TABLE I

PER CENT x INTENSITY IN THE ABSORPTION BANDS OF $\alpha,\beta,\gamma,\delta$-TETRAPHENYLPORPHINE[a]

Band	x intensity based on uncorrected fluorescence data, %	x intensity based on corrected fluorescence data, %[b]
I	70	100
II	45	55
III	27	22
IV	46	57
SI	54	71
SII	35	37

[a] Derived from data of ref. 7a. [b] Corrected as suggested in ref. 15, using a "randomization factor" of 0.44.[16]

Although detailed quantitative analysis based on simple inspection of reflection spectra is difficult except in favorable cases, the molecular dichroic ratios listed in Table I seem consistent with the crystal dichroism noted in Fig. 1, and certainly the largely y-character of band III is in accord with the appearance of the R_{min} curve.

If, accepting the usual interpretation of the porphine spectrum, one takes bands I and II as one electronic transition and bands III and IV as another, and further assumes that bands I and III indicate the intrinsic polarizations, one is now led to assign the first TPP transition x and the second y. Figure 1 and Table I also provide confirmation that the Soret absorption contains two distinct regions of polarization, that of low energy being x and that of high energy y.

The mixed polarizations of bands II, III, and IV indicate that, contrary to the commonly held view, each of these bands is not a single vibronic component, since this latter situation would require unique polarizations. The complex nature of these bands, which has been noted by earlier workers,[7b,17] is also supported by the additional structure that appears in low temperature solution absorption spectra.[18]

This work will be reported in more detail at a later date, as will emission and single crystal absorption work currently being undertaken. It is also intended to extend these investigations to include selected metal porphyrins.

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(19) National Institutes of Health Predoctoral Fellow.

STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT 06520

BASIL G. ANEX
ROBERT S. UMANS[19]

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Introduction of Covalent Cross-Linkages into Lysozyme by Reaction with α,α'-Dibromo-p-xylenesulfonic Acid

Sir:

Extensive studies on the cross-linking of proteins have been carried out. Fibrous proteins[1-4] and globular proteins[5-7] have been subjected to cross-linking. In no case were the reagents entirely satisfactory from the point of view of determining intramolecular distances under conditions simulating in part the conditions the proteins are exposed to in their native milieu.

The concept of using a "solubilized" bifunctional reagent of known dimensions was exploited first with various naphtholic and phenolic disulfonyl chlorides[8-10] and fluorides.[11] It was demonstrated that such compounds can react with lysozyme under conditions of homogeneity and that intramolecular bonding had occurred.[12] A tentative assignment of certain intramolecular distances was made.[10,12]

We wish to report here the synthesis and use of α,α'-dibromo- and α,α'-diiodo-p-xylenesulfonic acid (DBX and DIX). These and their S^{35}-labeled counterparts were synthesized by a variation of the procedures of Karslake and Huston,[13] and of Schmid and Karrer.[14]

The tritiated material was prepared by exposure of the unlabeled material to tritium by the Wilzbach procedure.[15] DBX (500 γ/ml.) was allowed to react with lysozyme (250 γ/ml. in 0.1 M borate buffer, total volume 1000 ml.) at 37° for 48 hr. at pH 9.1. The treated lysozyme was separated from unreacted DBX on a column of Sephadex G-25 using 0.1 M acetic acid as eluent and was found to emerge as a single peak.

It was homogeneous by ultracentrifugal analysis with essentially the same molecular weight as the native lysozyme. The enzymic activity was not diminished by a significant amount.

Two peaks were apparent by analysis by the Tiseluis technique, neither of which corresponded to lysozyme itself. The relative amount of material in each peak was 70 and 30%. The difference in mobilities between lysozyme and the peak nearest it in mobility was essentially equal to the difference between the mobilities of the two reacted species. This suggests that one and two residues of DBX have been introduced into lysozyme changing the charge on the molecule by two equal increments. Since the peptides A and B were of about equal radioactivity it would appear that all of the lysozyme had reacted with at least one DBX at one of two sites and a smaller fraction had reacted to give the least cationic species (30% of the material) seen in the electrophoretic analysis.

Examination of the tryptic digest of the lysozyme treated with performic acid by peptide mapping revealed some deletions and three additions (peptides A, B, and C) when compared to the lysozyme not exposed to DBX. The new peptides were radioactive when either DBX-S^{35} or DBX-H^3 were used.

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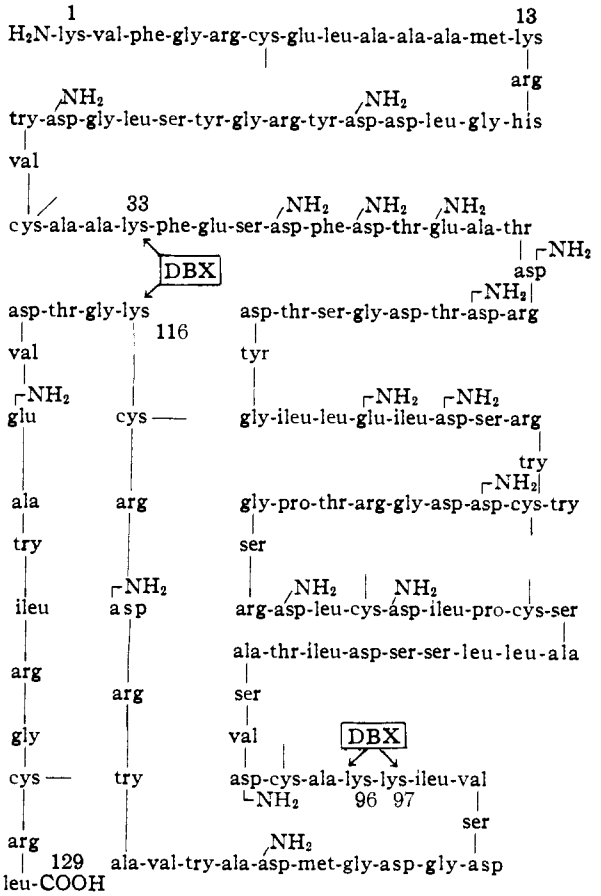

Fig. 1.—Schematic representation of points of attachment of α,α'-dibromo-*p*-xylenesulfonic acid.

Further purification of the labeled peptides was then followed by removal of the cross-linking residues under conditions used to hydrogenolyze benzyl amines.[16]

TABLE I

QUALITATIVE ANALYSIS OF PEPTIDES INVOLVED IN CROSS LINKAGES

Peptide	Cross linkage,[a] lysine residue	Amino acids
A_{11}	96 → 97	Calcd.: ala, arg, asp, gly, leu, met, ser, val
		Found: ala, arg, asp, gly, ileu,[b] met, ser, val
A_{12}	96 → 97	Calcd.: lys
		Found: lys
A_{13}	96 → 97	Calcd.: ala, asp, cys, ileu, leu, lys, pro, ser, thr, try, val
		Found: ala, asp, cys, ileu, leu, lys, pro, ser, thr, . . .,[c] val
B_{11}	33 → 116	Calcd.: ala, asp, cys, gly, leu, lys, ser, try, tyr, val
		Found: ala, asp, cys, gly, leu, lys, ser, . . .,[c] tyr, val
B_{12}	33 → 116	Calcd.: ala, arg, asp, glu, phe, ser, thr
		Found: ala, arg, asp, glu, phe, ser, thr
B_{21}	33 → 116	Calcd.: cys, lys
		Found: cys, lys
B_{22}	33 → 116	Calcd.: ala, arg, asp, glu, gly, ileu, thr, try, val
		Found: ala, arg, asp, glu, gly, ileu, thr, . . .,[c] val

[a] According to Canfield's sequence.[16] [b] Analysis ambiguous with respect to differentiating between leucine and isoleucine. [c] Acid hydrolysis left no detectable tryptophan residues.

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Peptide A afforded only one nonradioactive peptide (A_1) upon hydrogenolysis and peptide B gave two nonradioactive peptides (B_1 and B_2). Peptide C was present in such small amounts that it was neglected. Peptide A_1 upon trypsin digestion yielded three new peptides (A_{11}, A_{12}, and A_{13}) the analysis of which (Table I) showed that they were derived from amino acids 74 to 112 in the primary sequence announced by Canfield[17] (*cf.* Fig. 1). The three tryptides in this region correspond to Canfield's T_{11}, T_{12}, and T_{13}. Peptides B_1 and B_2 upon tryptic digestion each led to two new peptides (B_{11}, B_{12}, B_{21}, and B_{22}), respectively. These were analyzed and shown to correspond to Canfield's tryptides T_6, T_7, T_{15}, and T_{16}, respectively (see Fig. 1 and Table I). It follows, then, that DBX introduced cross-links into lysozyme and in particular between the ϵ-amino groups of lysine residues 96–97 and 33–116. The link between residues 33 and 116 corresponds to an assignment made by Herzig, *et al.*,[12] for phenol-2,4-disulfonyl chloride, a reagent of nearly identical span. The possible significance in the difference in patterns of cross-linking by reagents of nearly identical dimensions of greatly different reactivities will be discussed elsewhere.[18]

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(18) We acknowledge the generous support provided by the National Science Foundation (G-9794 and G-16161) and the American Cancer Society (In-79 and P-343) for this research.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CINCINNATI
CINCINNATI 21, OHIO

C. B. HIREMATH
RICHARD A. DAY

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Radical Scavenging in the Radiolysis of Liquid Methanol–Benzene Mixtures

Sir:

Radiolysis of liquid methanol containing increasing concentrations of benzene results in a sharp decrease in both hydrogen[1] and ethylene glycol[2] yields similar to that observed for the hydrogen yield in cyclohexane–benzene mixtures.[3] This phenomenon has been attributed to radical scavenging[1] or to energy transfer.[3] An examination of the radiolysis products of the cyclohexane–benzene system[4] has shown that phenylcyclohexane is formed with a *G*-value which reaches a maximum at ≈10% v./v. benzene, and then falls linearly with benzene concentration. It was also found that the *G*-values of both cyclohexylcyclohexene and bicyclohexyl show a sharp initial decrease with benzene concentration. Burr and Goodspeed have[5] recently discussed the formation of phenylcyclohexane and "polymers" from the radiolysis of benzene–cyclohexane mixtures and have indicated the importance of radical-scavenging mechanisms.

We now wish to report analogous observations for the methanol–benzene system. A number of radiolysis products have been separated and identified including

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