TABLE II

EXTINCTION COEFFICIENTS AND EQUILIBRIUM CONSTANTS AT 25° FOR HALOGEN COMPLEXES OF BENZENE AND *m*-XYLENE

Benzene	Cl_2	Br_2[a]	I_2[b,c]	ICl[d]
ϵ_c	9090	13400	15400	9900
K	0.33	1.04	1.72	4.76
m-Xylene				
ϵ_c	6340	10100	.....	9000
K	0.62	2.16	.....	16.0

[a] Ref. 4. [b] Ref. 3(b). Data taken at room temperature. [c] Cromwell and Scott, THIS JOURNAL, 72, 3825(1950), report a refinement in the mathematical treatment of the spectrophotometric data for iodine–benzene systems which leads to somewhat different values than those listed here. [d] Ref. 5.

K values. For this reason plans to investigate an extended series of aromatic–chlorine complexes by this method were abandoned.

While no uniform trends in ϵ_c values with changes in the halogen were observed for the benzene and *m*-xylene complexes, the K values increased in the order $Cl_2 < Br_2 < I_2 < ICl$. This series is regarded as representative of the relative acid strengths of the halogens since the role of the aromatic nucleus in forming the complex seems best represented as that of an electron donor.[3,4,5]

The authors have postulated[5] a tentative structure for these complexes in which the axis of the halogen molecule has been located on the sixfold symmetry axis of the ring. The proposed electronic structure is based on the many resonance structures corresponding to $C_6H_6^+ \; :\ddot{X}:\ddot{X}:^-$ In the case of the chlorine complexes an ion pair structure $C_6H_6^+ :\ddot{Cl}: \; :\ddot{Cl}:^-$ may be preferable since it avoids a central chlorine atom with ten electrons in its valence shell.[6] Since however the complexes form even at high dilution in the nonpolar carbon tetrachloride medium the expanded halogen valence shell picture seems attractive. The magnitude of the K values for the chlorine complexes is indeed considerably smaller than those for the iodine containing complexes for which the octet rule restrictions very likely do not apply.[7]

(6) *Cf.* (a) Buckles, Hausmann and Wheeler, *ibid.*, **72**, 2494 (1950). (b) Williams, *Trans. Faraday Soc.*, **37**, 761 (1941).

(7) See, for example, Luder and Zuffanti, "The Electronic Theory of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 32.

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Lanthionine in Subtilin

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Subtilin,[2] a peptide with antibiotic activity produced by a particular strain of *Bacillus subtilis*, contains 4.2% sulfur but no disulfide or sulfhydryl groups as judged by nitroprusside test with and without NaCN treatment. Analyses of subtilin hydrolyzates for cystine and for methionine were negative.

Subtilin gives a strong and rapid plumbite test for labile sulfur. Heating subtilin with concentrated sulfuric acid at 100° converts approximately 40% of the sulfur-bearing constituents to cystine as measured by the Sullivan[3] method.

meso-Lanthionine was isolated from subtilin hydrolyzates by fractionation with phosphotungstic acid and later by the method of Horn, Jones and Ringel,[4] in a yield accounting for approximately 10% of the sulfur. It was identified through the benzoyl and carbobenzoxy derivatives, degradation of the benzoyl derivative with Raney nickel, and X-ray powder photographs. The literature contains divergent reports of the melting points of the benzoyl derivatives of both *dl*- and *meso*-lanthionine. The melting points of benzoyl derivatives of our preparations, both from alkali-treated hair and from subtilin, have been found to vary widely with the rate of heating and to exhibit a previously unreported preliminary melting and resolidification at 110–115°. Refluxing of dibenzoyl-*meso*-lanthionine in 80% alcohol with Raney nickel as described by Mozingo, Wolf, Harris and Folkers[5] for general catalytic desulfurization caused a reduction of the benzenoid ring to give cyclohexylformyl-*dl*-alanine rather than the expected benzoyl-*dl*-alanine. Similar treatment of lanthionine isolated from subtilin hydrolyzates gave the same product. Benzoyl-*dl*-alanine was also reduced to cyclohexylformylalanine under the same conditions. A short digestion at low temperature was used in the preparation of the nickel catalyst.

Lanthionine has been isolated from alkali-treated hair and several other proteins after alkali treatment[4] and has also been found in small amounts in the tips of virgin wool by paper chromatography.[6] However, demonstration of its presence as a major constituent of protein material not previously conditioned with alkali is uncommon. The *p*H of the *B. subtilis* cultures from which subtilin was recovered remained below 7 during both incubation and isolation.

Experimental

Analyses for Cystine, Methionine and Sulfhydryl.—Concentrated solutions of subtilin gave negative nitroprusside tests for SH before and after reduction with sodium cyanide and with and without urea as a denaturing agent. Subtilin was hydrolyzed by refluxing overnight as a 20% solution in 6 *N* HCl. Analysis for cystine by the Sullivan[3] method and for methionine by the Sullivan–McCarthy[7] method showed zero or trace amounts of these amino acids, while recoveries of cystine and methionine added to the hydrolyzates were satisfactory. Heating a solution of 200 mg. of subtilin in 1 ml. of concentrated sulfuric acid for 15 hours on a steam-bath formed 5.8% cystine (equivalent to 38% of the sulfur of the peptide) as measured by the Sullivan method. Van Veen and Hyman[8] have reported that djenkolic acid is cleaved to cysteine and formaldehyde by similar treatment. Later trials with *meso*-lanthionine indicated that it may be converted almost quantitatively to cystine (as measured

(1) Quartermaster Food and Container Institute, Chicago, Illinois.

(2) Dimick, Alderton, Lewis, Lightbody and Fevold, *Arch. Biochem.*, **15**, 1 (1947).

(3) Sullivan, Hess and Howard, *J. Biol. Chem.*, **145**, 621 (1942).

(4) Horn, Jones and Ringel, *ibid.*, **144**, 87 (1942).

(5) Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

(6) Consden, Gordon and Martin, *Biochem. J.*, **40**, 580 (1946).

(7) McCarthy and Sullivan, *J. Biol. Chem.*, **141**, 871 (1941).

(8) Van Veen and Hyman, *Rec. trav. chim.*, **54**, 493 (1935).

by the Sullivan method) by heating as a 10% solution in concd. sulfuric acid for 10 minutes at 180°. Only very slight browning of the heated sulfuric acid solution occurred.

Isolation of Lanthionine from Subtilin.—When 6 *N* HCl hydrolyzates of subtilin were fractionated with phosphotungstic acid under the conditions of Van Slyke, *et al.*,[9] for optimum separation of the basic from the monoamino acids, the sulfur was about equally distributed between the precipitate (basic amino acids) and the filtrate (monoamino acids). Resuspension of the precipitate and chilling of the filtrate yielded phosphotungstates with a molar ratio for N:S of 2.3 with essentially all of the nitrogen in the amino form. After removal of the phosphotungstic acid several recrystallizations by solution in ammonium hydroxide and neutralization with acetic acid yielded a small amount of a substance tentatively identified as lanthionine by N analysis of it and its benzoyl derivative (m. p. 192–194°).

Subsequent isolations were made by the method of Horn, Jones and Ringel[4] (without prior alkali treatment of the subtilin). From 10 g. of active subtilin 345 mg. of recrystallized lanthionine (accounted for 10% of the S) were isolated; another similar isolation from 10 g. of the peptide yielded 300 mg. of the three times crystallized lanthionine. A third isolation from 120 g. of subtilin samples of low bacteriostatic activity gave 2.2 g.

Anal. Calcd. for $C_6H_{12}SN_2O_4$: N, 13.46; S, 15.47; C, 34.6; H, 5.81. Found: N, 13.4; S, 15.3; C, 34.2; H, 5.85; $[\alpha]^{24}D = 0 \pm 0.6°$ (*c*, 10 in 2.4 *N* NaOH).

The dibenzoyl derivative was prepared, m. p. 193–195°, with slow decomposition and a preliminary melting and solidification at 100–115°. The m. p. of dibenzoyl-*meso*-lanthionine is variously reported at 205–206°,[10] 210–211°[11] and 201[12] without specification of a preliminary melting point of decomposition. The dibenzoyl derivatives of our *meso*-lanthionine preparations both from subtilin and from Na_2CO_3-treated hair by the method of Horn, Jones and Ringel[4] (recrystallized several times from NaCN solution according to the method of Brown and du Vigneaud[13] for elimination of disulfide) are lower than literature values, vary with the rate of heating, and exhibit two melting points as mentioned above. Repeated recrystallizations of either the dibenzoyl derivatives or the untreated lanthionine have not changed this behavior. In baths of constant temperature, melting time varies from 24 min. at 180° to 5 seconds at 210°. Quite reproducible m. p.'s may be obtained by inserting the capillary in a bath with a 6°/min. rise at 185–190° under which conditions the melting points of the dibenzoyl derivatives of lanthionine from subtilin and of *meso*-lanthionine from alkali-treated hair, singly or mixed are 198–200°.

Anal. Calcd. for $C_{20}H_{20}O_6N_2S$: N, 6.73. Found: N, 6.71.

The dicarbobenzoxy derivative was prepared, m. p. 139–140°; reported[11] 138–140°.

Anal. Calcd. for $C_{22}H_{24}SN_2O_8$: N, 5.88. Found: N, 5.84.

A mixed melting point with dicarbobenzoxy-*meso*-lanthionine (m. p. 140–140.5°) from alkali-treated hair was 140–140.5°.

Hydrogenolysis with Raney Nickel.—A solution of 280 mg. of dibenzoyl-*meso*-lanthionine from alkali-treated hair in 80% alcohol was refluxed with about 10 g. of Raney nickel (prepared by the method of Mozingo[14] with a digestion period of 1 hour at 70°) by the method of Mozingo, *et al.*,[5] the product (160 mg.) melted at 176–177°. Recrystallization by solution in *N* NaOH, filtration, and acidification with HCl gave 120 mg., m. p. 176–177°.

Anal. Calcd. for $C_{10}H_{17}O_3N$: C, 60.3; H, 8.54; N, 7.04. Found: C, 60.1; H, 8.54; N, 6.99.

This compound was identified as cyclohexaneformyl-*dl*-alanine (reported m.p. 171–171.5°[15]) by synthesis from cyclohexane carbonyl chloride and *dl*-alanine.

The ethyl ester was prepared by refluxing in absolute ethanol containing 1% by volume of 11 *N* aq. HCl, m.p. 77–78°; reported 77–78°.[16]

Anal. Calcd. for $C_{12}H_{21}O_3N$: N, 6.17. Found: N, 6.08.

The same treatment of lanthionine from subtilin gave the same product in 60% yield, m.p. 176–177°.

Anal. Calcd. for $C_{10}H_{17}O_3N$: C, 60.3; H, 8.54; N, 7.04. Found: C, 60.4; H, 8.59; N, 7.00.

Refluxing benzoyl-*dl*-alanine with Raney nickel under similar conditions gave the same product in 71% yield.

X-Ray Powder Diagrams.—X-Ray powder diagrams of *meso*-lanthionine isolated from alkali-treated hair by the method of Horn, Jones and Ringel,[4] *meso*-lanthionine from active subtilin, and *meso*-lanthionine from low-potency subtilin were all identical and differed from a powder diagram of *dl*-lanthionine from alkali-treated hair.

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(14) *Org. Syntheses*, **21**, 15 (1941).
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The Action of Direct Current on Aqueous Solutions of S-Benzyl- and S-1-Naphthylmethylthiuronium Chlorides

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While widely studied in other connections, the S-aralkylthiuronium salts have never been electrolyzed. To gain information on this point, we have studied the effect of direct current on aqueous solutions of S-benzyl- and S-1-naphthylmethylthiuronium chlorides.

When a dilute aqueous solution of S-benzylthiuronium chloride is electrolyzed using platinum electrodes, hydrogen is evolved at the cathode and oxygen at the anode in a volume ratio 2:1. Further, the cathode becomes rapidly coated with a white solid characterized as S-benzylthiourea. The acidity which builds up during electrolysis prevents further deposition of S-benzylthiourea after a short period. An aqueous solution of S-1-naphthylmethylthiuronium chloride behaved similarly, S-1-naphthylmethylthiourea precipitating over the cathode.

Since it is well known[1] that alkali liberates the free base when acting on solutions of S-benzylthiuronium salts, it is reasonable to formulate our electrode processes as

Cathode: $2H_2O + 2e^- \rightarrow 2OH^- + H_2$

$$2[PhCH_2S{-}C(NH_2)_2]^+ + 2OH^- \rightarrow 2PhCH_2S{-}\overset{\overset{NH}{\|}}{C}{-}NH_2 + 2H_2O$$

Anode: $3H_2O \rightarrow 2H_3O^+ + {}^1/_2O_2 + 2e^-$

In an analytically inadequate paper Bernthsen and Klinger[2] claim the preparation of S-benzylthiourea, m. p. 70–71°, by action of alkali on the hydrochloride. Werner[1] repeated this, reporting the base, unpurified but analyzing correctly, to melt at 88°. On recrystallization of the 88° product from ethanol–water mixtures, the 70–71°

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(2) A. Bernthsen and H. Klinger, *Ber.*, **12**, 575 (1879).