

The Degradation Product from Male Sperm Antagglutin with Molten Potassium Bisulphate

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The degradation product from male sperm antagglutin with molten potassium bisulphate has been compared with the corresponding products from some model compounds, especially tocopherols and hydroquinone. Some reactions taking place in the molten potassium bisulphate have been studied. The chemical structure of the antagglutin product has been discussed.

Male sperm antagglutin, a substance which in mammalian spermatozoa^{1,2}, prevents head-to-head agglutination, is a proteid containing sugar², sulphuric acid residues³, and a further component which, probably in a somewhat modified form has been sporadically extracted with ethyl ether from antagglutin preparations. Judging from its ultra-violet spectrum this extractable artefact seems to be an aromatic compound of diphenolic character. It is easily oxidized to a quinone-like substance^{4,5}.

Heating of male sperm antagglutin in molten potassium bisulphate at 220°C gives a product which is soluble in ethanol and water, and which after oxidation shows a rather high optical density in ultra-violet light with a maximum at 256 m μ . The transformation of the antagglutin into this oxidized compound has been used as a method for quantitative determination of male sperm antagglutin⁶. This oxidized product is very similar to the oxidized form of the above mentioned aromatic substance which was extracted from the antagglutin solutions. As this degraded substance is rather easily formed under controlled conditions, experiments have been performed in order to obtain information about its properties and its precursor in the antagglutin molecule. Unfortunately the formed product is very easily further oxidized and therefore difficult to isolate. For this reason some model substances have been treated in the same way as the antagglutin, and the properties of the obtained products compared with those of the product from antagglutin. Some information about the chemical reactions that may occur in the molten potassium bisulphate have also been obtained from this model experiments.

EXPERIMENTAL AND RESULTS

The treatment of the male sperm antagglutin and the model substances with molten potassium bisulphate has been performed according to the method of quantitative determination of male sperm antagglutin⁶, but with some modifications: about 1–3 μg male sperm antagglutin or 0.1–1 mg of the model substance is mixed with a large quantity (1–2 g) of dried, ether-extracted potassium bisulphate. This is done in a test tube with a diameter of one cm and a length of six cm. This reaction tube is placed in a bigger tube in which it is held upright by a layer of glass beads at the bottom. The bigger tube is immersed for one hour into an oil bath of 220°C. After cooling the reaction tube is cleaned externally with ethyl ether, and then reduced to powder in a mortar together with its contents. The powder is extracted with 3 ml of absolute ethanol in a centrifuge tube with a glass stopper. The centrifugated ethanol extract is evaporated under vacuum at 50°C, and the residue extracted several times with 3 ml of freshly distilled, dried ethyl ether. The residue in the distillation flask is taken up in 3 ml of absolute ethanol or distilled water, and its absorption spectrum in ultra-violet light read, especially between 250 and 260 $m\mu$, where the degradation product from male sperm antagglutin has a maximum at 256 $m\mu$. In some cases manganese dioxide is added to the powder during the extraction in order to act as an oxidizing agent, but in other cases the oxidation is performed through aeration in presence of platinum black. A Beckmann DU spectrophotometer was used for the absorption readings.

Hydroquinone disulphonic acid has been synthesized according to v. Hesse⁷. The identity and purity of the obtained product was ascertained spectrophotometrically. The other chemicals used are commercial preparations of *pro analysi* purity.

Besides the male sperm antagglutin only few of the tested compounds give products after treatment with molten potassium bisulphate which have absorption maxima at 256 $m\mu$ (Table 1), *viz.* α - and δ -tocopherol on the one side, and hydroquinone and some substances which in molten potassium bisulphate may be transformed into hydroquinone, on the other.

The absorption maximum at 256 $m\mu$ of the degradation product from male sperm antagglutin disappears, when the solution is made alkaline, but reappears again when the solution is acidified (Fig. 1). The absorption maximum also disappears after hydrogenation with hydrogen and a quantity of platinum black, but reappears after aeration of this solution. The compound with absorption maximum at 256 $m\mu$ is very easily oxidized, and the maximum is then

Table 1. Compounds which have been tested with reference to the absorption maximum at 256 $m\mu$ after treatment with molten potassium bisulphate.

Aromatic compounds.		Aliphatic compounds.	
Phenol	—	Quinic acid	+
Hydroquinone	+	Inositol	+
Pyrocatechol	—	1,4-Cyclohexadion	—
Resorcinol	—	Glucose	—
Durohydroquinone	—	Fructose	—
Phloroglucinol	—	Arabinose	—
<i>p</i> -Benzoquinone	+		
2,5-Dihydroxybenzoic acid	+		
<i>p</i> -Dimethoxybenzene	—		
α -Tocopherol	+		
δ -Tocopherol	+		
Tyrosine	—		
Tryptophane	—		
Coumarine	—		

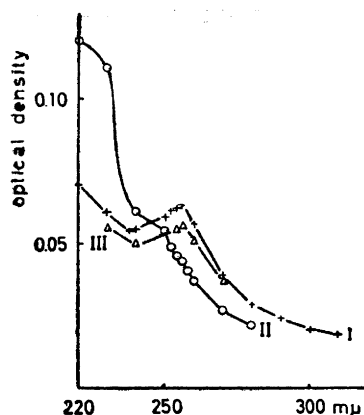


Fig. 1. The absorption in ultra-violet light of the degradation product of male sperm antagglutin with molten potassium bisulphate. I, pH about 12; II, pH about 4; III, pH restored to 4. Solvent distilled water.

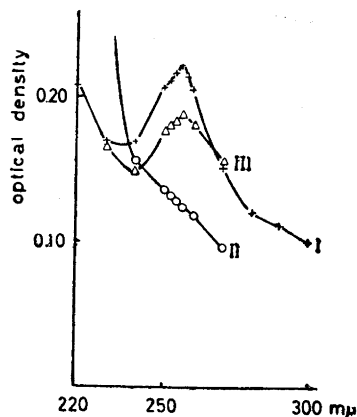


Fig. 2. The absorption in ultra-violet light of the product of α -tocopherylphosphate and molten potassium bisulphate. I, pH about 12; II, pH about 4; III, pH restored to 4. Solvent distilled water.

irreversibly lost. The degraded product from antagglutin is soluble in water and ethanol, but not in ethyl ether (Table 2).

The products from α -tocopherol and its acetate and phosphate-esters and δ -tocopherol after treatment with molten potassium bisulphate have the same properties as those of the degradation product from male sperm antagglutin (Fig. 2 and Table 2). The yields of the products are, however, very poor, only about one hundredth (w/w) of that of the degraded product from antagglutin. Trials to isolate the product from α -tocopherol have failed owing to its sensitivity to further oxidation.

From Table 1 it is obvious that hydroquinone as well as compounds that in molten potassium bisulphate may be transformed into hydroquinone, like quinic acid, inositol, and 2,5-dihydroxybenzoic acid, give a product with an absorption maximum at 256 μ (Fig. 3:I). With these compounds also a maximum at 312 μ is obtained which has never been observed with the products from antagglutin or the tocopherols. This absorption maximum at 312 μ is identical with that of hydroquinone disulphonic acid (Fig. 3:II) which after oxidation with manganese dioxide in 80 % ethanol gives a compound with an absorption maximum at 256 μ (Fig. 3:III).

This compound is *p*-benzoquinone disulphonic acid, the dipotassium salt of which in aqueous solution at pH 4 has a molar extinction at 256 μ of about 14 500. Hydroquinone is hence sulphonated in the molten potassium bisulphate which explains the non-solubility of the reaction product in ethyl ether (Table 2).

The product obtained from hydroquinone, although not identical with the product obtained from antagglutin, shows qualities which are similar to those of the latter (Table 2). Thus the absorption maximum at 256 μ disappears,

Table 2. Some properties of the compounds that give products with an absorption maximum at 256 m μ after treatment with molten potassium bisulphate.

Before treatment			After treatment					
Compound	Absorption maximum	Solubility	Absorption maximum	Absorption maximum after reduction	The pH-dependence of the absorption maximum	Solubility	Oxidability	The resulting product
I Male sperm antagglutin	None	Water	256 m μ	None	The maximum disappears in alkaline solution	Water, ethanol; insoluble in ether	Great	
II α -Tocopherol	292m μ	Ethanol, ether	›	›	›	›	›	
δ -Tocopherol	297m μ	Ethanol, ether	›	›	›	›	›	
III Hydroquinone	290m μ	Ethanol, ether	›	312 m μ	›	›	›	
Quinic acid	None	Water	›	312 m μ	›	›	›	<i>p</i> -Benzoquinone disulphonic acid
Inositol	None	Water	›	312 m μ	›	›	›	›

when the solution is made alkaline, and reappears again, when the solution is acidified. The synthetic *p*-benzoquinone disulphonic acid likewise shows the same qualities (Fig. 4). These substances also agree in being very easily further oxidized, even at room temperature. By this reaction the absorption maxima are lost (Table 2).

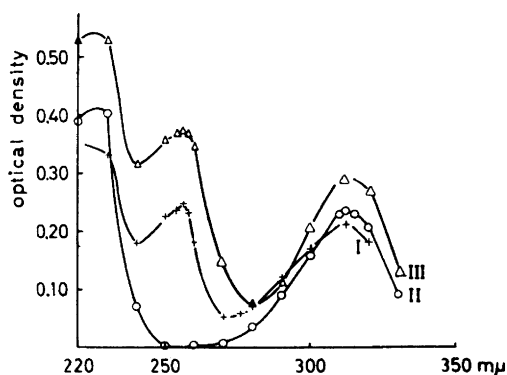


Fig. 3. The absorption in ultra-violet light of the product from hydroquinone and molten potassium bisulphate, I; synthetic hydroquinone disulphonic acid, II; partly oxidized synthetic hydroquinone disulphonic acid, III. Solvent distilled water.

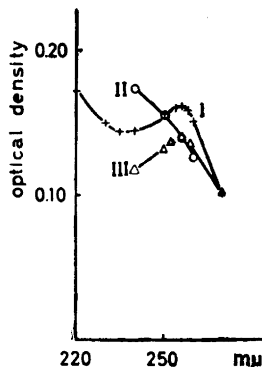


Fig. 4. The pH dependence of the ultra-violet spectrum of quinone disulphonic acid. I, pH about 4; II, pH about 12; III, pH restored to 4. Solvent distilled water.

DISCUSSION

The experiments with the hydroquinone group yield some information of the reactions that can take place in the molten potassium bisulphate. Sulphonation of hydroquinone in this milieu always gives the disulphonic acid. The wellknown dehydration reaction (compare the acrolein formation from glycerol in molten potassium bisulphate) occurs, when inositol and quinic acid are treated with the molten potassium bisulphate. Thus, by this treatment, an aliphatic carbocyclic structure with several hydroxylic groups can thus be transformed into an aromatic one. Sugars do not seem to react in this way (Table 1). The decarboxylation of 2,5-dihydroxybenzoic acid is certainly effected by the rather high temperature in the molten potassium bisulphate.

Of the tested compounds the products from α - and δ -tocopherol are most similar to the antagglutin degradation product. Yet it is surprising that both α - and δ -tocopherol give the same product, although there are three methyl groups in the benzene nucleus in the former, and only one in the latter substance. Durohydroquinone, that has the same benzene nucleus as α -tocopherol, does not give after corresponding treatment any product similar to the degradation product of antagglutin. It is therefore probable that the chromane structure of the α -tocopherol is at least partly retained in the product from α -tocopherol after heating in molten potassium bisulphate.

The aromatic compound of the male sperm antagglutin has been compared with tocopherols^{4,5}, and it has been shown that the derivative extracted from antagglutin is not identical with any of the known tocopherols⁵. It also appears most probable that this aromatic compound is an artefact originating from an aliphatic precursor constituent bound in the antagglutin molecule⁵. In fact, the intact antagglutin has no specific absorption in ultra-violet light like that of aromatic compounds.

The many resemblances between quinone disulphonic acid and the antagglutin degradation product point (Table 2) to a quinone-like structure, but no absorption maximum after reduction related to a hydroquinone has been detected. The situation of the absorption maximum of the antagglutin degradation product at 256 $m\mu$ in connection with the fact that an oxidation

is necessary in order to obtain this maximum makes it reasonable to propose that the chemical structure underlying the spectrum may be a carbonylic substance with two conjugated ethylenic bounds. The transformation in the molten potassium bisulphate of carbocyclic compounds like quinic acid and inositol into hydroquinone disulphonic acid suggests that the precursor constituent of the antagglutin degradation product and also of the extractable aromatic compound mentioned above is a carbocyclic residue with some hydroxylic groups.

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