Ozonolysis. V. Reaction of an Ozonide with the Isopropyl Grignard Reagent

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It has been reported[2] that treatment of the reaction mixture resulting from the ozonation of *trans*-alkenes in ether solution at −112° with the isopropyl Grignard reagent gave rise to an α-diol and carbinols. Supposedly, the α-diol arose from reaction of the Grignard reagent with the molozonide, and the carbinols, which were obtained in much smaller quantity than the α-diol, were formed by reaction of the Grignard reagent with a small amount of ozonide in the reaction mixture.

There are in the literature several instances of the treatment of ozonation reaction mixtures with Grignard reagents. Criegee and Schröder[3] described the reaction of methyl and isopropyl Grignard reagents with the reaction mixture resulting from the ozonation of di-*t*-butylethylene, and Sparks and Knobloch[4] reported the reaction of ethyl and phenyl Grignard reagents with reaction mixtures obtained by the ozonation of several 1-alkenes. In each case the products were carbinols which would result from the reaction of the Grignard reagent with the cleavage products of the ozonized alkene.

Ozonation of an alkene in solvents devoid of active hydrogen atoms gives rise to polymeric peroxidic material, carbonyl compounds, and ozonide, all of which will react with the Grignard reagent. Indeed, the ozonide may be formed in least amount. There are no reports of the reaction of a pure ozonide with the Grignard reagent, and it was thought desirable to ascertain the products resulting from these reactants.

The ozonide was obtained in excellent yield by the ozonation of *cis*-3-hexene in *n*-pentane solution at −70°. The ozonide distilled with no temperature range; vapor phase chromatography (v.p.c.) indicated a single component, and the infrared spectrum confirmed the absence of carbonyl- or hydroxy-containing impurities. In order to make comparisons with earlier experiments[2] the reaction of the ozonide (26.6 mmoles) with excess isopropyl Grignard reagent was carried out as were the earlier reactions; *i.e.*, the reactants were mixed at −112° and the reaction mixture was allowed to warm slowly. The temperature behavior of the reaction mixture in the early and later stages of the addition of the Grignard reagent indicated that some reaction did occur at −112°. The reaction mixture was eventually refluxed, and during the reflux period propane (17.1 mmoles) and propene (10.4 mmoles) were evolved. The ozonide and Grignard reagent reacted in the molar ratio 1.00:3.89. The other identifiable products of the reaction were isopropyl alcohol (12.8 mmoles) and ethylisopropylcarbinol (36.7).

The products resulting from the treatment of the solution which was obtained by the ozonation of *cis*-3-hexene in ether solution at −112° with isopropyl Grignard reagent have been reported.[2] To compare the results of this experiment with those described above, one must correct for the larger amount of ozonide in the earlier experiment. After this correction the products were propane (17.7 mmoles), propene (11.8 mmoles), isopropyl alcohol (18.9 mmoles), and ethylisopropylcarbinol (36.0 mmoles). Other than the discrepancy in the amounts of isopropyl alcohol, there is good agreement between the two experiments.

It is clear that ozonides react with the Grignard reagent to give carbinols which would result from reaction of the Grignard reagent with the cleavage products of the ozonide. Also, it is clear that not all of the carbon atoms of the ozonide react in this fashion, for the yield of ethylisopropylcarbinol is but 69% of what one would expect if all of the ozonide reacted to give this carbinol.

Experimental

Chemicals.—The *n*-pentane and isopropyl bromide were purified as described earlier.[2] The *cis*-3-hexene was an API standard sample (Carnegie Institute of Technology). Ether was an anhydrous grade which was stored over sodium ribbon.

Ozonide Preparation.—A solution of 3.26 g. (38.7 mmoles) of *cis*-3-hexene in 250 ml. of *n*-pentane was ozonized with 36.8 mmoles of ozone at −70°. The ozone–oxygen flow rate was 13.0 l./hr., and the ozone was added at the rate of 583 mg./hr. The ozonation flask was placed in a −50° bath, and the pentane was removed through a rotary evaporator under a pressure of 0.1 mm. The pentane (243 ml.) was condensed in a liquid nitrogen cooled trap. The −50° bath was replaced by one at 25°, and 4.22 g. of ozonide (87% yield) was collected in a clean trap. A small residue (0.40 g.) remained in the reaction flask. The ozonide was distilled through a Vigreux column to give 3.98 g. (30.2 mmoles, 82% yield) of ozonide, b.p. 45° (22 mm.), n^{25}D 1.4012, mol. wt. 132 (cryoscopic, benzene). The nuclear magnetic resonance spectrum of the ozonide (35 wt. % in carbon tetrachloride) had two overlapping triplets centered at 4.92, a multiplet centered at 1.55, and two, unresolved, overlapping triplets centered at 0.82 p.p.m. The integrated areas of these three peaks were in the ratio 1.00:2.04:3.12 and, accordingly, were assigned to the methine, methylene, and methyl protons, respectively. For the ozonide of *trans*-3-hexene, Criegee[5] reported b.p. 46.5° (26 mm.), n^{20}D 1.4050.

V.p.c. of a pentane solution of the ozonide on a silicon GE SF 96 column showed only one component. An infrared spectrum (Perkin-Elmer Model 421) of the ozonide (neat) indicated the absence of carbonyl- or hydroxy-containing impurities. Comparison of the spectrum with that of di-*n*-propyl peroxide showed the similarities to be very strong absorption at 950, 1380, and 1460 for the ozonide and 955, 1375, and 1453 cm.$^{-1}$, peroxide.

Reaction of the Ozonide with Isopropyl Grignard Reagent.—In a flask fitted with a stirrer, thermometer well, and dropping funnel was placed a solution of 3.52 g. (26.6 mmoles) of ozonide in 350 ml. of ether. To this solution was added 100 ml. (pipet) of isopropylmagnesium bromide (1.72 *M*) at such a rate that the temperature of the reaction mixture did not rise above −110°. After standing 3 days, the reaction mixture had warmed to 20°. The dropping funnel was replaced by a condenser which was attached to a gas-collecting bottle. Stirring and refluxing (1 hr.) the reaction mixture until no more gas was evolved gave 1183 ml. (STP) of gas, which was found by mass spectroscopy[6] to contain 17.1 mmoles of propane and 10.4 mmoles of propene. After cooling to −10° the reaction mixture was hydrolyzed by the addition of a solution of 9.31 g. of concentrated sulfuric acid in 100 ml. of water. Refluxing the mixture liberated the propane from the excess Grignard reagent. Determination of this propane showed that 104 mmoles of Grignard reagent had reacted with the ozonide; ozonide–Grignard reagent molar ratio, 1.00:3.89.

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[2] F. L. Greenwood, *J. Org. Chem.*, **29**, 1321 (1964).

[3] R. Criegee and G. Schröder, *Chem. Ber.*, **93**, 689 (1960).

[4] J. W. Sparks and J. O. Knobloch, U. S. Patent 2,671,812 (1954); *Chem. Abstr.*, **49**, 5508 (1955).

[5] R. Criegee, A. Kerchow, and H. Zinke, *Chem. Ber.*, **88**, 1878 (1955).

[6] We are indebted to the Texas Research Laboratories, Beacon, N. Y., for the mass spectrometric analysis.

The aqueous layer from the hydrolysis mixture was extracted in a continuous ether extractor. The ether extract was combined with the ether layer from the hydrolysis mixture, and the ether was removed from the dried (sodium sulfate) ether soution through a Fenske column. The residue was distilled *in vacuo* (5 mm.) through a Vigreux column. The distillate (4.69 g.) was condensed in a receiver which was in a −70° bath. A residue of 0.43 g. remained in the distilling flask. The constituents of this distillate were identified by comparison with authentic samples by v.p.c. (cyanosilicone column). The quantitative v.p.c. analysis of the distillate was carried out by the method of internal normalization.[7] The distillate contained isopropyl alcohol (12.8 mmoles), ethylisopropylcarbinol (36.7 mmoles, 69% yield on the basis of 1 mole of ozonide giving 2 moles of carbinol), and unidentified material (0.14 g.).

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(7) R. L. Pecsok, "Principles and Practice of Gas Chromatography," John Wiley and Sons, Inc., New York, N. Y., 1959, p. 143.

A New Synthesis of *p*-Methylaminobenzoyl-L-glutamic Acid[1]

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The preparation of *p*-methylaminobenzoyl-L-glutamic acid, an intermediate in the synthesis of N^{10}-methylfolic acid and analogs, by the aminolysis of sodium *p*-iodobenzoyl-L-glutamate with methylamine at pH 8–9 has been reported.[2] The desired acid failed to crystallize, and the crude sodium salt was isolated in unspecified yield.

We were unsuccessful with this procedure until the use of sodium hydroxide during the condensation was eliminated. The *p*-methylaminobenzoyl-L-glutamic acid was then isolated in low yield as the dihydrated barium salt from which the free acid was obtained as an oil. There was also a possibility of racemization of the optically active compound in the alkaline solution at elevated temperature. In a procedure here described, *p*-methylaminobenzoyl-L-glutamic acid hydrobromide has been synthesized in high purity and yield suitable for the synthesis of N^{10}-methylfolic acid and derivatives.

Starting with *p*-methylaminobenzoic acid, the methylamino group was protected by carbobenzyloxylation before conversion of the acid into its chloride. The N-*p*-[(carbobenzyloxy)methylamino]benzoyl chloride was allowed to react with diethyl L-glutamate as in the synthesis of derivatives of aminobenzoylglutamic acid.[3] The resulting ester was hydrolyzed by alkali to afford N-{*p*-[(carbobenzyloxy)methylamino]benzoyl}-L-glutamic acid. The carbobenzyloxy group was removed by 40% hydrogen bromide in glacial acetic acid,[4] and the *p*-methylaminobenzoyl-L-glutamic acid was isolated as the hydrobromide. Attempts to remove the hydrogen bromide from the dipeptide with pyridine, triethylamine, or sodium hydrogen carbonate yielded an oily product which could not be reconverted to the crystalline hydrobromide by treatment with hydrogen bromide in ether. The diethyl ester was prepared by the decarbobenzyloxylation of diethyl N-{*p*-[(carbobenzyloxy)methylamino]benzoyl}-L-glutamate and it showed optical rotation identical with that reported.[2] This indicates that no racemization occurred in the earlier aminolysis procedure.[2]

Experimental[5]

Barium *p*-Methylaminobenzoyl-L-glutamate from *p*-Iodobenzoyl-L-glutamic Acid.—*p*-Iodobenzoyl-L-glutamic acid,[2] $\lambda_{max}^{H_2O}$ 252 mμ, 2 g. (5.3 mmoles), 5 ml. of 30% aqueous solution of methylamine, and 10 mg. of fine copper powder was heated in a sealed tube at 150° for 10 hr. The reaction mixture was diluted with 10 ml. of methanol and filtered. Upon concentration under nitrogen, a golden oil resulted, which defied attempts at crystallization and which gave unreproducible refractive indices and erroneous elementary analyses. The oil was redissolved in 5 ml. of methanol and treated with 5 ml. of 0.5 *M* barium chloride in 50% methanol. The precipitated barium *p*-methylaminobenzoyl-L-glutamate was collected and washed with methanol; yield 0.6 g. (24%), $\lambda_{max}^{H_2O}$ 291 mμ.

Anal. Calcd. for $C_{13}H_{14}BaN_2O_5 \cdot 2H_2O$: C, 34.6; H, 4.0; N, 6.2. Found: C, 34.1; H, 4.0; N, 6.0.

Treatment of the barium salt with 1 *N* sulfuric acid to pH 6 afforded the free dipeptide as an oil which again gave unreproducible refractive indices and erroneous elementary analyses.

N-*p*-[(Carbobenzyloxy)methylamino]benzoic Acid.—*p*-Methylaminobenzoic acid (2 g., 13.3 mmoles) purified by the nitrosation procedure[6] was dissolved in 10 ml. of 2 *N* sodium hydroxide. To this solution was added carbobenzyloxy chloride (2.3 ml., 16 mmoles) alternately with 17 ml. of 2 *N* sodium hydroxide at 0°. Upon acidification to pH 3, the crude compound precipitated; it was collected and washed with cold water. The pure N-*p*-[(carbobenzyloxy)methylamino]benzoic acid was obtained after two recrystallizations from glacial acetic acid; yield 66%, m.p. 149.5–151.5°.

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.4; H, 5.3; N, 4.9. Found: C, 67.3; H, 5.4; N, 4.9.

Diethyl N-{*p*-[(Carbobenzyloxy)methylamino]benzoyl}-L-glutamate.—The N-*p*-[(carbobenzyloxy)methylamino]benzoyl chloride was prepared by treating N-*p*-[(carbobenzyloxy)methylamino]benzoic acid (1.4 g., 5 mmoles) with phosphorus pentachloride (1.2 g., 5.5 mmoles) in 20 ml. of anhydrous ether. The acid chloride was not isolated; the ether solution was washed with 10 ml. of ice-cold water and immediately poured into a mixture of diethyl L-glutamate hydrochloride[7] (1.2 g., 5 mmoles) and sodium hydrogen carbonate (3.3 g., 40 mmoles) in 20 ml. of ethyl acetate and 20 ml. of water. This was stirred at 0° for 15 min. and then at 25–30° for 1 hr. The organic phase was separated and washed successively with 10 ml. each of water, 2 *N* hydrochloric acid, and water. The crude product obtained after evaporation of the solvent was recrystallized from ethyl acetate–*n*-hexane (1:5) and acetone–*n*-hexane (1:5); yield 79%, m.p. 85°, sintered at 64–66°.

Anal. Calcd. for $C_{25}H_{30}N_2O_7$: C, 63.8; H, 6.4; N, 6.0. Found: C, 63.7; H, 6.2; N, 6.0.

N-{*p*-[(Carbobenzyloxy)methylamino]benzoyl}-L-glutamic Acid Hydrate.—Diethyl N-*p*-[(carbobenzyloxy)methylamino]benzoyl-L-glutamate (2.4 g., 5 mmoles) was hydrolyzed in 1 *N* sodium hydroxide (11 ml., 11 mmoles) and 11 ml. of methanol, first at 0° for 10 min. and then at 25–30° for 1 hr. The resulting

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(1) Supported in part by grants from the National Institutes of Health, U. S. Public Health Service, No. CY-3335 and C-6516.

(2) D. B. Cosulich and J. M. Smith, Jr., *J. Am. Chem. Soc.*, **70**, 1922 (1948).

(3) S.-C. J. Fu, *J. Med. Pharm. Chem.*, **5**, 33 (1962).

(4) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(5) All melting points are corrected. Optical rotation was measured with a Bellingham and Stanley polarimeter. Ultraviolet absorption spectra were recorded with a Cary Model 15 spectrophotometer. The elementary analyses were performed by Dr. C. K. Fitz, Needham Heights, Mass.

(6) (a) A. R. Surrey and H. F. Hammer, *J. Am. Chem. Soc.*, **66**, 2127 (1944); (b) F. Klaus and O. Baudisch, *Ber.*, **51**, 1036 (1918).

(7) $[\alpha]^{25}_{D}$ +23.0° (absolute ethanol). $[\alpha]_{D}$ +22.4° (water) is given in J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 932.