mg., m. p. 85–87°. Recrystallization gave a product, m. p. 90–91°, identical with that obtained by esterifying 3α,12α-diacetoxy-*nor*-cholanic acid.

Ethyl 3β-Hydroxy-Δ^5-thiolcholenate (VII).—The acid chloride prepared from 30.0 g. (0.075 mole) of 3β-formoxy-Δ^5-cholenic acid was dissolved in 200 ml. of benzene and treated with 37 ml. (0.50 mole) of ethyl mercaptan and 10 ml. of pyridine. After standing overnight at room temperature the reaction mixture was diluted with 250 ml. of water and extracted with 250 ml. of ether in portions. The ether layer was washed with 1% sodium hydroxide, 1% hydrochloric acid and water. After drying the ether was distilled and the residue crystallized from 300 ml. of alcohol and 50 ml. of water, giving 23 g. (72%) of ethyl 3β-hydroxy-Δ^5-thiolcholenate, m. p. 98–100°. Several recrystallizations from hexane–benzene raised the m. p. to 108.5–109.5°.

Deformylation of Ethyl 3β-Formoxy-Δ^5-thiolcholenate (VI) over Alumina.—Two hundred mg. of ethyl 3β-formoxy-Δ^5-thiolcholenate, m. p. 78.5–81.5°, was dissolved in 8 ml. of benzene and passed through a 10-g. alumina[8] column. The column was eluted, using the free flow method, with 8-cc. portions of benzene, benzene + 0.4% methanol, benzene + 1% methanol, benzene + 2% methanol, benzene + 4% methanol, benzene + 8% methanol and methanol. The benzene eluate contained 35 mg.% of crystalline material of m. p. 78–82° (starting material). The methanol fraction contained 135 mg. of crystalline material of m. p. 95–100°. After several recrystallizations from 3A alcohol[9] and from hexane (Skellysolve "B") the m. p. became constant at 105–108°. An admixture with a sample of ethyl 3β-hydroxy-Δ^5-thiolcholenate, m. p. 108.5–110°, melted at 108–110°. An admixture with starting material melted at 63–100°.

Formylation of Ethyl 3β-Hydroxy-Δ^5-thiolcholenate (VII).—A mixture of 710 mg. of ethyl 3β-hydroxy-Δ^5-thiolcholenate, m. p. 108–109.5°, 25 ml. of formic acid (87%) and 12.5 ml. of methyl formate was heated under reflux on the steam-bath for two and one-half hours; then the reaction mixture was evaporated *in vacuo* and the residue dissolved in ether. The ether solution was washed with 0.5% sodium hydroxide, then with water, and was dried over sodium sulfate. Evaporation *in vacuo* gave 452 mg. of ethyl 3β-formoxy-Δ^5-thiolcholenate (VI), m. p. 78–79.5.° Several recrystallizations from methanol, water and from alcohol brought the m. p. up to 80–82°. A mixture m. p. with authentic formoxy ester was not depressed.

Acetylation of Ethyl 3β-Hydroxy-Δ^5-thiolcholenate.—Three hundred mg. of ethyl 3β-hydroxy-Δ^5-thiolcholenate, m. p. 108–109.5°, was mixed with 5 ml. of acetic anhydride and 5 ml. of pyridine. After refluxing for two and one-half hours the mixture was evaporated to dryness on the steam-bath *in vacuo*. The residue was taken up in alcohol. The alcohol solution was diluted with water and extracted with ether. After washing with 1% sodium hydroxide and water the ether solution was dried over sodium sulfate and evaporated to dryness. The residue was crystallized from 3A alcohol to yield 290 mg. of ethyl 3β-acetoxy-Δ^5-thiolcholenate (VIII), m. p. 101–104°. After several recrystallizations, the melting point became constant at 101–102.5°. An admixture with a sample of authentic ethyl 3β-acetoxy-Δ^5-thiolcholenate, m. p. 101–103°, showed no melting point depression. An admixture with starting material melted at 79–82°.

Hydrolysis of 300 mg. of the thiol ester (VIII) with 1 g. of sodium hydroxide in 2.5 ml. of water and 15 ml. of alcohol gave an almost quantitative yield of 3β-hydroxy-Δ^5-cholenic acid.

Summary

A number of thiol esters of steroid acids have been prepared and characterized using ethyl, isopropyl, *t*-butyl, *n*-hexyl, benzyl and phenyl mercaptans; and the following acids: 3β-hydroxy-Δ^5-cholenic, 3β-hydroxy-5-chloro-*nor*-cholenic, 3-β-hydroxy-Δ^5-*bisnor*-cholenic, desoxycholic, *nor*-desoxycholic and lithocholic.

KALAMAZOO, MICHIGAN RECEIVED SEPTEMBER 2, 1947

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(9) 3A alcohol is commercial 95% alcohol denatured by the addition of 5% methanol.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Chemical Interactions of Amino Compounds and Sugars. III.[1] The Conversion of D-Glucose to 5-(Hydroxymethyl)-2-furaldehyde[2]

BY M. L. WOLFROM, R. D. SCHUETZ[3] AND LIEBE F. CAVALIERI[3]

It is well established that D-glucose and other hexoses are converted to 5-(hydroxymethyl)-2-furaldehyde on heating with acids.[4] Scallet and Gardner[5] have previously demonstrated that 5-(hydroxymethyl)-2-furaldehyde is formed from D-glucose on refluxing in water alone.

The purpose of the work herein reported was to determine whether ultraviolet spectroscopy would give any insight into the mechanism and intermediates in the conversion of D-glucose to 5-(hydroxymethyl)-2-furaldehyde. Hydrochloric acid was the catalyst chosen for these studies.

When an aqueous solution (initial *p*H 6.5) of D-glucose was prepared from triply distilled water and refluxed, a series of changes could be detected in the ultraviolet absorption spectrum; these are shown in Figs. 1 and 2. After three and one-half hours (curve 1, Fig. 1) a distinct band with a maximum at 228 mμ was evident. After eight hours of

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(1) Previous communication in this series: M. L. Wolfrom, L. F. Cavalieri and Doris K. Cavalieri, THIS JOURNAL, **69**, 2411 (1947).

(2) The subject matter of this paper has been undertaken in coöperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces under a contract (W11-009-Q-M-70183 and W44-109-QM-1027) with The Ohio State University Research Foundation. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

(3) Research Associate of The Ohio State University Research Foundation, Projects 278 and 238, respectively.

(4) G. Düll, *Chem. Ztg.*, **19**, 216 (1895); J. Kiermayer, *ibid.*, 1003; W. Alberda van Ekenstein and J. J. Blanksma, *Chem. Weekblad*, **6**, 217 (1909); *Ber.*, **43**, 2355 (1910); W. N. Haworth and W. G. N. Jones, *J. Chem. Soc.*, 667 (1944); B. Singh, G. R. Dean and S. M. Cantor, THIS JOURNAL, **70**, 517 (1948).

(5) B. L. Scallet with J. H. Gardner, THIS JOURNAL, **67**, 1934 (1945).

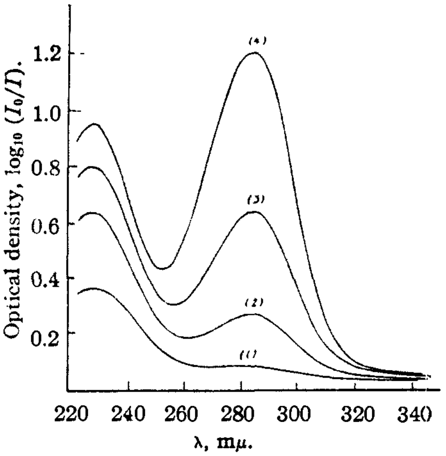


Fig. 1.—Absorption spectra of a 0.0500 molar aqueous D-glucose solution, initial *p*H 6.5, after refluxing for various time intervals: curve 1, after three and one-half hours; curve 2, after eight hours; curve 3, after twelve hours; curve 4, after seventeen hours. Beckman spectrophotometer (Model DU), 1-cm. cell, slit width 0.30–0.35 mm., optical densities 0.05–1.405.

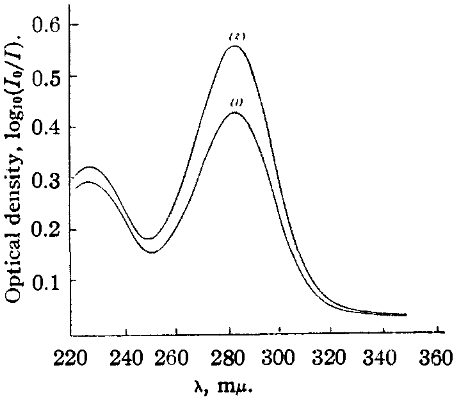


Fig. 2.—Absorption spectra of a 0.0500 molar aqueous D-glucose solution, initial *p*H 6.5, after refluxing for various time intervals. Samples were diluted four to one for absorption measurements: curve 1, after twenty hours; curve 2, after twenty-three hours; Beckman spectrophotometer (Model DU), 1-cm. cell, slit width 0.30–0.35 mm., optical densities 0.05–0.57.

heating (curve 2, Fig. 1), the maximum at 228 mμ was more pronounced and a second band was developing in the region of 285 mμ. At the end of twelve hours (curve 3, Fig. 1) the maximum at 228 mμ had increased and that at 285 mμ was increasing at an even greater rate. On continued heating for seventeen hours (curve 4, Fig. 1) the ratio of the substance producing the band at 285 mμ to the other (producing the band at 228 mμ) became very large. Finally, after a period of heating somewhat greater than twenty and twenty-three hours (curves 1 and 2, Fig. 2), respectively, the typical absorption spectrum of 5-(hydroxymethyl)-2-furaldehyde (Fig. 5) was developing.

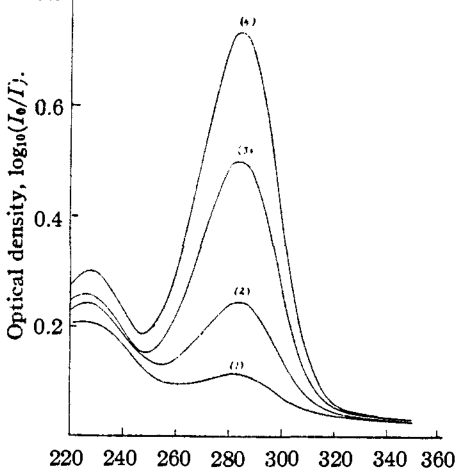


Fig. 3.—Absorption spectra of a 0.0500 molar aqueous D-glucose solution adjusted to an initial *p*H of 4.3 with hydrochloric acid, after refluxing for various time intervals: curve 1, after three and one-half hours; curve 2, after eight hours; curve 3, after twelve hours; curve 4, after fifteen hours. Beckman spectrophotometer (Model DU), 1-cm. cell, slit width 0.30–0.35 mm., optical densities 0.05–0.75.

When an aqueous solution of D-glucose was adjusted to an initial *p*H of 4.3 with hydrochloric acid and refluxed, a similar series of curves was obtained (Fig. 3). Here both bands are evident after three and one-half hours (curve 1) and again the band at 228 mμ was more pronounced. After eight hours (curve 2) both maxima, at 228 mμ and at 285 mμ, were of about equal intensity. At the end of twelve hours (curve 3) the ratio of

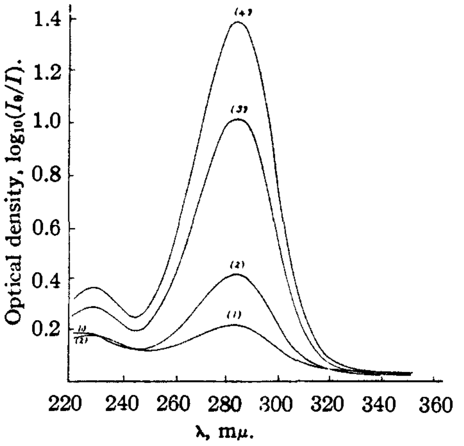


Fig. 4.—Absorption spectra of a 0.0500 molar aqueous D-glucose solution adjusted to an initial *p*H of 2.0 with hydrochloric acid, after refluxing for various time intervals: curve 1, after three and one-half hours; curve 2, after eight hours; curve 3, after twelve hours; curve 4, after fifteen hours. Beckman spectrophotometer (Model DU), 1-cm. cell, slit width 0.30–0.35 mm., optical densities 0.05–1.405.

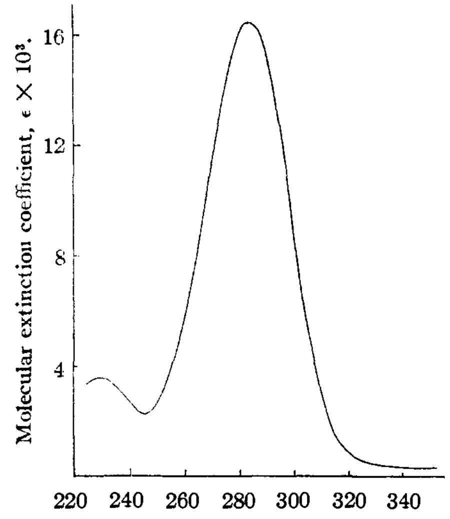


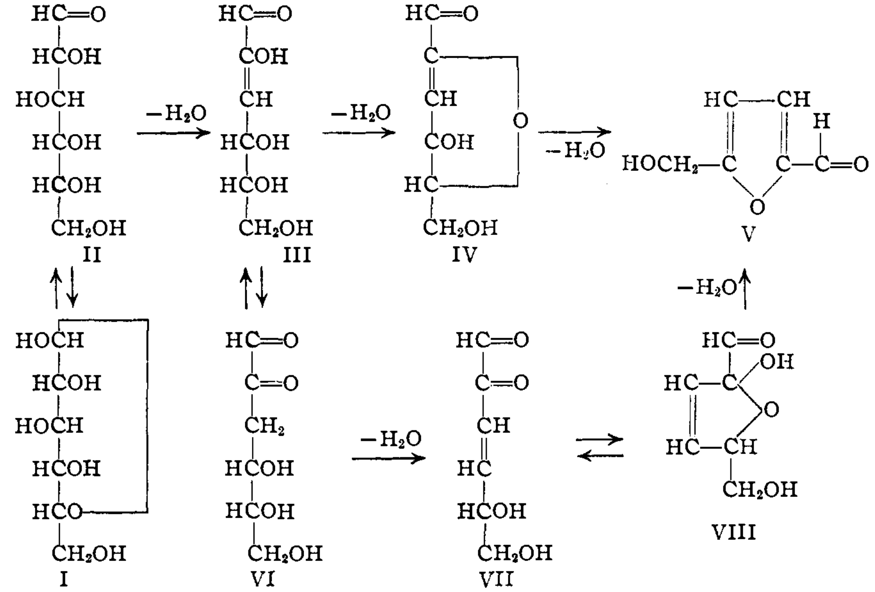
Fig. 5.—Absorption spectrum of pure 5-(hydroxymethyl)-2-furaldehyde[1] in water; concentration, 6.3627 × 10^{-5} mole per liter; $\epsilon = 1/c \ln (100/T)$ wherein c is the molar concentration and T is the percentage transmission; ϵ = 16,500, max. 285 mμ (major peak), ϵ = 3,620, max. 228 mμ (minor peak); slit width 0.32–0.35 mm., optical densities 0.016–1.048.

material producing the band at 285 mμ to that causing the band at 228 mμ had again become quite large. Finally, after heating for a period of fifteen hours (curve 4), the general absorption curve for 5-(hydroxymethyl)-2-furaldehyde (Fig. 5) was well developed.

The series of absorption curves (Fig. 4) resulted when an aqueous solution of D-glucose, adjusted with hydrochloric acid to an initial *p*H of 2.0, was refluxed. In curve 1, Fig. 4, after three and one-half hours, the maximum at 285 mμ was more pronounced than that at 228 mμ. After eight hours (curve 2, Fig. 4), the ratio of the compound responsible for absorption at 285 mμ to the one causing absorption at 228 mμ had again become large, and after twelve hours of continued refluxing, this ratio had further increased (curve 3, Fig. 4). Finally, after fifteen hours of heating, the typical absorption curve for 5-(hydroxymethyl)-2-furaldehyde resulted (curve 4, Fig. 4).

It is to be noted that the ultraviolet absorption spectra presented, at various time intervals, and at increasing initial *p*H values, during the conversion of D-glucose to 5-(hydroxymethyl)-2-furaldehyde are all very similar. Such differences as exist lie only in the rate at which the bands at 228 mμ and 285 mμ are developed. This indicates that the reaction mechanism involved in the formation of 5-(hydroxymethyl)-2-furaldehyde from D-glucose is independent of the initial *p*H value in the range studied (*p*H 6.5 to *p*H 2). These absorption curves, however, show that the reaction rate is dependent upon the acidity of the reaction medium, increasing with increasing acidity.

Further, it can be pointed out (curve 1, Fig. 1) that after three and one-half hours where the reaction is slowest (due to no added acid), there is absorption only at 228 mμ. That the substance producing this band is not 5-(hydroxymethyl)-2-furaldehyde is apparent from the fact that the major peak at 285 mμ is missing. In addition, the intensity of absorption at 228 mμ increased to a maximum after seventeen hours (curve 4, Fig. 1) where both peaks at 228 mμ and 285 mμ are present and then decreased to a corresponding value for 5-(hydroxymethyl)-2-furaldehyde as the reaction proceeded. It is probable that the intermediate producing the high absorption at 228 mμ is a conjugated acyclic diene as (or enal) since, Mulliken[6] and also Booker, Evans and Gillam[7] have shown, the cyclic dienes, which are necessarily oriented *cis*, would have shown a much weaker absorption in this region.



The absorption intensity at 228 mμ does not reach a very large value when aqueous solutions of D-glucose are refluxed at the lower initial *p*H.

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[6] R. S. Mulliken, *J. Chem. Phys.*, **7**, 121 (1939).

[7] H. Booker, L. K. Evans and A. E. Gillam, *J. Chem. Soc.*, 1453 (1940).

This is due to the increased rate of conversion through the intermediates to 5-(hydroxymethyl)-2-furaldehyde. This is shown by curves 1 in Figs. 1, 3, 4, and curve 3 in Fig. 4.

To explain these absorption curves we can postulate that D-glucose, represented by I (other anomers and ring structures than I are possible), is transformed first into II or its aldehydrol. The intermediate III then results from II by loss of water, producing a conjugated enal. Evidence for III is found in both the region and the magnitude of absorption shown in curve 4, Fig. 1. Evans and Gillam[8] have demonstrated that α,β-unsaturated aldehydes ($R-CH=CR'CHO$) show high absorption in the general region of 230 mμ. In this intermediate (III), R is $(CHOH)_2CH_2OH$ and R′ is the hydroxyl group. A similar type of intermediate was shown to be present in the conversion of 2,3,4,6-tetramethyl-D-1,2-glucoseen into 5-(methoxymethyl)-2-furaldehyde[9] in acid solution. There the 6-methyl ether of the conjugated enonal VII was established as an intermediate in this conversion by isolation as its crystalline phenylosazone.

The postulated intermediate III could produce IV by cyclic dehydration. The 5-(hydroxymethyl)-2-furaldehyde (V) could result from IV by a final dehydration producing a third double bond in conjugation with the two already present. The mechanism proposed here for the conversion of D-glucose to 5-(hydroxymethyl)-2-furaldehyde through the intermediates II, III and IV, is based upon that originally suggested by Hurd and Isenhour,[10] who, however, offered no experimental evidence to support it. The route through the intermediates VI, VII and VIII receives experimental support in the work of Wolfrom, Wallace and Metcalf.[9] The dehydrations postulated are the well-established ones of an hydroxyl group in the β position to a carbonyl. The resulting dehydrated products are α,β-unsaturated carbonyl compounds and we offer spectroscopic evidence for such intermediates.

Experimental

Materials.—The D-glucose used in this work was a very pure grade of the monohydrate.[11] It was crystallized from triply distilled water.

Absorption Measurements.—The D-glucose was weighed into a 500-ml. round-bottom Pyrex flask with a ground glass joint and connected to a reflux condenser. The solvent was added, and in the experiments where hydrochloric acid was used, the *p*H was adjusted at this point. The aqueous D-glucose solutions were refluxed by means of a Glas-col type electric heater. At definite time intervals aliquots were removed, cooled rapidly to room temperature, and immediately read in the spectrophotometer.

Acknowledgment.—The assistance of Clare B. Spitler and Doris K. Cavalieri is gratefully acknowledged.

Summary

The course of the formation of 5-(hydroxymethyl)-2-furaldehyde from D-glucose in the absence and presence of hydrochloric acid has been followed by ultraviolet absorption spectra measurements and on the basis of this, structures are proposed for several intermediates.

COLUMBUS, OHIO RECEIVED[12] OCTOBER 4, 1947

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[8] L. K. Evans and A. E. Gillam, *J. Chem. Soc.*, 565 (1943).

[9] M. L. Wolfrom, E. G. Wallace and E. A. Metcalf, THIS JOURNAL, **64**, 265 (1942).

[10] C. D. Hurd and L. L. Isenhour, *ibid.*, **54**, 317 (1932).

[11] We are indebted for this material to Dr. S. M. Cantor of the Corn Products Refining Co., Argo, Illinois.

[12] The original manuscript with all essential data was received on September 23, 1946.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CORN PRODUCTS REFINING COMPANY]

The Role of 5-(Hydroxymethyl)-furfural in the Discoloration of Sugar Solutions

BY BHAGAT SINGH,[1] G. R. DEAN AND SIDNEY M. CANTOR[2]

The acid catalyzed hydrolysis of starch at elevated temperature for the commercial production of D-glucose results in the formation of considerable coloring matter.[3] Under the same conditions of temperature and concentration the decomposition of pentoses to yield furfural and of hexoses to yield 5-(hydroxymethyl)-furfural (hereafter abbreviated in most cases to HMF) and levulinic and formic acids are well known phenomena. Various hypotheses have been advanced for the mechanism of color formation. Porst[4] believed that the color change might be due to furfural derived from the sugars and to traces of phenols and other derivatives which come from the decomposition of the small amount of protein contained in the starch. The possible participation of furfural and levulinic acid to form coumarone has been suggested as the cause of "humins" and color by Meunier.[5] The

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[1] Corn Industries Research Foundation Fellow at Corn Products Refining Company 1943–1946.

[2] Present address: The American Sugar Refining Co., Philadelphia 48, Pa.

[3] During the course of this investigation this laboratory became associated with the coöperative project on non-enzymatic browning sponsored by the Committee on Food Research of the Quartermaster Corps of the U. S. Army. The relationship between this work and the general problem of color development in sugar containing systems was called to the attention of other laboratories in the project and a portion of the data was presented in the symposium on Non-Enzymatic Browning of the Division of Food and Agricultural Chemistry at the Chicago Meeting of the American Chemical Society in September, 1946, in an introductory paper entitled, "A Review of Some Sugar Reactions which Give Rise to Color," by Sidney M. Cantor and Charles D. Hurd.

[4] Porst, Orig. Com. 8th Intern. Congr. Appl. Chem., **13**, 205 (1912).

[5] Meunier, *Chemie et Industrie*, Special No. 583, February (1929).