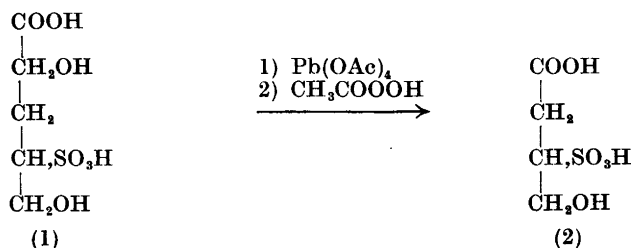# Reaction between D-Glucose and Sulphite

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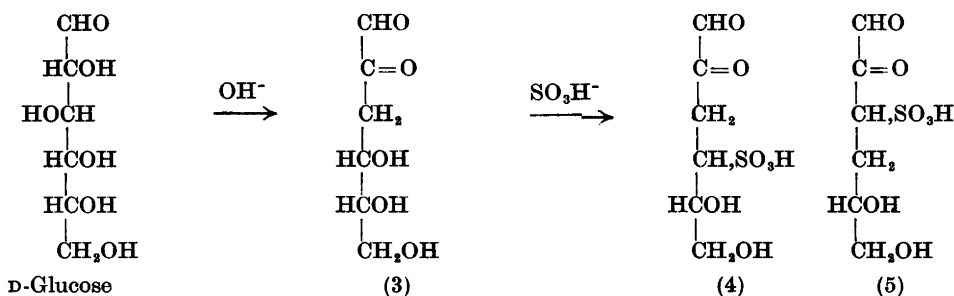*Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm Ö, Sweden*

The structures of three acids, previously isolated from the products obtained when glucose is treated, at 100° or 135°, with sulphite solutions of pH 6.5, have been determined by degradations and interconversions. The first step in these reactions is the formation of 3-deoxy-D-glucosone, which then reacts with sulphite to give a sulphonic acid (4). This acid is then either oxidised to a ketocarboxylic acid (6) or rearranged to a hydroxycarboxylic acid (7).

Hägglund and Johnson observed two types of reactions between D-glucose and sulphite. Under the conditions of acidic sulphite cooking, D-glucose was oxidised to D-gluconic acid.[1] At about pH 6 and 130° the reaction was more complex and carbohydrate sulphonic acids [2] could be isolated from the reaction mixture. Adler [3] studied the last reaction and was able to isolate two acids, A and B, with the compositions $C_6H_{10}O_8S$ and $C_6H_{12}O_8S$, respectively, both containing a sulpho group and a carboxyl group. The former also contained a carbonyl group, believed to be an aldehyde group because of its reaction with hypoiodite. Adler gave partial structures for these acids and drew attention to their similarity with the saccharinic acids. Yllner [4] studied the reaction between D-xylose and sulphite at pH 6.5 and 135° and isolated an optically inactive sulphonic acid (as its brucine salt) from the reaction mixture, which also contained other strong acids. He showed it to have the structure of a 1,4-dihydroxy-3-sulpho-pentanoic acid (1) by degrading it to 3-hydroxy-2-sulpho-butanoic acid (2), characterised as the crystalline pyridinium salt of its lactone. The configuration

```
COOH
|
CH₂OH            1) Pb(OAc)₄           COOH
|                2) CH₃COOOH           |
CH₂             ————————————→         CH₂
|                                      |
CH,SO₃H                               CH,SO₃H
|                                      |
CH₂OH                                 CH₂OH
  (1)                                    (2)
```

was not determined and the isolated acid probably represents one of the two possible anomeric pairs with the structure 1. The same acid was later isolated by Cordingly [5] after similar treatment of D-xylose or L-arabinose.

Ingles [6] obtained β-sulphopropionic acid by the treatment of D-glucose or D-fructose with sulphite at 100° and pH 9.6. He also obtained D-glucose-6-sulphate and smaller amounts of D-gluconic acid after treatment of D-glucose with sodium hydrogen sulphite at 100°.[7] From the product of the reaction at 100° and pH 6.5 he isolated another acid,[8] which he showed to have one of two structures (4 or 5).

CHO
|
HCOH
|
HOCH   OH⁻
| ⟶
HCOH
|
HCOH
|
CH₂OH
D-Glucose

CHO
|
C=O
|
CH₂   SO₃H⁻
| ⟶
HCOH
|
HCOH
|
CH₂OH
(3)

CHO
|
C=O
|
CH₂
|
CH,SO₃H
|
HCOH
|
CH₂OH
(4)

CHO
|
C=O
|
CH,SO₃H
|
CH₂
|
HCOH
|
CH₂OH
(5)

From the suggested reaction mechanism, with the intermediate formation of 3-deoxy-D-glucosone (3), the structure of a 3-deoxy-4-sulpho-hexosone (4) was considered to be the most probable. This is further supported by the analogy between 4 and the acid 1, which should be formed in a similar reaction, followed by a benzilic acid rearrangement of the 3-deoxy-4-sulpho-pentosone. For convenience this acid will be referred to as acid 4.
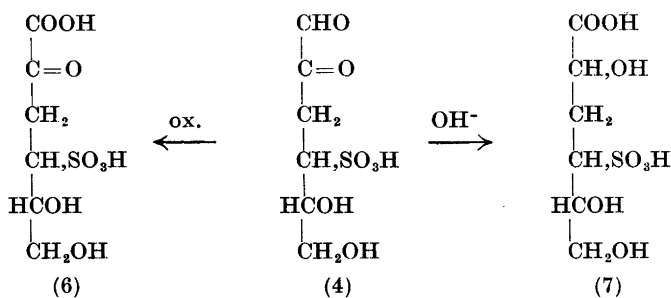
In a recent publication from this Department [9] it was reported that mannitol is oxidised to hexoses in sulphite solutions of different pH-values at higher temperatures and that, at pH-values above 4, reactions typical for alkaline solutions, such as epimerisation, fragmentation and saccharinic acid rearrangement of sugars take place.

In the present paper studies which support the above reaction mechanism and prove the structures of acid 4 and the two acids isolated by Adler [3] are reported.

D-Glucose and 3-deoxy-D-glucosone, prepared according to Kato,[10] were treated with sulphite at 100° and pH 6.5. From both reaction mixtures an acid was isolated having the same paper chromatographic and paper electrophoretic properties, and giving the same derivatives with 2,4-dinitrophenylhydrazine as reported by Ingles for acid 4. The yield of this acid from 3-deoxy-D-glucose was higher (48 %) than from D-glucose (13 %). It was observed, however, that different preparations of acid 4 differed in their optical rotations, for which values of $[\alpha]_D$ between −6° and + 9° were obtained. For the pyridinium salt of the 2,4-dinitrophenylosazone higher values, +41° and + 124°, were obtained. Acid 4 has two asymmetric carbon atoms and it is obvious that is it not pure but is a mixture of isomers, which are not separated by paper chromatography or paper electrophoresis, but may have been partially separated during the ion exchange chromatography used for fractionating the reaction product.

The present result, however, strongly supports the assumption that 3-deoxy-D-glucosone is an intermediate in the formation of the sulpho-acid.

The treatment of D-glucose with sulphite at 130° and pH 6.5, the conditions used by Hägglund et al.,[2] was repeated, the sulphonic acids isolated as their barium salts and further separated by reaction with phenylhydrazine as described by Adler.[3] The barium salts of the free acids, which formed phenylhydrazones, were regenerated by treatment with benzaldehyde. The carbonyl-free acid was isolated from the mother liquors by cellulose column chromatography. The two acids were chromatographically and electrophoretically indistinguishable from the two acids isolated by Adler. (Samples of these were kindly supplied by his coworker in these studies, Ing. S. Häggroth). The carbonyl group in acid A was shown not to be an aldehyde group, as the acid was unchanged after treatment with chlorite of pH 3.5, a reagent known to oxidise aldehydes to carboxylic acids.[11] It seemed most probable that acids A and B should be structurally related to the acid 4 obtained by Ingles. Two possible reactions of the latter in the sulphite medium are oxidation of the aldehyde, and alkaline rearrangement of the dicarbonyl structure to a hydroxy carboxylic acid. The two acids which should be formed in these reactions, 6 and 7, have the same compositions as the acids A and B.

$$
\begin{array}{ccccc}
\text{COOH} & & \text{CHO} & & \text{COOH} \\
| & & | & & | \\
\text{C}=\text{O} & & \text{C}=\text{O} & & \text{CH,OH} \\
| & & | & & | \\
\text{CH}_2 & \xleftarrow{\text{ox.}} & \text{CH}_2 & \xrightarrow{\text{OH}^-} & \text{CH}_2 \\
| & & | & & | \\
\text{CH,SO}_3\text{H} & & \text{CH,SO}_3\text{H} & & \text{CH,SO}_3\text{H} \\
| & & | & & | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
(6) & & (4) & & (7)
\end{array}
$$

They were prepared by chlorite oxidation, and treatment with aqueous calcium hydroxide, respectively, of acid 4 and proved to be chromatographically and electrophoretically indistinguishable from acids A and B. Further, as required by the hypothesis, an acid indistinguishable from B was obtained by borohydride reduction of either A or the chlorite oxidation product (6) of 4.

The acid resulting from the borohydride reduction of A, and acids B and 7 (prepared from 4 by treatment with alkali) were all subjected to periodate oxidation, followed by borohydride reduction and yielded an acid chromatographically and electrophoretically indistinguishable from acid 1, obtained by Yllner[4] from D-xylose. Electrophoretograms of Yllner's acid showed that the spot for the free acid was stronger than that of the lactone, but for the other acids the lactone spot was the stronger. This indicates that another isomer predominates in the acid obtained by degradation. Alkaline treatment of the last acid did not change these proportions but after such treatment of Yllner's acid, the lactone spot increased in intensity. The three acids obtained by degradation of borohydride reduced A, and degradation of B and 7 were further degraded by lead tetraacetate oxidation, followed by oxidation with peracetic

acid to 3-hydroxy-2-sulpho-butanoic acid (2), essentially as described by Yllner.[4] Because of the unfavourable proportion of free acid to lactone in the starting material, the lead tetraacetate oxidation was sluggish and the yield of 2 not as good as that obtained from Yllner's acid. Acid 2 was characterised as the pyridinium salt of its lactone and indistinguishable (m.p., IR) from authentic material. It crystallised in two different forms, m.p. 161—162° and 156—160°, which could be transformed into each other. From this degradation the structures of the acid isolated by Ingles and the two acids (A and B) isolated by Adler are shown to be 4, 6, and 7, respectively.

All the acids are most probably mixtures of isomers and as they have not been separated into the pure isomers, nothing can be said about the configuration at the asymmetric carbon atoms. It seems possible, however, that the configuration at C-5 is retained, as this centre does not take part in the reactions occurring during the treatment with sulphite. Some of the brucine salts from these acids probably represent pure or considerably enriched chemical individuals.

EXPERIMENTAL

Melting points are corrected.

Paper chromatograms were run on Whatman No. 1 and 3 MM papers in the following solvent systems:

A. Ethyl acetate, acetic acid, water, 3:1:1.
B. Ethanol, conc. aqueous ammonia, water, 6:1:1.
C. Ethyl acetate, pyridine, water, 8:2:1.

Paper electrophoresis was carried out on Whatman No. 1 papers in a buffer of pH 3 containing acetic acid (400 ml), pyridine (45 ml) and water (3000 ml). The migrations given (R_{GA}- and M_{GA}-values) are relative to that of D-gluconic acid.

Spraying reagents. In addition to conventional spraying reagents for acids, polyols and carbonyl compounds, the hydroxylamine-ferric chloride reagent [12] proved to be valuable in these studies. It gave violet spots with the lactones and yellow, slowly appearing spots with the free sulphonic acids, possibly due to lactonisation of the separated acids on the paper.

Preparation of acid 4

(a) *From* D-*glucose.* Ingles' experiment [8] was repeated, starting from D-glucose (45 g). The yield of acid 4, as the barium salt, was 9.8 g (13 %). Chromatographic and electrophoretic investigation of the reaction product revealed the presence of several other acids, including gluconic acid, sulphonic acids and acids containing carbonyl groups.

(b) *From 3-deoxy-*D-*glucosone.* 3-Deoxy-D-glucosone was prepared from D-glucose and butylamine according to the method of Kato [10] and isolated from the reaction mixture by cellulose column chromatography as described by Anet.[13] This substance (2.03 g), sodium hydrogen sulphite (2.17 g) and sodium sulphite (2.50 g) were dissolved in water (100 ml) and kept at 100° for 4 h. The pH was 6.5 before and after this treatment. The reaction mixture was converted into the free acids by ion exchange and the resulting solution introduced onto a column (2.8 × 20 cm) of Dowex 1—X4 (50—100 mesh) in the acetate form. This was eluted, first with water and then with 0.2 M sulphuric acid. The fractions containing pure acid 4 were combined and converted to the barium salt (1.78 g, 48 %).

The acids obtained in the two experiments gave identical IR-spectra (KBr), both as free acids and as their barium salts, and were chromatographically and electrophoretically indistinguishable from each other. They each gave two spots (M_{GA} 1.91 and 2.58, R_{GA} 0.42 and 0.80 in solvent A) indicating a slow equilibrium between different forms. The material eluted from the zones corresponding to either of these spots gave the same two spots on paper chromatography or electrophoresis. Two samples of the free acid obtained from D-glucose and two obtained from 3-deoxy-D-glucosone showed $[a]_D^{20} + 9, + 6, - 6°,$

and + 3, respectively (c 2.0, water). The acid and its salts are not very stable and become discoloured on keeping and on concentrating their solutions.

The pyridinium salt of the 2,4-dinitrophenylosazone of acid 4 was prepared as described by Ingles.[8] The product derived from D-glucose had m.p. $225-229°$ (decomp.) and $[a]_D^{20} + 124°$ (c 0.5 dimethylformamide, equilibrium value), that from 3-deoxy-D-glucosone had m.p. $224-228°$ (decomp.) and $[a]_D^{20} + 41°$. They gave identical IR-spectra (KBr) and were chromatographically indistinguishable from each other (solvent systems A and C). They also gave intense blue colour reactions with 2 N sodium hydroxide, as observed by Ingles.[8]

Preparation of the two acids (A and B), described by Adler[3]

D-Glucose (30 g), sodium pyrosulphite (41 g) and hydrated ($7H_2O$) sodium sulphite (120 g) were dissolved in water (600 ml), heated at 130° for 75 min and the crude barium salts (42 g) of the resulting sulphonic acids were isolated as described [2] previously, except that sodium ions were removed by ion exchange, after which the solution was degassed and neutralised at 50° by the addition of barium carbonate over a period of 30 min.

To the barium salts (24 g) dissolved in water (120 ml) 50 % aqueous acetic acid (9.0 ml) and phenylhydrazine (7.0 ml) were added, and the mixture kept at 50°. A precipitate slowly formed and after 2 h water (300 ml) was added and the mixture left at room temperature for 24 h. Filtration followed by washing of the residue successively with water, ethanol and ether afforded a pale yellow material (6.1 g). The phenylhydrazone of the free acid was prepared by treating a suspension of the barium salt, in water, with cation exchange resin. It appeared as a single round spot on paper electrophoresis, $M_{GA} = 1.29$, and gave a characteristic grey colour with the lactone spray. When the acidic solution was adjusted to pH 7 with barium hydroxide the difficulty soluble barium salt crystallised out again.

A further amount of the phenylhydrazone of the barium salt of the acid (2.32 g; purity > 80 %) was obtained by adding ethanol to the mother liquors to a concentration of about 30 %. The combined filtrate and washings after filtrations of the precipitate were concentrated to dryness and the product extracted with water (300 ml) at 40°. The solution was treated with carbon (80 g) at room temperature for 30 min, filtered, concentrated to 40 ml and added to ethanol (400 ml). The filtered precipitate after washing with 90 % aqueous ethanol, anhydrous ethanol and ether, amounted to 8.75 g. This product, as revealed by paper electrophoresis, still contained some phenylhydrazone, but the main spot (corresponding to more than 50 % of the product) was indistinguishable from that given by an authentic sample of Adler's acid B.

Acid A. To a suspension of the barium salt (2.00 g) in ethanol (60 ml) and water (100 ml) freshly distilled benzaldehyde (2 ml) and acetic acid (0.8 ml) were added, and the mixture boiled under reflux for 3.5 h in an atmosphere of nitrogen. The volume of the solution was then reduced to 50 ml, and the benzaldehyde phenylhydrazone removed by filtration. The filtrate, after treatment with carbon, was concentrated to 10 ml and addition to ethanol (100 ml) yielded a precipitate of the barium salt, which was filtered off (1.10 g) and found to be chromatographically and electrophoretically pure and so was used directly for further reaction. Further purification, however, could be carried out by crystallisation from water. The free acid, obtained from the barium salt by ion exchange, was chromatographically and electrophoretically indistinguishable from an authentic sample of Adler's acid A, and had the following properties: $[a]_D^{21} - 25°$ (c 2, water); M_{GA} 3.16 (free acid); R_{GA} 0.7 (elongated, solvent A) and 1.05 (solvent B). Brucine salt, m.p. $206-210°$ (decomp.); $[a]_D^{20} - 23°$ (c 1, water).

Acid B. The acid corresponding to the crude barium salt obtained as described above, could be purified *via* repeated recrystallisation of the brucine salt. The acid used for the degradation studies, however, was purified by cellulose column chromatography (solvent system B) of the ammonium salt. From the crude barium salt (3.33 g) pure acid B (0.58 g) was obtained and comparable amounts of the acid, contaminated with other acids, were present in surrounding fractions. The acid, which was chromatographically and electrophoretically indistinguishable from an authentic sample of Adler's acid B, had the follow-

ing properties: $[a]_D^{22}$ + 13° (c 2, water): M_{GA} 2.33 (lactone): R_{GA} 0.7 (elongated, solvent A) and 0.94 (solvent B). Brucine salt, m.p. 155−160° (decomp.): $[a]_D^{22}$ −30° (c 1, water).

Chlorite oxidation of acid 4 to acid 6. Acid 4 (2.00 g), prepared from D-glucose, was dissolved in an aqueous solution (100 ml) of 1.5 M acetate buffer of pH 3.5, sodium chlorite (4.5 g) added and the resulting solution kept at room temperature for 24 h. The reaction mixture was successively treated with sulphur dioxide, cation exchange resin, silver carbonate, hydrogen sulphide, barium carbonate and cation exchange resin as previously reported [14] for an analogous oxidation. The main acid in the product was chromatographically and electrophoretically indistinguishable from Adler's acid A. It was isolated in a pure state by cellulose column chromatography (solvent system B) and converted to the barium salt (0.67 g). Comparable amounts of this acid, contaminated with other acids were present in surrounding fractions. The phenylhydrazone was electrophoretically indistinguishable from the corresponding derivative of Adler's acid A. Free acid: $[a]_D^{21}$ −3° (c 2, water). The brucine salt of this acid had m.p. 178−182° (decomp.) and $[a]_D^{19}$ −33° (c 1.0, water). The corresponding values for the brucine salt of Adler's acid A are m.p. 206−210° (decomp.) and $[a]_D^{19}$ −23° (c 1.0, water).

Preparation of acid 7

(a) From acid 4. To an aqueous solution of the barium salt of acid 4 (1.5 g in 50 ml), prepared from D-glucose, was added calcium oxide (3.0 g) and the mixture kept under an atmosphere of nitrogen for 4 h at 50°, filtered, converted to the free acids by ion exchange and concentrated to a syrup (0.93 g), which was fractionated on a cellulose column (solvent system B). The main component, chromatographically and electrophoretically indistinguishable from Adler's acid B, was obtained as the ammonium salt (pure top-fraction: 0.39 g). Free acid: $[a]_D^{22}$ + 17° (c 1, water). Part of the product was converted to the brucine salt, m.p. 157−161° (decomp.).

(b) From Adler's acid A. The barium salt of Adler's acid A (1.40 g) was reduced with borohydride and the product isolated as the ammonium salt (0.68 g). The acid was chromatographically and electrophoretically indistinguishable from Adler's acid B. Free acid: $[a]_D^{21}$ + 20° (c 1, water). Part of it was converted to the brucine salt, m.p. 154−158° (decomp.) $[a]_D^{20}$ −19° (c 1, water).

(c) From acid 6. The barium salt of acid 6, prepared from acid 4 by chlorite oxidation, as described above, was reduced with borohydride and the acid obtained shown to be chromatographically and electrophoretically indistinguishable from Adler's acid B; $[a]_D^{22}$ + 9° (c 1, water).

Preparation of 3-hydroxy-2-sulpho-butanoic acid by degradation of the C_6-acids

(a) From acid 4. Acid 4 was converted to acid 7 by treatment with lime water (see above). A solution of the latter acid (0.90 g, 3.69 mmole) in water (200 ml) was adjusted to pH 4 with sodium hydrogen carbonate, sodium metaperiodate (1.20 g) added and the solution kept in the dark at room temperature. The consumption of periodate was followed by titration of aliquots. After 24 h when the consumption was 2.75 mmole, excess of ethylene glycol was added, and after a further hour the solution was adjusted to pH 9 with barium hydroxide. The precipitate was removed by filtration and excess of borohydride added to the solution. After 24 h the solution was filtered through a column of cation exchange resin and the acids adsorbed on a column of Dowex 3 (free base) and recovered as their ammonium salts (0.80 g). This product was dissolved in acetic acid (400 ml), containing lead tetraacetate (7.00 g) and potassium acetate (7.00 g) and kept in the dark at room temperature. After four days the consumption of oxidant was 2.5 mmole, compared with a calculated value of 3.2, assuming that the starting material was pure acid 1. The lead tetraacetate was then destroyed by addition of ethylene glycol and the mixture oxidised with peracetic acid and worked up as described by Yllner.[4] The resulting syrup, which should contain the pyridinium salt of the lactone of acid 2 was extracted at 50° with a 1 % solution of pyridine in butanol saturated with water (3 × 30 ml). By this treatment some salts and discoloured products were left undissolved, and these were shown by chromatography not to contain the pyridinium salt of the lac-

tone of acid 2. The syrup (0.48 g) obtained on concentrating the solution was subjected to repeated fractionations on thick filter papers (solvent: ethyl acetate, pyridine, water, 3:8:2) and a pure product (35 mg) was obtained which crystallised spontaneously and after two recrystallisations from ethanol melted at 159 — 161°. It was optically inactive and the m.p. was not depressed on admixture with the authentic pyridinium salt of the lactone of 3-hydroxy-2-sulpho-butanoic acid and the IR-spectrum was identical with that of the last substance.

(b) From Adler's acids A and B. Similar treatments of Adler's acids A (first reduced with borohydride as described above) and B gave amounts of the crystalline pyridinium salt, comparable with that obtained from acid 4. Its identity with the authentic material was confirmed by m.p. determinations and IR. The salt sometimes crystallised as prisms, m.p. 159 — 161° and sometimes as rods, m.p. 156 — 160° (probably gradually transforming into the other modification during the m.p. determination). The substance always crystallised as the higher melting form from a melt. The two forms were found to have slightly differing IR-spectra.

The individual steps of the degradation were studied in separate experiments by fractionation of the mixtures on thick filter paper. After periodate oxidation of either of the three C_6-acids about 20 % of material with the same chromatographic and electrophoretic properties as the starting material was obtained. After the lead tetraacetate oxidation an acid and (predominantly) a lactone, chromatographically and electrophoretically indistinguishable from acid 1 and its lactone amounted to more than 40 % of the reaction product. Another acid, with the same chromatographic and electrophoretic properties as sulphosuccinic acid, was also present in considerable amounts. The same products, but in smaller proportions, were also present when acid 1, from D-xylose, was subjected to the same treatment.

In one experiment the ammonium salt of Adler's acid B (234 mg) was subjected to periodate oxidation and borohydride reduction as described above and the C_5-acid isolated as the ammonium salt (120 mg) by chromatography on thick filter paper (solvent system A). It was converted to the brucine salt, which after three recrystallisations from aqueous acetone was obtained as small needles, m.p. 173 — 176° (decomp.), $[a]_D^{20} - 20°$ (c 2.0, water). The brucine salt of the acid prepared from D-xylose by Yllner[4] crystallised as rods, m.p. 185 — 189° (decomp.) and showed $[a]_D^{20} - 17°$. Cordingly[5] reports for the brucine salt of the main C_5-acid $[a]_D^{20} - 26°$. The two former brucine salts were converted to the free acids, which had negligible optical rotations. They gave a similar picture on paper electrophoresis with one spot for the free acid (M_{GA} 3.26) and one for the lactone (M_{GA} 2.56). For Yllner's acid the spot corresponding to the free acid was stronger than that of the lactone, but for the other acid the spot corresponding to the lactone was the stronger. The acids were treated with 0.5 N sodium hydroxide at 100° for one hour and transformed into the acid-lactone mixtures. After this treatment the proportion of lactone in Yllner's acid had increased but the ratio of free acid to lactone in the other was virtually unchanged.

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