lutidine with aliphatic esters gave rise to both ketones and carbinols as had been observed earlier[10] when 2-picoline was acylated with aliphatic esters. It should be noted that while those ketones derived from 2-picoline and 2,6-lutidine appeared to give copper salts when treated with copper(II) acetate solution, the salts could not be obtained in crystalline form. Furthermore, the ketones derived from quinaldine did not exhibit a visible reaction when treated similarly.[15]

Experimental

Starting Materials.—The tar bases and the methyl esters were obtained from commercial sources with the exception of the methyl pyridinecarboxylates which were prepared by the method of Levine and Sneed.[16]

Operating Procedure for Conducting Condensations.—The syntheses were carried out by the interaction of the lithium derivatives of the tar bases with the appropriate esters as described earlier.[10]

Acknowledgment.—The authors gratefully acknowledge the support of the U. S. Atomic Energy Commission during the course of the investigation.

(15) The reaction between divalent cations and these ketones is being studied by Dr. W. C. Fernelius and his co-workers at the Pennsylvania State College. Their results will be reported at a later date.

(16) R. Levine and J. K. Sneed, THIS JOURNAL, **73**, 5614 (1951).

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An Agent from *E. Coli* Causing Hemorrhage and Regression of an Experimental Mouse Tumor. II. The Component Monosaccharides[1]

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RECEIVED MAY 19, 1952

It has been shown previously[3] that the agent, isolated from cultures of *E. coli*, which produces a hemorrhagic response in and causes the regression of the experimental mouse sarcoma 180 is a complex polysaccharide which contains both a peptide and a phospholipide component. An acid hydrolysate of the above polysaccharide was found to possess a reducing power of 52–55 equivalent % glucose and a hexosamine content of 15–17 equivalent % glucosamine.

Ultraviolet absorption spectra of solutions of the experimental mouse tumor hemorrhagic agent in 79% sulfuric acid[4,5] are given in Fig. 1. The lack of any appreciable absorption at 25° is indicative of the absence of ketoses and nucleic acids. The character of the spectrum of the heated solution suggests the probable absence of 6-desoxyaldehexoses (no maximum in the 327 mμ region), aldopentoses (low extinction value at 300 mμ), aldohexuronic acids (low extinction values at 220 and 294 mμ), and mannose (no maximum in the 250 mμ region). Paper chromatography, with phenol and collidine as solvents,[6] of an acid hydrolysate of the hemorrhagic agent gave evidence of the presence of glucosamine and of either or both glucose and galactose. Of the qualitative carbohydrate tests applied directly to the agent, the Scherer test for inositol[7] was negative, the mucic acid test for galactose, or galacturonic acid, positive, and the Morgan–Elson test for apparent N-acetylhexosamine,[8] negative. The phenylhydrazone test for mannose, performed on a hydrolysate of the agent, was negative. The presence of hexosamine has been commented upon previously.[3] By drastic hydrolysis of the hemorrhagic agent D-glucosamine was isolated and identified as the hydrochloride and the N-carbobenzoxy derivative. With milder conditions of hydrolysis D-glucose and D-galactose were isolated and identified as the diethyl mercaptals,[9] the substituted benzimidazoles[10] and the picrates thereof.[10] The properties of the above derivatives are summarized in Table I.

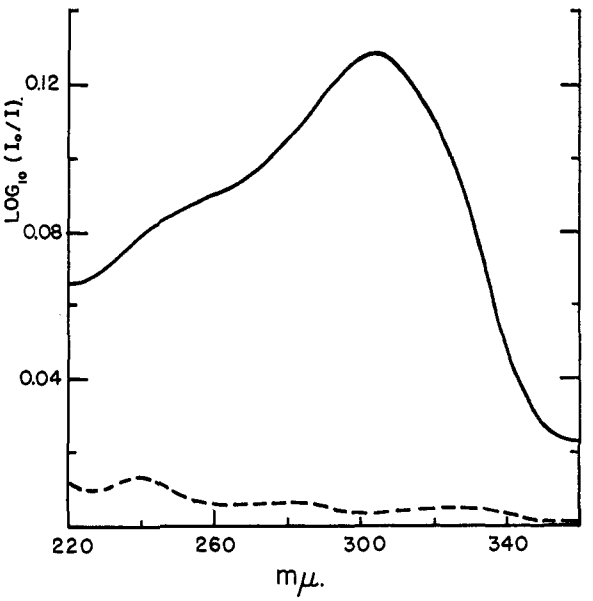

Fig. 1.—Ultraviolet absorption spectra of solutions of the mouse tumor hemorrhagic agent from *E. coli* in 79% sulfuric acid; solid line, after 15 min. at 100°; dotted line, after 2 hours at 25°.

The observation that the component monosaccharides of the experimental mouse tumor hemorrhagic agent from *E. coli* are D-glucosamine, D-glucose and D-galactose serves to differentiate this substance from the corresponding agent obtained from *B. prodigiosus* which was reported to contain hexosamine, a methylpentose, and presumably an aldehexose.[11]

Experimental

Hemorrhagic Agent.—All experiments were conducted on the ethanol-fractionated material designated in our previous communication[3] as fraction B_1.

(1) Supported from 1938 to 1943 by grants from the Argonaut Foundation and from 1948 onwards by grants from the National Cancer Institute of the U. S. Public Health Service.

(2) To whom inquiries regarding this article should be sent.

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(10) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 297, 303 (1940).

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TABLE I

PROPERTIES OF THE CARBOHYDRATE DERIVATIVES OBTAINED FROM THE MOUSE TUMOR HEMORRHAGIC AGENT FROM *E. coli*.

Sugar	Derivative	Melting point, °C. Obsd.[a]	Lit.	Mixed[b]	$[\alpha]_D$ Obsd.[c]	Lit.
D-Glucosamine	Hydrochloride[d]	.....	.....	.....	+73[e]	+72.5[e]
	N-Carbobenzoxy	213–214[f]	214[f]	214–215[f]		
D-Glucose	Diethylmercaptal	128.5–130	127–128	128–130		
	Benzimidazole	211–212[f]	215[f]	210–211[f]	+10[g]	+ 8.7[h]
	Benzimidazole picrate	203[f]	203[f]			
D-Galactose	Diethylmercaptal	143.5–145	140–142	144–145		
	Benzimidazole	242[f]	245[f]	241[f]	+42[g]	+45.1[h]
	Benzimidazole picrate	214–215[f]	217[f]			
	Mucic acid	217–220[f]	222	218–220[f]		

[a] All melting points are corrected. [b] Mixed melting point with an authentic sample. [c] At 25°. [d] N, calcd. 6.5, found 6.4. [e] Final value in water. [f] With decomposition. [g] In 1.0 *N* hydrochloric acid. [h] In 1.0 *N* hydrochloric acid at 20°, *cf.* N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, 64, 1612 (1942).

Ultraviolet Absorption Spectra in 79% Sulfuric Acid.—One ml. of a solution of fraction B_1 in water (100 γ/ml.) was added to 9 ml. of 84% sulfuric acid as previously described[4,5] and the ultraviolet absorption spectra determined after 2 hours at 25° or 15 min. at 100°.

Paper Chromatography.—A small sample of fraction B_1 was hydrolyzed with 1 *N* sulfuric acid for 1.5 hours, the hydrolysate neutralized with barium hydroxide, the barium sulfate removed, and the clear solution evaporated to dryness *in vacuo*. The residue was redissolved in water and the procedure of Partridge[6] followed.

Isolation of Mucic Acid.—Fraction B_1 (365 mg.) was heated with 5 ml. of nitric acid (sp. gr. 1.15) on a steam-bath until the mixture was transformed into a thick yellow sirup. The sirup was triturated with 0.3 ml. of water, extracted with ether to remove fatty material, and allowed to stand overnight. The solid that had formed was collected, and washed successively with a small amount of water and ethanol. The crude mucic acid so obtained was dissolved in a small amount of dilute sodium hydroxide, the solution filtered, and acidified with dilute nitric acid to give 7.7 mg. of mucic acid, *cf.* Table I.

Isolation of D-Glucosamine Hydrochloride.—A sample of 169 mg. of fraction B_1 was heated, under refluxing conditions, with 25 ml. of 5 *N* hydrochloric acid for 4 hours. The cooled solution was filtered, extracted with chloroform, and concentrated to dryness *in vacuo*. The resulting crystalline solid was washed with ethanol until no further colored substances were extracted. The solid was then dissolved in water, the insoluble material removed, and the solution again evaporated to dryness. The resultant colorless crystalline solid was washed with ethanol and dried to give 20.7 mg. of D-glucosamine hydrochloride, *cf.* Table I.

Isolation of N-Carbobenzoxy-D-glucosamine.—To 1.61 g. of carbohydrate mixture obtained as described in the isolation of the mercaptal derivatives (see below) was added 25 ml. of 5 *N* hydrochloric acid and the solution heated, under refluxing conditions, for 4 hours. The black insoluble material which had formed was removed and the filtrate evaporated *in vacuo* to dryness. A crystalline residue was obtained which was washed with absolute ethanol giving 245 mg. of solid. This material was treated with carbobenzoxy chloride according to Chargaff and Bovarnick[12] to give, after two recrystallizations from 30% aqueous methanol, 98 mg. of N-carbobenzoxy-D-glucosamine, *cf.* Table I.

Isolation of Diethylmercaptal Derivatives.—To a solution of 558 mg. of fraction B_1 in 100 ml. of water was added 3 ml. of concd. sulfuric acid and the solution heated under refluxing conditions for one hour. The hydrolysate was cooled, extracted with chloroform, the aqueous phase neutralized with barium hydroxide, the barium sulfate removed, and the filtrate evaporated to dryness *in vacuo* at 40°. This residue was dissolved in one ml. of concd. hydrochloric acid and mercaptalated with ethyl mercaptan according to Wolfrom and Karabinos.[9] However no crystalline material was obtained until after acetylation and deacetylation.[9] The crystalline material so obtained was fractionally recrystallized from acetone to give the diethylmercaptals of D-glucose and D-galactose, *cf.* Table I.

(12) E. Chargaff and M. Bovarnick, *J. Biol. Chem.*, **118**, 421 (1937).

Isolation of Substituted Benzimidazole Derivatives.—Fraction B_1, 2.2 g., was hydrolyzed as described in the preceding section to give 1.28 g. of a mixture of monosaccharides. This mixture was oxidized with potassium hypoiodite in methanol as directed by Moore and Link[10] to give 0.38 g. of a potassium aldonate fraction and 1.29 g. of a barium aldonate fraction. The former fraction was converted into the corresponding benzimidazoles[10] and, when no crystalline material was obtained directly from the reaction mixture, the benzimidazoles were precipitated and purified as the copper salts.[10] From these latter salts a small amount of D-galactobenzimidazole was obtained. The barium aldonate fraction also gave no crystalline benzimidazoles directly, but after purification through the copper salts and fractional crystallization from water and ethanol there was isolated 32 mg. of the less soluble D-galactobenzimidazole and 38 mg. of the more soluble D-glucobenzimidazole. Upon further recrystallization from water and aqueous ethanol 25 mg. of each of the above products were obtained in a relatively pure state, *cf.* Table I. For further confirmation these latter benzimidazoles were converted into the corresponding picrates, *cf.* Table I.

CONTRIBUTION NO. 1693 FROM THE
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4β-Acetoxy-Δ⁵-cholestene-3β,7α-diol

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RECEIVED JUNE 5, 1952

In a recent paper[3] the isolation of Δ⁵-cholestene-3β,4β,7α-triol monoacetate (Ia or Ib) from the reaction of cholesterol acetate and N-bromosuccinimide followed by chromatography was reported. The acetate group was tentatively assigned to C-3 since the starting material was cholesterol acetate. However, it was recognized[3] that acetyl migration during chromatography was possible and the product may have been the isomeric 4β-acetoxy-Δ⁵-cholestene-3β,7α-diol (Ib). Evidence that acetyl migration had indeed occurred is now presented.

It has previously been shown[3] that of the two free hydroxyl groups in I, only that on C-7 is oxidized by chromic acid or N-bromosuccinimide to yield

(1) Abstracted from the thesis of R. W. Jailer presented in partial fulfillment for the degree of Master of Science in Chemistry at New York University.

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(3) S. Lieberman and D. K. Fukushima, THIS JOURNAL, **72**, 5211 (1950).