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.

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A Test for Enzymatic Transpeptidation Reactions

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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	.11	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]

(5) M. Brenner, R. H. Mueller and R. W. Pfister, *Helv. Chim. Acta*, **33**, 568 (1950).

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

(7) M. Brenner, E. Sailer and K. Rufenacht, *ibid.*, **34**, 2096 (1951).

(8) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **117**, 189 (1937); **118**, 405 (1937).

(9) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(10) H. Tauber, THIS JOURNAL, **74**, 847 (1952).

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

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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.

mm., $n^{25}D$ 1.4386, 192 g.; and fraction 3, b.p. 141–151° at 4 mm., $n^{25}D$ 1.4644, 45 g.

Fraction 1 was shown to be recovered malonic ester (63%). Its infrared spectrum was identical with that of an authentic sample, and was distinctly different from that of an authentic sample of γ-butyrolactone. Other physical properties are in agreement with the literature values.[3] It should be pointed out that malonic ester and γ-butyrolactone boil only two degrees apart. The refractive indices, $n^{20}D$, are 1.4143 and 1.4354, respectively. Raha reports n^{35} 1.3760 for his product, but does not indicate the wave length of light. Fraction 1, with concentrated ammonia, gave malonamide, m.p. 168–169° (lit. value[4] 170°). A mixed melting point with an authentic sample showed no depression.

It should be pointed out that malonic ester ($C_7H_{12}O_4$) and γ-butyrolactone ($C_4H_6O_2$) have nearly the same percentage composition and saponification equivalent, possibly accounting for Raha's error in interpretation of the elemental analyses. The higher boiling fraction 2, however, should have had a distinctly different analysis, and we cannot account for Raha's claim that all fractions had the same carbon and hydrogen content, and saponification equivalent.

Fraction 2 was shown to be β-chloroethyl ethyl malonate. Although the elemental analysis is not entirely satisfactory, it is given here to demonstrate that the compound contained chlorine.

Anal.[5] Calcd. for $C_7H_{11}O_4Cl$: C, 43.2; H, 5.7; Cl, 18.2. Found: C, 43.8; H, 5.7; Cl, 17.2.

The high carbon and low chlorine can be explained satisfactorily by about 5% of malonic ester as an impurity. Fraction 2, with urea,[6] gave a good yield of barbituric acid, m.p. 256° (lit. value[7] 245°).

Anal. Calcd. for $C_4H_4N_2O_3$: C, 37.5; H, 3.11; N, 21.9. Found: C, 37.5; H, 2.82; N, 21.8.

Fraction 2 (10 g.) was shaken for 15 minutes with 60 ml. of concentrated ammonia. After standing for one hour, the crystalline solid was filtered. After recrystallization from hot water there was obtained 4.5 g. (85.6%) of malonamide, m.p. and mixed m.p. 168–169°.[4] A small sample of this malonamide was further identified by bromination,[8] which gave dibromomalonamide, m.p. 201–202° (lit. value[8] 203°).

Raha claimed that the two fractions which he obtained as product, although they boiled as far as 45° apart, gave identical derivatives. It is now obvious why this was so, since both materials differed only in the alcohol portion of the ester.

Fraction 2 accounted for 20% of the original malonic ester.

Fraction 3 was shown to be bis-β-chloroethyl malonate. With ammonia, using the same procedure described above, malonamide was again obtained. The boiling point was consistent with the literature value.[9] Fraction 3 accounted for 4% of the original malonic ester. Thus, without including intermediate fractions, 87% of the original malonic ester is accounted for as recovered ester, or the two ester-interchange products. If any γ-butyrolactone was formed at all, it must have been (including intermediate fractions) in less than 6% yield.

Finally, authentic β-chloroethyl ethyl malonate and bis-β-chloroethyl malonate were synthesized according to the procedure of Michael and Weiner[10] from malonic ester, ethylene chlorohydrin and hydrogen chloride. The products had identical physical constants and infrared absorption spectra with fractions 2 and 3, respectively.

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(3) See I. Heilbron, "Dictionary of Organic Compounds," Vol. 1f, Oxford University Press, New York, N. Y., 1953, p. 186.

(4) I. Heilbron, *ibid.*, Vol. III, p. 206.

(5) Analyses were by Clark Microanalytical Laboratory, Urbana, Ill.

(6) The procedure was analogous to that in "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 60.

(7) H. Biltz and H. Wittek, *Ber.*, **54**, 1035 (1921).

(8) J. V. Backes, R. W. West and M. A. Whiteley, *J. Chem. Soc.*, **119**, 364 (1921).

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(10) A. Michael and N. Weiner, THIS JOURNAL, **58**, 999 (1936).

A New Synthesis of Neopentyl Alcohol[1]

BY JOSEPH HOFFMAN[2] AND CECIL E. BOORD

RECEIVED NOVEMBER 24, 1954

In the course of an investigation on the preparation of 2,4,4-trimethyl-2-pentyl (isoöctyl) hydroperoxide by mixing diisobutylene[3] with an aqueous solution consisting of sulfuric acid and hydrogen peroxide,[1] it was found that the hydroperoxide could be prepared if the acid concentration of the aqueous phase was less than 45%. When the percentage of acid in the aqueous phase was increased to 65–70, no hydroperoxide was found at the end of a run (as evidenced by lack of active oxygen) even though most of the diisobutylene had disappeared during the course of mixing. When the aqueous acid layer was poured onto cracked ice, a viscous oil separated. This suggested that the hydroperoxide although formed was being degraded almost as rapidly.

The acid-catalyzed degradation of hydroperoxides has been observed previously.[4–8] It has been postulated to proceed through the initial scission of the oxygen–oxygen bond followed by rearrangement.[6–8] It is reasonable to assume that the extent of rearrangement as well as the nature of the group or groups participating in it will vary with the structure of the molecule. One might predict that the decomposition of isoöctyl hydroperoxide (involving only the migration of a neopentyl group) would result in the formation of neopentyl alcohol.

$$(CH_3)_3CCH_2C(CH_3)_2OOH \xrightarrow{H^+} (CH_3)_3CCH_2OH + CH_3COCH_3$$

It was suspected, therefore, that the unidentified oil was crude neopentyl alcohol, and subsequently this was confirmed.

It was thought that perhaps a new synthetic method for the preparation of this alcohol might be developed by further study of the reaction. A series of experiments was performed in which hydrogen peroxide and diisobutylene (DIB) were allowed to react in varying ratios in sulfuric acid solution. As the H_2O_2/DIB ratio was increased, a point of diminishing returns was reached at about 2/1. In each experiment an organic product was recovered by dilution of the aqueous acid phase; this was identified as being mainly neopentyl alcohol. The acetone produced by the degradation of the hydroperoxide reacted with hydrogen peroxide to form acetone peroxide. A small amount of methyl neopentyl ketone was identified in the unreacted DIB phase; this ketone can be accounted for by the

(1) This work was presented before the Division of Organic Chemistry at the 121st Meeting of the American Chemical Society, at Milwaukee, Wis., April 2, 1952. It was taken in part from the dissertation submitted by Joseph Hoffman to the Graduate School of The Ohio State University, in 1951, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Firestone Tire and Rubber Co. Fellow, 1949-1951.

(3) Commercial diisobutylene comprises approximately 85% 2,4,4-trimethyl-1-pentene and 15% 2,4,4-trimethyl-2-pentene.

(4) H. Hock and S. Lang, *Ber.*, **77**, 257 (1944).

(5) R. Criegee and H. Dietrich, *Ann.*, **560**, 135 (1948).

(6) R. Criegee, *ibid.*, **560**, 127 (1948).

(7) M. S. Kharasch, A. Fono and W. Nudenberg, *J. Org. Chem.*, **15**, 748 (1950).

(8) M. S. Kharasch and J. G. Burt, *ibid.*, **16**, 150 (1951).