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Treatment of D-Erythrose and D-Xylose with Sulphite

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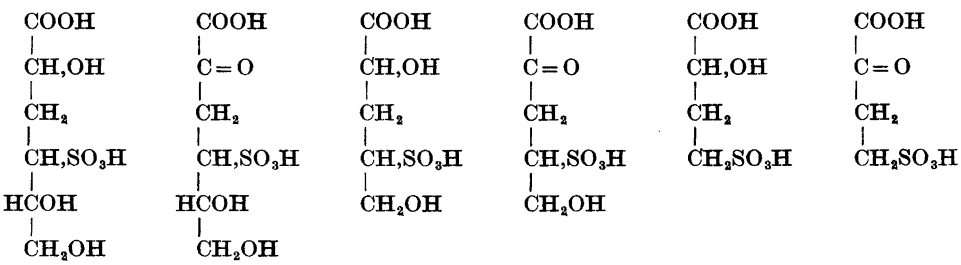
From the treatment of D-erythrose with aqueous sulphite at pH 6.5 and 135° 3-sulpho-propanoic and 2-hydroxy-4-sulpho-butanoic (V) acids were isolated as the main sulphonic acids, together with smaller amounts of a keto acid (probably VI). Acid V was synthesised. A reinvestigation of the sulphonic acids from D-xylose, treated similarly, revealed that 5-hydroxy-2-oxo-4-sulpho-pentanoic (IV) and 2,5-dihydroxy-4-sulpho-pentanoic (III) acids were the main acids, with the first one predominant. NMR-studies of the sulphonic acids isolated are presented.

In a previous investigation [1] it was proved that the two main acids formed by treatment of D-glucose in aqueous sulphite solution at pH 6.5 and 130° were the two sulphonic acids I and II. It was also shown that they were formed by a benzilic acid rearrangement and oxidation, respectively, of a rather labile intermediate, a 3-deoxy-4-sulpho-hexosone. The latter acid had previously been isolated by Ingles [2] after a similar treatment of D-glucose at 100°. Some years earlier, Yllner [3] and Cordingly [4] reported the formation of a five-carbon homologue to I, *i. e.* compound III, by treatment of D-xylose [3,4] and L-arabinose with aqueous sulphite of pH 6.5 at 130 – 135°.

In the present investigation, D-erythrose was treated with aqueous sulphite at pH 6.5 and 135°, as a continuation of previous studies on the higher monosaccharides.



I II III IV V VI

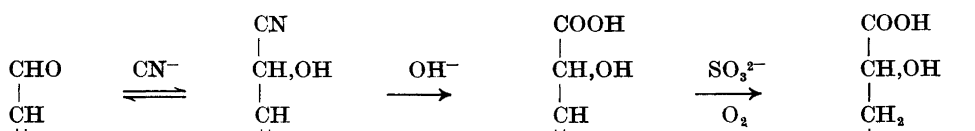
The reaction mixture from D-erythrose was treated with 2,4-dinitrophenylhydrazine (DNP) to detect keto acids. Examination of the product by paper chromatography and electrophoresis indicated only small amounts of keto acids and one dominating spot, which was later shown to correspond to two sulphonic acids. An attempt to isolate a difficultly soluble barium phenylhydrazone of a keto acid, by analogy with the isolation of acid II from glucose directly from the crude barium salts, failed.

By a chromatographic separation of the DNP-treated reaction product on a cellulose column three main fractions were collected. The first yellow one was rather small and was, after subfractionation on paper, obtained in pure state and in an amount corresponding to *ca.* 5 % of the sulphonic acid mixture. It showed similar properties (paper chromatographic and electrophoretic) as the corresponding DNP-derivative of acid IV (isolation and identification, see below). This indicated that it was probably a DNP-derivative of acid VI, and that the latter acid thus was a minor product.

From the second fraction, a sulphonic acid was isolated as a crystalline brucine salt. The NMR-spectrum of its disodium salt indicated that the acid is 3-sulpho-propanoic acid. The two symmetrical multiplets at $\delta = 2.6$ and $\delta = 3.1$ ppm are assigned to the methylene protons next to the carboxylate group and the sulphonate group, respectively. 3-Sulpho-propanoic acid was synthesised,[6] and the two acids were found to be indistinguishable (IR, NMR, and by paper chromatography and electrophoresis).

From the third fraction and the mother liquor from the second fraction, another sulphonic acid was isolated as a crystalline barium salt, precipitating from warm aqueous ethanol. The IR-spectrum showed peaks for sulphonate and carboxylate groups, and the elemental analysis indicated the formula $C_4H_6O_6SBa$, which is in accordance with a barium salt of 2-hydroxy-4-sulphobutanoic acid (V). No optical activity of the acid was observed. The structure was confirmed by NMR. The NMR-spectrum of the disodium salt shows a quartet at $\delta = 4.2$ ppm (1H), which must be assigned to the proton α to the carboxylate group, and attached to the same carbon atom as the hydroxyl group. The proton is coupled to the non-equivalent adjacent methylene protons (J 5 and 6 cps.) These and the remaining methylene protons appear as two complex multiplets (both geminal and vicinal couplings) at $\delta = 2.2$ ppm (2H) and $\delta = 3.1$ ppm (2H), respectively.

The 2-hydroxy-4-sulphobutanoic acid was also synthesised by the addition of sulphite to vinyl glycolic acid (VII) in the presence of oxygen (anti-Markovnikov addition) and subsequent chromatographic purification.



VII V

The NMR- and IR-spectra and chromatographic properties were identical for the synthesised and isolated acid V.

In connection with the NMR-studies of the acids from D-erythrose, a further study was made on the brucine salt of the acid previously isolated from sulphite treatment of D-xylose.[3] The NMR-spectrum of the barium salt obtained from this brucine salt clearly showed that the acid could not have the structure III previously reported,[3] as a signal from a C_2-proton was missing, but the data indicated that the previously isolated acid was instead the keto acid IV. The IR-spectrum of its barium salt showed absorption for carboxylate and sulphonate groups, but no absorption in the range expected for the keto group. This indicates either a hydrated or a cyclicised keto group. A borohydride reduction of the acid gave almost quantitatively an acid, whose NMR-spectrum agreed with that expected for acid III.

The NMR-spectrum of the barium salt of acid IV shows three groups of signals: a broad doublet ($\delta = 2.6$ ppm, 2H) for the methylene protons at carbon 3; a multiplet ($\delta = 3.9$ ppm, 1H) for the proton at carbon 4; and a quartet ($\delta = 4.3$ ppm, 2H) for the carbinol methylene protons.

The NMR-spectrum of the barium salt of acid III shows four groups of signals: a multiplet ($\delta = 2.2$ ppm, 2H) for the methylene protons at carbon 3; a quintuplet ($\delta = 3.2$ ppm, 1H) for the proton at carbon 4; a quartet ($\delta = 3.9$ ppm, 2H) for the carbinol methylene protons; and a quartet ($\delta = 4.4$ ppm, 1H) for the proton at carbon 2.

It should be pointed out that both structures (III and IV) give the same end products by the degradation sequence used in the previous study.[3] The acids were not chromatographically separable in the solvent systems used, but were separable by electrophoresis, as the keto acid is a stronger acid.

The above findings made it of interest to reinvestigate the products obtained by treatment of D-xylose with sulphite at pH 6.5 and 135°. The reaction product seemed electrophoretically to contain two main sulphonic acids, and after treatment with DNP and chromatographic fractionation, a DNP-derivative of an acid was obtained as a difficultly soluble barium salt in good yield. An identical derivative could be obtained from the acid which was obtained from the previous[3] investigation. The corresponding acid was obviously acid IV, as supported by the NMR-spectrum discussed above and the elemental analysis of the derivative.

From a later fraction another acid was isolated as its brucine salt. The elemental analysis and the NMR-spectrum for this compound prove that the corresponding acid was acid III. The amount of the two acids was about 80 % of the non-volatile acids in the reaction product, the keto acid (IV) predominating, *i.e.* it was formed in a considerably higher yield than the lower homologue (VI) from erythrose. The brucine salt of IV was the only one which could be obtained crystalline directly from the crude acid mixture. (No corresponding salt of III was crystallised, however, in spite of seeding.)

Later work showed that the two acids also could be separated by anion exchange chromatography using aqueous hydrogen chloride as eluent. It is also of note that acid III is the main acid in the liquor from the treatment of cellulose with sulphite of pH 7 at 180°.[5]

EXPERIMENTAL

General methods. Concentrations were done under reduced pressure at a bath temperature not exceeding 45°.

Paper chromatography was run on Whatman No. 1 and 3 mm papers in the following systems: A, ethyl acetate-acetic acid-water 3:1:1; B, ethanol-conc. aqueous ammonia-water 12:1:2.

Paper electrophoresis (run on the same papers) was carried out in a buffer of pH 3 (C) containing acetic acid (400 ml), pyridine (45 ml), and water (3000 ml). The migration values given are relative to that of sulphuric acid ($M_{H_2SO_4}$). The acids were detected by bromophenolblue-indicator (0.04 %) with sodium acetate (0.05 %) in 96 % ethanol after drying and subsequent steaming of the papers.

Reaction between D-*erythrose and sulphite.* D-Erythrose (4.0 g), sodium pyrosulphite (7.4 g), and sodium sulphite heptahydrate (19.6 g) were dissolved in water (100 ml total volume), and the solution was heated in a stainless steel autoclave for 1.5 h at 135° in a glycol bath. The colour and pH of the solution did not change during the treatment.

The reaction mixture was treated with cation exchange resin (Dowex 50W, H^+) and most of the sulphur dioxide was removed by evaporation of the solution to a smaller volume. Saturated barium hydroxide solution was added to give pH 7.5, and the precipitated barium sulphate and sulphite was filtered off. (The soluble part in the combined filtrate and washings amounted to 7.8 g.) The decationised solution was passed through an anion exchanger (Dowex 3, free base), and the neutral fraction was not further examined. The acids were eluted from the column with conc. ammonia-water 1:1, and the solution was evaporated and decationised. The acids thus obtained were treated with DNP (0.2 g) in a 5 % aqueous methanol solution for 2 h on a boiling water bath. The excess of DNP was removed by shaking the solution with ethyl acetate, and the water solution was evaporated. The product was fractionated on a cellulose column (l = 80 cm, d = 5 cm) using solvent A as irrigant, and three main fractions were collected.

The *first* fraction (0.4 g) contained a yellow acid, which was purified by thick paper chromatography in solvent A (0.1 g obtained). On paper chromatograms and electropherograms it appeared with the same characteristic colour as the well-identified DNP-derivative of IV, obtained from the xylose treatment (see below) and with about the same migrations. These properties together with the mode of its formation support that it is a DNP-derivative of acid VI. The $M_{H_2SO_4}$-value (buffer C) for DNP-VI was 0.51 (compare for DNP-IV: 0.50).

The *second* fraction (1.1 g) showed one main spot on electrophoresis (buffer C), $M_{H_2SO_4} = 0.63$. The barium salt did not crystallise, and no purification was obtained by fractionated precipitation in ethanol-water. After removal of the barium ions by cation exchange resin, the product was converted into brucine salts by adding brucine until about pH 5. A brucine salt (1.2 g) crystallised from a water-acetone solution. It was found by electrophoresis that the crystals represented one compound ($M_{H_2SO_4} = 0.64$) and that the mother liquor mainly consisted of another component ($_{H_2SO_4} = 0.62$). After several recrystallisations of the brucine salt from aqueous acetone and a final recrystallisation in water, pure brucine salt (0.34 g) was obtained (m.p. 153 – 156°). The IR-spectrum of the free acid showed absorption for the sulpho group (1045, 1170, and 1190 cm^{-1}) and the carboxyl group (1265, 1420, and 1710 cm^{-1}). The NMR-spectrum (see the text) of the disodium salt strongly indicated that the acid was 3-sulpho-propanoic acid. This acid was synthesised by oxidation of 3-mercapto-propanoic acid[6] with hydrogen peroxide, and the IR- and NMR-spectra were identical for the isolated and synthesised compounds.

The *third* fraction (0.51 g) and the mother liquor from the second were converted into barium salts. The solution was evaporated to a concentration of *ca.* 5 % and heated on a water bath. Ethanol was added dropwise until a slight cloudiness arose, and on prolonged heating small heavy crystals formed. The salt was filtered off and the procedure was repeated several times on the filtrate. The barium salt (0.60 g) was purified by conversion to its acid and reneutralisation with barium hydroxide. The pure barium salt obtained amounted to 0.33 g. The IR-spectrum showed absorption for sulphonate and carboxylate groups and indicated a hydroxyl function. Elemental analysis was consistent with the formula $C_4H_6O_6SBa$ (Found: C 15.1; H 2.33; S 9.91; Ba 42.7. Calc.: C 15.0; H 1.88; S 10.0; Ba 43.0.) The NMR-spectrum (see p. 878) of the disodium salt supports structure V for the corresponding acid. The compound was synthesised (see below) and found to

be identical with that isolated (NMR, IR, electrophoresis). The free acid of V showed no optical rotation. The migration ($M_{H_2SO_4}$) in buffer C was 0.62.

Synthesis of 2 hydroxy-4-sulphobutanoic acid (V). The acid V was synthesised, starting from acrolein. The first step was the preparation of vinyl glycolic acid (VII) according to Glattfeld and Hoen.[7] The zinc salt of this acid, 6.7 g (0.05 mol), was dissolved in water, and the solution was passed through a Dowex 50 W (H^+) column. Sodium sulphite, 6.3 g (0.05 mol) in water (50 ml), was added dropwise to the vinyl glycolic acid solution (100 ml), while a stream of oxygen passed through the solution. The temperature during the reaction was kept under 25° (final pH *ca* 6). The sodium ions were exchanged for barium ions (cation exchange and neutralisation with barium hydroxide), and the barium sulphite which precipitated was filtered off. The solution was evaporated to a smaller volume, and attempts to obtain the barium salt of the expected acid crystalline (as described above) failed, probably because of impurities present. The barium salts were therefore converted into ammonium salts, and the mixture was fractionated on a cellulose column ($l = 70$ cm, $d = 4$ cm), using solvent B as irrigant. The R_F-values of the ammonium salts of vinyl glycolic acid and acid V are 0.77 and 0.66, respectively.

A narrow top fraction (1.20 g), containing the pure ammonium salt of the expected acid, was collected. This was converted to the corresponding barium salt which could easily be crystallised as described above.

Reaction between D-*xylose and sulphite.* Xylose (2.5 g), sodium pyrosulphite (3.7 g), and sodium sulphite heptahydrate (9.8 g) were dissolved in water (50 ml total volume), and the solution was heated in a stainless steel autoclave for 1 h at 135° on a glycol bath. The reaction mixture was worked up in the same way as described for the erythrose.

The neutral fraction (0.68 g) was not examined any further, and the ammonium salts amounted to 2.16 g. The decationised acids were treated with DNP (1.0 g) in a 5 % aqueous methanol solution for 1.5 h on a boiling water bath. After cooling, the mixture was extracted with ethyl acetate to remove the excess of DNP (0.5 g). The acids were separated on a cellulose column ($l = 80$ cm, $d = 5$ cm), using solvent A as irrigant.

The *first* fraction (0.77 g), containing a yellow coloured acid was neutralized with barium hydroxide, and a difficultly soluble barium salt (yellow-brown) was obtained (0.57 g). The elemental analysis was consistent with a barium-DNP-derivative of acid IV: $C_{11}H_{10}O_{10}NS$ Ba. (Found: C 25.0; H 2.12; N 10.5; S 5.94; Ba 25.1. Calc.: C 25.0; H 1.91; N 10.6; S 6.08; Ba 26.0.) The migration of the DNP-derivative of the acid in buffer C ($M_{H_2SO_4}$) was 0.50. An identical derivative could be obtained from the previously[3] isolated brucine salt.

To a *second* fraction (0.75 g) brucine was added to give pH 5, and from this a crystalline brucine salt could be obtained from aqueous acetone. After recrystallisations and conversion to the corresponding barium salt the elemental analysis was consistent with the formula $C_5H_8O_7SBa$. (Found: C 17.0; H 2.44; S 9.08; Ba 39.1. Calc.: C 17.2; H 2.31; S 9.17; Ba 39.3.) This fact, and the NMR-spectrum discussed above, prove that the compound was the barium salt of acid III.

The proportions between the acids III and IV in the reaction mixture was estimated by paper electrophoresis (buffer C) to 2:3, and these main products were about 80 % of the non-volatile acids. The $M_{H_2SO_4}$-values were 0.56 (acid III) and 0.70 (acid IV), respectively.

Fractionation of the reaction mixture on anion exchanger. Crude barium salts (2.0 g) from D-xylose, treated with sulphite as described above, were dissolved in water (20 ml) and applied to an anion exchange column ($l = 50$ cm, $d = 4$ cm; Dowex 1-X8, 100–200 mesh, Cl^-). The column was first eluted with water, until no chloride could be detected in the eluate, and then with a gradient of hydrochloric acid (0 – 0.4 N, total volume 1200 ml) to fractionate the acids.

Two main fractions, containing mainly acid III (0.37 g) and acid IV (0.65 g), respectively, were collected. The main part of the hydrochloric acid had been removed by evaporation to dryness, and the remaining precipitated as silver chloride. These two fractions together amounted to 80 % of the non-volatile acids collected.

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