aliphaticum will preferentially attack the *d* or *l* form of a racemic hydrocarbon mixture.

Materials and Methods

The original pure culture of *Bacterium aliphaticum* was isolated with the aid of petroleum ether enrichment from the soil obtained at the base of a gasolene pump on a farm. The culture used in this investigation was obtained from a stock culture maintained on Söhngen's inorganic medium (0.05% ammonium chloride, 0.05% potassium hydrogen phosphate, a trace of calcium chloride) and on 5% petroleum ether (maximum boiling point 120°).

One of the hydrocarbons used was 3-methylheptane obtained from the American Petroleum Institute Ohio State University Manifest P-61 and was characterized by $n^{20}D$ 1.3998. The other hydrocarbon, 3-methylhexane, was obtained through the courtesy of Dr. J. O. Smith of the Research Laboratory of the Tidewater Associates Company. It was characterized by $n^{20}D$ 1.3884. Both of these hydrocarbons showed no rotation.

The optical activity was determined on a Franz Schmidt Research Polarimeter whose accuracy was +0.01°. A two-decimeter semi-micro polarimeter tube was used.

Two hundred milliliters of the nutrient solution and ten milliliters of the substrate hydrocarbon were placed in an 8-oz. prescription bottle and the thickness of the hydrocarbon layer was measured to a thirty-second of an inch. This mixture was then inoculated by means of a platinum loop with a stock culture growing on petroleum ether. All standard precautions were observed to prevent contamination. The prescription bottles were closed with screw caps and allowed to stand at room temperatures. Two or three days after inoculation a cloudiness appeared and this became more and more dense with time indicating ready metabolism of the hydrocarbons. A blank showed no growth. After three weeks the level of the hydrocarbon dropped to half its original value and the cultures were treated as follows: The hydrocarbon layer was separated from the aqueous layer by means of a separatory funnel and dried over anhydrous calcium chloride. The optical activity was found to be zero in the case of either of the two hydrocarbons. Another portion of the hydrocarbon layer was washed six times with concentrated sulfuric acid and subsequently six times with saturated magnesium sulfate solution. It was then twice washed with distilled water and dried over anhydrous magnesium sulfate. The optical activity was again found to be zero in both cases. The aqueous layer was centrifuged to precipitate the bacterial suspension, and the supernatant liquid was measured in the polarimeter. The optical activity was again zero in both cases. After these measurements the hydrocarbons were again used as substrate for the growth of *bacterium aliphaticum*. After three weeks the same procedure as above was used on the residual hydrocarbon which represented one-third of the volume of the original sample. The optical activity in both cases, that of 3-methylhexane and 3-methylheptane, was again zero.

Discussion.—It has been abundantly established since Pasteur's discovery that certain organisms especially molds, yeasts and bacteria metabolize preferentially one of the two optical antipodes of a racemic mixture.[2]

The results of the present investigation show that the *bacterium aliphaticum* does not attack preferentially one of the two optical isomers of 3-methylhexane and of 3-methylheptane. P. A. Levene and R. E. Marker[3] report an observed rotation of $[\alpha]_D$ +1.64° and a maximum rotation

(2) *Cf.* H. Scheibler in J. Houben's "Die Methoden der organischen Chemie," Vol. 2, Part 2, Leipzig, 1925, p. 1087; R. L. Shriner, R. Adams and C. S. Marvel in Gilman's "Organic Chemistry," **1**, 263 (1943).

(3) Levene and Marker, *J. Biol. Chem.*, **95**, 13 (1932).

of $[\alpha]_D$ 9.67° for 3-methylhexane while we observe less than 0.01°. It, therefore, follows that if there is a preferential action in the rate of attack of the *bacterium aliphaticum* it is less than one part in two thousand. This result is not surprising in view of the variety of substrates that this organism can metabolize, *e. g.*, aliphatic hydrocarbons and sugars. It is further consistent with the results of F. H. Johnson and H. W. Schwarz[4] who found that this bacterium metabolized both *d* and *l* arabinose though no attempt was made to determine whether the rate of metabolism was the same.

It is, however, unfortunate that there is no preferential action on optical antipodes, for if there were, it would offer a method of obtaining a wide variety of optically active hydrocarbons for the study of the mechanism of hydrocarbon reactions.

(4) F. H. Johnson and H. W. Schwarz, *J. Bact.*, **47**, 373 (1944).

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Addition of Secondary Amines to Dibromopropanol

BY FRANK C. WHITMORE, HARRY S. MOSHER, DAVID P. SPALDING, ROBERT B. TAYLOR, GEORGE W. MOERSCH AND WILLIAM H. YANKO

We reported[1] that 2,3-dibromopropanol (I) reacts with secondary amines such as piperidine, diethylamine or morpholine to give the metathetical product (II).

$$HOCH_2CHBrCH_2Br + HNR_2 \longrightarrow$$
(I)
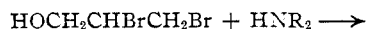
$$HOCH_2CH(NR_2)CH_2NR_2$$
(II)
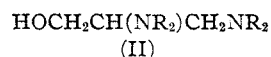
On the suggestion of Dr. P. H. Williams, of the Shell Development Company, Emeryville, California, we have re-investigated these products, and have found in at least one case, *i. e.*, piperidine, that the symmetrical compound

$$R_2NCH_2CHOHCH_2NR_2 \quad (III)$$

is formed as well.

Thus, when piperidine was treated with 2,3-dibromopropanol and the product distilled from a Claisen flask, the first portion of the amine fraction gave a picrate, m. p. 190–191°, which showed no depression of the melting point when mixed with the picrate from the reaction product of piperidine and epichlorohydrin. This must be the symmetrical 1,3-dipiperidino-2-hydroxypropane (III). The last fraction from the distillation gave a picrate melting at 174–175° and showed the same melting point when mixed with the picrate from the product of the Bouveault–Blanc reduction of ethyl 2,3-dipiperidion-propionate. This must be the picrate of the metathetical reaction product, 2,3-dipiperidino-

(1) Whitmore, *et al.*, THIS JOURNAL, **67**, 664 (1945).

1-hydroxypropane (II) reported in the above reference.

The formation of the symmetrical isomer probably arises from the intermediate ethylene oxide.

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The Uronic Acid Component of Heparin

BY M. L. WOLFROM AND F. A. H. RICE

Sulfuric acid and D-glucosamine[1] have been the only identified hydrolytic products of heparin, the blood anticoagulant isolable from animal tissues. Qualitative[2] and quantitative data[3] indicating the presence of a uronic acid in heparin have been obtained. It has been demonstrated[4] that the hydrolytic conditions which will liberate the uronic acid can also readily destroy it since it is decomposed by acidity. We have accordingly subjected crystalline barium acid heparinate to oxidative hydrolysis, considering that any uronic acid liberated might be stabilized as the acid-resistant dibasic acid. From the reaction mixture there was isolated D-glucosaccharic acid (as the crystalline potassium acid salt) and crystalline D-glucosaminic acid. Under similar non-oxidative hydrolytic conditions, no D-glucosaccharic acid was isolable. These results therefore establish the uronic acid component of heparin as D-glucuronic acid. We wish to remark that the optical rotation of our isolated D-glucosaccharic acid was ascertained, a significant point generally overlooked in most isolations of this substance.

Experimental

An amount of 200 mg. of crystalline barium acid heparinate was dissolved in 2 cc. of water and cooled to near 0°. Five drops of bromine were added, followed by the gradual addition, over a period of ten minutes, of a total of 5 cc. of concentrated sulfuric acid. The mixture was allowed to stand at *ca.* 3° for one week. From time to time, as the solution became less colored, a few drops more of bromine were added. The solution was finally kept at room temperature for *ca.* five hours, aerated to effect bromine removal, and poured into 75 cc. of cold (near 0°) water. The sulfuric acid was neutralized in the cold with barium carbonate and the mixture filtered. Concentration (30-40°) of the filtrate under reduced pressure yielded a thick sirup.

The sirup was acidified with a drop of concentrated hydrochloric acid and extracted at room temperature with 95% ethanol. The extract was neutralized to *ca.* *p*H 6 with 10% aqueous potassium hydroxide, filtered and concentrated under reduced pressure to a thick sirup. The sirup was treated with 10 cc. of absolute ethanol, filtered and again concentrated under reduced pressure to a sirup. This sirup was dissolved in 1 cc. of water, neutralized with solid potassium carbonate and 1 cc. of glacial

(1) E. Jorpes and S. Bergström, *Z. physiol. Chem.*, **244**, 253 (1936).
(2) W. H. Howell, *Bull. Johns Hopkins Hosp.*, **42**, 199 (1928).
(3) M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos, W. H. McNeely and J. McLean, THIS JOURNAL, **65**, 2077 (1943).
(4) M. L. Wolfrom and J. V. Karabinos, *ibid.*, **67**, 679 (1945).

acetic acid added. Crystals formed overnight that had the appearance of potassium acid saccharate when viewed under the microscope. A further quantity of like crystals were obtained by extracting the barium sulfate (formed above in the neutralization of the sulfuric acid) at room temperature with 10 cc. of 1% aqueous potassium hydroxide. The neutralized (with acetic acid) extract was concentrated (30-40°) under reduced pressure to a volume of 1 cc. and acidified with an equal volume of glacial acetic acid. Crystals formed on standing overnight; total yield 29.9 mg., $[\alpha]^{20}$D +10° (*c* 2.5 as dipotassium salt, water). The polarization was effected by solution in an equivalent (to phenolphthalein) amount of aqueous potassium carbonate solution. A known sample of potassium acid D-glucosaccharate gave the same value, $[\alpha]^{20}$D +10°, under the same conditions. The solutions were colored slightly yellow by the neutralization procedure.

Anal. Calcd. for $C_6H_9O_8K$: K, 15.72. Found: K, 15.82.

The crystalline product was therefore identified as potassium acid D-glucosaccharate.

The insoluble material remaining after the ethanol extraction described above was treated with a small amount of silver carbonate and extracted at room temperature with 25 cc. of 95% ethanol. The extract was concentrated to 5 cc., filtered and ether added to incipient opalescence. Crystals (long needles) separated on standing; yield 26 mg., dec. 250-260°, $[\alpha]^{21}$D -19 ±2° (*c* (as weighed) 1.0, 2.5% hydrochloric acid, twelve hours). The crystals were acid toward litmus and contained amino nitrogen (by soda-lime fusion). A crystalline copper salt (bluish-green crystals) was formed with cupric carbonate. These data identify the substance as D-glucosaminic acid,[5] for which Fischer and Leuchs[6] cite the constants: dec. 250°, $[\alpha]^{18}$D -17° → -15° (*c* 10, 2.5% hydrochloric acid, thirty hours).

On repeating the above described hydrolysis of crystalline barium acid heparinate but omitting the bromine, no potassium acid D-glucosaccharate was formed.

(5) E. Fischer and F. Tiemann, *Ber.*, **27**, 138 (1894).
(6) E. Fischer and H. Leuchs, *ibid.*, **35**, 3787 (1902); **36**, 24 (1903).

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NEW COMPOUNDS

3-Trichloromethyl-6-hydroxy-7-chlorophthalide and its Acetyl Derivative

The hydroxyphthalide was prepared by Chattaway and Calvet's method.[1] Three grams of 2-chloro-3-hydoxy-benzoic acid and 4 g. of U. S. P. chloral hydrate were dissolved in 30 g. of concentrated sulfuric acid. After standing twenty-four hours, the solution was poured into cracked ice and water, and the precipitate, which formed, when washed with water and dried weighed 5.2 g. and melted at 190-192°. One crystallization from benzene and two from ethanol-water raised the melting point to 195.5-196°. The compound forms a precipitate when warmed with alcoholic silver nitrate, is readily soluble in 5% aqueous sodium hydroxide solution, and produces a green fluorescence with resorcinol and sulfuric acid.

Anal. Calcd. for $C_9H_4Cl_4O_3$: Cl, 46.97. Found: Cl, 46.91, 46.94.

The acetyl derivative was prepared by the method of Pratt and Robinson.[2] Five-tenths gram of the hydroxyphthalide gave 0.48 g. of a product melting at 175-177°.

(1) Chattaway and Calvet, *J. Chem. Soc.*, 1092 (1928).
(2) Pratt and Robinson, *ibid.*, **127**, 1184 (1925).