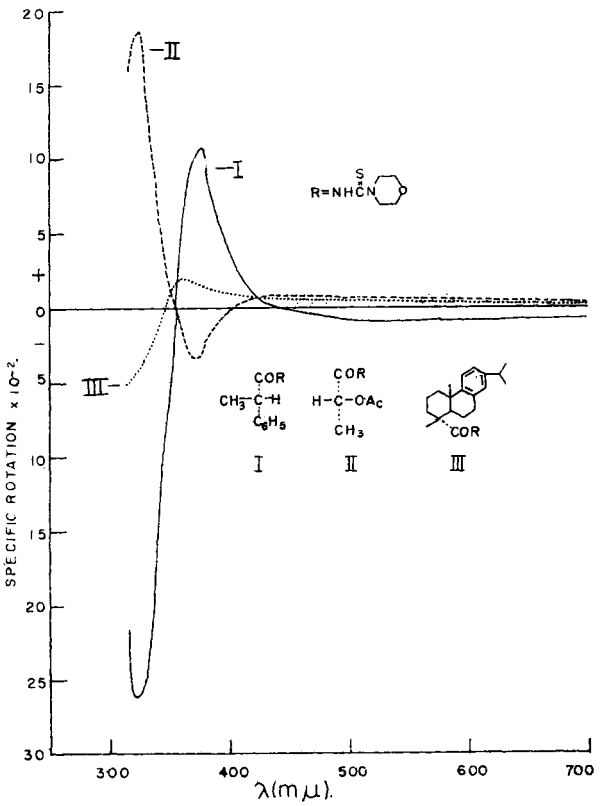


Fig. 1.

portion of the spectrum. Thus dithiocarbamates and xanthates of α-amino and α-hydroxy acids, respectively, have lent themselves admirably to this approach[4] and we now wish to record a satisfactory solution to the problem of converting a carboxyl group into a "chromophoric" derivative[5] exhibiting a Cotton effect.

After an extensive search, we have found acylthioureas ($RCONHC(=S)NR'_2$), notably of morpholine (acyl morpholinothiocarbamides), to be the most desirable derivatives because of their ease of preparation (treatment of acid chloride with potassium thiocyanate and then morpholine) and their low intensity ultraviolet absorption maximum near 340 mμ, which is "optically active." Most importantly, *the resulting Cotton effect curves apparently can be used for absolute configurational assignments of the α-asymmetric center, members of the* L-*series exhibiting a positive Cotton effect and of the* D-*series, a negative one.* Typical curves are shown in Fig. 1.

The nature of the α-substituent does not appear to be critical: the morpholinothiocarbamides of L-(−)-hydratropic acid (I) and L-(+)-α-methylbutyric acid show positive Cotton effects, while negative ones are associated with the corresponding derivatives of D-(−)-acetoxylactic acid (II) and D-(+)-α-(2-naphthyl)-propionic acid. It should be noted that the confusion associated with opposite sign rotations at the sodium D-line by members of the same series is eliminated when the Cotton effects of acyl thioureas are considered.

The presently described approach also may prove useful in the diterpene field for configurational assignments of the carboxyl group, since dehydroabietyl morpholinothiocarbamide (III) shows a positive Cotton effect (Fig. 1), in contrast to the negative one exhibited by acetoxypodocarpyl morpholinothiocarbamide. The only stereochemical difference between these two resin acid derivatives resides in the asymmetric center adjacent to the carboxyl group.

As an example of a typical preparation, 120 mg. of dehydroabietic acid was heated for two hours with 2.5 cc. of thionyl chloride, excess reagent was removed, the residual acid chloride was dissolved in 2 cc. of anhydrous acetone and 44 mg. of freshly dried potassium thiocyanate in 2 cc. of acetone was added. After heating under reflux for one hour, 0.6 cc. of morpholine was added, heating continued for ten minutes and the mixture let stand overnight. Removal of the solvent, addition of water and dilute acid, followed by ether extraction afforded 86% of III, m.p. 102–104° (from hexane), $\lambda_{max}^{MeOH}$ 282 and 340 mμ, log ε 4.16 and 2.41.

Rotatory dispersion curves of additional acid derivatives and further details of this approach will be discussed in detail in our complete paper.[6]

(4) B. Sjöberg, A. Fredga and C. Djerassi, THIS JOURNAL, **81**, 5002 (1959).

(5) One possible derivative is the methyl ketone available by the reaction of methyl lithium with a carboxylic acid. Rotatory dispersion curves of such ketones already have been examined by C. Djerassi and L. E. Geller, *ibid.*, **81**, 2789 (1959), and by B. Sjöberg, *Arkiv Kemi*, **15**, 473 (1960).

(6) We are indebted to Mrs. Ruth Records for the rotatory dispersion measurements.

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## SYNTHESIS OF TRIPHOSPHONITRILIC DIAMIDETETRAAZIDE

*Sir:*

As part of a study involving the synthesis and the chemical and physical properties of phosphonitrilic chloride and cyanuric chloride derivatives we wish to report the preparation and isolation of a mixed triphosphonitrilic amidoazido derivative, $P_3N_3(NH_2)_2(N_3)_4$, which to our knowledge is the first crystalline azide of the phosphonitrilic system to be synthesized. The triphosphonitrilic hexaazide was prepared by Grundmann and Rätz[1] and reported to be an unstable oil. Related triphosphonitrilic derivatives which have been reported by other investigators as solid products are the hexahydrazide,[2] $P_3N_3(N_2H_3)_6$; the hexathiocyanate,[3] $P_3N_3(CNS)_6$; the hexaamide,[4] $P_3N_3(NH_2)_6$; and the tetraanilidediamide,[5] $P_3N_3(NHC_6H_5)_4(NH_2)_2$.

The triphosphonitrilic diamidetetrachloride starting material was obtained readily by the reaction of an ether solution of $P_3N_3Cl_6$ with aqueous am-

(1) C. Grundmann and R. Rätz, *Z. Naturforsch.*, **10b**, 116 (1955).

(2) R. J. A. Otto and L. F. Audrieth, THIS JOURNAL, **80**, 3575 (1958).

(3) G. Tesi, R. J. A. Otto, F. G. Sherif and L. F. Audrieth, *ibid.*, **82**, 528 (1960).

(4) L. F. Audrieth, R. Steinman and A. D. F. Toy, *Chem. Revs.*, **32**, 109 (1943).

(5) H. Bode, K. Butow and G. Lienau, *Chem. Ber.*, **81**, 547 (1948).

monia.[6] Triphosphonitrilic diamidetetraazide was then prepared by treating the triphosphonitrilic diamidetetrachloride with $NaN_3$ for one hour at 25–30° in 1:3 water–acetone solution.

$$P_3N_3(NH_2)_2Cl_4 + 4NaN_3 \longrightarrow P_3N_3(NH_2)_2(N_3)_4 + 4NaCl$$

A white solid product separated when the reaction mixture was poured into cold water. However, this product was unstable and has not as yet been identified. Upon evaporation of the acetone in the filtrate, a second solid product was obtained which later was identified as triphosphonitrilic diamidetetraazide. Recrystallization of the latter from an acetone–water mixture gave white needles in 20% yield of triphosphonitrilic diamidetetraazide, m.p. 81–82°.

Identification of the compound is based on infrared absorption data and elemental analysis. Absorption peaks at 2160 cm.$^{-1}$ and 3400 cm.$^{-1}$ suggest the presence of azido and amino groups[7] in the product and strong absorption at 1200 cm.$^{-1}$ indicates retention of the trimeric $P_3N_3$ ring system.[8] Calcd. for $P_3N_{17}H_4$: P, 27.72; N, 71.06. Found: P, 27.67; N, 67.93; Cl, neg.

We are grateful to Mrs. P. Wheeler for the microanalysis and to Mr. A. S. Tompa for the infrared analysis.

(6) H. Moureu and A. M. de Ficquelmont, *Compt. rend.*, **213**, 306 (1941).

(7) L. J. Bellamy, "Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, pp. 214, 230.

(8) L. W. Daasch, THIS JOURNAL, **76**, 3403 (1954).

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## SEPARATION OF AMINO ACID-SPECIFIC "SOLUBLE" RIBONUCLEIC ACIDS BY PARTITION CHROMATOGRAPHY

*Sir:*

The separation of amino acid-specific "soluble"-fraction ribonucleic acids by countercurrent distribution has been described.[1,2,3,4] This communication describes the separation of these ribonucleic acids by partition chromatography.

The solvent system was prepared by dissolving 278 g. of dipotassium hydrogen phosphate, 435 g. of sodium dihydrogen phosphate monohydrate, and 506 mg. of magnesium chloride hexahydrate in glass-distilled water to give a total volume of 2500 ml., and mixing this solution at room temperature (25°) with 1000 ml. of 2-propanol (Mallinckrodt, reagent) and 250 ml. of formamide (Fisher, reagent). Silicic acid (Mallinckrodt, reagent, 100 mesh, for chromatographic analysis) was washed with 0.001 *M* potassium ethylenediaminetetraacetate, *p*H 7, and with glass-distilled water (fine particles which did not settle rapidly were removed by decantation), and dried overnight at 110°. At room temperature, 300 g. of this silicic acid was mixed thoroughly in a mortar with 200 ml. of the aqueous phase (lower layer) of the solvent system. The resulting free-flowing powder was suspended in the organic phase (upper layer) of the solvent system, and poured into a column 4 cm. by 50 cm. After the silicic acid had settled by gravity, a suspension of 40 mg. of yeast "soluble"-ribonucleic acid[4] in 20 ml. of the organic phase, was placed on the column. A total of 3400 ml. of the organic phase then was allowed to flow through the column (100 ml. per hour). The effluent was collected in fractions totalling 125 ml. each, and these fractions were dialyzed and evaporated.[4] The absorbancies of the fractions at 260 mμ were determined, and the fractions were assayed for amino acid-acceptor activity by standard methods.[4,5]

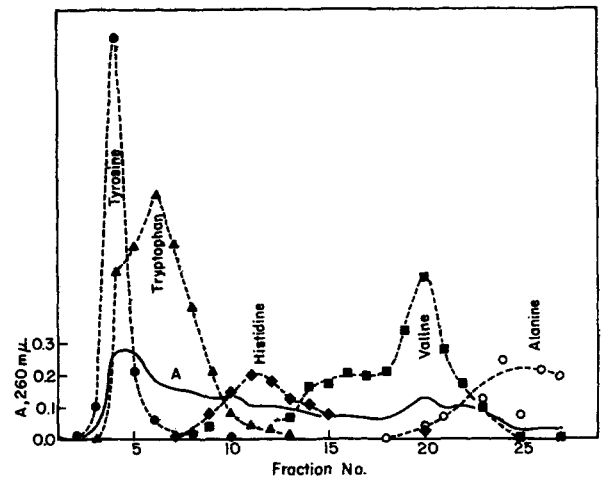


Fig. 1.—Partition chromatography of yeast "soluble"-ribonucleic acids; —, absorbancy at 260 mμ; - - -, amino acid-acceptor activity for the amino acids shown. With the exception of tyrosine, the activities are plotted on a scale such that the activity of the starting material would coincide with its absorbancy, and the specific activities of the fractions relative to the starting material are given by the ratio of activity to absorbancy. The activity for tyrosine is plotted at one-half the scale of the others. The increase in the specific activities of the peak fractions, relative to the starting material, was: tyrosine, nine; tryptophan, four; histidine, two; valine, four; alanine, five. (The starting material gave approximately these counts per minute per mg. under the assay conditions used: tyrosine, 1000; tryptophan, 1000; histidine, 1500; valine, 2000; alanine, 1500.)

The results (Fig. 1) show conclusively that amino acid-specific ribonucleic acids can be separated by partition chromatography. This is the first demonstration that partition chromatography can be used for the fractionation of nucleic acids. The specific activities of the different ribonucleic acids were considerably increased relative to the starting material.

In comparison with countercurrent distribution, the chief disadvantage of this partition chromatographic procedure is the high loss of material (approximately 60%), presumably by adsorption on the silicic acid, but this disadvantage is minimized by the ready availability of the yeast

(1) R. W. Holley and S. H. Merrill, THIS JOURNAL, **81**, 753 (1959).

(2) R. W. Holley, B. P. Doctor, S. H. Merrill and F. M. Saad, *Biochim. et Biophys. Acta*, **35**, 272 (1959).

(3) R. W. Holley, J. Apgar and B. P. Doctor, *Ann. N. Y. Acad. Sci.*, **88**, 745 (1960).

(4) R. W. Holley, J. Apgar, B. P. Doctor, J. Farrow, M. A. Marini and S. H. Merrill, *J. Biol. Chem.*, in press.

(5) M. B. Hoagland, P. C. Zamecnik and M. L. Stephenson, *Biochim. et Biophys. Acta*, **24**, 215 (1957).