sulfide was obtained from thietane and a reflux time of 185 hr with triethyl phosphite yielded 10% of triethyl thionophosphate.

Comparison of Reactivity of Chloromethylthiirane and 3-Chlorothietane.—A solution of chloromethylthiirane (4.0 g, 0.036 mole) and triphenylphosphine (9.5 g, 0.036 mole) in 100 ml of benzene was refluxed. Aliquots of 10 ml were taken at various times, the solvent was removed under vacuum, and the residue was chromatographed on an alumina column with cyclohexane and benzene. The weight of triphenylphosphine sulfide eluted from the column was converted into per cent yield. The same procedure was followed with 3-chlorothietane. The results are shown in Table I.

TABLE I

COMPARISON OF REACTIVITY OF 3-CHLOROTHIETANE AND CHLOROMETHYLTHIIRANE WITH TRIPHENYLPHOSPHINE IN REFLUXING BENZENE

3-Chlorothietane		Chloromethylthiirane	
Time, hr	% $(C_6H_5)_3PS$	Time, hr	% $(C_6H_5)_3PS$
8.5	19	9.5	77
22.5	26	22.25	82
46.5	40	46.25	84
60.5	49	70.25	81
84.5	58	94.25	85
163.5	72	162.25	85

Registry No.—Triphenylphosphine, 603-35-0; triethyl phosphite, 122-52-1; 3-chlorothietane, 6013-95-2; 3-hydroxythietane, 10304-16-2; chloromethylthiirane, 3221-15-6.

Equilibration and Acid Hydrolysis of Biotin Sulfoxides[1a,b]

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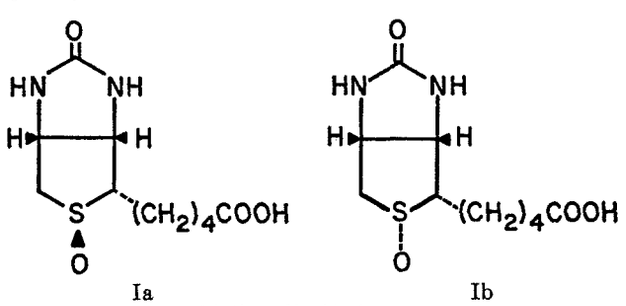
Biotin sulfoxides (Ia and b) have been considered to be intermediates in the microbiological degradation of biotin.[2] To supplement an understanding of the metabolism of these compounds, studies on the acid hydrolysis of biotin sulfoxides were carried out.



Ia Ib

biotin sulfoxide isomers

Whereas treatment with 1 *N* formic acid and 0.1 *N* hydrochloric acid did not affect biotin *d*-sulfoxide, partial epimerization of the sulfoxide grouping of the compound was obtained in 1 *N* hydrochloric acid (Figure 1). Acid-catalyzed racemization of other sulfoxides in 97% sulfuric acid[3] and in 12 *M* aqueous HCl–dioxane (1:2)[4] has been reported previously. Analogous results have been obtained by treatment of the *l*-sulfoxide under the same conditions. Equilibration of the *d*-sulfoxide follows clean pseudo-first-order kinetics in the beginning, with deviations starting after about 16 hr, mainly resulting from competition of hydrolysis reactions with the inversion. Under the conditions used the initial rate constants were $k_d = 1.38 \times 10^{-5}$ sec^{-1} for inversion of the *d*-sulfoxide and $k_l = 0.47 \times 10^{-5}$ sec^{-1} for the *l*-sulfoxide. The products formed showed R_f values which were in agreement with those found for biotin *l*-sulfoxide or biotin *d*-sulfoxide, respectively, in both solvent systems used (see Table I). The ratio of *l*-sulfoxide to *d*-sulfoxide was determined to be 3:1 after a reaction time of 66 hr, which is in good agreement with the value for the equilibrium ratio calculated from initial rate constants k_d and k_l. The reaction showed a remarkable specificity for catalysis by hydrogen chloride since both 1 *N* formic acid and 1 *N* sulfuric acid did not invert the configuration of biotin *d*-sulfoxide. This specificity is in agreement with similar results obtained by Mislow, *et al.*[4] These investigators postulated a mechanism for the HCl-catalyzed inversion of configuration of sulfoxides involving a symmetrical sulfur dichloride intermediate or transition state: $R_2SO + 2HCl \rightarrow R_2SCl_2 +$

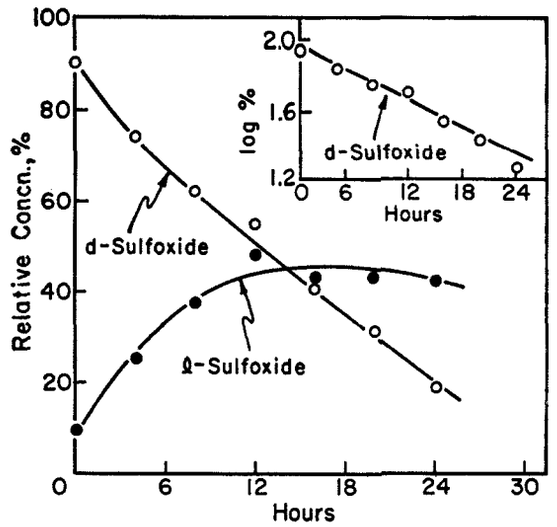


Figure 1.—Partial inversion of configuration of biotin *d*-sulfoxide (c_0 = 2 mg/ml of 1 *N* HCl, 100°, concentration given as % of radioactive material present).

TABLE I

PAPER CHROMATOGRAPHY OF BIOTIN ANALOGS

Compd	R_f values A[a]	B[b]
Biotin	0.67	0.68
Biotin sulfone	0.28	0.20
Biotin *d*-sulfoxide	0.40	0.45
Biotin *l*-sulfoxide	0.25	0.38
Diaminocarboxylic acid sulfate of biotin	0.13	0.51

[a] Water-saturated 1-butanol. [b] 1-Butanol–methanol–benzene–water (2:1:1:1).

(1) (a) Presented, in part, at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966; (b) supported, in part, by Research Grant AM-08721 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service; (c) recipient of a Fulbright Travel Scholarship.

(2) R. N. Brady, H. Ruis, D. N. McCormick, and L. D. Wright, *J. Biol. Chem.*, **241**, 4717 (1966).

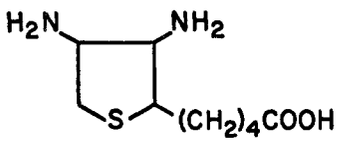
(3) S. Oae, T. Kitao, and Y. Kitaoka, *Chem. Ind.* (London), 291 (1961).

(4) K. Mislow, T. Simmons, J. T. Melillo, and A. L. Ternay, Jr., *J. Am. Chem. Soc.*, **86**, 1452 (1964).

H_2O. The same mechanism may be effective in the reactions described in this paper, although the considerably higher water concentration in the medium used in the present work may decrease the probability of formation of a dichloride intermediate.

A synthetic method was developed to obtain biotin *l*-sulfoxide in considerably better yield than possible with the method used up to now.[5] Biotin was oxidized with an equimolar amount of hydrogen peroxide and the resulting mixture of sulfoxides was treated with 1 *N* hydrochloric acid.

As was indicated above, hydrolysis of biotin sulfoxides can be observed to compete with racemization after longer reaction times. To increase the rate of hydrolysis, 2 *N* hydrochloric acid was used for further experiments. The major hydrolysis product was the diaminocarboxylic acid of biotin (3,4-diaminothiophane-2-pentanoic acid, II). To isolate this compound



II

and possibly other reaction products, hydrolysis products were separated on a Dowex-1 column after reaction times of 6, 12, and 24 hr. Typical elution patterns are shown in Figure 2. Small amounts of biotin were also formed in the reaction. Attempts were made to hydrolyze biotin and biotin sulfone under similar conditions, but both were much less susceptible to hydrolysis than were the sulfoxides; biotin gave less than 1% of its diaminocarboxylic acid after a reaction time of 12 hr. Upon hydrolysis, the diaminocarboxylic acid of biotin sulfoxide is probably formed and disproportionated to the diaminocarboxylic acid of biotin and several oxidation products. Several attempts were made to isolate and characterize some of the oxidation products, but the low yield of any of these prevented any success in this direction. Apparently disproportionations of sulfoxides under acidic conditions do not simply lead to the formation of the corresponding sulfide and sulfone, but result in a mixture of oxidation products. This also has been shown previously in the case of DL-methionine sulfoxide.[6] The unhydrolyzed biotin sulfoxide seems to disproportionate analogous to the diamino compound, but at a much lower rate, as could be shown by the isolation of biotin from the reaction mixture.

Experimental Section[7]

Materials.—*d*-Biotin was purchased from Nutritional Biochemicals Corp. Carbonyl-^{14}C-biotin (32.4 mcuries/mmole) was from Nuclear-Chicago. Carboxy-^{14}C-biotin (2.05 mcuries/mmole) was from Nuclear Research Chemicals. The *d*- and *l*-sulfoxides of carbonyl- and carboxyl-labeled biotin were made essentially following the method of Melville.[5] Carboxy-^{14}C-biotin sulfone was made according to the method of Hofmann, *et al.*[8] Carboxyl-^{14}C-diaminocarboxylic acid sulfate of biotin was made by hydrolysis of biotin according to Hofmann, *et al.*[8]

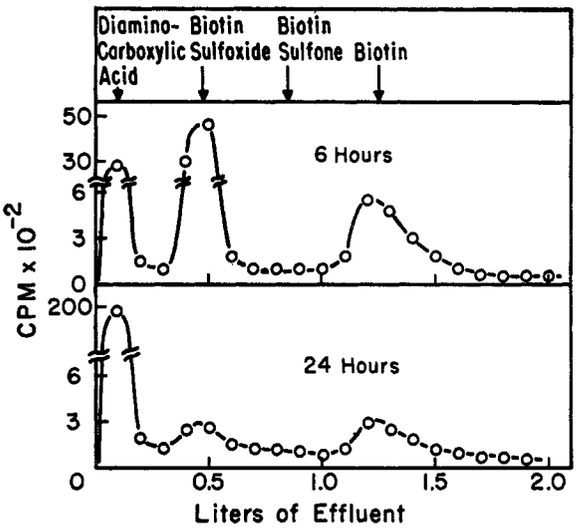


Figure 2.—Elution patterns from column chromatography of hydrolysis products of biotin *d*-sulfoxide on Dowex-1 (formate). Products were eluted with a linear gradient of water to 1 *M* ammonium formate. Retentions of several biotin analogs are given for comparison.

Racemization Experiments. 1. 1 *N* Hydrochloric Acid.—To 8 mg of carbonyl- or carboxy-^{14}C-biotin *d*-sulfoxide (0.08 μcuries/mg for carbonyl- and 1 μcurie/mg for carboxyl-labeled material) or carbonyl-^{14}C-biotin *l*-sulfoxide (0.08 μcurie/mg) was added 4 ml of 1 *N* HCl. The solution was heated under N_2 to 100° in a 10-ml, three-necked flask equipped with gas inlet tube, thermometer, and reflux condenser. Reaction times were between 8 and 24 hr, and 10-μl samples were taken at 1-, 2-, or 4-hr intervals and spotted on paper for chromatography.

2. 1 *N* Sulfuric Acid.—Experiments were carried out with carboxyl-^{14}C-biotin *d*-sulfoxide for 10 hr, taking samples every 2 hr, under the above conditions, but with 1 *N* H_2SO_4 instead of HCl.

Paper Chromatography.—Chromatography was carried out on Whatman No. 1 paper. The chromatograms were developed in descending water-saturated 1-butanol or 1-butanol–methanol–benzene–water (2:1:1:1, upper phase). The papers were counted in a radiochromatogram scanner and relative amounts of compounds were determined by means of a planimeter.

Synthesis of Biotin *l*-Sulfoxide.—Biotin (1 g) was dissolved in 100 ml of warm glacial acetic acid. The solution was cooled to room temperature and 0.5 ml of 30% H_2O_2 was added. The solution was kept at room temperature for 24 hr and then the solvent was evaporated *in vacuo*. The residue was dissolved in 100 ml of 1 *N* HCl and the solution refluxed under N_2 for 10 hr. The solvent was then evaporated *in vacuo*, the residue was dissolved in water, and the solution was treated with Norit, filtered, and evaporated to dryness. The crude sulfoxide was recrystallized from water to yield 750 mg of material melting at 239–243° dec (lit.[5] mp 238–241° dec): $[\alpha]^{20}D$ −39.4° (*c* 1.00, 0.1 *N* NaOH) [lit.[5] $[\alpha]^{20}D$ −39.5° (*c* 1.01, 0.1 *N* NaOH)].

Hydrolysis Experiments. Biotin *d*-Sulfoxide.—Carboxy-^{14}C-biotin *d*-sulfoxide (500 mg, 0.01 μcurie/mg) in 10 ml of 2 *N* HCl was refluxed under N_2 for 6, 12, and 24 hr in subsequent experiments. The acid was evaporated *in vacuo*, the products were than dissolved in 15 ml of water, and the solutions were neutralized with 5% ammonia and applied to a 2 × 35 cm column of 100–200 mesh Dowex-1-X8 (formate) for elution with a linear gradient from 1 l. of water to 1 l. of 1 *M* ammonium formate. Fractions (12.5 ml) were collected, and radioactivity was determined in 0.2-ml aliquots. Bray's solution[9] was used for scintillation counting. Fractions 3–10 were then combined, the volume was reduced *in vacuo*, the concentrate was applied to a 2 × 35 cm column of 100–200 mesh Dowex-50-X8 (H^+), and material was eluted with 1 *M* NH_4OH. The fractions containing radioactive material were combined, evaporated to dryness *in vacuo*, and dissolved in 1 ml of water. The solutions were acidified to pH 2 with 1 *N* sulfuric acid and, after addition of

(5) D. B. Melville, *J. Biol. Chem.*, **208**, 495 (1954).

(6) N. F. Floyd, M. S. Cammaroti, and Th. F. Lavine, *Arch. Biochem. Biophys.*, **102**, 343 (1963).

(7) Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Analyses were by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(8) K. Hofmann, D. B. Melville, and V. du Vigneaud, *J. Biol. Chem.*, **141**, 207 (1941).

(9) G. A. Bray, *Anal. Biochem.*, **1**, 279 (1960).

methanol, gave a crystalline precipitate which was collected and recrystallized from water–methanol (yields: 6 hr, 6 mg; 12 hr, 30 mg; 24 hr, 130 mg), mp 240–250° dec (diaminocarboxylic acid sulfate of biotin lit.[5] mp 245–255°). The infrared spectra were completely identical with that of an authentic sample of diaminocarboxylic acid sulfate of biotin.

Anal. Calcd for $C_9H_{20}N_2O_6S_2$: C, 34.16; H, 6.37; N, 8.85; S, 20.27. Found: C, 34.29; H, 6.47; N, 8.69; S, 20.21.

In a similar way, fractions 90–105 of the 6-hr experiment were applied to a Dowex-50 column and eluted with 1 *M* NH_4OH, the solvent was evaporated, and the product was recrystallized from water. A product (30 mg) melting at 228–230° was obtained (biotin lit.[10] mp 230–232°). The infrared spectrum of this product was identical with that of an authentic sample of biotin.

Biotin.—Carboxyl-^{14}C-biotin (200 mg, 0.01 μcurie/mg) in 4 ml of 2 *N* HCl was refluxed for 12 hr under N_2. The material was treated further as given for hydrolysis of biotin *d*-sulfoxide. The elution pattern of the Dowex-1 column showed that less than 1% of the biotin was hydrolyzed to its diaminocarboxylic acid.

Biotin Sulfone.—Carboxyl-^{14}C-biotin sulfone (80 mg, 0.01 μcurie/mg) in 4 ml of 2 *N* HCl was refluxed under N_2 for 12 hr. The solvent was evaporated and the remaining material was recrystallized from water. Biotin sulfone (71 mg) of unchanged melting point was recovered.

Registry No.—Biotin, 58-85-5; biotin sulfone, 786-79-8; biotin *d*-sulfoxide, 10406-89-0; biotin *l*-sulfoxide, 10406-90-3; diaminocarboxylic acid sulfate of biotin, 10349-21-0.

(10) V. du Vigneaud, K. Hofmann, D. B. Melville, and J. R. Rachele, *J. Biol. Chem.*, **141**, 763 (1941).

The Iodine–Cycloheptatriene System[1,2]

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Oxidation of cycloheptatriene with iodine in ether solvent affords excellent yields of tropenium salts; iodine alone gives tropenium triiodide of high purity; and if mercuric iodide is also present tropenium triiodomercurate or pentaiododimercurate salts are obtained.

Dauben and Harmon[3] have reported the preparation of a tropenium triiodomercurate[4] by reaction of *t*-butyl iodide, mercuric iodide, and cycloheptatriene in ether. They proposed a mechanism involving ionization of *t*-butyl iodide by mercuric iodide, followed by hydride transfer from cycloheptatriene to the trimethylcarbonium ion so formed, but were unable to isolate isobutane from the reaction. We now find that this reaction is not an ionization–hydride transfer process, but proceeds *via* oxidation of cycloheptatriene by iodine produced by photochemical decomposition of *t*-butyl iodide. Mixtures of *t*-butyl iodide, mercuric iodide, and cycloheptatriene react to a very limited extent in the dark, but reaction proceeds quantitatively when the solution is exposed to sunlight. A series of controlled-atmosphere experiments confirms that no appreciable reaction occurs in the absence of light or oxygen, and that light is the more potent catalyst (Table I).

TABLE I

REACTIONS OF CYCLOHEPTATRIENE WITH *t*-BUTYL IODIDE AND MERCURIC IODIDE IN ETHER UNDER CONTROLLED CONDITIONS[a]

Reaction	Light	Oxygen	Water	% yield of $C_7H_7^+$
1	0	0	0	0.39
2	Trace	0	0	1.04
3	0	+	0	1.63
4	+	0	0	5.50
5	+	+	0	17.5[b]

[a] Identical concentrations, temperature, and times. [b] Yield (5)/yield (1) = 45.

We have recently found that tropenium ion is reduced to cycloheptatriene by hydrogen iodide in nonpolar solvents[5] with concurrent formation of iodine; this reduction was unexpected in light of the oxidations noted above. These conflicting observations are in accord with the existence of a mobile oxidation–reduction equilibrium (eq a). In ether solvent the reaction

$$C_7H_8 + I_2 = C_7H_7^+ + I^- + HI \qquad (a)$$

would normally be shifted to the right by formation of insoluble tropenium salts; however, treatment of tropenium ion with excess hydrogen iodide would drive the reaction to the left by mass action. In contrast to tropenium ion, hydroxytropenium ion is not reduced by hydrogen iodide; presumably resonance interaction of nonbonding oxygen electrons with the π system of the ring lends sufficient stabilization to prevent such reduction.

In a solvent where tropenium salts are soluble, the equilibrium should be directly observed. In methylene chloride solution the reaction between iodine and cycloheptatriene proceeds rapidly to produce a limited amount of tropenium ion; after this time no further change occurs. The reaction is more complex in methylene chloride than is represented by eq a, since an additional, colorless, iodine-containing species is also present (see the Experimental Section). The nature of this species is not known at this time; one possibility is a diene–diiodide similar to the diene–dibromide which occurs as an intermediate in the oxidation of cycloheptatriene by bromine.[7,8] Whatever the nature of this colorless compound, its formation does not irreversibly remove cycloheptatriene from the reaction system, since addition of mercuric iodide to methylene chloride solutions of cycloheptatriene and iodine greatly increases the yield of tropenium ion by formation of insoluble, complex mercuric iodide salts.

(1) Work supported by the Petroleum Research Fund and the National Science Foundation.

(2) Reported in part at the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 9, 1964.

(3) (a) H. J. Dauben, Jr., and K. M. Harmon, 134th National Meeting of the American Chemical Society, Chicago, Ill., Sept 8, 1958, Abstracts, p 35P; (b) Ph.D. Thesis of K. M. H., University of Washington, 1958; *Dissertation Abstr.*, **19**, 1563 (1959).

(4) This material was a less stable, lower melting (157°) crystal modification of that reported herein; on long standing it slowly changed to the higher melting form.

(5) Hydrogen iodide does not reduce tropenium ion in sulfuric acid, in accord with the conclusion of Deno[6] that in this solvent hydrogen iodide only reduces carbonium ions with a pK_{R+} less than −5; pK_{R+} for tropenium ion is 4.75.

(6) N. C. Deno, *et al.*, *J. Am. Chem. Soc.*, **84**, 4713 (1962).

(7) W. von E. Doering and L. H. Knox, *ibid.*, **76**, 3203 (1954); **79**, 352 (1957).

(8) H. J. Dauben, Jr., and D. L. Pearson, unpublished work in Ph.D. Thesis of D. L. P., University of Washington, 1955; *Dissertation Abstr.*, **15**, 978 (1955).