Chemistry. — "*Influence of different compounds on the destruction of monosaccharids by sodiumhydroxide and on the inversion of sucrose by hydrochloric acid. Constitution-formula of α-amino-acids and of betain*". By Dr. H. I. WATERMAN. (Communicated by Prof. J. BOESEKEN).

(Communicated in the meeting of April 27, 1917).

The destruction of monosaccharids, such as glucose, galactose etc., by basic substances, is accompanied by a decrease of polarisation of the solution in question whilst at the same time the colour becomes brown.

The action of hydroxylions is measured by the rapidity of diminution of polarisation and by the colour-intensity of the solution. I have noticed that different substances belonging to a series of compounds which generally have no or only a slightly acidic character, for instance amino acetic acid and α-aminopropionic acid, are able to neutralize the destructive action on glucose of considerable concentrations of alkali [1]).

In order to increase the general importance of my observations I have repeated the experiments with solutions of galactose instead of glucose. The results obtained are quite the same as with glucose (See table I). The action of hydroxylions on galactose is retarded too by different "neutral" substances. Whereas the addition of 5 cm³. 1,06 normal NaOH-solution in the experiments mentioned in table I after three hours has lowered polarisation from + 12,4 to + 9,3 and + 9,4, in the presence of 500 milligrams of alanin the same concentration of alkali has lowered polarisation only to + 11,1. After 24 hours and especially after 48 hours the phenomenon could be observed much better still. So, after 48 hours, without the addition of alanin the polarisation had diminished from 12,4 to 4,0 respectively 4,3; in the presence of alanine the polarisation had only diminished to 9,7.

The difference in colour-intensity of the solutions examined was in accordance with these facts. After ± 48 hours, in presence of sodium hydroxide, but without alanine, the colour was brown yellow, with alanine only pale-yellow.

[1]) H. I. WATERMAN, Chemisch Weekblad *10*, 739 (1913); *14*, 119 (1917).

From these and other experiments described in previous communications it follows that the quantity of alkali, which is fixed by glycine and alanine is very important.

TABLE I. *Retarding action of alanine on the destruction of galactose by alkali.*

		A	B	C	D
		40 cm³ of a solution containing ± 3,5 % galactose [1])			
Quantity of alanine added		0	0	0	500 milligram
Number of cm³ 1,06. normal NaOH-solution added		0	5	5	5
		Filled up with H_2O to 50 cm³			
		A, B, C and D were at the same time placed in a thermostat with watermantle (temperature: 33°)			
Polarisation in grades VENTZKE (length of the polarisation-tube 2 dm.)	At the beginning (temp. of the polarisation-solution: 18,5°)	+ 12,3	+ 11,1	+ 11,1	+ 12,3
	After ± 3 hours (temp. of the polarisation-solution: 20°)	+ 12,4	+ 9,3	+ 9,4	+ 11,1
	After ± 24 h. (temp. of the polarisation-solution: 18,5°—19°)	+ 12,5	+ 5,6	+ 5,8	+ 9,9
	After ± 48 h. (temp. of the polarisation-solution: 18°)	+ 12,4	+ 4,0	+ 4,3	+ 9,7
Colour of the solution	At the beginning	colourless	colourless	colourless	colourless
	After ± 3 hours	colourless	pale yellow	pale yellow	colourless
	After ± 24 hours	colourless	yellow	yellow	colourless
	After ± 48 hours	colourless	brown-yellow	brown-yellow	scarcely pale yellow

The number of cm³. alkali used for titration of the same quantity of these amino acids dissolved in water (with phenolphtalein as indicator) can practically be neglected with regard to the above.

By further experiments it has appeared that glycine and alanine behave under the circumstances belonging to these researches as one-basic acid.

[1]) Before using this solution, it was boiled for a moment and afterwards cooled.

Some of the observations referring to this are united in table II.

TABLE II. *Determination of the quantity of alkali fixed by glycine and alanine.*

		A	B	C	D	E	F	G	H	I	J
		80 cm³ of a solution containing ± 5 % glucose									
Number of cM³ 1,06 normal NaOH-solution added		0	2	3	4	5	6	10	10	10	10
Added									500 milligr. glycine		500 milligr. alanine
		Filled up to 100 cm³ and placed in thermostat with watermantle (temperature 33°)									
Polarisation in grades VENTZKE (2 dm. tube)	At beginning	+ 11,0							+ 10,6	+ 9,7	+ 10,5
	After ± 2 h.	+ 10,9	+ 10,5	+ 10,1	+ 9,9	+ 9,6	+ 9,3	+ 8,3	**+ 10,0**	+ 8,5	**+ 9,6**
Colour of the solution	After 40 hours	colourless	scarcely pale yellow	pale yellow	pale yellow (< C)	yellow	yellow-brown	brown	pale yellow (little deeper than D)	yellow-brown deeper than F.	pale yellow (= D)

A solution of glucose in water of fixed concentration was under comparable circumstances submitted to the action of different quantities of alkali. The number of cm³. normal NaOH-solution added varied between 0 and 10 cm³. per 100 cm³. solution.

The most important decrease and the darkest yellow colour occurred there, where most alkali had been added (experiments G and I). After about two hours the polarisation of G and I had diminished from + 10,9 to + 8,3 and + 8,5. By the experiments H and J was proved once more the protective influence of glycine and alanine; the polarisation was respectively + 10,0 and + 9.6.

The polarisation of H lay between that of C and D. From this it is apparent that 500 milligram glycine has fixed $10 - 3\frac{1}{2} = 6{,}5$ cm³. 1,06 N.NaOH = almost 7 cm³. N.NaOH. The intensity of colour was in accordance with this. In an analogous way it was demonstrated that 500 milligrams of alanine had fixed about $5 \times 1{,}06 = 5{,}3$ cm³. N.NaOH.

Glycine and alanine regarded as monobasic acid, 500 milligrams

of these compounds would fix respectively $\frac{500}{75} = 6{,}7$ cm³. and $\frac{500}{89} = 5{,}6$ cm³. N. alkali, so the agreement is sufficient.

On the other hand amino acetic acid and α-aminopropionic acid behave in hydrochloric acid containing solution as monacidic alkali, so that these aminoacids slacken the velocity of inversion of sucrose by hydrochloric acid considerably (Table III).

TABLE III. *Slackening influence of glycine and alanine on the inversion of sucrose by hydrochloric acid.*

		130 Gr. sucrose was dissolved in H_2O and filled up to 500 cm³ (solution R.)								
		A	B	C	D	E	F	G	H	I
		50 cm³ of solution R								
Added							500 milligr. glycine	500 milligr. alanine	500 milligr. phenol	
Number of cm³ 1,01 Normal hydrochloric acid added		0	2	4	6	10	10	10	10	10
		Filled up with H_2O to 100 cm³ and placed in thermostat with watermantle (temperature 33°)								
Polarisation in grades VENTZKE (2dm tube)	At the beginning of the experiments	+49,6	+49,4	+49,4	+49,5	+49,3	+49,4	+49,1	+49,0	+49,0
	after about 2 hours	+49,8	+48,5	+47,6	+46,3	+43,9	**+47,2**	**+46,4**	+43,1	+43,4
	after ± 43 hours	not determined because the sucrose was attacked by organisms	+8,3 (temp. 13°)	−8,3	−13,8 (temp. 13°,5	−16,7 (temp. 13°)	**−6,0**	**−10,5**	−16,6 (temp. 14,5)	−16,1 (temp. 14°)
	after ±3×24 hours		−2,9 (temp. 17°,5)	−13,9 (temp. 18°,5)	−16,1 (temp. 17°,5)	−16,5 (temp. 17°,5)	**−13,1** (temp. 18°,5)	**−14,9** (temp. 18°,5)	−16,4 (temp. 18°)	−16,4 (temp. 18°)
	after ±4×24 hours		not determined	−14,8 (temp. 21°,5)	not determined	not determined	**−14,7** (temp. 21°,5)	**−15,2** (temp. 21°)	not determined	not determined
	after ±6×24 hours		not determined	−16,0 (temp. 18°,5)	not determined	not determined	**−16,0** (temp. 18°,5)	**−15,9** (temp. 18°)	not determined	not determined

As follows from the survey given in table III the protective action of glycine and alanine on sucrose corresponds with respectively ± 6 cm³. and ± 5 cm³. N.HCl.

It is thus proved that under the circumstances of these experiments both aminoacids behave as monacidic basic substances.

Phenol has no protective influence; from the results obtained the opposite would sooner be inferred.

In acidic solution the properties of phenol differ widely from those of glycine and alanine, in alkalic solution on the contrary phenol behaves as monobasic acid just as glycine and alanine.

TABLE IV. *Influence of phenol on the destruction of glucose by alkali.*

		A	B	C	D	E	F	G
		80 cm³ of a solution containing ± 5% glucose						
cm³ 1,06 normal NaOH-solution added		0	3	5	10	10	10	10
quantity of phenol added		0	0	0	0	500 milligr.	1500 milligr.	0
		Filled up with H_2O to 100 cm³. Placed in thermostat with watermantle (temperature 33°).						
Polarisation in grades VENTZKE (2 dm-tube)	At beginning of the experiments	+ 11,1	+ 10,6	+ 10,3	+ 9,8	+ 10,2	+ 10,9	not determined
	After ± 3½ hours	+ 11,1	+ 9,6	+ 8,7	+ 6,9	**+ 8,5**	**+ 10,6**	+ 6,9
	Temp. of the polarisation-solution	(19°,5)	(19°)	(19°)	(19°)	(19°)	(18°)	
	After ± 24 hours	+ 11,0	+ 3,0	+ 0,4	— 1,0	+ 0,6	+ 9,5	— 0,7
	Temp. of the polarisation-solution			(20°)	(20°)	(19°,7)		
	After ± 2 × 24 h.	not determined	+ 0,3	— 0,2	— 0,7	— 0,6	+ 8,1	— 0,6
	Temp. of the polarisation-solution				(17°,5)		(17°,5)	
Colour	After ± 24 hours	colourless	pale yellow	yellow	deep yellow	yellow	almost colourless	deep yellow

500 milligram phenol neutralizes the action of about 5 cm³. 1,06 N. NaOH-solution = ± 5,3 cm³. N. NaOH.

Regarding phenol as monobasic acid 500 milligram corresponds with $\frac{500}{94} = 5{,}3$ cm³. N.acid. So the agreement is sufficient.

In the above we have made acquaintance with two sensible methods, which enable us to determine the acidic or basic character

of a compound in another way than this has usually happened up to now.

Besides they give us an important indication about the condition of the amino acids in watery solutions.

These amphoter electrolytes have apparently a neutralising influence in two directions. This can only be understood well, when we suppose that the action of the alkalic substances and of the acids on the destruction of the monosaccharids and on the inversion of sucrose is caused by molecules or molecule-fractions, which can be fixed by the amino acid.

From these results we may conclude that with these reactions under the influence of strong acids the amino acids assume the character of rather strong basic substances and under influence of strong alkali they act as rather strong acids.

These *two* opposite properties of the amphoter substance come very distinctly to the front, which can best be symbolized by the supposition of *the open chain* as well in strong acidic as in alkalic medium.

When we suppose the ring-constitution in pure water, against which no decisive difficulty exists because the electrice conductive power is so small, the above can also be defined as follows:

By strong alkali the carboxyl-side

$$\begin{matrix} CH_2{-}NH_2 \\ | \\ CO-OK \end{matrix} \quad \leftarrow \quad \begin{matrix} CH_2{-}NH_2 \\ | \quad | \\ CO-O \end{matrix} \quad \rightarrow \quad \begin{matrix} CH_2{-}NH_3Cl \\ | \\ CO-OH \end{matrix}$$

of the ring is opened, by strong acids the ammonium-side.

Now it was interesting to know how betain should behave; this compound has no doubt in pure water the ring-constitution and it could be expected that this ring should not open on the two sides as easily, at least not in an equally strong degree, as this proved the case with the amino-acids.

Really, the experiments joined in tables V^a and V^b show that betain does not hinder the destructive action of alkali on glucose, whereas betain acts as monacidic alkali on hydrochloric acid during the inversion of sucrose. Accordingly in presence of alkali the ring-formula must be assigned to betain; in presence of strong acids this compound has an open chain.

$$\begin{matrix} CH_2{-}N(CH_3)_3 \\ | \quad | \\ CO-O \end{matrix} \quad \rightarrow \quad \begin{matrix} CH_2{-}N(CH_3)_3Cl \\ | \\ CO-OH \end{matrix}$$

Betain in neutral and *alkalic* solutions. — Betain in strong *acidic* solutions.

TABLE Va. *Behaviour of betain in alkalic solution.*

		A	B	C	D	E
		40 cm³ of a solution containing ± 5% glucose				
Quantity of betain-hydrochloric acid added					499 milligr.	
Number of cm³ 1,06 Normal NaOH-solution		0	2,5	5	5	5
		Filled up to 50 cm³ and placed in thermostat with watermantle (temperature 33°)				
Polarisation in grades VENTZKE (2 dm. tube)	At beginning of the experiment	+12,4	+11,8	+11,0	+11,8	+11,2
	After ± 3 hours	+12,5	+ 9,7	+ 7,5	+10,2	+ 7,6
	After ± 6 hours	+12,5	+ 7,4	+ 4,1	+ 8,0	+ 4,1
	After ± 24 hours	+12,5	+ 1,8	− 0,8	+ 3,2	− 0,5
Colour of the solution	After ± 3 hours	colourless	hardly pale yellow	pale yellow	hardly pale yellow	pale yellow
	After ± 24 hours	colourless	pale yellow	yellow	hardly pale yellow	yellow

499 milligram betain-hydrochloric acid correspond with $\frac{499}{153,5} =$ almost 3,3 cm³. normal HCl. If in alkalic medium betain behaves as a neutral compound, the action of the added 5 cm³. 1,06 N. NaOH = 5,3 cm³.* N. NaOH must be diminished by that of 3,3 cm³.; then 5,3—3,3 = 2 cm³. N. NaOH remains. From the experiments it follows indeed that 499 milligrams of betain-hydrochloric acid and 5 cm³. 1,06 N. NaOH act together as something less than 2,5 cm³. 1,06 N. NaOH.

Whilst in alkalic solution betain behaves neutral, in acidic solution it acts like one-acidic alkali.

From this results that the betain-hydrochloric acid-complex behaves as practically neutral.

Hence the inversion of sucrose by hydrochloric acid is accelerated but little by betain-hydrochloric acid.

TABLE V*b*. *Behaviour of betain in acidic solution.*

		130 Gr. sucrose was dissolved in H_2O and filled up to 500 cm³ (R)							
		A	B	C	D	E	F	G	H
		50 cm³ of liquid R							
Quantity of betain-hydrochloric acid added						500 milligr.		500 milligr.	
Number of cm³ 1,01 normal HCl-solution added		0	3	5	7	7	10	10	10
		Filled up with H_2O to 100 cm³, placed in thermostat with watermantle (temperature: 33°)							
Polarisation in grades VENTZKE (2 dm) tube	At beginning	+ 49,8	+ 49,4	+ 49,3	+ 49,2	+ 49,3	+ 49,2	+ 49,1	+ 49,1
	Temperature of the polarisation liquid			(17°)	(17°)	(17°)	(18°)	(18°)	(18°)
	After ± $2^3/_4$ hours	+ 49,7	+ 46,7	+ 45,0	+ 42,5	+ 41,9	+ 40,2	+ 39,5	+ 39,4
	Temperature of the polarisation liquid			(19°)	(19°)	(19°)	(19°)	(19°)	(18°)
	After ± $3^1/_2$ hours	+ 49,8	+ 45,5	+ 43,1	+ 39,9	+ 39,1	+ 36,7	+ 35,8	+ 36,0
	Temperature of the polarisation liquid			(19°)	(19°)	(19°)	(18,5°)	(19°)	(19°)
	After ± 72 hours	not determ-ined	not determ-ined	— 16,2	— 16,4	— 16,2	— 16,6	— 16,3	not determ-ined
	Temperature of the polarisation liquid			(17°)	(17°)	(17°)	(17°)	(17°,5)	

The summary of the above mentioned results becomes as follows:

1. Amino acetic acid and α amino propionic acid retard the destruction of glucose by sodiumhydroxide.

2. This phenomenon is independent of the presence and the quality of the monosaccharid, for the destruction of galactose by sodium-hydroxide is retarded too by the substance mentioned.

3. Amino acetic acid and α-aminopropionic acid behave in alkalic medium as acids. By further examination it was demonstrated that they behaved as about one-basic acid.

4. Just on the other hand these aminoacids in presence of hydrochloric acid behave as monacidic alkali, so that they considerably

retard the rapidity of inversion of sucrose by hydrochloric acid.

5. The behaviour of glycine and alanine deserves special attention because these compounds behave by the usual way of titration as practically neutral.

The number of cm^3. alcali necessary for colouring pink a solution of glycine or alanine, which contains phenolphtalein, is insignificant if compared with the quantity of alkali which would be necessary when both compounds should behave in this case as one-basic acids. The same holds for phenol (Compare 7°).

6. The destructive influence of sodium-hydroxide on monosaccharids and the inversion of sucrose by hydrochloric acid can be used for the edification of two sensible methods, which enable us to judge in another way than was usual up to now, whether a compound has acidic or basic properties [1]).

7. Remarkable too is the behaviour of phenol in alkalic solution. Phenol acts then as about a one-basic acid, whilst this compound practically has no influence on the inversion of sucrose by hydrochloric acid.

8. The pure amphoter behaviour of glycine in alkalic and in acidic solution, together with the behaviour of betain, which compound in alkalic solution is practically neutral and in acidic solution acts as one-acidic basic substance, make it probable that glycine as well as alanine possess in alkalic and acidic solution the open formula of constitution. In entirely neutral solution the ring formula is sufficient. To betain the ring-formula must be granted in neutral and alkalic solution, in acidic solution the open constitution-formula.

This research will be continued in different directions in order to study the acidic and basic character of the substances and at the same time to determine how far their usual constitution-formula corresponds with this character.

Dordrecht, February 1917.

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[1]) These methods can only be used if we know with certainty that the substance to be examined is not destroyed in acidic or alkalic solution and has for the rest no disturbing influence.