in the organic acid and its concentration should aid in the separation of relatively large cleavage products of other proteins. The need for such separations recently has been emphasized.[18,19]

Acknowledgments.—The author is indebted to Prof. A. C. Chibnall and Dr. O. K. Behrens for samples of insulin. The assistance of Burton D. Wilson and Daniel E. Ott is gratefully acknowledged.

(18) A. C. Chibnall, in "Les Proteines, Rapports et Discussions," R. Stoops, Ed., Institut International de Chimie Solvay, Brussels, 1953, p. 128.

(19) R. L. M. Synge, *ibid.*, p. 153.

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The Resolution of DL-*threo*-1-*p*-Methylsulfonylphenyl-2-amino-1,3-propanediol

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The preparation of D-*threo*-1-*p*-methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol by the oxidation of D-*threo*-1-*p*-methylmercaptophenyl-2-dichloroacetamido-1,3-propanediol has been reported by Cutler, Stenger and Suter.[1] Although the synthesis of the racemate of the former compound from 4-methylsulfonylacetophenone was described by Suter, Schalit and Cutler[2] in a subsequent publication, no resolution of the final product or of its intermediates has appeared in the literature. Because of the high order of antibacterial activity of the D-*threo*-*p*-methylsulfonyl compound, it is of interest to describe a method for resolving DL-*threo*-1-*p*-methylsulfonylphenyl-2-amino-1,3-propanediol developed in our laboratory during a series of studies of N-acylated compounds of this type.[3]

The recovery of racemic *threo*-1-*p*-methylsulfonylphenyl-2-amino-1,3-propanediol from the hydrolysis of the corresponding acetamide or dichloroacetamide presented a problem because of the extreme solubility of this compound in water. Suter, *et al.*,[2] described recovery of a 19.5% yield of base from the acid hydrolysis of the acetamide. The product was isolated by extracting a strongly alkalinized solution of the hydrolysate with butanol and evaporating the butanol extract. In our laboratory it was found that the recovery of the base could be substantially improved by passing the acid hydrolysate over an XE 98[4] basic resin column and evaporating the eluate containing the liberated base to dryness under reduced pressure. This method has been used also to recover the resolved bases from the optically active mandelate salts. In the latter case the salt was first treated with an equivalent amount of hydrochloric acid to liberate the mandelic acid and the resolving agent was removed by ether extraction. The aqueous residue containing the optically active hydrochloride salt was then passed over an XE 98 column and the D- or L-*threo* base isolated as above.

The resolution of DL-*threo*-*p*-methylsulfonylphenyl-2-amino-1,3-propanediol was achieved by fractional crystallization of the appropriate active mandelic acid salt from ethanol. When (+)mandelic acid was used, the mandelate salt of the levorotatory base was less soluble in ethanol and could be isolated in the optically pure state by repeated crystallization. The dichloroacetamide of this base proved to be identical with the product obtained by oxidizing D-*threo*-1-*p*-methylmercaptophenyl-2-dichloroacetamido-1,3-propanediol. The antibacterial activity was twice that of the racemate. On the other hand, the dichloroacetamide of the dextrorotatory base obtained when (−)-mandelic acid was used as the resolving agent had substantially no *in vitro* antibacterial activity.

Experimental

The Preparation of DL-*threo*-1-*p*-Methylsulfonylphenyl-2-amino-1,3-propanediol.—A 5-g. sample of DL-*threo*-1-*p*-methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol[5] was treated with 40 ml. of 1.0 *N* hydrochloric acid for 2.5 hours on the steam-bath. The chilled hydrolysate was diluted with 125 ml. of distilled water and put over a basic XE 98[4] column which had been washed previously to *p*H 9.4 with distilled water. The combined alkaline eluates were evaporated under reduced pressure and the residue dried by repeated evaporation with ethanol and benzene to a crystalline solid. The base was recrystallized from ethanol to give 2.23 g. of product (m.p. 121–122°).

Anal. Calcd. for $C_{10}H_{15}NO_4S$: C, 48.96; H, 6.16; N, 5.71. Found: C, 49.14; H, 6.21; N, 5.73.

The Resolution of DL-*threo*-1-*p*-Methylsulfonylphenyl-2-amino-1,3-propanediol.—A 2-g. sample of DL-*threo*-1-*p*-methylsulfonylphenyl-2-amino-1,3-propanediol was treated with 1.24 g. of (+)mandelic acid in 20 ml. of absolute ethanol. After standing overnight at room temperature, 2.7 g. of crystalline salt melting at 138–140° was obtained. The mandelate salt was then dissolved in 100 ml. of absolute ethanol and allowed to stand for 48 hours at room temperature while the resolved product slowly separated. It was found that separation could be materially hastened when a few crystals of D base (+)mandelate were added as seeds. The 630 mg. of crystalline product which was obtained in this manner melted at 145–147°. Recrystallization from 20 ml. of methanol raised the melting point to 151–152°. The salt was filtered after two hours. A final recrystallization yielded 430 mg. of salt with no change in melting point, $[\alpha]^{22}D$ +24.8° (*c* 1.37% in water).

Anal. Calcd. for $C_{18}H_{23}NO_7S$: C, 54.39; H, 5.83; N, 3.52. Found: C, 54.55; H, 5.68; N, 3.41.

The 430 mg. of resolved base mandelate salt was dissolved in 13 ml. of distilled water containing 0.8 ml. of 2 *N* hydrochloric acid. The aqueous solution containing the base hydrochloride was passed over a basic XE 98 column and the resolved base isolated from the eluates in the same manner as was the racemic base. The recrystallized product melted at 140–142°, $[\alpha]^{22}D$ +20° (*c* 2% in ethanol).

Anal. Calcd. for $C_{10}H_{15}NO_4S$: C, 48.96; H, 6.16; N, 5.71. Found: C, 49.10; H, 6.46; N, 5.78.

The above product was converted to the dichloroacetamide by heating with excess methyl dichloroacetate in ethanol solution for 1 hour on the steam-bath. The product was worked up in the usual manner.[6] The dichloroacetamide melted at 164–165°, $[\alpha]^{22}D$ +12.8° (*c* 2% in ethanol), $[\alpha]^{22}D$ −16.2° (*c* 11.3% in dimethylacetamide). The resolved product was twice as active in *in vitro* antibacterial

(1) R. A. Cutler, R. J. Stenger and C. M. Suter, THIS JOURNAL, **74**, 5475 (1952).

(2) C. M. Suter, S. Schalit and R. A. Cutler, *ibid.*, **75**, 4330 (1953).

(3) We are indebted to Dr. A. S. Schlingman, Mrs. Della Fox and Miss Mary Manning and co-workers for detailed antibacterial studies of these compounds. The antibacterial activity found independently by these workers is qualitatively and quantitatively in agreement with that reported by Cutler, *et al.*[1]

(4) Rohm and Haas Company.

(5) DL-*threo*-1-*p*-Methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol was prepared by the same method used by Suter, *et al.*[2]

(6) M. C. Rebstock, THIS JOURNAL, **72**, 4800 (1950).

tests as the racemate. These data are in agreement with the properties ascribed by Suter, *et al.*,[1] to the compound obtained by oxidation of D-*threo*-1-*p*-methylmercaptophenyl-1,2-dichloroacetamido-1,3-propanediol. The corresponding L-*threo*-1-*p*-methylsulfonylphenyl-2-dichloroacetamido-1,3-propanediol was prepared from the base obtained when (−)mandelic acid was used as the resolving agent.

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Observations on the Rate of Autoxidation of *d*-Limonene

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During the course of a more general investigation of the chemical behavior of *d*-limonene, we have had occasion to study the influence of several variables on its rate of autoxidation. The autoxidation of *d*-limonene from the standpoint of the products formed has been studied by several investigators[1]; *d,l*-carveol, *d,l*-carvone, and 1-methyl-4-isopropenyl-1,2-cyclohexanediol have been reported as reaction products. In our own work, we have isolated the above products as well as limonene-1,2-epoxide and an unsaturated hydrocarbon corresponding in molecular weight to a dimer of limonene. In order to isolate definite products from the autoxidation, it was necessary to first destroy peroxidic materials; this was accomplished by steam distillation of the autoxidation reaction mixtures or by treatment with aqueous sodium hydroxide. Even under the most favorable conditions, the identified autoxidation products accounted for only a small percentage of the limonene undergoing reaction; unworkable tars constituted the major reaction product in all cases. It has been observed previously[2] that the polymerization of *d*-limonene is accelerated by the process of autoxidation.

We were primarily interested in the influence of variables on the rate of autoxidation of *d*-limonene rather than on the products formed. Our technique was to measure the rate of oxygen-uptake by a standard sample of 5 ml. of carefully purified *d*-limonene using a carefully standardized experimental procedure. The course of the oxygen-uptake was similar to that observed for other alkene autoxidations[3]; at 71° there was observed a definite induction period lasting about 2 hours, followed by a period of rapid oxygen absorption for 6–8 hours, and finally a period of decreasing oxygen absorption. Hydroquinone was observed to greatly prolong the induction period and delay the attainment of maximum oxygen absorption, while such substances as manganous, ferrous, ferric and chromic stearates and iron phthalocyanine eliminated the induction and greatly increased the rates of oxygen absorption.

It is generally agreed that the process of hydrocarbon autoxidation is a free radical chain reaction involving hydroperoxides as initial products,[3] and that there should be a correlation between the rate of oxygen uptake and the hydroperoxide content of an autoxidation reaction mixture. We have found that the presence of catalytic amounts of either *d*-limonene hydroperoxide (introduced by exposure of the sample to air prior to the oxygen absorption measurements) or *t*-butyl hydroperoxide had the effects of virtually eliminating the induction period during autoxidation of limonene, hastening the attainment of a maximum oxygen absorption rate, and slightly increasing the maximum rate of oxygen absorption. Furthermore, we have demonstrated experimentally (Fig. 1), a definite correlation between the rate of oxygen absorption and the hydroperoxide content of an autoxidizing sample of *d*-limonene; the maximum hydroperoxide content was attained at about the same time that maximum absorption was observed, and both decreased thereafter.

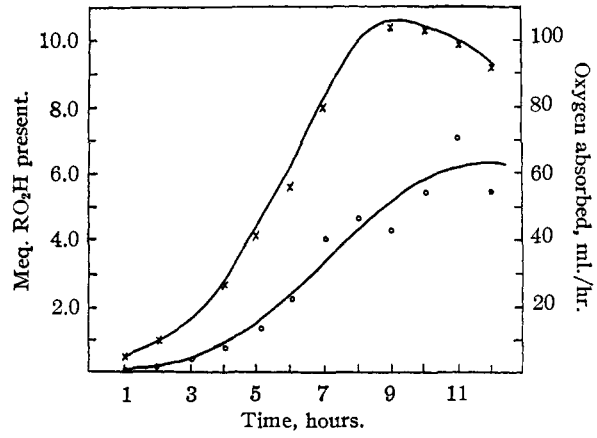

Fig. 1.—Correlation of rate of oxygen absorption of *d*-limonene and hydroperoxide content: O, RO_2H content; ×, rate of O_2 absorption.

The most interesting feature observed relating to the rate of autoxidation of *d*-limonene is a marked retardation by acids and by bases. The data are shown in Fig. 2. Since water itself exerts a retarding effect on oxygen uptake by *d*-limonene, comparison was made in each case with the oxygen uptake of a mixture of 5 ml. of limonene and 1 ml. of water. Various acids were shown to exert a retarding effect on the autoxidation of limonene, the magnitude of the effect increasing with the strength of the acid as measured by its dissociation constant. Thus, the order of various acids in retarding the oxygen absorption by limonene was observed to be: sulfuric > phosphoric > citric > benzoic > water. The base, sodium carbonate, exerted a similar retarding effect about equal in magnitude to that of citric acid at a comparable concentration. The general effect of acids and bases was to diminish the rate of maximum oxygen absorption and to delay the attainment of the maximum rate. This effect is to be contrasted with that of hydroquinone, which simply increases the induction period without serious effect on the magnitude of the maximum absorption rate. It appears quite probable that acids and bases exert their retarding effect on limonene autoxidation by

(1) See, for example, A. Blumann and O. Zeitschell, *Ber.*, **47**, 2323 (1914); R. Dupont, *Ind. Chim. belge*, **11**, 3 (1940); H. Schmidt, *Chem. Ber.*, **82**, 11 (1949).

(2) H. Staudinger and L. Lautenschläger, *Ann.*, **488**, 1 (1931).

(3) See, for example, C. E. Frank, *Chem. Revs.*, **46**, 155 (1950), and previous references there given.