

Ewald is presented in the fifth column. In general the agreement is good, with differences in the direction expected from the revision of Nier's cadmium data. That is, low abundance nuclides are moderately reduced with higher abundance nuclides correspondingly increased. There is a moderately large percentage difference at Ru⁹⁸. This was a weak line in Ewald's spectrum and most susceptible to error in plate calibration and densitometry. Variations in the natural abundance of Ru⁹⁸ are of interest as a possible means of detection of natural Tc⁹⁸.

The atomic weights computed from these data and Ewald's are in good agreement. Using the packing fraction listed by Mattauch⁸ and the chemical conversion factor of 1.000275 a value of 101.08 is obtained. This agrees with the value of 101.08 obtained by Gleu and Rehm⁹ from studies on the decomposition of RuCl₃·5NH₃ but seriously diverges from the accepted value, 101.7, obtained from studies on oxide decomposition.

The authors wish to thank Prof. Geoffrey Wilkinson for his cooperation in supplying us with a sample of pure ruthenocene.

(8) T. Mattauch and S. Fluegge, "Nuclear Physics Tables," Interscience Publishers, Inc., New York, N. Y., 1942.

(9) K. Gleu and K. Rehm, *Z. anorg. Chem.*, **235**, 352 (1938).

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Competitive Interaction between Proteins and Surface Active Anions Demonstrated by Electrophoresis¹

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Both ovalbumin² and serum albumin³ possess a strong affinity for anionic detergents which is manifested in the formation of an "all-or-none"⁴ complex. This complex formation is thought to involve denaturation of the protein and the denatured complex can be resolved from the native protein electrophoretically. The authors have shown recently⁵ that ovalbumin (O) yields the all-or none reaction at lower detergent concentrations than does bovine serum albumin (A).

It occurred to the authors that it should be possible to demonstrate this difference in reactivity directly through electrophoretic studies of mixtures of the proteins. The technique used was to mix one of the native proteins with the dodecylbenzene

sodium sulfonate complex of the other. After allowing the mixed solution to stand for two days at 2° it was dialyzed for two days against the electrophoretic buffer (either glycine-NaCl, pH 10.0, $\Gamma/2$ 0.1 or veronal-NaCl, pH 8.5, $\Gamma/2$ 0.1) and subjected to electrophoresis. Some typical results are summarized in Fig. 1.

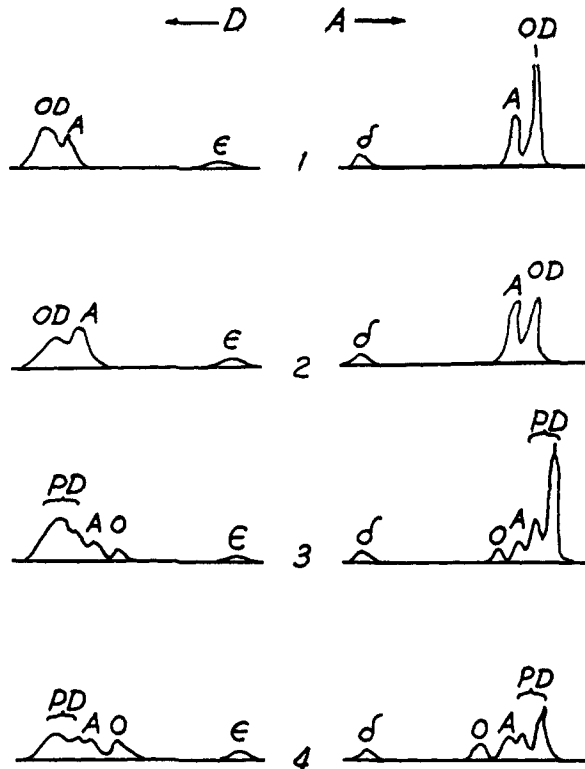


Fig. 1.—Electrophoretic analyses of albumin-SDBS mixtures in glycine-NaCl buffer (pH 10.0, $\Gamma/2$ 0.10). Runs 1 and 2, ovalbumin-SDBS (OD) plus bovine serum albumin (A); O/A ratio 66/34 and 48/52, respectively. Runs 3 and 4, bovine serum albumin-SDBS (AD) plus ovalbumin (O); A/O ratio 66/34 and 49/51, respectively.

It will be seen in Fig. 1 that when A is added to the detergent complex of O (OD) only two components are observed and these have the mobilities characteristic of A and OD. Furthermore the relative areas under the boundaries are close to the relative composition in the original mixture. In other words the pattern is an additive function of the separate patterns on the two components. On the other hand when O is added to AD interaction is clearly revealed. Thus the area corresponding to O is greatly reduced from that which would be expected on the basis of the mixing ratio and a boundary corresponding in mobility to A appears. Similar results were obtained in the veronal buffer; however, in this buffer the patterns were complicated by a split in the protein-detergent boundary and by the appearance of a fast moving boundary anomaly in the descending pattern.

In a study of the electrophoretic analysis of detergent extracts of the corn proteins Foster, Yang and Yui⁶ concluded that zein preferentially binds most or all of the detergent present. It was

(6) J. F. Foster, J. T. Yang and N. H. Yui, *Cereal Chem.*, **37**, 477 (1950).

(1) Journal Paper No. J-2334 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 978. Supported in part by a grant from Swift and Company. Taken from a thesis submitted by Jen Tsi Yang in partial fulfillment of the requirements for the degree Doctor of Philosophy, Iowa State College, 1952.

(2) H. P. Lundgren, D. W. Elam and R. A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943).

(3) F. W. Putnam and H. Neurath, *ibid.*, **159**, 195 (1945).

(4) The term "all-or-none" as applied to this reaction is in some respects perhaps unfortunate. It is now well known that different complexes are formed in the case of horse serum albumin³ and that there may be stepwise binding both prior to and following the so-called "all-or-none" step or steps.⁶ The term is used in the absence of a better one with these reservations to distinguish binding which leads to new components which are readily resolvable by electrophoresis.

(6) J. T. Yang and J. F. Foster, *THIS JOURNAL*, **75**, 5560 (1953).

thus of interest to study the behavior of mixtures of zein-detergent complex (ZD) and either O or A. Figure 2 shows that O removes a part of the detergent from ZD yielding an OI boundary whereas A yields only an additive pattern. This is again in complete agreement with the idea that O forms the all-or-none complex much more readily than A. Again similar results were obtained in the two buffer systems; however, glycine yielded better resolution of ZD and A whereas resolution of ZD and O was superior in veronal.

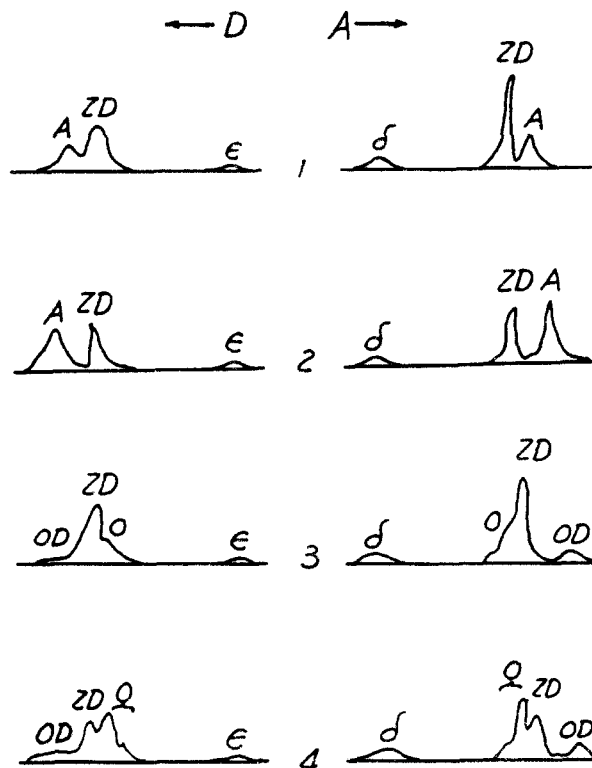


Fig. 2.—Electrophoretic analyses of mixtures of zein-SDBS (ZD) and ovalbumin (O) or bovine serum albumin (A). Runs 1 and 2, ZD plus A in glycine-NaCl buffer (pH 10.0, $\Gamma/2$ 0.10); Z/A ratio 69/31 and 36/64, respectively. Runs 3 and 4, ZD plus O in veronal-NaCl buffer (pH 8.9, $\Gamma/2$ 0.10); Z/O ratio 67/33 and 34/66, respectively.

Experimental

The albumin-detergent complexes were prepared from O and A by mixing 3 parts O and 2 parts A, respectively, with 1 part dodecylbenzene sodium sulfonate⁷ (by weight) followed by isoelectric precipitation and redispersion of the precipitate in alkaline buffer. The complexes could also be prepared by adding a slight excess of detergent to O and A and removing the excess by prolonged dialysis against water.⁸ The electrophoretic properties of the complexes prepared by either technique were substantially the same.

The zein-detergent complex was prepared by dissolving excess zein in detergent solution. Since the zein is insoluble in water a complex is formed which corresponds to the minimum detergent ratio for solubility.⁹ This complex could also be prepared by dissolving zein in excess detergent and remov-

ing the excess detergent by dialysis. This procedure proved to be time consuming and tedious, however.

Electrophoretic analyses were carried out in an 11-ml. cell of the Tiselius type using a Philpot-Svenson type optical system. In the glycine-NaCl buffer O typically had a mobility of $7.4-8.3 \times 10^{-5}$, A $8.8-9.6 \times 10^{-5}$ and the detergent complexes $9.6-11.3 \times 10^{-5}$ cm.² volt⁻¹ sec.⁻¹.

Acknowledgment.—The authors are indebted to Armour and Company and to the Corn Products Refining Company for supplying the crystalline bovine serum albumin and the zein, respectively.

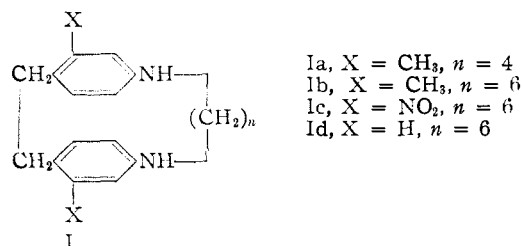
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Heterocycles Containing *p*-Phenylene Units. III. Substituted Amines

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Three substituted cyclic diamines of type I have been synthesized. The 2,2'-dimethyl-4,4'-(polymethylenediimino)-bibenzyls (Ia and Ib) were prepared from 4-nitro-1,2-dimethylbenzene following a sequence of reactions analogous to that previously reported.² Nitration of 4,4'-(hexamethylenediimino)-bibenzyl (Id) in sulfuric acid produced



the dinitrated heterocyclic compound (Ic). In each of the three syntheses, evidence for the formation of more than one isomer (*i.e.*, *cis* and *trans* forms) was lacking.

Experimental³

Cyclization of *p,p'*-Diphenylsulfonamidobibenzyl and Hexamethylene Bromide.—A solution of 9.20 g. (0.0187 mole) of *p,p'*-diphenylsulfonamidobibenzyl and 5.00 g. (0.0204 mole) of hexamethylene bromide in 200 ml. of dimethylformamide was added, in high dilution and with stirring, over a period of 12.5 hours, to a suspension of 60.0 g. (0.43 mole) of anhydrous potassium carbonate in 500 ml. of refluxing dimethylformamide. After the addition was complete, the mixture was boiled under reflux, with stirring, for an additional 12 hours and worked up as in the previous cases.² The residue, obtained from the chloroform extract, was recrystallized from acetic acid to give 3.18 g. (29.7%) of the cyclic disulfonamide as tan prisms melting at 201–205°. Two additional recrystallizations from acetic acid afforded the pure product as white prisms, m.p. 204–206°.

Anal. Calcd. for C₂₂H₂₄N₂O₂S₂: C, 66.87; H, 5.96; N, 4.86; S, 11.16. Found: C, 66.75; H, 5.75; N, 4.89; S, 11.11.

Cleavage of the Cyclic Disulfonamide.—A mixture of 2.21 g. of the cyclic disulfonamide, 5.0 g. of phenol and 75 ml. of 48% hydrobromic acid was boiled under reflux, with stirring, for 75 minutes. The isolation procedure was that outlined previously²; the cyclic diamine crystallized from a methanol-water mixture as white needles, m.p. 146–147°, yield 0.86 g. (76%).

(1) Atomic Energy Commission Predoctoral Fellow, 1951–1953.

(2) R. C. Fuson and H. O. House, *THIS JOURNAL*, **75**, 1327 (1953).

(3) All melting points are corrected. The infrared spectra were determined and interpreted by Miss Helen Miklas. The microanalyses are by Mrs. Katherine Pih, Mrs. Esther Fett and Mr. Joseph Nemeth.

(7) Santomerse No. 3, Monsanto.

(8) Neither the composition of the complexes nor their electrophoretic mobility was found to be appreciably dependent on the extent of this dialysis. It is felt that most of the ions removed are lower homologs of the detergent which are not strongly bound⁴ and that the activity of true detergent ions in equilibrium with the complex is so low that removal is negligible under these conditions.

(9) J. F. Foster, *J. Phys. Chem.*, **53**, 175 (1949).