[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Infrared Spectra of Some Proteins and Related Substances

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Though the infrared spectra of proteins have been examined by several groups of investigators,[1-6] the results reported either have been limited to the region below 4 μ, or have been carried out on samples of questionable homogeneity. The recent availability of several proteins in crystallized form has made it possible to obtain data, over a wide spectral region, on samples of high purity. With these homogeneous samples, it has been feasible also to examine profitably the spectra of synthetic complexes between proteins and small ions.

Experimental

Infrared absorption spectra were obtained with the Beckman Infrared Spectrophotometer, model IR2. Water from a thermostat at 25° was circulated through the instrument, which was operated in a room with a relative humidity below 45%. All reported absorptions were recorded manually, though some data were obtained also with an automatic recorder.

Spectra were obtained for these substances in the solid state by deposition of an appropriate solution on a supporting plate and evaporation in a vacuum desiccator containing phosphorus pentoxide. The most successful supports for the films were silver chloride plates made by the Harshaw Chemical Company. These slides of about 1 mm. thickness transmit over 80% of the incident radiation at least up to 10 μ. Satisfactory spectra were obtained also with polythene sheets of about 0.01 mm. thickness, but this material was less convenient because it has steep absorption bands at 3.45 and 6.90 μ due to C–H vibrations.

Samples of tyrocidine and gramicidin, kindly supplied by the Wallerstein Company, were converted to films by dissolving about 10 mg. of solid in 0.5 cc. of 95% ethanol, depositing the solution on the silver chloride plate, and allowing the alcohol to evaporate. The removal of any traces of water from the film was insured by drying the plate over phosphorus pentoxide.

A similar procedure was used in the preparation of a film of salmine, except that water was used as solvent. The salmine sulfate was supplied generously by Eli Lilly and Company. The polylysine film also was deposited from a water solution, but on a polythene base, because the peptide was obtained in the form of the hydroiodide, which attacked the silver chloride plates. We are indebted to Dr. E. Katchalski for the sample of this synthetic polypeptide.

Cytochrome c was purchased from the Treemond Company, which specified a purity of 97% as determined by spectrophotometric assay. The film in this case also was prepared by evaporation from an aqueous solution.

Lysozyme, pepsin and bovine serum albumin were crystallized samples purchased from Armour and Company. All were deposited as films from aqueous solution, but for the first two proteins the solvent had to contain an electrolyte. Solutions with sodium chloride were used with pepsin, phosphate buffer with lysozyme.

The sodium dodecyl sulfate was a specially purified sample generously supplied by the Fine Chemicals Division of E. I. du Pont de Nemours and Company.

Results and Discussion

1. Spectra of Single Substances.—Of all the polypeptides examined, the antibiotics tyrocidine and gramicidin have the smallest molecular weights,[7] about 2500 for the former and near 2800 for the latter. The spectra of these two substances are illustrated in Fig. 1.

In the 3 μ region one observes for tyrocidine a strong band at 3.05 μ and weak shoulders at 3.25 and 3.30 μ. The first of these bands is characteristic of the N–H vibration in amides.[8,9,10] The bands at 3.25 and 3.30 μ are probably C–H stretching vibrations, the low wave lengths being an expression of the relatively high content of aromatic amino acids, tyrosine, tryptophan and phenylalanine.[7] In the 6 μ region, the band at 6.15 μ while slightly more displaced than is usual in solid amides[8,11] nevertheless, is undoubtedly due to the C=O group. Similarly, the 6.65 μ absorption is to be attributed to an N–H bending frequency,[6,8] even though it is at a slightly higher wave length than is observed usually in substituted amides. The peak at 7.05 μ, in turn, is characteristic of the C–H bending vibration[9,10] with perhaps some contribution from the single ionized carboxyl group in the molecule, while that at 7.60 μ corresponds to a similar vibration found in long-chain aliphatic polymers,[12] and in some amino acids.[11] Finally the absorption band at 8.25 μ, characteristic of a C–O bond in an aromatic compound, is undoubtedly due to the tyrosine content of tyrocidine.

The absorption spectrum of gramicidin resembles that of tyrocidine quite closely. Bands at 3.05 and 3.25 μ presumably are due to the N–H and aromatic C–H vibrations, that at 3.40 to the aliphatic C–H frequency. It should also be mentioned, however, that some of the 3.25 μ absorption may be due to an N–H frequency[13,14] overlapping the aromatic C–H vibration. In the 6 μ region, bands occur at 6.21 and 6.61 μ, again somewhat displaced, but undoubtedly due to the C=O and N–H groups, respectively. The

(1) R. Stair and W. W. Coblentz, *J. Research Nat. Bur. Standards*, **15**, 295 (1935).

(2) A. M. Buswell, K. F. Krebs and W. H. Rodebush, *J. Phys. Chem.*, **44**, 1126 (1940).

(3) J. D. Bath and J. W. Ellis, *ibid.*, **45**, 204 (1941).

(4) A. M. Buswell and R. C. Gore, *ibid.*, **46**, 575 (1942).

(5) H. Lenormant, *Compt. rend.*, **221**, 58 (1945).

(6) S. E. Darmon and G. B. B. M. Sutherland, THIS JOURNAL, **69**, 2074 (1947).

(7) R. D. Hotchkiss, *Advances in Enzymology*, **4**, 153 (1944).

(8) R. E. Richards and H. W. Thompson, *J. Chem. Soc.*, 1248 (1947).

(9) H. W. Thompson, *ibid.*, 327 (1948).

(10) R. B. Barnes, R. C. Gore, R. W. Stafford and V. Z. Williams, *Anal. Chem.*, **20**, 402 (1948).

(11) I. M. Klotz and D. M. Gruen, *J. Phys. Colloid Chem.*, **52**, 961 (1948).

(12) H. W. Thompson and P. Torkington, *Proc. Royal Soc. (London)*, **A184**, 3, 21 (1945).

(13) A. M. Buswell, J. R. Downing and W. H. Rodebush, THIS JOURNAL, **62**, 2759 (1940).

(14) G. B. B. M. Sutherland, private communication.

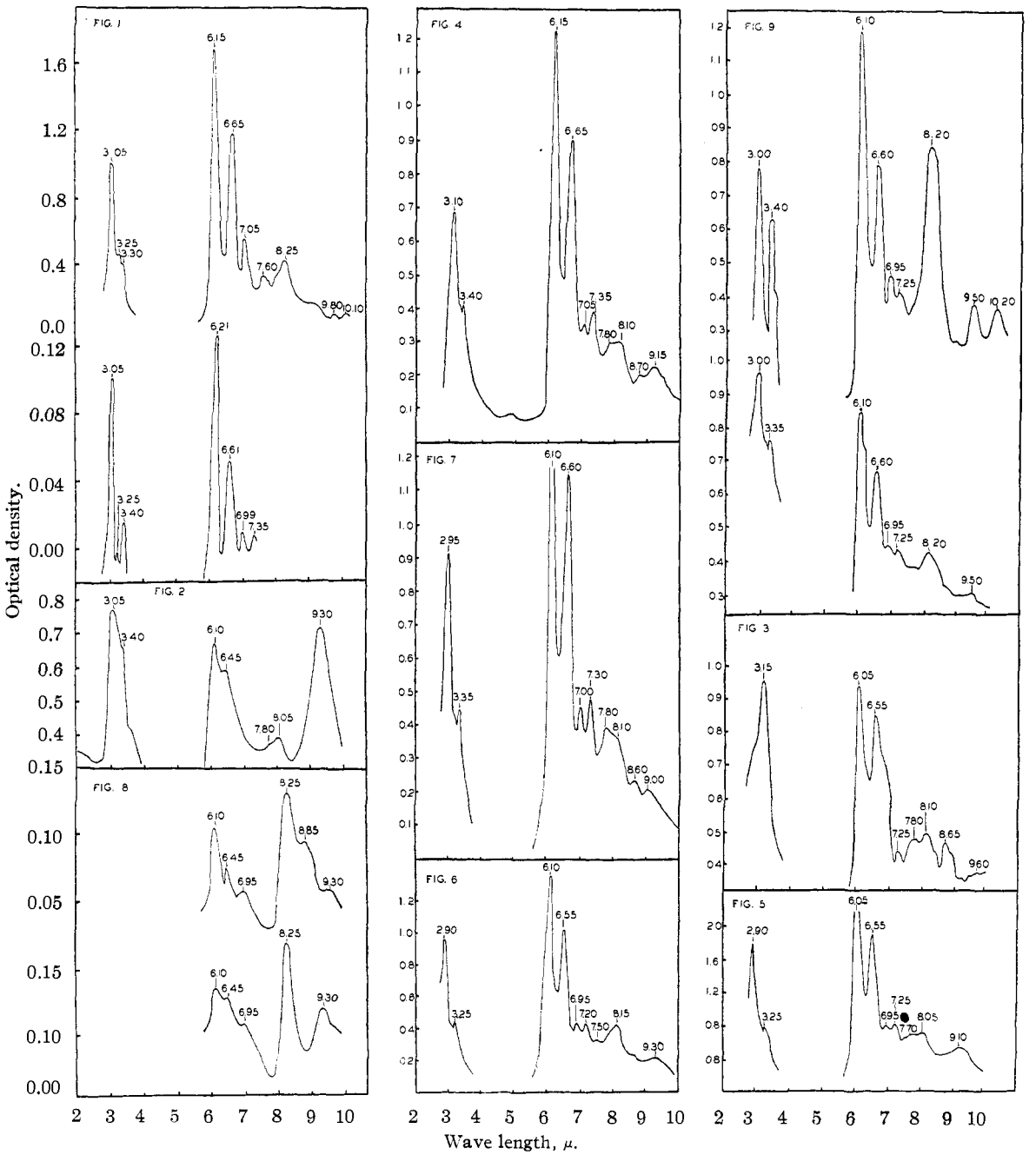


Fig. 1.—Infrared absorption spectra of tyrocidine (upper curve) and gramicidin (lower curve). Fig. 2.—Infrared absorption spectrum of salmine sulfate. Fig. 3.—Infrared absorption spectrum of polylysine. Fig. 4.—Infrared absorption spectrum of cytochrome c. Fig. 5.—Infrared absorption spectrum of lysozyme. Fig. 6.—Infrared absorption spectrum of pepsin. Fig. 7.—Infrared absorption spectrum of bovine serum albumin. Fig. 8.—Infrared absorption spectra of mechanical mixture of salmine with sodium dodecyl sulfate (lower curve) and of complex formed (upper curve) on exposure to water vapor. Fig. 9.—Infrared absorption spectra of lysozyme complexes with dodecyl sulfate. Top curve, protein: anion weight ratio of 2:1; bottom curve, protein: anion weight ratio of 3:1.

small absorption at 7.0 μ, due to CH_2 groups, is at a slightly lower wave length than is observed in tyrocidine, probably because of the absence of any ionized carboxyl group in gramicidin. An automatic recording also indicates a slight peak near 8.25 μ, which may be due to the unidentified hydroxyamino acid in this antibiotic.

Turning to a higher natural polypeptide, salmine, with a larger molecular weight, near 8000,[15]

(15) G. R. Tristram, *Nature*, **160**, 637 (1947).

one observes a spectrum (Fig. 2) even closer to that of the simple amides than is true for the antibiotics just described. The very strong N–H band, at 3.05 μ, perhaps a reflection of the very high content (89%) of basic amino acids in this substance, almost overwhelms the C–H frequency at 3.4 μ. The absence of any indication of a 3.25 μ band is consistent with the absence of aromatic amino acids in salmine.[15] At higher wave lengths, C=O and N–H bands are observed at 6.10 and 6.45 μ, respectively, remarkably close to the corresponding positions in the simple amides. The very strong peak at 9.30 μ is undoubtedly partially a reflection of the O–H groups of the seven serine residues in the protamine molecule. However, it is quite possible that some contribution is made also by the sulfate anions associated with salmine, since crystalline sodium sulfate has been found to have a strong absorption at 9.0 μ.

In view of the very high content of a basic amino acid, arginine, in the natural polypeptide, salmine, it has seemed pertinent to examine the spectrum of a synthetic polypeptide, polylysine[16] which is constructed entirely from a basic amino acid, and has a molecular weight near salmine. The absorption bands are illustrated in Fig. 3. In the 3 μ region, a more detailed examination than can be presented in Fig. 3, reveals small shoulders at 3.0 and 3.45 μ, in addition to the giant band clearly visible at 3.15 μ. The presence of two N–H bands, at 3.0 and 3.15 μ, may be related to the two types of N–H group present in the molecule, one in the peptide structure, the other as the quaternary ion, but no certain assignment can be made on the basis of these data alone. The C=O band of polylysine at 6.05 μ is at a wave length typical of the simple amides as well as of many proteins, and the N–H frequency at 6.55 μ is characteristic of most of the proteins to be described shortly. The small peak at 7.25 μ may be due to some C–H bending vibration, although it is a little high for such an assignment, since no methyl group is present in this substance. Some speculation is also possible in connection with the twin peaks at 7.8 and 8.1 μ, and will be discussed shortly.

Another natural protein with a high content of basic amino acids is cytochrome c, which has 22 lysine residues, as well as 3 histidines and 2 arginines, per molecule of 13,000 molecular weight.[17] The spectrum of cytochrome c (Fig. 4) in the 3 μ region is remarkably similar to that of polylysine, if proper account is taken of the presence of 4 tyrosine residues in the protein. Thus there is a very strong band at 3.10 μ, slightly displaced from the 3.15 position observed for polylysine, probably because of the presence of hydroxyamino-acid residues. Similarly the additional shoulder at 3.25 μ for cytochrome may be attributed to the aromatic C–H vibration of the tyrosine group. In the 6 μ region, cytochrome has its C=O and N–H peaks at 6.15 and 6.65 μ, respectively, each displaced about 0.1 μ from corresponding position in polylysine. The bands at 7.05 and 7.35 μ in the protein may be assigned to C–H vibration, with perhaps some contribution to the former frequency from carboxylate ions.

Concluding the consideration of basic polypeptides, we might examine the spectrum of lysozyme, which has a molecular weight[18] of 13,900 and an isoelectric point[19] near 11. Despite the high isoelectric point, the total content of arginine, lysine and histidine groups is only around 20%.[20] The basicity of the protein thus seems to be due to the relatively small quantity of dicarboxylic acids.[21] The spectrum of lysozyme (Fig. 5), is somewhat unusual in the 3 μ region, in that the primary peak is at 2.90 μ, a shorter wave length than that of any substance mentioned hitherto. Since absorption below 3 μ seems to be characteristic of O–H groups, even when hydrogen-bonded, this peak may indicate a high content of hydroxyamino acids. So far, however, only the tyrosine content of lysozyme has been determined, but it is substantial, being 4.4% to the total amino acid content[20] of the protein. Confirming this interpretation of the position of 2.9 μ band is the occurrence of the same band in pepsin (Fig. 6), a protein which is also rather distinctive in its relatively high content of hydroxyamino acids.[22] The absorption bands at 3.25, 6.05, 6.55, 6.95, and 7.25 μ can be interpreted along the same lines as discussed for the preceding substances. The band at 9.10 μ would fit the presence of an aliphatic hydroxyamino acid.

It is a point of interest that lysozyme, cytochrome c, polysine and salmine each give indications of a twin peak at approximately 7.8 and 8.1 μ. Absorption in this region might be attributed to the aromatic C–O of tyrosine residues, were it not for the complete absence of this amino acid in polylysine. It is perhaps more than a coincidence that each of these polypeptides does contain a high content of either lysine or arginine or both. For this reason, it seems worthwhile to consider the possibility that the presence of free quaternary nitrogen atoms of the guanidinium or ammonium type is accompanied by this particular feature in the infrared spectrum.

Turning to pepsin, a protein with low isoelectric point (<3) and with a molecular weight of 34,400, we find a spectrum (Fig. 6) which again is consistent with its amino acid composition.[22]

(16) E. Katchalski, I. Grossfeld and M. Frankel, THIS JOURNAL, **69**, 2564 (1947).

(17) H. Theorell and A. Akesson, *ibid.*, **63**, 1804 (1941).

(18) K. J. Palmer, M. Ballantyne and J. A. Galvin, *ibid.*, **70**, 906 (1948).

(19) G. Alderton, W. H. Ward and H. L. Fevold, *J. Biol. Chem.*, **157**, 43 (1945).

(20) E. P. Abraham, *Biochem. J.*, **33**, 622 (1939).

(21) H. Fraenkel-Conrat and H. S. Olcott, *J. Biol. Chem.*, **161**, 259 (1945).

(22) E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947).

Thus the peak at 2.90 μ fits the expectation for a protein with a high content of hydroxy-amino acids. The small hump at 3.25 μ also is consistent with the known presence of a substantial quantity of tyrosine. The C=O band at 6.10 μ and the N–H frequency at 6.55 μ are close to the positions found for the polypeptides discussed previously. Similarly the small peaks at 6.95 and 7.20 μ can be assigned to the C–H vibrations, the latter probably being that of the methyl group. A relatively strong absorption at 8.15 μ is to be expected from the C–O bond in tyrosine, whereas the 9.30 μ peak corresponds to the presence of the aliphatic hydroxy amino acids, serine and threonine. It is, perhaps, pertinent to point out the absence of a twin peak at 7.8 and 8.1 μ, found in the basic polypeptides discussed earlier. This lacuna is consistent with the presence of only a very small quantity of basic amino acid residues in pepsin.

The largest of the proteins examined in this study is bovine serum albumin. This protein, with a molecular weight of 69,000, contains[23] an appreciable quantity of tyrosine (5.5%), of serine (4.5%), and of threonine (6.5%) residues, as well as a large fraction (22.4%) of the basic amino acids, arginine, lysine and histidine. The position of the peak (Fig. 7) at 2.95 μ is fairly close to that of the other proteins investigated which have a large number of hydroxy-amino-acid residues. The peaks at 3.35, 6.10, 6.60, 7.00 and 7.30 μ can be interpreted adequately along lines already outlined. It is of particular interest to note, in addition, the presence of the two peaks at 7.8 and 8.1 μ, a characteristic to be expected, according to the suggestion mentioned earlier, in proteins containing a substantial number of cationic nitrogen groups. The remaining peaks at 8.6 and 9.0 μ are rather small. The former may be due to a C–H bending vibration, whereas the latter band is probably to be attributed to the aliphatic hydroxyamino acids.

The spectrum of bovine albumin was examined also in a film evaporated from an acid solution, in contradistinction to the isoelectric state described in Fig. 7. The C=O peak was found at 6.05 μ. The absence of any appreciable shift in albumin on going from the isoelectric condition to an acid state, offers convincing evidence that the absorption in the 6.1 μ region is due to C=O groups of the amide linkage and not of the carboxyl group, as might be suggested from the structure proposed by Wrinch[24]; for in the latter case one would expect the band to be shifted to 5.8 μ, the position characteristic of the neutral COOH group. To forestall possible criticism of this interpretation on the grounds that deposition of the albumin as a film denatures the protein, the ability of a sample film, of which the infrared spectrum had been taken, to bind organic anions was examined. As has been demonstrated previously,[25] a typical anion such as methyl orange changes its color when it is bound by albumin, and this binding affinity of the protein disappears on denaturation.[26,27] The film used for infrared investigations, however, when dissolved in phosphate buffer at *p*H 7.6 showed the usual ability of native albumin to cause a spectral shift in the visible spectrum of methyl orange (Table I). Thus, there would seem to be little room left for doubt that native, corpuscular proteins contain an amide-type carbonyl group.

TABLE I

CHANGE IN OPTICAL ABSORPTION OF METHYL ORANGE IN PRESENCE OF BOVINE SERUM ALBUMIN

Wave length, Å.	Depression in optical density: Protein from film (8 mg.)	Depression in optical density: Control protein (12 mg.)
4600	0.063	0.099
4700	.097	.138
4800	.117	.165
4900	.134	.175
5000	.123	.167

2. Spectra of Complexes with Sodium Dodecyl Sulfate.—Several proteins form complexes with sodium dodecyl sulfate,[28] soluble ones in high mole-ratios of detergent to protein, insoluble ones at lower ratios. The stability of these complexes seems to depend on electrostatic and van der Waals forces, although there is evidence of the formation of covalent bonds if the complex is heated mildly.[29] It has seemed appropriate, therefore to examine the effect of complex-formation on the infrared spectrum of the protein or polypeptide.

Gramicidin and tyrocidine are not sufficiently soluble in aqueous solution to enable investigation of complex formation with dodecyl sulfate. Salmine, on the other hand, is adequately soluble and precipitates readily when sodium dodecyl sulfate is added. The precipitate obtained is so granular, however, that scattering of the infrared radiation is very high, and a satisfactory spectrum could not be obtained. As an alternative, the following procedure was adopted. A sample of the protamine and dodecyl sulfate was mixed mechanically, and the spectrum was taken. This mechanical mixture, on the slide, was then exposed to water vapor from a steam-bath to permit the reaction to occur, the product dried, and the spectrum examined again. A comparison of the two absorptions above 6 μ is illustrated in Fig. 8. In the mechanical mixture, the significant differences from pure salmine (Fig. 2) are the absorption at 6.95 and 8.25 μ, both due, no

(23) E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

(24) D. M. Wrinch, *Proc. Royal Soc. (London)*, **A161**, 505 (1937).

(25) I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

(26) B. D. Davis, *Amer. Scientist*, **34**, 611 (1946).

(27) I. M. Klotz, H. Triwush and F. M. Walker, THIS JOURNAL, **70**, 2935 (1948).

(28) F. W. Putnam and H. Neurath, *ibid.*, **66**, 692 (1944); F. W. Putnam, *Advances in Protein Chemistry*, **4**, 79 (1948).

(29) K. G. A. Pankhurst and R. C. M. Smith, *Trans. Faraday Soc.*, **43**, 511 (1947).

doubt, to the added alkyl sulfate. The former band corresponds to the normal C–H frequency found in this region, the latter to the sulfate group.[11] The major change on exposure to water vapor lies in the appearance of the shoulder at 8.85 μ. The position of this peak suggests the formation of a covalent bond between the protamine and the dodecyl sulfate.[10,11] In view of the experience of Pankhurst and Smith,[29] it seems likely that this bond formation was brought about by the heat of the condensing water vapor from the steam, for in the complexes to be described shortly, formed at room temperature or lower, no such band appeared.

Polylysine also forms complexes readily with dodecyl sulfate, but here again a completely satisfactory film could not be obtained. The spectra which were taken, however, indicated that the C=O and N–H frequencies near 6 μ were displaced slightly toward higher wave lengths as compared to those for the pure polypeptide. Such a displacement probably signifies that the detergent is bound at the amide groups of polylysine, as well as at the cationic nitrogens.

In contrast to the preceding polypeptides, lysozyme formed complexes with sodium dodecyl sulfate which were very suitable for infrared analysis. The soluble complex, formed at a 2:1 weight ratio of protein:detergent, as well as the insoluble complex, formed at 3:1 ratio, was examined, and the results are shown in Fig. 9. For both complexes, the peaks are essentially at the same frequencies, except that the one with the higher ratio of dodecyl sulfate exhibits an additional band at 10.20 μ. The origin of this vibration is not clear. For both complexes there are small differences in the frequencies in the 3 and 6 μ regions which would tend to indicate that the dodecyl sulfate interacts in a rather general fashion with the protein molecule as well as at certain specific loci. In particular it is of interest to note that the peak at 9.1 μ in pure lysozyme is replaced by one at 9.5 μ. If the latter is also due to aliphatic hydroxy amino acids, the shift would indicate that the dodecyl sulfate anion interacts strongly with the O–H group. A similar demonstration of interaction with quaternary nitrogen groups would be highly desirable, in view of other evidence that cationic groups on the protein form the loci of attachment of anions.[30] Unfortunately the strong band of the sulfate radical at 8.20 μ seems to overwhelm the weak peaks near 8.1 and 7.8 μ, which, as indicated previously, seem to be characteristic of cationic nitrogens on the protein.

In view of the extensive work on serum albumin–dodecyl sulfate complexes reported in the literature,[28] it seemed appropriate to examine the infrared spectrum of this combination also. An "alkaline" complex was prepared by evaporation of a solution of protein and detergent having a *p*H of 7.05. A similar solution was adjusted to *p*H 4.8 with hydrochloric acid, the precipitate centrifuged, spread on the slide and dried. In both cases a weight ratio of 2:1 for albumin:detergent was used to parallel some of the electrophoretic investigations of Putnam and Neurath.[31] The results obtained are not illustrated, for the spectra do not differ significantly from those for the pure protein, except for the presence of the broad sulfate band at 8.15 μ and the introduction of a small peak near 9.5. The latter, again, may indicate that the detergent interacts with hydroxyl groups, as well as cationic loci, on the protein. With albumin, as with lysozyme, direct evidence for interaction with quaternary nitrogens could not be obtained because of the broad sulfate peak in the 8 μ region.

(30) I. M. Klotz and F. M. Walker, THIS JOURNAL, **69**, 1609 (1947).

(31) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **159**, 195 (1945).

Conclusions

Perusal of the results obtained with the homogeneous substances indicates that the spectra of polypeptides and proteins are very largely the summation of the contributions of the constituent amino acid residues. Each spectrum is dominated by the N–H bands in the 3 μ region and by the C=O amide peak in the 6 μ region, and the presence or absence of most of the other peaks below 10 μ is determined by the corresponding presence or absence of an amino acid residue containing the appropriate secondary functional group. Nevertheless it is true that small variations in the position of the N–H and C=O bands do occur from substance to substance, and these may reflect the specific configurational arrangements in each macromolecule.

Spectra of complexes of the polypeptides or proteins with sodium dodecyl sulfate show some differences from the corresponding absorptions of the simple macromolecule. A direct demonstration of interaction with cationic groups cannot be made, however, because the sulfate band overlaps the region where characteristic absorption of the charged nitrogens may occur. On the other hand, shifts in other bands indicate that the dodecyl sulfate interacts with hydroxyl and N–H groups, and may also cause some mild perturbations in C–H vibrations.

Acknowledgments.—This investigation was supported in part by grants from the Rockefeller Foundation and from the Abbott Fund of Northwestern University. The authors are also indebted to Lt. Henry Triwush for his assistance in obtaining the spectrum of gramicidin.

Summary

Infrared absorption spectra from below 3 μ to 10 μ have been obtained for tyrocidine, gramicidin, salmine, polylysine, cytochrome c, lysozyme, pepsin and bovine serum albumin, and for the complexes of several of these substances with sodium dodedyl sulfate. The spectra of the pure

polypeptides can be interpreted in terms of the constituent amino acid residues. The spectra of the complexes indicate that more than one group on the protein is perturbed by the combined dodecyl sulfate.

In the case of serum albumin, tests indicate that the protein is not denatured during the process of taking a spectrum. The presence of absorption characteristic of the amide linkage, therefore, offers conclusive evidence of the presence of such a structure in a native globular protein.

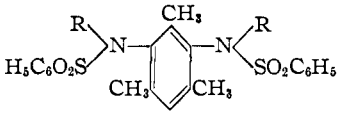
EVANSTON, ILLINOIS RECEIVED NOVEMBER 22, 1948

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

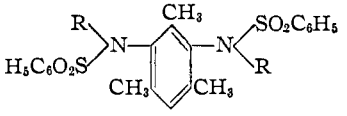
Restricted Rotation in Substituted Aromatic Amines. VII. Stereoisomers of N,N′-Dimethyl-N,N′-diarylsulfonyldiaminomesitylenes

BY ROGER ADAMS AND MORTON ROTHSTEIN

In the preceding paper,[1] pairs of isomers of various N,N′-dialkyl-N,N′-dibenzenesulfonyldiaminomesitylenes (I and II), in which R was ethyl, *n*-butyl, *n*-dodecyl, benzyl, carbethoxymethyl and carboxymethyl, were described. The isomerism was explained on the basis of two points of restricted rotation in the molecules.



I

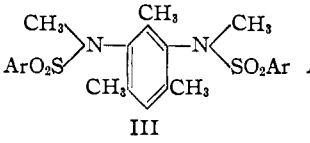


II

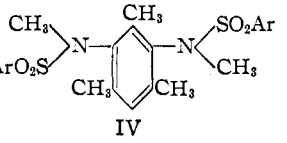
The method of preparation of these compounds consisted of alkylation in alkaline medium of N,N′-dibenzenesulfonyldiaminomesitylene. An alternative and unsatisfactory procedure, the benzenesulfonation of the N,N′-dialkyldiaminomesitylenes, was investigated in the case of the N,N′-diethyl derivative. The expected product was not obtained, but instead an ethyl group was eliminated and N-ethyl-N,N′-dibenzenesulfonyldiaminomesitylene was formed.

The present communication describes the results of a study of the synthesis of N,N′-dimethyl-N,N′-dibenzenesulfonyldiaminomesitylene by the two methods previously explored for the synthesis of the corresponding diethyl derivative. Replacement of benzenesulfonyl groups by substituted benzenesulfonyl groups has also been investigated.

Methylation of N,N′-dibenzenesulfonyldiaminomesitylene with methyl iodide proceeded smoothly to the N,N′-dimethyl-N,N′-dibenzenesulfonyldiaminomesitylene, and the *cis*- and *trans*-forms (III and IV) were readily isolated. Benzenesulfonation of N,N′-dimethyldiaminomesitylene resulted in the same two isomers. No methyl group was lost as was observed when the diethyl derivative was subjected to the same reaction. However, unidentified oily by-products accompanied the desired products and hindered the isolation of the latter in a pure state. The method was therefore not as satisfactory as the synthesis by methylation of dibenzenesulfonyldiaminomesitylene.



III



IV

Ar = phenyl, *p*-tolyl, *p*-bromophenyl or *p*-nitrophenyl

The N,N′-di-*p*-toluenesulfonyl, *p*-bromobenzenesulfonyl and *p*-nitrobenzenesulfonyl derivatives of diaminomesitylene were synthesized. These were in turn methylated and in all instances pairs of isomers (III and IV) were isolated. The reaction of *p*-toluenesulfonyl chloride and diaminomesitylene differed from that of the benzene or *p*-bromobenzenesulfonyl chlorides. A substantial quantity of the mono-*p*-toluenesulfonyldiaminomesitylene was found in the reaction product even though a large excess of reagent was used; this compound could be converted to the disubstituted derivative by further treatment. The preparation of the N,N′-di-*p*-nitrobenzenesulfonyldiaminomesitylene was not entirely satisfactory. An oily by-product contaminated the product and no method for its removal was found. However, methylation of the crude material gave two dimethylated isomers which appeared to be pure.

When diaminomesitylene was methylated with methyl iodide in aqueous or methanolic solution, the reaction proceeded stepwise. With one mole equivalent of methyl iodide the product was N-methyldiaminomesitylene contaminated with only minimum quantities of unmethylated or dimethylated homologs. With two mole equivalents or excess over that quantity, the N,N′-dimethyldiaminomesitylene was formed exclusively. Under the mild conditions used, the introduction of additional methyl groups is apparently inhibited by the crowded condition of the atoms in the molecule. This dimethyl derivative is identical with that described previously,[2] formed from the action

(1) Adams and Tjepkema, THIS JOURNAL, **70**, 4204 (1948).

(2) Adams and Chase, *ibid.*, **70**, 4202 (1948),