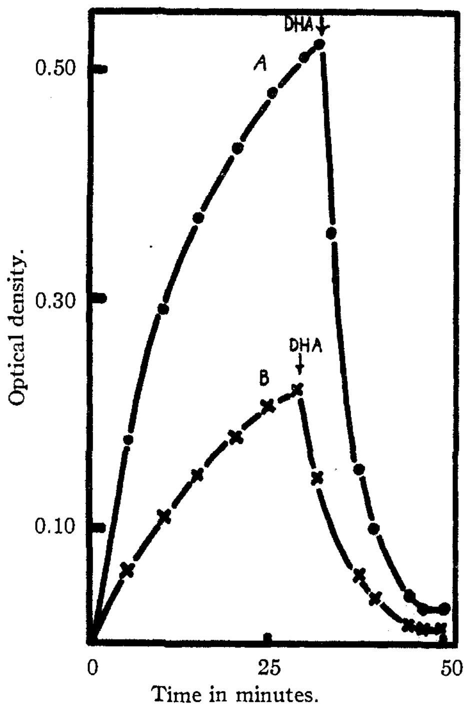

Fig. 1.—The reversible reaction catalyzed by glycerol dehydrogenase: curve A, the reaction mixture contained 100 μM. of potassium phosphate buffer, 0.5 μM. of DPN, 0.6 mg. of dialyzed *A. aerogenes* glycerol dehydrogenase preparation, and 500 μM. of glycerol in a total volume of 3.0 ml. at *p*H 9. The change in optical density was measured at 340 mμ with a Beckman model DU spectrophotometer at 23°. At the arrow 100 μM. of DHA was added. Curve B, the details for curve A apply here except 100 μM. of glycerol was used with 10 μM. of DHA added at the arrow.

During the course of this investigation an interesting non-enzymatic reaction between DHA and DPN was observed.[6] The product of this reaction has an ultraviolet spectrum identical to that of DPNH and possesses other properties of DPNH. That this product is not DPNH is shown by its failure to reduce acetaldehyde in the presences of yeast alcohol dehydrogenase. Further study has shown that the basic requirements for the reaction are an N-substituted nicotinamide structure (as in DPN), and α,β-hydroxy-keto-structure (as in DHA), and an alkaline medium. The details of this investigation and a discussion of the reaction mechanism will be reported elsewhere.

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(6) A somewhat similar reaction using glyceraldehyde had been noticed previously by D. M. Needham, L. Siminovitch, and S. M. Rapkine, *Biochem. J.*, **49**, 113 (1951), who presumed that DPNH and glyceric acid were formed.

(7) Public Health Service Research Fellow of the National Heart Institute.

ADDITIONAL COMMENTS ON THERMODYNAMIC FUNCTIONS FOR SURFACES OF CRYSTALS

Sir:

In the preceding letter, S. H. Bauer has criticized a recent article by us. It appears that we were too brief in our discussion of the work which was presented. Our belief was that the data which we had obtained could not be considered final. We were careful to point out several factors which might change the final results. It was the hope of the senior author to reserve the discussion of some of the questions raised in Dr. Bauer's letter until better data were available. Since we knew that several years would be essential for a complete solution to all of the experimental problems involved in the determination, we thought that it would be advisable to publish that which we had done in the hope that these preliminary results would be of value and that others would think and work actively on this problem.

First, concerning the pragmatic test of the invariance of the results with respect to particle size and shape. For the past two years we have engaged in the design and construction of a calorimeter for the sole purpose of making these measurements with respect to the enthalpy. We have hopes that in a year we will be able to make a report of the results of this work. After this project is finished, we hope to make similar measurements of the heat capacity. These of course, will take even more time since the necessary experimental procedures are even more difficult than those involved in the enthalpy determination.

Bauer states that the results should be independent of size and shape. This, of course, will be true only if two conditions are fulfilled: (1) only one crystallographic face is present, and (2) if the particle is sufficiently large that the surface properties are not dependent on particle size. The first of these considerations was an overwhelming factor in the choice of MgO, since the structure is such that we would expect only the 100 face to appear at the temperatures at which the experiments are performed. The few poor electron photomicrographs that we have obtained with our sample of MgO indicated that this was so. Too few individual particles were observed, however, to state that this was certainly true for all of the particles. Since we considered these results as preliminary, we made no really serious efforts to obtain really good pictures. Actually, it can be shown that under certain circumstances, even if more than one crystallographic face appears, it is possible to obtain the surface tension of each face from the same measurements and a knowledge of the crystal habit; provided that the future work shows that the proposed scheme of measurements is valid. The size at which it may be expected that the thermodynamic functions become size dependent, can be readily computed by the method of Lennard-Jones and Dent.[1]

The question naturally arises as to the possible specific effects mentioned by Bauer. First, the contribution to these functions of the gross motion of the particles studied by Jura and Pitzer[2]: at very low temperatures, say to about 10°K., this effect would be appreciable. However, at room temperature, this quantity is several orders of magnitude less than the observed experimental effects. There is complete justification in its neglect.

(1) J. E. Lennard-Jones and B. E. Dent, *Phil. Mag.* [7] **8**, 530 (1929).

(2) G. Jura and K. S. Pitzer, THIS JOURNAL, **74**, 6030 (1952).

The authors feel that the effect of defects on the measured entropy is also small by several orders of magnitude. The effect of edges and corners depends on the actual structure of the surface. It is simple to consider those systems in which only atomically plane surfaces are present. For the particles that were used in the experiment, assuming an average cube size, it is found that in round numbers there are 8 corner to 600 edge to 15,000 surface ions. The corners, obviously can be neglected. If it is assumed that the effect of the edge is twice that of the surface, then in order to detect the effect of edges with certainty, it would be necessary to make calorimetric measurements by a factor of ten better than now possible. If the surface is heterogeneous, then estimates of the effect of heterogeneity are impossible. However, if the degree of heterogeneity is independent of particle size, then the results which are obtained are valid. For very small particles, such as those which are used as catalysts, there is probably variation in the heterogeneity of the surface. These particles are smaller than those used in these experiments. It appears reasonable that as the particles grow, the distribution of energy over the surface would become independent of size.

In view of the preceding considerations the authors still feel that the ideas presented in the original paper are valid.

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RECEIVED JANUARY 30, 1953

SYNTHESIS AND CONFIGURATION OF DIHYDROSPHINGOSINE[1,2]

Sir:

Dihydrosphingosine is one of the four stereoisomeric forms of 1,3-dihydroxy-2-aminoöctadecane.[3] Recently several papers have appeared describing synthetic methods for obtaining such compounds.[4,5,6,7] However, none of these methods provides evidence as to the stereochemistry of the product and indeed most of them would be presumed to yield a mixture of the two racemic forms.

We have approached the synthesis by a different route. The two racemic α-amino-β-hydroxystearic acids were prepared and characterized as the *threo* and *erythro* isomers by comparison of the properties of a number of derivatives with the corresponding derivatives of threonine (*threo*) and allothreonine (*erythro*). The assignment of configuration was based on the complete agreement in the relative behavior of the C_{18} isomers as compared to the C_4 isomers.[8] Thus the characteristic shifts in infrared spectra from threonine to allothreonine and the relative melting point and solubility behavior of the two isomers and their derivatives are duplicated exactly in the C_{18} series. Furthermore the interconversion and isomerization of the 2-phenyloxazoline derivatives of threonine and allothreonine[9] are given by the C_{18} isomers under identical conditions. These data leave little or no doubt as to the configuration of the two α-amino-β-hydroxystearic acids. This work will be reported in detail shortly.

The methyl esters of the two amino acids were reduced to the corresponding 1,3-dihydroxy-2-aminooctadecanes with lithium aluminum hydride. The N-benzoyl methyl esters under similar conditions yielded the corresponding N-benzyl derivatives, which were readily debenzylated by catalytic reduction. The melting points of these substances are summarized in Table I.

TABLE I

Each of the compounds listed gave C, H, and N analyses agreeing with the theoretical.

	Threo series m.p., °C.	*Erythro* series m.p., °C.
α-Amino-β-hydroxystearic acid	205–206	217–220
N-Benzoyl	92–95	174–176
Methyl ester	77–78	71–73
Methyl ester, N-benzoyl-	86–88	97–99.5
1,3-Dihydroxy-2-aminoöctadecane	99.5–100.5	84–86
N-Benzyl-	55–56	62.5–63.5
Triacetyl-	65–66	90–92
Tribenzoyl-	. . .	144–45
N-Acetyl-	104.5	120–121

Reduction of the methyl α-amino-β-hydroxysterates over Raney nickel also gave excellent yields of the corresponding bases with no evidence of inversion. These results thus establish with some certainty the configuration of the two DL-1,3-dihydroxy-2-aminoöctadecanes.

Determination of the configuration of the natural compound was made possible by a striking difference in properties of the two racemic bases. The *erythro* base gave readily a tribenzoyl derivative (m.p. 144–145°) in quantitative yield on treatment with benzoyl chloride and pyridine. Under similar conditions natural dihydrosphingosine gives a tribenzoyl derivative melting at 144–145°. In marked contrast to this behavior we have found that the *threo* base gives only a dibenzoyl derivative and have not been able to obtain a tribenzoyl derivative with pyridine and benzoyl chloride under any of a variety of conditions. When a mixture of the *threo* and *erythro* bases was benzoylated only the tribenzoyl derivative of the *erythro* form was obtained. This result affords an explanation for the fact that all of the synthetic 1,3-dihydroxy-2-aminoöctadecane preparations described in the literature gave a tribenzoyl derivative melting at about 146°.

On the basis of these data it is tentatively con-

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(1) This investigation was supported in part by a research grant (RG 2031) from the National Institutes of Health, Public Health Service.

(2) Part of the material in this paper was taken from the thesis submitted to the Graduate College of the University of Illinois by J. Bradley Harrison in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

(3) H. E. Carter, F. J. Glick, W. P. Norris, and G. E. Phillips, *J. Biol. Chem.*, **170**, 285 (1947).

(4) G. I. Gregory and T. Malkin, *J. Chem. Soc.*, 2453 (1951).

(5) N. Fisher, *Chem. and Ind.*, 130 (1952).

(6) C. A. Grob, E. F. Jenny and H. Utzinger, *Helv. Chim. Acta*, **34**, 2249 (1951).

(7) C. A. Grob and E. F. Jenny, *ibid.*, **35**, 2106 (1952).

(8) J. Bradley Harrison, Ph.D. thesis, University of Illinois, 1952.

(9) D. F. Elliott, *J. Chem. Soc.*, 62 (1950).