Anal. Calcd. for $C_9H_{18}O_2$: C, 68.33; H, 11.47. Found: C, 68.48; H, 11.37.

In confirmation, XIII was prepared from 1,2-hexanediol (prepared from 1-hexene) by shaking 2.0 g. (0.017 mole) of the diol with 2 g. of sodium sulfate and 20 ml. of 1% hydrogen chloride in acetone for 6 hr. followed by standing overnight. Distillation gave 1.2 g. (45%) of XIII, b.p. 62°/15 mm., n_{D}^{25} 1.4351.

1,2-Hexanediol (XIV). A mixture of 300 mg. (0.0019 mole) of XIII and 10 ml. of 2% sulfuric acid was refluxed for 2 hr., neutralized with barium hydroxide and centrifuged. Evaporation of the supernatant and distillation gave 150 mg. (67%) of 1,2-hexanediol, b.p. 140°/760 mm., n_{D}^{25} 1.4400 (lit.[11] b.p. 110–113°/6 mm.).

Anal. Calcd. for $C_6H_{14}O_2$: C, 60.96; H, 11.94. Found: C, 60.75; H, 11.79.

A sample prepared from 1-hexene by conditions described[21] for the hydroxylation of 1-octene gave a 29% yield of XIV, b.p. 107–108°/4 mm., n_{D}^{25} 1.4414.

Infrared results. A Perkin-Elmer Model 21 Spectrophotometer was used with rock salt, variable thickness absorption cells for liquid compounds. Solids were examined by the pressed plate technique using potassium chloride as the carrier. The results are summarized in Table I.

TABLE I

INFRARED ABSORPTION CHARACTERISTICS (Cm^{-1})

Compound	C=O	C=C	OH
2,3-Dihydro-4*H*-pyran-2-carboxaldehyde	1742	1653	Absent
2-Hydroxymethyl-2,3-dihydro-4*H*-pyran (I)	Absent	1653	3367
2-Methyl-2,3-dihydro-4*H*-pyran (IV)	Absent	1647	Absent
6-Methyltetrahydropyran-2-ol (V)	Absent	Absent	3367
4-Hydroxypentanal (VII)	1776	Absent	3401
Reducing "disaccharide" (VIII)	1718	Absent	3378
1,2,6-Hexanetriol (X)	Absent	Absent	3311
2,2-Dimethyl-4-(4-hydroxybutyl)dioxolane (XI)	Absent	Absent	3378
p-Toluenesulfonate (XII) of XI	Absent	Absent	Absent
4-Butyl-2,2-dimethyldioxolane (XIII)	Absent	Absent	Absent

Acknowledgment. We wish to thank Dr. H. V. Knorr of the Charles F. Kettering Foundation, Yellow Springs, Ohio, for the infrared analyses.

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(21) D. Swern, G. N. Billen, and J. T. Scanlan, *J. Am. Chem. Soc.*, 68, 1504 (1946).

[CONTRIBUTION FROM RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Effect of Aqueous Sulfuric Acid on Reducing Sugars. V. Infrared Studies on the Humins Formed by the Action of Aqueous Sulfuric Acid on the Aldopentoses and on the Aldehydes Derived from Them[1]

F. A. H. RICE

Received May 16, 1957

Studies on the infrared absorption spectra of the polymeric materials, formed by the treatment of aldopentoses with sulfuric acid, indicated that the chemical structure of the polymer did not depend on the conditions of acid concentration or temperature under which it was formed. The ethanol-soluble and -insoluble fractions into which the polymeric material could be separated were found, so far as could be determined from the infrared spectra, and by carbon and hydrogen analysis, to be identical.

A comparison between the infrared spectrum of the polymeric material obtained by the treatment of L-arabinose with sulfuric acid and the infrared spectra of the polymeric materials prepared by the sulfuric acid treatment of either furfural alone, or furfural admixed with crotonaldehyde, or mixtures of furfural, crotonaldehyde, acetaldehyde, and formaldehyde strongly suggested that furfural alone was not responsible for the insoluble material obtained when a pentose is treated with sulfuric acid. The polymeric material obtained by the treatment of mixtures of furfural, crotonaldehyde, acetaldehyde, and formaldehyde with sulfuric acid were almost identical with the polymers prepared by treating L-arabinose with sulfuric acid.

This finding can explain the fact that the ultraviolet absorbance of an aldopentose in sulfuric acid reaches a steady state after an interval of time that depends on the concentrations of aldopentose and acid and the temperature.

In previous communications on the effect of sulfuric acid on the aldosugars[2,2a] it was shown that the ultraviolet spectrum which develops when an aldopentose or aldohexose is treated with sulfuric acid, depends on the formation of certain specific compounds, and that these compounds can be extracted from aqueous acid into ether solution. It was found possible to isolate and identify the compounds in the ether solution by forming and chromatographing the mixture of their 2,4-dinitrophenylhydrazones. The separated hydrazones gave crystalline products which were identified. Furfuraldehyde, crotonaldehyde, acetaldehyde, and formaldehyde were found to be formed from the action of acid on the pentose series of reducing sugars.[2]

Examination of the ultraviolet spectra as they developed in acid solution of reducing sugars

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(1) Published with permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the author.

(2) F. A. H. Rice and L. Fishbein, *J. Am. Chem. Soc.*, **78,** 1005 (1956).

(2a) F. A. H. Rice and L. Fishbein, *J. Am. Chem. Soc.*, **78,** 3731 (1956).

showed that after a period of time a steady state was reached. The time required and the optical density of the solution at the steady state depended on the concentration of the sugar, the concentration of the acid, and the temperature. The steady state, when it was reached under the same conditions, was characteristic of the sugar. When the aqueous acid solution of the sugar was continuously extracted with ether, the concentration in the ether phase of those compounds, which are primarily responsible for the ultraviolet absorption spectrum of the sugar, steadily increased. A steady state, however, was not reached over a period of time several times greater than that required by the sugar when the ether-soluble products were not continuously removed by extraction. Undoubtedly the continuous increase in the optical density at 290 mμ of the ether extract, is due to the continuous shifting of the equilibrium in the aqueous phase by the extraction of the aldehydes.

It was suggested,[2] that at the steady state, the rate of formation of ether-soluble products from the sugar was the same as that at which these same compounds polymerized to form insoluble humin materials. The humins do not show any absorption maxima in the ultraviolet[3] and hence would not contribute to the ultraviolet absorption spectrum.

It has been suggested that the formation of humins from a hexose under the influence of acid, is due to the formation and polymerization of either 5-hydroxymethyl-2-furaldehyde or acid degradation products of 5-hydroxymethyl-2-furaldehyde.[4] The yield of polymeric material from the hexose under the influence of either hydrochloric or sulfuric acid is relatively high[5] (37 to 39 per cent) and depends on both the configuration of the sugar[6] and the concentration of the acid.[7]

Although it has been claimed that the polymers formed by the action of strong mineral acid on carbohydrate material are definite chemical entities[8] the analyses that have been reported for carbon and hydrogen[6,8,9] on various preparations show considerable disagreement.

Marcussen[10] treated furan with hydrochloric acid and obtained a polymeric product. Since the analyses of the product were lower than would be expected (C, 65.5; H, 2.5) from a polyfuran (C, 70.6; H, 5.9), Marcussen suggested that the furan ring had opened and the resulting unsaturated dialdehyde had polymerized.

In our studies on the effect of sulfuric acid on reducing sugars, three compounds, in addition to furfural, were isolated from the reaction of a pentose with acid.[2] Since the formation of a steady state, as measured by ultraviolet absorption, requires the continuous removal of at least two of these compounds (furfural and crotonaldehyde which make the greatest contribution to the ultraviolet spectrum), it was suggested[2] that these two compounds polymerized as they were formed at a rate which depended on the concentration of the acid and the temperature. If such were the case, the polymer formed through the action of acid on a pentose should be different from that formed from furfural alone but identical with the one formed from a mixture of the compounds responsible for the ultraviolet absorption spectrum. If compounds other than those which show ultraviolet absorption and are extractable from aqueous acid into ether, entered into the constitution of the polymer formed from the pentose, the polymer obtained from the pentose should have distinct characteristics of its own.

It was thought that a comparison of the infrared spectra of the polymers formed by the action of acid on a pentose with the infrared spectra of polymers formed by the action of acid on mixtures of the compounds responsible for the ultraviolet absorption spectrum of the pentose might establish the identity or dissimilarity of the two polymeric materials.

Accordingly, humin material was prepared from L-arabinose by treating the sugar with various concentrations of sulfuric acid at the temperature of the boiling water bath and at room temperature. The polymers which formed were isolated by filtration on a fritted glass filter, washed with water, ethanol, and ether and dried at 80° in a high vacuum, over anhydrous calcium chloride.

Furfural, and mixtures of furfural and the other aldehydes isolated from the reaction of a pentose with sulfuric acid,[2] were treated in the same manner as were the pentoses. The polymeric material which formed was isolated, washed in the same way as the polymers obtained from the pentose, and dried in a high vacuum over anhydrous calcium chloride.

The infrared spectrum of each polymer pressed at the same concentration into a potassium bromide disc,[11] was recorded and the spectra were compared (Fig. 1 and 2).

The infrared spectra of the insoluble humins obtained from L-arabinose at various concentrations of acid, from $5N$ to $20N$, were found to be identical. A small fraction of the humin which was soluble in

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(3) V. B. Evstigneer and V. N. Nikiforova, *Biokhimiya*, **15**, 86 (1950); *Chem. Abstr.*, **44**, 5212 (1950).

(4) V. E. Bookly, *J. Agr. Sci.*, **11**, 69 (1921); J. J. Blanksma and G. Egmond, *Rec. tray. chim.*, **65**, 309 (1946); J. Marcussen, *Z. Angew. Chem.*, **34**, 437 (1921); J. Marcussen *Mitt. Materialprüfungsamt.*, **40**, 591 (1923).

(5) Jeiso Takahashi, *J. Agr. Chem. Soc., Japan*, **20**, 553 (1944).

(6) W. B. Bottomley, *Biochem. J.*, **9**, 260 (1915).

(7) T. Ploetz, *Naturwissenschaften*, **29**, 707 (1941).

(8) A. Schweizer, *Rec. trav. chim.*, **57**, 345, 956 (1938).

(9) W. Eller, *Ann.*, **431**, 133 (1923).

(10) J. Marcussen, *Ber.*, **54**, 542 (1921).

(11) M. M. Stimson and M. J. O'Donnel, *J. Am. Chem. Soc.*, **74**, 1805 (1952); V. Schiedt and H. Reinwein, *Z. Naturforsch.*, **7b**, 270 (1952).

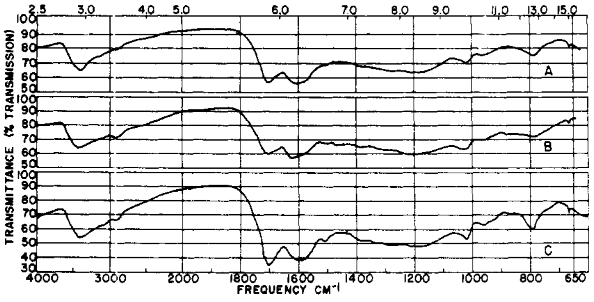

Fig. 1. Infrared spectra of humins obtained from L-arabinose under the influence of hot aqueous sulfuric acid. *A*, from reaction with 10*N* acid, ethanol-insoluble; *B*, from reaction with 5*N* acid, ethanol-soluble; *C*, from reaction with 5*N* acid, ethanol-insoluble. Concentrations were 2.0 per cent in KBr

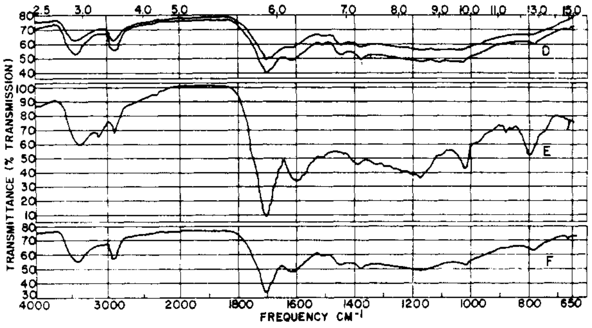

Fig. 2. Infrared spectra on the products obtained by: *D*, action of 10*N* sulfuric acid on a mixture of furfural, crotonaldehyde, acetaldehyde, and formaldehyde. Upper curve, ethanol-insoluble fraction; lower curve, ethanol-soluble fraction. *E*, action of 10*N* sulfuric acid on furfural, ethanol-insoluble. *F*, action of 10*N* sulfuric acid on a mixture of furfural and crotonaldehyde, ethanol-insoluble. Concentrations were 2.0 per cent in KBr

ethanol also showed the same infrared spectrum as the ethanol insoluble material. Fig. 1 shows the infrared spectra obtained on polymeric material prepared from L-arabinose by treatment with 10*N* (*A*) and 5*N* (*C*) sulfuric acid. The ethanol-soluble fraction (*B*) of the polymer obtained from L-arabinose by treatment with 5*N* acid is included for comparison. No essential differences will be observed. It is possible that the difference between the ethanol-insoluble and -soluble material is one of molecular weight. In agreement with the results of the infrared analyses, the analytical values for carbon and hydrogen on *A*, *B*, and *C* are essentially the same.

Fig. 2 shows the infrared spectra of polymers prepared from mixtures of furfural, crotonaldehyde, acetaldehyde, and formaldehyde (*D*). The upper curve in *D* is the spectrum of the ethanol-insoluble fraction, while the lower curve in *D* is the spectrum of that portion of the material which is soluble in ethanol. *E* is the spectrum of the polymer prepared from furfural alone and *F* is the spectrum of the polymeric material prepared from mixtures of furfural and crotonaldehyde. Although 10*N* sulfuric acid was used in the preparation of the materials whose infrared spectra are shown, other concentrations of acid gave polymeric material with identical spectra.

A comparison of the two curves in *D* shows that as far as can be determined by the infrared spectra, both the ethanol-soluble and ethanol-insoluble fractions are identical. The differences in solubility could be due to differences in molecular weight rather than differences in the chemical structure of the repeating units. Comparisons between the ethanol-soluble and -insoluble fractions of each of the other polymeric materials indicated the same identity between the ethanol-soluble and the -insoluble product.

If the spectra *D*, *E*, and *F* are compared, although certain features are common to the three spectra, *E*, the product from furfural alone seems to be quite different from the other two. The intensity of the absorption in the 1400 to 1800 cm.$^{-1}$ region compared to the 2500 to 3000 cm.$^{-1}$ region is much increased over that found in either *D* or *F*, and when it is remembered that all the spectra were run under as near as possible the same conditions and we also find that under these conditions spectra *A*, *B*, and *C* are essentially superimposable, considerable importance can be attached to this finding. In addition there are additional absorption bands in the 800 to 1200 cm.$^{-1}$ region. The analytical values for carbon and hydrogen obtained on the polymeric material from furfural are also lower than the values obtained on the other two polymers, the spectra of which are given in *D* and *E*.

No unequivocal conclusions can be drawn from a comparison of the infrared spectrum of the humin obtained from L-arabinose and the infrared spectra of the polymeric material obtained from the various mixtures of saturated and unsaturated aldehydes. In general, however, particularly if the importance of the relative intensities of the absorption bands is stressed, the infrared absorption spectrum of the humin from L-arabinose is almost identical with the spectrum of the product formed by the action of acid on the two unsaturated aldehydes (*F*) which indeed is essentially the same as the spectra of the product formed when the four aldehydes were subjected to the action of sulfuric acid (*D*). The chief difference in the infrared spectra of humin from L-arabinose and the polymeric material formed by the action of acid on the four aldehydes is in the intensity of absorption at 2900 cm.$^{-1}$ On the basis of this absorption at 2900 cm.$^{-1}$ it is possible that additional compounds, which are not extractable from aqueous acid by means of ether, enter into the formation of the humin materials formed from the reducing pentoses under acid conditions.

It is of interest to note that the infrared spectra indicate[12] that hydroxyl (3440 cm.$^{-1}$), carbonyl

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(12) L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York, N. Y.

(1705 cm.$^{-1}$) and possibly carbon-carbon double bonds (1620 cm.$^{-1}$) are present in each one of the polymeric materials investigated.

EXPERIMENTAL

Infrared spectra. The infrared spectra were recorded on a Perkin-Elmer, model 21, double beam infrared spectrophotometer. The sample for analysis (200 mg.) was ground in a mortar with anhydrous potassium bromide (1 g.) and the mixture (300 mg.) was pressed into a disk in a high vacuum under 18,000 p.s.i.

Preparation of humins from L-arabinose. Four 10-g. samples of L-arabinose ($[\alpha]^{21}_{D}$ + 104°) were respectively dissolved in 200 ml. of 5*N*, 10*N*, 15*N*, and 20*N* sulfuric acid contained in 1-liter flasks. The solutions were heated on the steam bath for a period of 12 hr. During this period a heavy brownish black precipitate formed. An equal volume of ice cold distilled water was then added to the contents of each flask and the precipitate removed by filtration on a fritted glass funnel. The precipitate was washed on the funnel with several liters of distilled water and then extracted with 1 liter of boiling ethanol, followed by 200 ml. of ether. The insoluble material was then dried at 80° in a high vacuum over anhydrous calcium chloride.

The ethanol extract was concentrated to dryness on the steam bath and then washed with ether and dried in a high vacuum over anhydrous calcium chloride. No differences were found in the carbon and hydrogen content of the polymers formed under the influence of the several concentrations of acid.

Anal. Found: C, 64.2 to 67.8; H, 4.2 to 4.9.

No analytical differences could be found between the ethanol-soluble and -insoluble material.

Anal. (ethanol-soluble). Found: C, 65.8 to 67.5; H, 4.1 to 4.9.

Preparation of humin from furfural. An amount of 0.96 g. of furfural was dissolved in 200 ml. of 10*N* sulfuric acid. The solution was heated on the steam bath in a flask equipped with a reflux condenser. The resulting polymeric material was then isolated in the same way as was the humin from L-arabinose (above).

Anal. (ethanol-soluble). C, 68.6; H, 4.6; (ethanol-insoluble), C, 68.4; H, 4.5.

Preparation of humin from furfural and crotonaldehyde. An amount of 0.95 g. of furfural together with 0.7 g. of crotonaldehyde was dissolved in 200 ml. of 10*N* sulfuric acid. The rest of the procedure was identical with the above.

Anal. (ethanol-soluble) C, 69.8; H, 6.6; (ethanol-insoluble), C, 70.4; H, 6.4.

Preparation of humin from a mixture of furfural, crotonaldehyde, acetaldehyde, and formaldehyde. A mixture consisting of 0.96 g. furfural, 0.7 g. of crotonaldehyde, 0.44 g. of acetaldehyde, and 0.30 g. of formaldehyde was dissolved in 200 ml. of 10*N* sulfuric acid.

Further treatment was the same as for furfural (above).

Anal. (ethanol-soluble). C, 63.0; H, 4.3; (ethanol-insoluble), C, 63.4; H, 4.7.

Acknowledgments. I should like to thank Mrs. P. P. Wheeler for the microanalyses, Mrs. N. F. Lyons for running the infrared spectra, and Mr. A. H. Johnstone for technical assistance in preparing the polymeric materials.

INDIAN HEAD, MD.

[CONTRIBUTION NO. 473 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

Synthetic Tuberculostats. XII. Structure of the Reaction Product of Isoniazid and Formaldehyde

H. HERBERT FOX

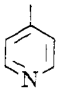
Received July 22, 1957

The compound prepared by the interaction of isoniazid with formaldehyde contains a triazane ring and is probably the monohydrate of hexahydro-1,3,5-triisonicotinamido-*s*-triazine.

During the course of an investigation into the tuberculostatic activity of isoniazid and its derivatives, an effort was made to synthesize 1-isonicotinyl-2-methylidenehydrazine according to the following scheme:

$$\mathrm{PyCONHNH_2 + CH_2O \longrightarrow PyCONHN{=}CH_2}$$

where Py =



The reaction was carried out by refluxing a mixture of isoniazid, aqueous formaldehyde (35%), and isopropyl alcohol. As the reaction proceeded, a fine white crystalline precipitate appeared which proved to be practically insoluble in all of the common solvents with the exception of dilute hydrochloric acid and dilute sodium hydroxide. Since the anticipated methylidene derivative was expected to have solubility characteristics quite similar to those of the parent isoniazid, the marked insolubility of the product (hereinafter called Ro 2–4969) strongly suggested that it was not the desired compound at all. This was subsequently confirmed by elementary analysis.

In view of its marked tuberculostatic activity, it was decided to investigate the structure of the compound in some detail.

One obvious line of approach was to determine the molecular weight of the compound. Every attempt in this direction failed—principally because of its insolubility, although instability and possibly the presence of water of hydration were also contributory factors. Most of these attempts involved modifications of the Rast procedure with a variety