Anal. Calcd. for C_3H_7OCl: Cl, 37.5. Found: Cl, 34.8.

Attempts to prepare quaternary ammonium salts as solid derivatives were not successful. Even at 0°, N,N-dimethylbenzylamine, dimethylaniline or pyridine and the chloroether gave only the amine hydrochloride.

α,β-Dibromoethyl Methyl Ether.—A portion of the distilled condensate from the Dry Ice traps was brominated.[11] The yield was 75%, boiling 69–71° (22 mm.).

Anal. Calcd. for $C_3H_6OBr_2$: Br, 73.5. Found: Br, 73.2.

Generalized Procedure.—The directions for making 3,5-dinitrobenzoate derivatives are as follows. A mixture of 1.0 g. of 3,5-dinitrobenzoyl chloride and 2–3 ml. of acetal or ketal in a 25-ml. round-bottom flask is heated by an oil-bath at gentle reflux for 5–60 minutes. The time depends on the reflux temperature—if the acetal boils below 60°, use 60 min. For boiling points between 60 and 100°, 30 minutes is sufficient, and over 100° the reaction requires only 5–10 minutes. If the mixture turns dark, heating should be stopped because the yield at this point will be adequate. After cooling to room temperature, 10 ml. of aqueous 5% sodium carbonate is added, and the mixture is solidified by cooling. This is crushed in a mortar, and an additional 10 ml. of sodium carbonate solution added. After heating in a beaker with stirring at 45–50° for 10 minutes, the crude ester is collected, washed with water, dried in air, and crystallized from 95% ethanol.

DEPARTMENT OF CHEMISTRY
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

A Test for Enzymatic Transpeptidation Reactions

BY FELIX HAUROWITZ[1] AND JACK HOROWITZ[2]

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In investigations on the action of enzymes on proteins, peptides and amino acids, it is important to decide whether or not transfer of amino acid residues takes place under the conditions of the experiment. This problem can be solved by adding small amounts of radioactive substrates of the respective enzyme, and determining whether the radioactivity is incorporated into the reaction products. In our laboratory this method has been used to investigate the mechanism of plastein formation.

A peptic digest of ovalbumin was prepared according to Tauber.[3,4] Thirty ml. of the neutralized and concentrated digest was placed in each flask and mixed with the substrates shown in Table I. After 36 hours incubation with 9.6 mg. of crystalline chymotrypsin (Armour), at *p*H 7.30 and 37°, the insoluble plastein formed was washed, dried, plated and counted in a gas flow counter. Table I shows that the isolated plastein was radioactive after incubation with phenylalanine ethyl ester, but practically free of activity after incubation with phenylalanine, glycine or glycine ethyl ester. Evidently, the formation of plastein involves transpeptidation, *i.e.*, the transfer of phenylalanyl residues from ethanol to peptides of the peptic digest. This is in agreement with results of Brenner, *et al.*,[5–7] obtained with chymotrypsin.

TABLE I

RADIOACTIVITY OF PLASTEIN FORMED IN THE PRESENCE OF VARIOUS C^{14} SUBSTRATES

Type	Substrates[a] Wt., g	Counts/min.	Plastein Counts/min. per mg.
Glycine	0.50	82,500	0.13
Glycine ethyl ester-HCl	.94	69,500	.22
Phenylalanine	.11	134,000	.10
Phenylalanine ethyl ester-HCl	.14	132,000	28.4 (28.6[b])

[a] Labeled by C^{14} in 2-position. [b] After extraction with acetone.

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(2) Predoctorate fellow of the National Science Foundation, 1952–1954.

(3) H. Tauber, THIS JOURNAL, **73**, 1298 (1951).

(4) H. Tauber, *ibid.*, **73**, 4005 (1951).

The fact that only traces of glycine ester are incorporated is in accordance with the substrate specificity of chymotrypsin.[8,9] Since the radioactivity of the insoluble material is not extracted by acetone,[10] it cannot be due to contamination by phenylalanylphenylalanine.

Obviously, the method described in the preceding paragraphs also can be used for other enzymes. While the esters of isotopically labeled phenylalanine, tyrosine or methionine are suitable substrates for chymotrypsin, or cathepsin C, labeled lysine or arginine ester or amide would have to be used as test substrates for trypsin or cathepsin B.[11]

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(6) M. Brenner and R. W. Pfister, *ibid.*, **34**, 2085 (1951).

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(11) H. H. Tallan, M. E. Jones and J. S. Fruton, *J. Biol. Chem.*, **194**, 793 (1952).

DEPARTMENT OF CHEMISTRY
INDIANA UNIVERSITY
BLOOMINGTON, INDIANA

On the $AlCl_3$-catalyzed Reaction between Ethylene Oxide and Malonic Ester

BY HAROLD HART AND OMER E. CURTIS, JR.

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It was claimed recently[1] that malonic ester can be alkylated by ethylene oxide, using anhydrous aluminum chloride, to give a *quantitative* yield of γ-butyrolactone. Because of an interest in lactones as intermediates in dicycloalkyl ketone syntheses[2] and because we were unaware of any phenomenon of "dimorphism" which would cause γ-butyrolactone to have two different boiling points 45° apart, as was claimed,[1] we reinvestigated the reaction.

We have found that the products described by Raha are, in fact, recovered malonic ester and the ester-interchange product, β-chloroethyl ethyl malonate. In addition, a third product, bis-β-chloroethyl malonate, was obtained. We isolated no γ-butyrolactone from the reaction.

Experimental

The "alkylation" was carried out following Raha's procedure identically, and also on a larger scale, except that the ethylene oxide was obtained from a cylinder (Matheson) rather than generated from chlorohydrin. From five moles each of malonic ester, aluminum chloride and ethylene oxide there was obtained, upon distillation through an efficient column, three main fractions: fraction 1, b.p. 60–61° at 1 mm., $n^{25}D$ 1.4130, 504 g.; fraction 2, b.p. 104–105° at 4

(1) C. Raha, THIS JOURNAL, **75**, 4098 (1953).

(2) H. Hart and O. E. Curtis, Jr., abstracts of papers presented at Cincinnati, Ohio, April 1955, p. 46 N.