POLARIZED INFRARED SPECTRA FOR SILKWORM-GUT AND OTHER FIBROUS PROTEINS

Sir:

Infrared spectra observed with anisotropic specimens in polarized light depend upon their orientations relative to both direction of incidence and plane of polarization of the light. Spectra for different orientations of the same specimen can be correlated with structure and arrangement of its molecules. We have been employing this technique to study fibrous proteins. A recent account[1] of results obtained in this way by others encourages us to report some of our own findings at this time.

Polarized spectra for silkworm-gut, a material giving[2] the X-ray diffraction pattern characteristic of fibroin, are shown in Fig. 1. Each spectrum was traced from original instrumental records of *per cent.* transmission through two different samples, one about twenty, another about five microns thick, at left and right, respectively.

These spectra indicate an arrangement that is highly ordered in some respects. The degree of polarization exhibited by several bands approaches the best observed here for single crystals of favorably arranged, simple molecules, exceeding that for the other fibrous proteins we have examined.

The *rachis* of seagull feather exhibited many features appearing in Fig. 1 while quill from pigeon feather was polarized perceptibly at the lower frequencies only. Several natural collagens gave spectra similar to ones described above, with polarizations varying vicariously like ones for feather keratins. Films of rabbit myosin, oriented[3] so as to produce the α-keratin structure, and porcupine quill, showed only small effects of different sort.

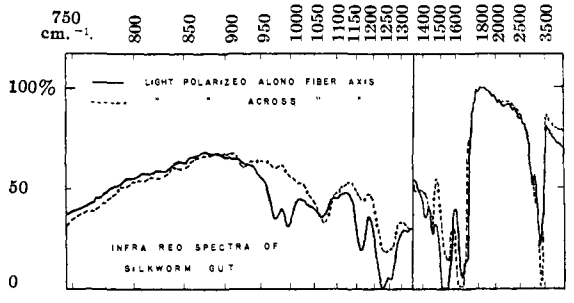

Fig. 1.

Our findings are confirmed in part by those of Ambrose, Elliott and Temple,[1] who worked just in the limited spectral range between 2800 and 3500 cm.$^{-1}$. There they found only slight differences for myosin (91% *vs.* 87% absorption, recalculated from their optical densities) and even smaller ones for porcupine quill and tropomyosin. They report larger effects, however, with feather keratin, as do we.

A full report will be submitted when our work has been completed.

(1) E. J. Ambrose, A. Elliott, and R. B. Temple, *Nature*, **163**, 859 (1949).

(2) R. S. Bear, THIS JOURNAL, **66**, 2043 (1944).

(3) W. T. Astbury and S. Dickinson, *Proc. Roy. Soc.* (*London*), **B129**, 307 (1940).

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NEW YORK, N. Y. RALPH S. HALFORD

RECEIVED JULY 25, 1949

USE OF *p*H INDICATORS WITH ION EXCHANGE RESINS

Sir:

I wish to report the use of *p*H indicators as a means of detecting acids adsorbed on a strong base type resin, Amberlite I.R.A 400.[1]

When the basic form of this resin is treated with phenolphthalein the indicator is quantitatively removed from solution and the resin becomes the characteristic deep pink. Washing has no effect and even strong acid or base will not elute the indicator. Acid decolorizes the resin but base restores the color. If the resin is left for a few days in neutral solution, however, the color is destroyed and base will not restore it. When methyl orange is adsorbed the characteristic color is given with hydrogen ion. Washing removes the color. Again neither strong acid or base will elute the indicator.

The following experiment on the determination of I.R.A 400 capacity for aspartic acid will serve to illustrate the use of a column indicator: Aspartic acid solution was exchanged on the basic form of the resin. The column was washed and 50–100 drops of 1% phenolphthalein passed through. The top of the column remained colorless and a pink zone formed at the junction of the aspartic acid and unreacted resins. The columns were micro burets containing 5–6 g. of 20–30 mesh resin. The acid was 4 mg./ml. and a flow rate of 0.2 ml./min. was used. The value obtained was: 1.5 cc. wet R:NOH resin = 100 mg. aspartic acid.

The method gave a visual illustration of resin efficiency. Not one colored particle was located in the aspartic resin. The pink zone was perfectly even. There was no measurable displacement of aspartic by phenolphthalein.

(1) Rohm & Haas Co.

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RECEIVED SEPTEMBER 19, 1949

THE HYDROLYSIS OF NICOTINYL-L-TYROSYL-HYDRAZIDE BY CHYMOTRYPSIN

Sir:

The recent report that benzoyl-L-tyrosylhydrazide is ineffective as an inhibitor in the chymotrypsin catalyzed hydrolysis of benzoyl-L-tyrosylamide or ethyl ester[1] would lead one to infer

(1) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

that the hydrazide analogs of the specific chymotrypsin amide or ester type substrates[2-5] are not hydrolyzed by this enzyme. We therefore wish to point out that at least one of the acylated α-amino acid hydrazides possessing the structural characteristics required of ester or amide type specific chymotrypsin substrates,[2-5] *i. e.*, nicotinyl-L-tyrosylhydrazide is hydrolyzed by this enzyme (Table I).

TABLE I

HYDROLYSIS OF NICOTINYL-L-TYROSYLHYDRAZIDE BY CHYMOTRYPSIN

t, min.	Hydrolyis, %	$\frac{1}{t}\log\frac{s_0}{s}$
1.2	1.2	0.064
6.6	7.0	.064
10.6	10.0	.061
20.3	21.8	.067
61.0	49.4	.064
91.0	63.0	.063

Nicotinyl-L-tyrosylhydrazide, m.p. 242–243° (cor.)(*Anal.* Calcd. for $C_{15}H_{16}O_3N_4$; C, 60.0; H, 5.4; N, 18.7. Found; C, 59.9; H, 5.3; N, 18.6) was prepared from nicotinyl-L-tyrosine ethyl ester, m.p. 147–149° (cor.) obtained by the acylation of L-tyrosine ethyl ester with nicotinyl azide.[6] The enzymatic hydrolysis was conducted at 25° and *p*H 7.9 (0.02 *F* ethylenediamine–hydrochloric acid buffer) with an initial substrate concentration, s_0 of 5.0 micromoles per ml. reaction mixture and an initial enzyme concentration, E_0, of 0.075 mg. protein nitrogen per ml. reaction mixture. A formol titration was used to determine the extent of hydrolysis.

(2) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 405 (1937).
(3) J. S. Fruton and M. Bergmann, *ibid.*, **145**, 253 (1942).
(4) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).
(5) S. Kaufman and H. Neurath, *ibid.*, **21**, 437 (1949).
(6) T. Curtius and E. Mohr, *Ber.*, **31**, 2493 (1898).

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RECEIVED SEPTEMBER 29, 1949

SEPARATION OF COLUMBIUM AND TANTALUM WITH ANION EXCHANGE RESINS[1]

Sir:

In a previous communication[2] an experiment was described indicating a partial separation of zirconium and hafnium on an anion exchange column in HCl–HF mixtures. While this separation was unusually difficult, the separation of the adjacent elements columbium and tantalum by the same method, under similar conditions, was very efficient.

The experiments were carried out with a 12.5-cm. column (0.0226 sq. cm. cross-section) of the anion exchange resin Dowex-1 using columbium[95] (β-emitter $T_{1/2}$ = 35 days[3]) and tantalum[182] (β-emitter $T_{1/2}$ = 117 days[3]). The columbium was carrier-free fission product and the tantalum was prepared by a neutron bombardment of tantalum metal. In a typical experiment, the tracers were added to the column in a mixture of 9 *M* HCl and 0.05 *M* HF and elution carried out in the same medium at an average flow rate of *ca.* 0.3 ml./sq.cm./min.

The results are shown in Fig. 1 which represents a transcribed continuous record of the activity of the eluent. The bands were identified by standard radiochemical procedures.

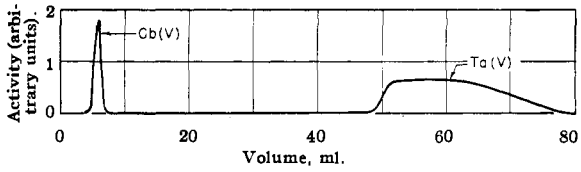

Fig. 1.—Separation of columbium (V) and tantalum (V) by anion exchange: dowex-1 column 12.5 cm. length 0.0226 sq. cm. cross-section, average flow rate; 0.3 ml./sq. cm./min.

Columbium eluted relatively rapidly in a sharp, well-shaped band and the tantalum very much more slowly in a somewhat diffuse band with a sharp front edge. The separation appears to be complete and could probably be achieved with better than 99% purity of the fractions using columns of considerably shorter length.

The experiments prove that both columbium and tantalum can form negatively charged complexes in this medium with probable negative charge minus two or greater. From the slower elution rate of the tantalum one can conclude that the average negative charge on this element is greater than that on columbium.

The very large difference in elution behavior of these two elements is somewhat surprising since both elements, in some complexes at least, show practically the same size. Thus Hoard[4] found no significant difference in the lattice constants of the complex fluorides K_2CbF_7 and K_2TaF_7. The rather large difference in the behavior of these elements on anion-exchangers may thus be due to comparatively large differences in their polarizability, causing considerable differences in the chloride complex constants, or to small differences in the value of each stability constant with a resulting large difference in the negatively charged series due to the fact that for these the product of a considerable number of such constants is involved.

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RECEIVED AUGUST 20, 1949

(1) This document is based on work performed under Contract Number W-7405 eng 26 for the Atomic Energy Project at Oak Ridge National Laboratory.
(2) K. A. Kraus and G. E. Moore, THIS JOURNAL, **71**, 3263 (1949).
(3) Information from G. T. Seaborg and I. Perlman, "Table of Isotopes," *Rev. Mod. Phys.*, **20**, 585 (1946).
(4) J. L. Hoard, THIS JOURNAL, **61**, 1252 (1939).