acid with calcium carbonate, on the water-bath, a violent evolution of carbonic acid takes place, and, after some time, a soluble calcium

salt can be filtered off from the excess of calcium carbonate. If the solution is at all colored it can be purified by animal charcoal. When suitably concentrated, this solution on standing several days, changes into a mass of irregular white globules, which are aggregations of fine microscopic needles.

The air-dried salt, when heated to 100° C. loses only hygroscopic water, and, when heated above 100° C. turns brown, which would indicate decomposition. The salt after crystallization is difficultly soluble in cold water; readily soluble in hot water, and is precipitated from its aqueous solution by alcohol.

The analysis of the salt gave the formula:  $C_6H_{11}Ca'O_7$ .

- I. 0.2097 grams of the salt dried at 100° C. gave .2552 grams  $CO_2$  and .1015 grams  $H_2O$ .
- II. 0.317 grams of the dried salt gave .3851 grams  $CO_2$  and .1508 grams  $H_2O$ .
- III. 0.3305 grams of the dried salt gave .0429 grams CaO.
- IV. 0.272 grams of the dried salt gave .036 grams CaO.

	Calculated.	Found.			
		1.	2.	3.	4.
$C_6$	33.49	33.18	33.12	----	----
$H_{11}$	5.12	5.37	5.28	----	----
$Ca'^*$	9.35	----	----	9.27	9.45
$O_7$	52.05	----	----	----	----

*Barium Salt.*—On treating a portion of the acid on the water-bath with barium carbonate, the latter salt is decomposed, and a soluble barium salt of the acid results. The salt thus formed does not crystallize readily from this solution. Alcohol is then added in excess to the fluid, when a heavy white precipitate forms, flocculent at first, but soon becoming gummy. This precipitate is washed with alcohol, and after drying has the appearance of a hard yellow gum. The gum-like mass is dissolved in water, filtered through animal charcoal, and evaporated to a small bulk, when, after standing a week, the fluid is converted into a mass of quite large, white, glassy prisms, which contain water of crystallization.

The analysis of the salt dried at 100° C. gave the formula:  $C_6H_{11}Ba'O_7$ . The air-dried salt gave the formula:  $C_6H_{11}Ba'O_7 + 1\frac{1}{2}H_2O$ .

- I. 0.347 grams of the salt dried at 100° C. gave .3488 grams  $CO_2$  and .1402 grams  $H_2O$ .
- II. 0.2778 grams of the dried salt gave .2793 grams  $CO_2$  and .1102 grams  $H_2O$ .
- III. 0.498 grams of the dried salt gave .1872 grams  $BaCO_3$ .
- IV. 0.3117 grams of dried salt gave .1175 grams  $BaCO_3$ .
- V. 1.2257 grams of the air-dried salt gave by drying at 100° C. .11542 grams  $H_2O$ .

\*  $Ca' = 20$ .

	Calculated.	Found.				
		1.	2.	3.	4.	5.
C <sub>6</sub>	27·32	27·41	27·41	----	----	----
H <sub>11</sub>	4·17	4·48	4·40	----	----	----
Ba <sup>*</sup>	25·99	----	----	26·14	26·21	----
O <sub>7</sub>	42·54	----	----	----	----	----
1½H <sub>2</sub> O	9·64	----	----	----	----	9·41

The crystals of this salt are very readily soluble in hot and cold water, but insoluble in alcohol.

The air-dried crystals when placed over concentrated sulphuric acid lose 6·42 per cent of water. The calculated amount for one molecule of water is 6·74 per cent. On drying the crystals at 100° C. the remaining one-half molecule is driven off. Heated at 120° C. the crystals turn brown and swell up.

*Cadmium Salt.*—On treating an aqueous solution of the acid with cadmium carbonate, on the water-bath, a soluble cadmium salt is obtained which does not crystallize. The salt is precipitated from its solution by three or four volumes of alcohol, then redissolved in water, filtered through animal charcoal, and reprecipitated by alcohol. It is thrown down from its solution as a flocculent precipitate which soon becomes gummy, and when hard yields on trituration a perfectly white powder.

The analysis of the salt dried at 100° C. gave the formula: C<sub>6</sub>H<sub>11</sub>CdO<sub>7</sub>.

- I. 0·3924 grams of the salt dried at 100° C. gave ·4105 grams CO<sub>2</sub> and ·1618 grams H<sub>2</sub>O.
- II. 0·3544 grams of the dried salt gave ·3702 grams CO<sub>2</sub> and ·1412 grams H<sub>2</sub>O.
- III. 0·344 grams of the dried salt gave ·0989 grams CdS.
- IV. 0·3568 grams of the dried salt gave ·1023 grams CdS.

	Calculated.	Found.			
		1.	2.	3.	4.
C <sub>6</sub>	28·68	28·52	28·48	----	----
H <sub>11</sub>	4·38	4·58	4·42	----	----
Cd <sup>†</sup>	22·31	----	----	22·36	22·29
O <sub>7</sub>	44·70	----	----	----	----

*Cobalt Salt.*—On heating an aqueous solution of the acid with cobaltic carbonate, on the water-bath, a cherry-red solution of a cobalt salt of the acid, is obtained, which does not crystallize readily from the aqueous solution. On the addition of alcohol to a concentrated or only moderately dilute solution of this salt, a heavy pink colored precipitate forms which soon becomes gummy.

This precipitate, after being washed with alcohol and dried at 100° C. gave by analysis the formula: C<sub>6</sub>H<sub>11</sub>CoO<sub>7</sub>.

\* Ba' = 68·5.

† Cd' = 56.

- I. 0.279 grams of the salt dried at 100° C. gave .325 grams CO<sub>2</sub> and .1213 grams H<sub>2</sub>O.  
 II. 0.283 grams of the dried salt gave .3325 grams CO<sub>2</sub> and .1200 grams H<sub>2</sub>O.  
 III. 0.2005 grams of the dried salt gave .0268 grams Co.

	Calculated.	Found.		
		1.	2.	3.
C <sub>6</sub>	32.07	31.76	32.04	----
H <sub>11</sub>	4.89	4.83	4.71	----
Co <sup>1/2</sup> *	13.14	----	----	13.36
O <sub>7</sub>	49.89	----	----	----

From a very dilute solution in water, the cobalt salt is precipitated by alcohol in the form of pink flocks. On allowing this precipitate to stand several weeks in the alcoholic fluid, it will be found to have changed its form, and under the microscope, will be seen to consist of fine needle-shaped crystals. These crystals dried at 100° C. gave by analysis the formula: C<sub>6</sub>H<sub>11</sub>CoO<sub>7</sub>+H<sub>2</sub>O.

- I. 0.219 grams of the salt dried at 100° C. gave .2372 grams CO<sub>2</sub> and .108 grams H<sub>2</sub>O.  
 II. 0.2992 grams of the dried salt gave .3225 grams CO<sub>2</sub> and .1484 grams H<sub>2</sub>O.  
 III. 0.2052 grams of the dried salt gave .0254 grams Co.

	Calculated.	Found.		
		1.	2.	3.
C <sub>6</sub>	29.69	29.53	29.40	----
H <sub>13</sub>	5.36	5.47	5.51	----
Co <sup>1/2</sup> *	12.16	----	----	12.37
O <sub>8</sub>	52.78	----	----	----

*Manganese Salt*—A solution of the acid treated, as in the preceding methods, with manganic carbonate, forms a soluble manganese salt which separates from the suitably concentrated fluid in masses of fine microscopic feather-like crystals. These crystals upon close examination are seen to be made up of radiating needles. When agitated in water they have a brilliant silky luster. They are slightly yellow, soluble in water, but insoluble in alcohol. The salt dried at 100° C. gave by analysis the formula: C<sub>6</sub>H<sub>11</sub>MnO<sub>7</sub>.

- I. 0.305 grams of the salt dried at 100° C. gave .362 grams CO<sub>2</sub> and .142 grams H<sub>2</sub>O.  
 II. 0.353 grams of the dried salt gave .062 grams Mn<sub>3</sub>O<sub>4</sub>.

	Calculated.	Found.	
		1.	2.
C <sub>6</sub>	32.35	32.36	----
H <sub>11</sub>	4.94	5.17	----
Mn†	12.35	----	12.65
O <sub>7</sub>	50.33	----	----

\* Co'=29.5.

† Mn'=27.5.

*Lead Salt.*—On treating an aqueous solution of the calcium salt with basic lead acetate, best with the application of heat, a heavy white gelatinous precipitate is obtained, which after washing with hot water and drying at 100° C., yielded by analysis the formula:  $C_6H_8Pb_2O_7$ .

- I. 0.280 grams of the salt dried at 100° C. gave .115 grams  $CO_2$  and .0358 grams  $H_2O$ .
- II. 0.3855 grams of the dried salt gave .1645 grams  $CO_2$  and .0505 grams  $H_2O$ .
- III. 0.436 grams of the dried salt gave .3212 grams PbO.
- IV. 0.1522 grams of the dried salt gave .1122 grams PbO.

	Calculated.	Found.			
		1.	2.	3.	4.
$C_6$	11.88	11.20	11.63	----	----
$H_8$	1.32	1.42	1.45	----	----
$Pb''_2$	68.31	----	----	68.38	68.43
$O_7$	18.49	----	----	----	----

On adding a solution of neutral lead acetate to an aqueous solution of the acid, a white flocculent precipitate is obtained. A similar precipitate is obtained with basic lead acetate. These precipitates, after washing with water and drying at 100° C., gave by analysis the following results:

Precipitate produced by neutral lead acetate.		Precipitate produced by basic lead acetate.	
C	11.60	C	11.42
H	1.42	H	1.55
Pb	68.19	Pb	68.66
O	18.79	O	18.37

A silver salt was also obtained as a flocculent precipitate. This was not analyzed.

By a backward glance we see that in all the salts obtained, with the exception of the lead salt, the acid acts as a monobasic acid. In the case of the lead salt, however, four atoms of hydrogen are replaced by two atoms of the metal. Hlasiwetz in an article upon the basicity of "lactonsäure" and "gluconsäure,"\* in which he shows that these acids are not only monobasic but also dibasic, gives a method whereby he obtained a dibasic barium salt from a monobasic calcium salt. On treating an aqueous solution of the monobasic calcium salt of this acid in the same manner, viz: with baryta water, and heating to boiling, a white flocculent precipitate is obtained, which after washing with hot water and drying at 100° C. gave by analysis the formula:  $C_6H_{10}Ba''O_7$ .

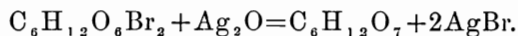
- I. 0.282 grams of the salt dried at 100° C. gave .2192 grams  $CO_2$  and .0905 grams  $H_2O$ .
- II. 0.1585 grams of the dried salt gave .1235 grams  $CO_2$  and .0505 grams  $H_2O$ .
- III. 0.3255 grams of the dried salt gave .192 grams  $BaCO_3$ .

\* *Annalen der Ch. u. Pharm.*, clviii, 253.

	Calculated.	1.	Found.	2.	3.
C <sub>6</sub>	21.75	21.19	21.24	----	----
H <sub>19</sub>	3.02	3.56	3.54	----	----
Ba <sup>9</sup>	41.38	----	----	----	41.01
O <sub>7</sub>	33.83	----	----	----	----

The formation of this salt, together with the lead salt, shows that from this acid both monobasic and dibasic salts can be formed.

The formula of the acid is C<sub>6</sub>H<sub>12</sub>O<sub>7</sub> and in the oxidation of the glycogen we can assume that the following reactions take place: C<sub>6</sub>H<sub>10</sub>O<sub>5</sub> + H<sub>2</sub>O + 2Br = C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>Br<sub>2</sub>. Adding silver oxide to the bromine compound we have:



From analogy, it would seem proper to apply to this acid the name glycogenic acid.

The preceding analyses and reactions show conclusively that by the action of bromine, water and silver oxide on glycogen, an acid is formed which bears the same relation to glycogen as "dextronsäure" to dextrin. On comparing this acid with the descriptions of "gluconsäure"\* and "dextronsäure,"† we see that the glycogenic acid differs from the two no more than the two differ from each other. There is also the same relationship existing between glycogenic acid and the acid or acids obtained by the oxidation of amyllum and paramyllum‡ by Haberman, which latter show but few points of difference from "gluconsäure" and "dextronsäure."

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