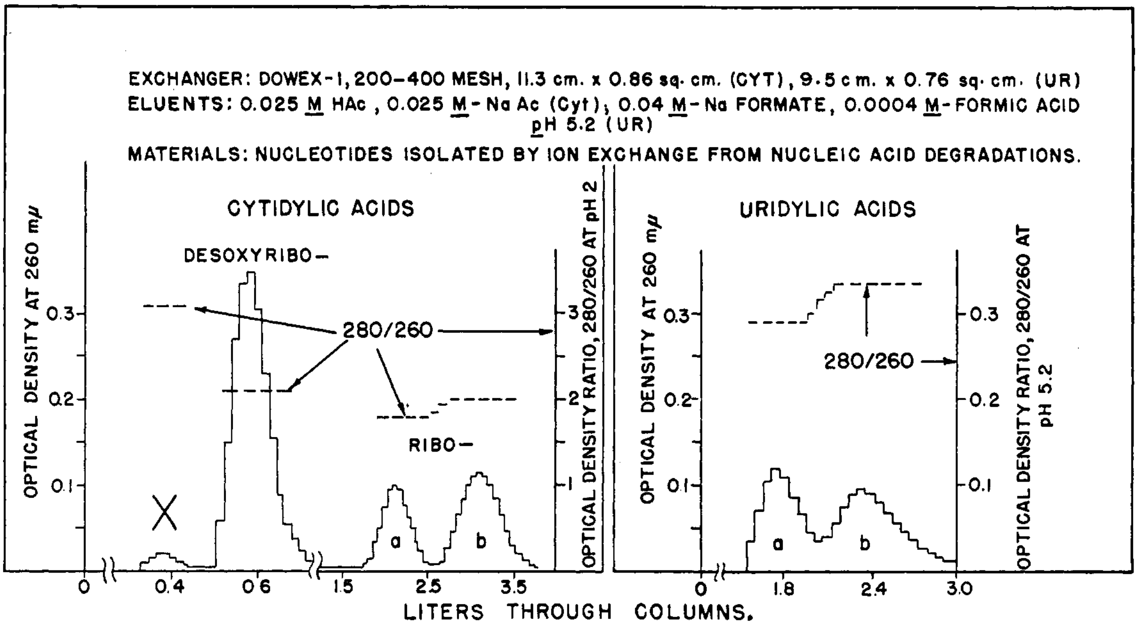

Fig. 1.—Separation of cytidylic acids and uridylic acids by ion exchange.

lated and characterized by accepted procedures,[5] in which no trace of purine compounds could be detected. This, together with small but significant differences in spectral properties, is taken as evidence of heterogeneity, if not true isomerism, in the pyrimidine nucleotides, thus raising the number of isolatable mononucleotides from yeast nucleic acid to eight. Isomerism is favored by the finding that heating for a short period in 0.1 *N* HCl is sufficient to produce an equimolecular mixture of both members of a given pair from either one.

In addition, careful analysis of the cytidylic acid fraction isolated by our usual methods[2,3,6] from an enzymatic hydrolysate of desoxyribonucleic acid[7] (which yields the four desoxyribonucleotide fractions expected) has resulted in the isolation of a small amount of material ("X" in Fig. 1) possessing spectral properties similar to, but differing significantly from, desoxycytidylic acid (280/260 *ca.* 3.1; maximum, 287 mμ at *p*H 2). The base derived from this nucleotide has an ion-exchange behavior like that of cytosine and spectral properties practically identical with those reported by Hitchings[8] for 5-methylcytosine.

Among the tentative conclusions supported by this and previously reported[2,3,4] data are that the number of constituents isolatable from nucleic acid hydrolysates may continue to increase as more refined techniques are developed and as the methods and yields of the degradation of nucleic acid (acid, alkali, enzymatic) are more carefully explored.[9]

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(5) W. E. Cohn and C. E. Carter, This Journal, **72**, 2606 (1950).

(6) J. X. Khym, W. E. Cohn and C. E. Carter, to be published.

(7) E. Volkin, W. E. Cohn and J. X. Khym, to be published.

(8) G. H. Hitchings, *et al.*, *J. Biol. Chem.*, **177**, 357 (1949).

(9) C. E. Carter and W. E. Cohn, This Journal, **72**, 2604 (1950).

CONFIGURATION OF ISOMERIC ALLOTHREONINES BY ENZYMATIC RESOLUTION

Sir:

Kidney acylase and pancreatic carboxypeptidase act only on N-acylated-L-amino acids and do not affect the corresponding D-isomers. On the basis of this absolute optical enzymatic specificity, a general method of resolving the racemates of many α-amino acids has been developed.[1-5] When applied to the case of DL-allothreonine, it should be possible to uniquely and simply distinguish the configuration of each of the enantiomorphs.

DL-Allothreonine was converted to the N-chloroacetyl derivative, and recrystallized from acetone-ether; m. p. 90–92° (uncor.); N calcd. 7.2, found 7.2; yield 60%. The hydrolysis rate of the susceptible L-isomer by crude hog kidney homogenate at *p*H 7.0 and 37° is 73 μM per hour per mg. N. This rate is very nearly the same as that for the corresponding L-threonine derivative (*i. e.* 80).[1] The N-chloroacetyl-DL-allothreonine was subjected to the action of a purified hog kidney acylase, and the resolution conducted as described previously for DL-threonine and other α-

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(1) Fodor, Price and Greenstein, *J. Biol. Chem.*, **178**, 503 (1949).

(2) Price, Gilbert and Greenstein, *ibid.*, **179**, 1169 (1949).

(3) Gilbert, Price and Greenstein, *ibid.*, **180**, 473 (1949).

(4) Greenstein, Gilbert and Fodor, *ibid.*, **182**, 451 (1950).

(5) Fodor, Price and Greenstein, *ibid.*, **182**, 467 (1950).

amino acids.[2] A maximum of 50% hydrolysis of the racemate was achieved in a few hours, which was not altered by addition of fresh enzyme and further incubation. The liberated L-amino acid was separated from the digest as usual, and recrystallized as glistening prisms from 50% alcohol; N calcd. 11.8, found 11.7; $[\alpha]^{25}\text{D}$ +10.0° (4.00% in H_2O); yield 80% on the chloroacetyl derivative. The chloroacetyl-D-allothreonine in the mother liquor was extracted, hydrolyzed with 2 *N* HCl, and isolated in 62% yield after neutralization and recrystallization from 50% alcohol; N found 11.7; $[\alpha]^{25}\text{D}$ −9.8° (4.00% in H_2O).

The optical rotation values of the allothreonine isomers are in agreement with those given by West and Carter[6] and by Elliott,[7] and the assignment of their configuration is in accord with that recently reported by Elliott[7] on the basis of organic chemical procedures.

We thank Dr. H. E. Carter for a gift of DL-allothreonine.

(6) West and Carter, *J. Biol. Chem.*, **122**, 611 (1938).

(7) Elliott, *J. Chem. Soc.*, 62 (1950).

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RECEIVED MAY 4, 1950

OBSERVATIONS OF THE MANNICH REACTION WITH OPTICALLY ACTIVE SUBSTITUTED MANDELIC ACIDS

Sir:

We have found that the Mannich reaction involving *d-o*-nitromandelic acid ($[\alpha]^{20}\text{D}$ +346° in ethanol), formaldehyde and piperidine, yielded the expected Mannich base α-piperidinomethyl-*o*-nitromandelic acid in an optically active form ($[\alpha]^{20}\text{D}$ +439° in ethanol). The *l*-acid gave a product whose rotation was $[\alpha]^{20}\text{D}$ −449° in ethanol. The lack of racemization appears to render inadmissible the enolization step postulated by Alexander and Underhill[1] for all compounds that undergo the Mannich reaction which have a methynyl group on a carboxyl group. Their investigation concerned the Mannich reaction with ethylmalonic acid, formaldehyde and dimethylamine.

The possibility of oxygen alkylation rather than carbon alkylation required by the Mannich reaction was eliminated by means of Zerewitinoff tests performed on the Mannich base.

A series of reactions with *d-o*-nitromandelic acid at various *p*H values and temperatures indicated that the condensation occurred too rapidly to permit a satisfactory study of its kinetics. The results obtained at 20° and 0° were virtually identical. Reaction occurred without apparent change in rate at *p*H values of 7.2, 8.5 and 9.3, while at *p*H 3.1 (piperidine hydrochloride), it failed to occur.

The investigation of this reaction and other similar reactions is continuing and will be reported in the near future.

(1) E. Alexander and E. Underhill, THIS JOURNAL, **71**, 4014 (1949).

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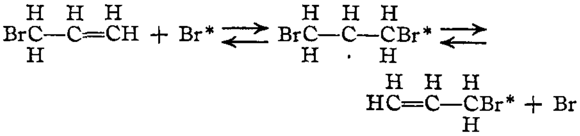
RECEIVED JANUARY 14, 1950

ISOTOPIC EXCHANGE OF ATOMIC BROMINE WITH ALLYLIC VINYLIC HALIDES[1,2]

Sir:

Recent work[3,4] indicates that most bromine atoms produced by slow neutron capture attain thermal energy before exchanging with gaseous hydrogen bromide. An efficient organic reagent for radioactive atoms is very desirable and it occurred to us that vinylic and allylic halides might serve this purpose by the following type of exchange



$$\overset{\text{H}}{\underset{\text{H}}{\text{BrC}}}-\overset{\text{H}}{\text{C}}=\overset{\text{H}}{\text{CH}} + \text{Br}^* \rightleftharpoons \overset{\text{H}}{\underset{\text{H}}{\text{BrC}}}-\overset{\text{H}}{\underset{\cdot}{\text{C}}}-\overset{\text{H}}{\underset{\text{H}}{\text{CBr}^*}} \rightleftharpoons \overset{\text{H}}{\text{HC}}=\overset{\text{H}}{\text{C}}-\overset{\text{H}}{\underset{\text{H}}{\text{CBr}^*}} + \text{Br}$$

Such reagents should be quite efficient since the exchange velocity depends primarily upon addition at the double bond, with a small or zero energy of activation.

We have found that neutron bombardment of gaseous mixtures consisting of 1–25 mm. of bromoolefin and 180 mm. of hydrogen bromide yields 80% organic activity. This high efficiency, which is practically independent of bromoölefin concentration over the range studied, has been interpreted elsewhere.[4]

Other experiments have demonstrated the efficiency of these olefins as reagents for Br* in the liquid state. Liquid ethyl bromide containing approximately one volume per cent. of a bromoölefin was subjected to thermal neutron bombardment. Additional bromoölefin and its addition product (*e.g.*, allyl bromide and 1,2-dibromopropane) were added after bombardment and separated by distillation. The addition product was invariably inactive, and the bromoolefin contained 25–50% of the total activity. This efficiency for the nascent Br* is maintained down to concentrations as low as 0.01 volume per cent. α,β-Dibromoethylene, 2-bromopropene and allyl chloride have been equally effective.

We have previously reported[2] the temperature coefficient of competitive exchange of nascent Br* with α,β-dibromoethylene and allyl bromide when both are present during neutron bombardment. We have recently found that the significance of this result has been obscured by a

(1) Work supported in part by A.E.C. Contract No. At(11-1)38.

(2) Presented at the 116th Meeting of the A.C.S., Atlantic City, September, 1949.

(3) W. H. Hamill and R. R. Williams, Jr., *J. Chem. Phys.*, **16**, 1171 (1948).

(4) R. R. Williams, Jr., and W. H. Hamill, *ibid.*, in press.