The specific volume was obtained from density, which was measured by flotation in carbon tetrachloride. The percentage increase in crystallinity was calculated using the values given by H. Mark[1] for the density of crystalline cellulose, 1.59 g./ml. (Sp V = 0.629). The density of amorphous cellulose was taken to be 1.50 g./ml. (Sp. V = 0.667) as estimated by P. H. Hermans.[2] The following relation was used to calculate the increase in crystallinity

$$\frac{\text{Sp. } V \text{ of untreated sample} - \text{Sp. } V \text{ of treated sample}}{\text{Difference in Sp. } V \text{ of amorphous and crystalline cellulose}}$$

A rayon of specific volume 0.653 showed a weight loss of 10% when treated with the reagent for five minutes. The specific volume of the treated sample was 0.647, indicating an increase of 16% in the crystallinity.

If the only process involved in the initial stages of this treatment is the attack and removal of the amorphous portion of the fiber, the density increase should predict a change of crystallinity of the same order of magnitude as the weight of material lost. However, the actual increase in density is much larger. This may indicate that with the rupture of a cellulose chain in an amorphous portion of the fiber a process of crystallization is initiated.

(1) H. Mark, "Physik und Chemie der Cellulose," Berlin, 1932.
(2) P. H. Hermans, "Contribution to the Physics of Cellulose Fibres," Elsevier Publishing Co., Inc., 1946.

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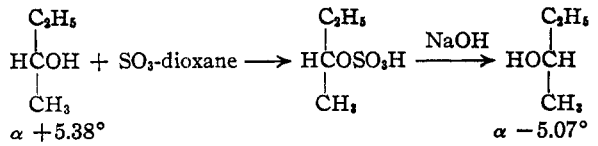
A NEW WALDEN INVERSION

Sir:

The following reaction sequence constitutes a new Walden inversion



The second step resembles the displacement of *p*-toluenesulfonate ion from secondary alkyl esters of the sulfonic acid by ethoxide or acetate ions, a reaction which has been shown to invert the configuration of the carbinol carbon.[1]

We previously[2] prepared (+)*s*-butylsulfuric acid by action of Suter's sulfur trioxide–dioxane reagent on (+)*s*-butyl alcohol and found that upon its acid hydrolysis a (+)alcohol was recovered whose rotation was 30% of that of the starting material. We had assumed that the racemization was confined to the hydrolytic step and that the formation of the alkylsulfuric acid proceeded with little if any racemization. This is now confirmed.

Since the action of chlorosulfonic acid and of sulfuric acid upon (+)alcohol gave (+)*s*-butylsulfuric acid, and since it was difficult to see how all these methods could involve breaking the carbon–oxygen bond of the alcohol, it was considered that the alkylsulfuric acid had the same configuration as the alcohol. If this is true, and its plausibility is increased by our finding that the sulfur trioxide–pyridine complex[3] also gives a (+)alkylsulfuric acid, then the saponification involves the displacement of a sulfate ion by a hydroxide ion with inversion of configuration. Since this reaction is one between two ions of like charge it is comparatively slow.

The alkylsulfuric acid was prepared by action of the sulfur trioxide–dioxane complex upon an alcohol of α +5.38. In one saponification, 8 g. of (+)sodium *s*-butylsulfate (from neutralizing the alkylsulfuric acid reaction mixture with sodium hydroxide, evaporating and extracting the sodium alkylsulfate with methanol) and 10 g. of sodium hydroxide were dissolved in 50 cc. of water. At 100° the reaction required two days for substantial completion. Alcohol was recovered in 54% yield with a rotation 6% below that of the starting alcohol and of opposite sign. About 8% of gas, apparently butylene, was evolved. A similar alcohol was obtained from the barium salt in a more concentrated potassium hydroxide solution, but several times as much butylene resulted.

Further investigation now under way at this laboratory should reveal the degree to which this reaction is common to secondary alcohols.

(1) This work of Kenyon, Phillips and co-workers is reviewed by Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, pp. 160–163.
(2) Burwell, THIS JOURNAL, **67**, 220 (1945).
(3) Sobel, Drekter and Natelson, *J. Biol. Chem.*, **115**, 381 (1936).

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RECEIVED DECEMBER 18, 1947

FORMYLFOLIC ACID, A FUNCTIONAL DERIVATIVE OF FOLIC ACID

Sir:

Previous studies from this Laboratory have indicated that a coenzyme containing *p*-aminobenzoic acid is involved in combining a single carbon unit into the pyrimidine ring of purines[1] and that folic acid functions in the biosynthesis of purines.[2] Seeking functional derivatives which could act as "carriers" of formate, we prepared *p*-aminobenzoylhistidine and condensed it with α,β-dibromopropionaldehyde and 2,4,5-triamino-6-hydroxypyrimidine to obtain pteroylhistidine. No pronounced activity was obtained with either of these histidine derivatives. The announcement of the structure of rhizopterin[3] which is *p*-[N-(2-amino-4-hydroxypyramido-[4,5-b]pyrazin-6-ylmethyl)-formamido]-benzoic acid gave a clue as

(1) Shive, *et al.*, THIS JOURNAL, **69**, 725 (1947).
(2) Rogers and Shive, *J. Biol. Chem.*, in press.
(3) Wolf, *et al.*, THIS JOURNAL, **69**, 2753 (1947).

to how formate may be carried by a functional derivative of folic acid. Accordingly, formylfolic acid was prepared by heating 2 cc. of 98% formic acid and 0.5 cc. of acetic anhydride with 26 mg. of folic acid (pteroylglutamic acid) for one hour at 100°. The volatile reactants were removed *in vacuo*, and the light yellow product was precipitated from an alkaline solution by acetic acid. For analysis, a sample was hydrolyzed, and formic acid determined (Calcd.: 9.8%. Found.: 9.5%). The formylfolic acid was as effective as folic acid in stimulating the growth of *Streptococcus faecalis R* and *Lactobacillus casei*.

However, in an inhibition analysis with 7-methylfolic acid,[4] formylfolic acid was approximately thirty times as active as folic acid in preventing the toxicity of the inhibitor for *S. faecalis R;* the antibacterial index was 3,000 for formylfolic acid as compared with 100 for folic acid over a range of inhibitor concentrations from 10 to 100γ per 10 cc. In 10 cc. of medium,[2] a mixture of 0.3 γ of folic acid with 0.03 γ of formylfolic acid was no more effective than 0.03 γ of formylfolic acid alone in preventing the toxicity of the inhibitor. Synthetic rhizopterin[3] was two to three times as effective as folic acid in preventing the toxicity of the inhibitor. The toxicity of methylfolic acid was increased several fold by treating with 98% formic acid at 100° for two hours. For *L. casei* formylfolic acid was only slightly more effective than folic acid in preventing the toxicity of the inhibitor.

These results indicate that formylfolic acid is a functional derivative of folic acid and is competitively inhibited by methylfolic acid. The activity of rhizopterin indicates that it probably is converted directly to formylfolic acid.

These and other unpublished experiments offer additional evidence that the biochemical functions of *p*-aminobenzoic and folic acid derivatives involve the introduction of the single carbon unit into purines, pyrimidines and probably histidine.

The possibility of enhanced activity and decreased toxicity for formylfolic acid as compared with folic acid in treatment of pernicious anemia indicates the desirability for clinical testing.

(4) Crude product from the condensation of α,β-dibromobutyraldehyde, 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoylglutamic acid obtained from Dr. E. L. R. Stokstad (Franklin, *et al.*, *J. Biol. Chem.*, **169**, 427 (1947)).

THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH, AUSTIN, TEXAS

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RECEIVED DECEMBER 29, 1947

CONCERNING THE STRUCTURE OF PHTHIOIC ACID

Sir:

Polgar and Robinson[1] have recently proposed, partly on the basis of the highly questionable degradative evidence of Wagner-Jauregg,[2] that phthioic acid,[3] isolated from tubercle bacillus, may be 3,13,19-trimethyltricosanoic acid. This acid was synthesized, but since it contains three asymmetric carbons the *dl*-mixture was of no value for comparison with the natural product. By use of the data which have now been collected concerning the optical rotation of fatty acids containing branching methyl groups, it is possible to estimate with some accuracy the maximum rotation to be expected of an acid such as 3,13,19-trimethyltricosanoic acid. Data of interest to this discussion appear in Table I.

TABLE I

Acid	$[\alpha]_D$	$[M]_D$
(+)-2-Methylhexacosanoic[4]	6.8°[a]	28°
(+)-2-Methyldodecanoic[4]	13.2°	28.3°
(+)-4-Methylhexanoic[5]	8.4°	11.0°
(+)-12-Methyltetradecanoic[6]	4.7°	11.4°
(+)-10-Methyloctadecanoic[7]	0.06°	0.2°
(+)-3-Methylhendecanoic[7]	5.0°	10.1°

[a] Taken in chloroform solution, remaining values obtained homogeneous.

As shown by the above data and elsewhere,[6] the molecular rotation remains reasonably constant for a given relationship of methyl group to the ends of the chain. Variables such as solvent and temperature have appreciable but not large effects in these compounds. For the case of 3,13,19-trimethyltricosanoic acid, it seems safe to assume that (a) the contribution of each asymmetric carbon to the rotation is unaffected by the presence of the other branching groups, (b) the contribution of carbon-19 is less than the rotation of the third or fourth acids listed in Table I, (c) the contribution of carbon-13 is similar to that of the fifth acid in Table I, (d) the contribution of carbon-3 is the same as the rotation of the sixth acid in Table I. It follows that the maximum molecular rotation for 3,13,19-trimethyltricosanoic acid should be approximately 22°, less than half the value of 49.7° observed for phthioic acid. Thus, it seems impossible that phthioic acid could have the structure suggested by Polgar and Robinson.

It is of interest that 2,3,21-trimethyltricosanoic acid has a calculated maximum molecular rotation of approximately 49.5° (ignoring any effect caused by the proximity of the 2- and 3-methyl groups), and all other trimethyltricosanoic acids would have a lower rotation. Thus, if phthioic acid is a long-chain acid with several branching methyl groups (evidence supporting this proposition seems doubtful), three of these groups are probably in the 2-, 3- and anteiso positions. Additional methyl groups between the 3- and anteiso positions should contribute little to the rotation.

(1) Polgar and Robinson, *J. Chem. Soc.*, 389 (1945).

(2) Wagner-Jauregg, *Z. Physiol. Chem.*, **247**, 135 (1937).

(3) Spielman and Anderson, *J. Biol. Chem.*, **112**, 759 (1936).

(4) Ställberg-Stenhagen, *Arkiv Kemi, Mineral. Geol.*, **23**, No. 15 (1946).

(5) Welt, *Compt. rend.*, **119**, 855 (1894).

(6) Weitkamp, THIS JOURNAL, **67**, 447 (1945).

(7) Prout, Cason and Ingersoll, *ibid.*, **69**, 1233 (1947).