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.

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4β-Acetoxy-Δ⁵-cholestene-3β,7α-diol

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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

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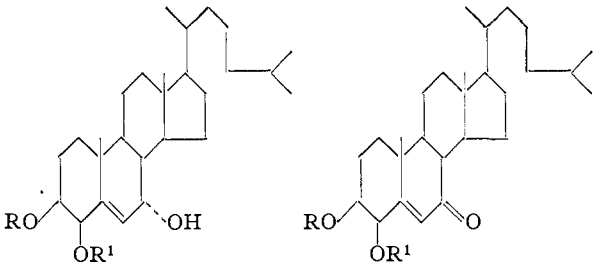
(3) S. Lieberman and D. K. Fukushima, THIS JOURNAL, **72**, 5211 (1950).



Ia, R = CH_3CO, R^1 = H
b, R = H, R^1 = CH_3CO

IIa, R = CH_3CO, R^1 = H
b, R = H, R^1 = CH_3CO
c, R = CH_3CO, R^1 = C_6H_5CO
d, R = C_6H_5CO, R^1 = CH_3CO

IIa or IIb. Benzoylation of the unoxidized hydroxyl group in IIa or IIb resulted in the acetoxybenzoxy-unsaturated-7-ketone (IIc or IId). This compound could not be obtained in crystalline form even after purification by chromatography and vacuum sublimation. All attempts at crystallization resulted in a gel which gave an amorphous white powder on drying. Formation of gels in this series of compounds has been found to occur frequently.[4] 3β,4β-Dihydroxy-Δ⁵-cholesten-7-one acetate benzoate thus obtained melted at 194–197°, $[\alpha]^{23}D$ −58.3° (chloroform), $\epsilon_{233m\mu}$ 23,000. In order to prove the structure of this compound, both isomers, IIc and IId were prepared by unequivocal methods.

Chromic acid oxidation of the known 3β-acetoxy-4β-benzoxy-Δ⁵-cholestene[5] yielded 3β-acetoxy-4β-benzoxy-Δ⁵-cholesten-7-one (IIc) which in contrast to the amorphous product described above readily crystallized, m.p. 163–164°, $[\alpha]^{23}D$ −58.1° (chloroform), $\epsilon_{233\ m\mu}$ 24,400. Its m.p., crystalline form and especially its characteristic infrared spectrum in the region of 1185–875 cm.$^{-1}$ [6] definitely established its non-identity with the amorphous ketone obtained from the triol monoacetate I.

Similar oxidation of 3β-benzoxy-4β-acetoxy-Δ⁵-cholestene[5] gave 3β-benzoxy-4β-acetoxy-Δ⁵-cholesten-7-one (IId) which could not be obtained crystalline. The product on drying gave an amorphous powder,[7] m.p. 185–188°, $[\alpha]^{23}D$ −57.1° (chloroform), $\epsilon_{233m\mu}$ 23,000.

These physical constants together with the infrared spectrum (1185–875 cm.$^{-1}$), identical in every respect with that of the product obtained from I, proved that the latter was the 3β-benzoxy-4β-acetoxy derivative (IId). It is evident from these results that the reaction of cholesterol acetate with N-bromosuccinimide followed by chromatography gave rise to the 4-acetoxy derivative Ib rather than Ia as previously formulated.

Experimental[8]

Benzoylation of 7-Keto-Δ⁵-cholestene-3β,4β-diol Monoacetate (IIb).—One hundred and eighty-six mg. of 7-keto-Δ⁵-cholestene-3β,4β-diol monoacetate (IIb) was benzoylated overnight at room temperature with pyridine and benzoyl chloride. The benzoylated product was chromatographed on alumina and the petroleum ether eluates gave 139 mg. of amorphous material. Attempts to crystallize the product from a number of solvents always led to the formation of a gel. Sublimation in a high vacuum yielded a hard glassy material and attempts at crystallization of this product also failed. The acetate-benzoate was finally purified by allowing a concentrated methanol solution to gel, filtering with vacuum and washing the gel with cold methanol. This procedure was repeated three times to give an amorphous powder of IId, m.p. 194–197° with previous sintering at 190°, $[\alpha]^{23}D$ −58.3 ± 1° (chloroform), $\epsilon_{233m\mu}$ 23,000 (ethanol).

Anal. Calcd. for $C_{36}H_{50}O_5$: C, 76.83; H, 8.96. Found: C, 76.10; H, 8.69.

3β-Acetoxy-4β-benzoxy-Δ⁵-cholesten-7-one (IIc).—To 600 mg. of 3β-acetoxy-4β-benzoxy-Δ⁵-cholestene[5] in 12 cc. of glacial acetic acid was added portionwise, a solution of 0.48 g. of chromic oxide, 0.4 cc. of water and 1.8 cc. of glacial acetic acid over a period of 2 hours. The oxidation mixture was maintained at 60° during this time and for an additional 2 hours. The excess chromic oxide was destroyed with ethanol and the solvent removed *in vacuo*. The residue was taken up in ether and washed with dilute hydrochloric acid, sodium carbonate solution and water. The ether solution was dried and the solvent evaporated to give 267 mg. of crystalline product. This material was chromatographed on silica gel and the petroleum ether–ether (4:1) eluates gave 188 mg. of crystalline 3β-acetoxy-4β-benzoxy-Δ⁵-cholesten-7-one (IIc). Recrystallizations from methanol and acetone gave IIc, m.p. 163–164°, $[\alpha]^{23}D$ −58.1 ± 1° (chloroform), $\epsilon_{233\ m\mu}$ 24,400 (ethanol).

Anal. Calcd. for $C_{36}H_{50}O_5$: C, 76.83; H, 8.96. Found: C, 77.09; H, 8.72.

The infrared spectrum in the region 1185–875 cm.$^{-1}$ was different from that of its isomer 3β-benzoxy-4β-acetoxy-Δ⁵-cholesten-7-one (IId) and the product obtained by the benzoylation of 7-keto-Δ⁵-cholestene-3β,4β-diol monoacetate.

3β-Benzoxy-4β-acetoxy-Δ⁵-cholesten-7-one (IId).—A suspension of 190 mg. of 3β-benzoxy-4β-acetoxy-Δ⁵-cholestene[5] in 4 cc. of glacial acetic acid was oxidized in the same manner as above with 1.1 cc. of a solution containing 0.48 mg. of chromic oxide, 0.4 cc. of water and 1.8 cc. of glacial acetic acid. Upon chromatographic separation on silica gel, the petroleum ether–ether eluates gave 87 mg. of amorphous material. All attempts at crystallization were unsuccessful.[7] The oxidation product was purified by allowing a concentrated solution in methanol to gel, filtering with vacuum and washing the amorphous material with cold methanol. This process was repeated three times to give an amorphous powder of 3β-benzoxy-4β-acetoxy-Δ⁵-cholesten-7-one (IId), m.p. 185–188° with previous sintering at 179.5°, $[\alpha]^{23}D$ −57.1 ± 1° (chloroform), $\epsilon_{233m\mu}$ 23,000 (ethanol), Petrow and Starling[4] reported m.p. 217–218°, $[\alpha]D$ −59.4° (chloroform).

Anal. Calcd. for $C_{36}H_{50}O_5$: C, 76.83; H, 8.96. Found: C, 76.85; H, 8.92.

The infrared spectrum in the region 1185–875 cm.$^{-1}$ was identical in every respect to the benzoylation product of 7-keto-Δ⁵-cholestene-3β,4β-diol monoacetate.

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(4) V. A. Petrow and W. W. Starling, *J. Chem. Soc.*, 749 (1946).

(5) V. A. Petrow, O. Rosenheim and W. W. Starling, *ibid.*, 135 (1943).

(6) We wish to express our gratitude to the late Dr. K. Dobriner and to Mrs. P. Humphries for their help in determining and interpreting the infrared spectra.

(7) Petrow and Starling[4] have reported the preparation of this compound in crystalline form, m.p. 217–218°, $[\alpha]D$ −59.4° (chloroform). However, attempts to crystallize IId by their methods were unsuccessful.

(8) All melting points are corrected.

The Heat of Fusion of Lithium

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The heat of fusion of lithium has been variously reported as 0.15,[1] 0.23,[2] 0.76[3] and 1.1[4] kcal./gram

(1) J. Sherman, *Chem. Revs.*, **11**, 93 (1932).

(2) A. Thum, Dissertation, Zurich, 1906.

(3) F. R. Bichowsky and F. D. Rossini, "The Thermochemistry of the Chemical Substances," Reinhold Publishing Corp., New York, N. Y., 1936.

(4) K. K. Kelley, U. S. Bur. Mines, Bull., No. 393, 166 p. (1936).