of the fractions it was calculated that they contain 1.6 g. (33%) of unreacted starting material and 0.86 g. (24%) of IIIf.

The distillation pot, from which these fractions were distilled, contained 0.75 g. (23%) of α-chloro-β-methylcrotonic acid.

(e) To a mixture of 15 ml. of 10% hydrochloric acid and 75 ml. of methanol was added 5.95 g. of IIf. The homogeneous mixture was stirred magnetically at room temperature for 24 hours then refluxed for 1 hour. Methanol was removed by vacuum distillation at room temperature. The residue in the flask separated into two layers. Water and ether were added, the aqueous layer separated and extracted twice with ether and the combined ether fractions dried (magnesium sulfate). Distillation yielded 3.5 g. (60%) of starting material contaminated with traces of the pyrolysis product IIIf. Distillation of the water layer from the separation yielded no organic material.

(f) To 1 g. of cyclopropanone diethylacetal (XVIII) was added 6 ml. of concentrated hydrochloric acid. The homogeneous solution was allowed to stand at room temperature for 0.5 hour and then diluted with 6 ml. of water which caused a second layer to separate. The mixture was extracted with ether and the ether extractions dried (magnesium sulfate). Distillation recovered 0.7 g. (70%) of unchanged XVIII.

(g) To 2 g. of XVIII was added 12 ml. of concentrated hydrochloric acid and the resulting solution heated on the steam-bath for 20 minutes. The mixture was then distilled under reduced pressure; no residue remained. The distillate was neutralized with solid sodium carbonate and extracted with ether. The ether fraction was dried (magnesium sulfate) and distilled to yield 0.185 g. (12%) of ethyl propionate and 0.476 g. (34%) of chloroacetone. The ethyl propionate was characterized by its infrared spectrum which was identical with that of an authentic sample. The chloroacetone was characterized by its infrared spectrum which was identical to that of an authentic sample, and by its 2,4-dinitrophenylhydrazone derivative, m.p. 124–125.5°, which on admixture with an authentic sample melted at 122–123°.

The aqueous fraction was acidified with sulfuric acid and continually extracted with ether for 24 hours. This extracted fraction was titrated with 0.1 *N* sodium hydroxide indicating 4.58 milliequivalents of an acid (30% from the cyclopropanone diethylacetal). The sodium salt was then isolated and converted to the *p*-toluide, m.p. 125–126°, which on admixture with the *p*-toluide of propionic acid melted at 125°.

(h) When XVIII was treated with anhydrous hydrogen chloride at room temperature for 1.5 hours, 0.3 of an equivalent was absorbed but infrared analysis disclosed no new product. Treatment of 1.15 g. of the acetal with hydrogen chloride at 95° produced a 38% yield of ethyl propionate, but no chloroacetone. Unchanged cyclopropanone acetal (21%) was recovered.

(i) To 20 ml. of concentrated hydrochloric acid was added 5 g. of 2,2-dimethyl-3-phenylcyclopropanone dimethylacetal (XXII). The heterogeneous mixture was refluxed for 2 hours and extracted with benzene. Distillation at 0.05 mm. yielded 1.85 g. (40%) of XXII, b.p. 54–58°, n^{25}D 1.5080, and 1.80 g. of a mixture, b.p. 58–76°, n^{25}D 1.5159. The latter fraction gave an infrared spectrum containing carbonyl and hydroxyl absorption peaks and was not investigated further.

Bromination of Cyclopropanone Diethylacetal (XVIII).—To 1.3 g. (0.01 mole) of cyclopropanone diethylacetal in 15 ml. of carbon tetrachloride was added a solution of 1.6 g. (0.01 mole) of bromine in 10 ml. of carbon tetrachloride *via* a dropping funnel over a period of 0.5 hour. The bromine color was dissipated immediately and the resulting solution was light yellow. The solvent was removed by distillation during which hydrogen bromide and some ethyl bromide were evident. Vacuum distillation (7 mm.) of the residue through a 30-cm. Podbielniak column gave the fractions: (a) 0.10 g., b.p. 66–75°, n^{25}D 1.4527; (b) 0.35 g., b.p. 75–85°, n^{25}D 1.4677; (c) 0.50 g., b.p. 85–87°, n^{25}D 1.4774; (d) 0.60 g., b.p. 87–88°, n^{25}D 1.4866; (e) 0.20 g., b.p. 89°, n^{25}D 1.4952.

Fraction a was mostly ethyl β-bromopropionate, characterized by its infrared spectrum.

Fractions d and e were mostly ethyl α,β-dibromopropionate. The infrared spectra of these fractions were superimposable upon that of authentic material prepared by action of bromine on ethyl acrylate.

Infrared analysis of fractions b and c indicated that they were mixtures of the monobromo and the dibromo esters. On the basis of the refractive indices of the fractions it was found that the monobromo ester was produced in 41% yield, the dibromo ester in 39% yield.

Bromination of 2,2-Dichlorocyclopropanone Diethylacetal (IIb).—To 10 g. (0.05 mole) of IIb was added a solution of 8 g. (0.05 mole) of bromine in 7 ml. of carbon tetrachloride *via* a dropping funnel. An ice–water-bath was necessary to cool the exothermic reaction which developed after approximately one-quarter of the bromine had been added. The bromine color was not dissipated completely until the mixture was stirred extensively after each addition of bromine solution. Removal of solvent and distillation yielded 9.65 g. (77.3%) of ethyl β,β-dichloro-β-bromopropionate (XXIII), b.p. 40–46° (0.07 mm.). A middle fraction boiling at 43–44° (0.07 mm.), n^{25}D 1.4857, d^{25}_4 1.6138 was analyzed.

Anal. Calcd. for $C_5H_7BrCl_2O_2$: C, 24.03; H, 2.83; total halogen, 60.35. Found: C, 24.51; H, 3.04; total halogen, 60.6.

A 6.65-g. sample of the ester XXIII was added to a solution of 0.027 mole of sodium ethoxide in absolute ethanol. An immediate precipitation occurred and an exothermic reaction was evident. The mixture was stirred for 3 hours at room temperature and then refluxed for 0.25 hour. Ether (20 ml.) was added, the mixture filtered and distilled to yield 0.80 g. (17.5%) of ethyl β,β-dichloroacrylate, b.p. 173–177°, n^{25}D 1.4621; chlorine content: 41.8% (calcd. 40.7); previously reported[13] b.p. 173–175°.

This ester was saponified by shaking with 5% sodium hydroxide in a separatory funnel until homogeneous (about 5 minutes). Isolation of the acid yielded β,β-dichloroacrylic acid, m.p. 75–76° after two recrystallizations from petroleum ether; previously reported[21] m.p. 76–77°.

---

(21) O. Wallach, *Ann.*, **193**, 20 (1878).

MADISON 6, WISC.

[CONTRIBUTION #1032 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Reaction of Some Methylene Derivatives with Ketene Diethylacetal[1]

BY MALCOLM F. DULL AND PHILLIP G. ABEND

RECEIVED NOVEMBER 24, 1958

The reaction of diazomethane with ketene diethylacetal (III) and phenylketene diethylacetal (V) has been found to give cyclopropanone diethylacetal (II) and phenylcyclopropanone diethylacetal (IV), respectively, the latter also being obtained by the reaction of phenyldiazomethane with III. Ethyl diazoacetate and III gave only carbethoxymethylketene diethylacetal (VI), while ethyl α-bromoacrylate was obtained from the reaction of bromoform and potassium *t*-butoxide with III. The formation of unstable 2,2-dibromocyclopropanone diethylacetal as a precursor is postulated.

McElvain and Weyna[2] have shown that when chloroform and benzal chloride are treated with sodium *t*-butoxide in the presence of a variety of ketene acetals the resulting dichloro- and phenylchlorocarbene adds to the ketene acetals to form

---

(1) Taken from the thesis of Phillip G. Abend submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Pittsburgh, 1958.

(2) S. M. McElvain and Philip L. Weyna, THIS JOURNAL, **81**, 2579 (1959).

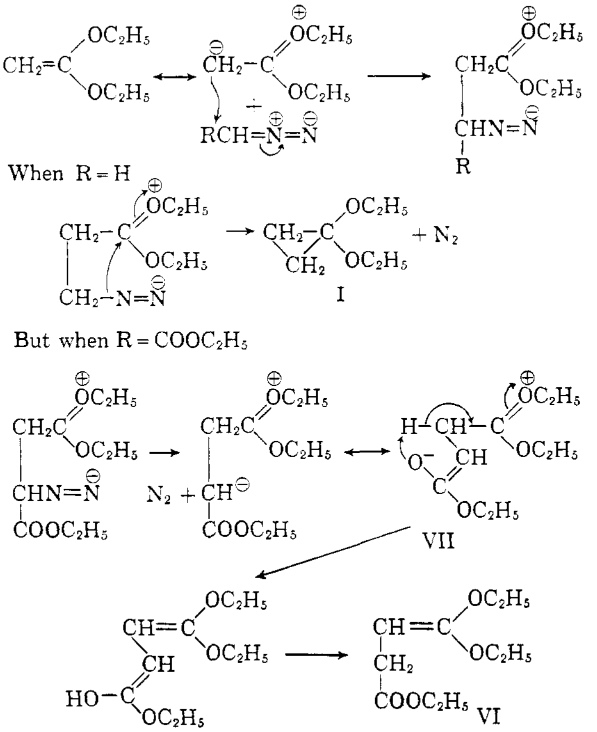
the correspondingly substituted cyclopropanone acetals. Rearrangement of the dichlorocyclopropanone acetals to the corresponding α-chloroacrylates was observed to occur on distillation at various pressures, although 2,2-dichlorocyclopropanone acetals distilled unchanged at low pressures. Reduction of 2,2-dichlorocyclopropanone diethylacetal (I) to cyclopropanone diethylacetal (II) in 27% yield by sodium in *t*-butyl alcohol was described. Other chlorocyclopropanone acetals were similarly reduced to dechlorinated substituted cyclopropanone acetals and their remarkable resistance to hydrolysis under acid conditions was noted. Hydrolysis under drastic conditions and bromination causes ring fission.

The preparation of the hydrate, methyl- and ethylhemiacetals of cyclopropanone by reaction of ketene with diazomethane in ether containing some water or the alcohol has been reported by Lipp, Buchkremer and Seeles.[3] These substances were observed to undergo slow rearrangement to propionic acid, its methyl and ethyl esters, respectively. In this Laboratory it has been found that reaction of diazomethane with ketene diethylacetal (III) in *n*-hexane solution catalyzed by cuprous bromide gives a yield of up to 50% (average yield 35%) of II, which was characterized by infrared and proton n.m.r. resonance spectra. It reacts readily with bromine in carbon tetrachloride but is resistant to aqueous permanganate and 50% sulfuric acid at room temperature. On refluxing with 2% permanganate the color is slowly discharged. Reaction also occurs on refluxing with 50% sulfuric acid to give ethanol and products which were not identified but gave a positive test with Tollens reagent and with dilute permanganate in the cold. McElvain and Weyna[2] found that when II is refluxed with concentrated hydrochloric acid it is converted to a mixture of chloroacetone, ethyl propionate and propionic acid, and McElvain[4] has suggested that refluxing with 50% sulfuric acid may lead to the formation of acetol which reduces both Fehling solution and Tollens reagent. Lipp, *et al.*,[3] stated that the cyclopropanone hydrate and hemiacetals are sensitive to Tollens reagent and dilute permanganate.

Phenylcyclopropanone diethylacetal (IV) was obtained by the reaction of phenyldiazomethane with III and of diazomethane with phenylketene diethylacetal (V) in 82 and 12% yields, respectively, although the latter are probably not optimum. Identity of IV was not as definitely established by infrared and n.m.r. spectra as was that of II, but its formation by alternate methods and its inertness to 33% sulfuric acid and dilute permanganate solutions at room temperature serve for characterization. Like II it reacts rapidly with bromine in carbon tetrachloride.

Reaction of ethyl diazoacetate with III in *n*-hexane, in contrast, gave only carbethoxymethylketene diethylacetal (VI) in about 55% average yields, characterized by its infrared spectrum and reactivity to bromine in carbon tetrachloride, aqueous permanganate and dilute sulfuric acid. The latter converted it to a succinic acid ester subsequently hydrolyzed to the acid.

The contrast in the reaction of diazomethane and ethyl diazoacetate with III, leading to formation of II and VI, respectively, is striking though not unique in the chemistry of aliphatic diazo compounds. The course of both reactions may be rationalized in terms of an electrophilic attack of the diazo compound upon the ketene acetal.



The charge distribution to the carbonyl oxygen in VII permits the electrophilic center to be neutralized by the proton shift converting VII to VI.[4]

Dibromocarbene, from the reaction of potassium *t*-butoxide with bromoform, reacted with III to give a product identified after distillation at reduced pressure as ethyl α-bromoacrylate in yields averaging 51%, together with ethyl bromide. It is probable that unstable 2,2-dibromocyclopropanone diethylacetal was formed and decomposed with rearrangement in the manner suggested by McElvain and Weyna[3] for the conversion of I to the α-chloroacrylate.

Experimental

Cyclopropanone Diethylacetal (II).—Into a 500-ml. two-neck round bottom flask, fitted with a constant pressure dropping funnel and a Dry Ice–acetone condenser protected with a calcium chloride drying tube, were placed 0.25 g. of cuprous bromide, 8.7 g. (0.075 mole) of III[5] and 20 ml. of *n*-hexane. A solution containing 0.096 mole of diazomethane[6] in 200 ml. of *n*-hexane was added dropwise with stirring provided by a magnetic stirring bar. Reaction was accompanied by a vigorous evolution of gas (nitrogen and some ethylene) and formation of some polymethylene in the dropping funnel. The reaction mixture was filtered and the solvent removed by distillation through a packed column from a water-bath. The residue was shaken in a

---

[3] P. Lipp, J. Buchkremer and H. Seeles, *Ann.*, **499**, 1 (1932).

[4] S. M. McElvain, personal communication.

[5] P. M. Walters and S. M. McElvain, THIS JOURNAL, **62**, 1482 (1940).

[6] F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

separatory funnel with 5% sulfuric acid to remove unreacted III, and the organic phase taken up in ether. The ether layer was separated, washed with three 30-ml. portions of 10% sodium carbonate solution, then with water and dried over anhydrous sodium sulfate. The solution was filtered and the ether distilled at atmospheric pressure through a small packed column from a water-bath. The residue was transferred to a 25-ml. modified Claisen flask and distilled at reduced pressure from an oil-bath. The yield of product boiling at 67.0–68.5° at 139 mm. was 4.0 g. (41%), $n^{25}\text{D}$ 1.3990; average yield 35%. *Anal.* Calcd. for $C_7H_{14}O_2$: C, 64.62; H, 10.77; mol. wt., 130.2. Found: C, 64.94; H, 10.27; mol. wt. (cryoscopically in nitrobenzene), 130.

A fraction boiling at 68.0–69.0° at 130 mm. was employed for infrared and nuclear magnetic resonance analysis. In the infrared spectrum bands at 1060 and 1130 cm.$^{-1}$ are indicative of a ketal structure.[7] No bands appeared in the region 1620 to 1680 cm.$^{-1}$, indicating the absence of a carbon–carbon double bond.[8] Presence of a small amount of carbonyl impurity was indicated by the appearance of a band of weak intensity at 1725 cm.$^{-1}$. The H^1 n.m.r. spectrum at 40 megacycles showed four peaks between 1.0 and 2.0 parts per million of the applied field relative to water due to the methylene groups in the ethoxy radicals. A triplet centered at about 4.1, with intensities about 1:2:1, is from the methyl groups. A single line at about 4.6 is from the ring hydrogens. Its position on the high field side of the methyl resonance is consistent with the spectra observed for other cyclopropane derivatives.[9] The presence of a single unsplit line indicates that all of the ring hydrogens are equivalent. The infrared and n.m.r. spectra are, therefore, completely consistent with the proposed structure.

Reaction of II with a 5% solution of bromine in carbon tetrachloride was immediate, eight drops being required to produce a permanent color in 0.5 ml. of II. There was no reaction at room temperature with aqueous permanganate or 50% sulfuric acid. On refluxing with 2% permanganate the color was slowly discharged. Refluxing 2 g. of II with 20 ml. of 50% sulfuric acid for two hours, followed by neutralization of the solution with 10% sodium carbonate, resulted in a positive iodoform test. Steam distillation of the solution gave a distillate exhibiting a positive test with Tollens reagent and with dilute permanganate.

Phenylcyclopropanone Diethylacetal (IV). (a) From III and Phenyldiazomethane.—Into a 250-ml. three-neck round bottom flask, fitted with two constant pressure dropping funnels and a reflux condenser connected through a calcium chloride drying tube to an inverted 2-l. graduated cylinder filled with water, were placed 0.25 g. of cuprous bromide and 20 ml. of *n*-hexane. Refluxing was maintained by means of an infrared heating lamp while being stirred with a magnetic stirring bar. In one addition funnel was placed a solution of 5.9 g. (0.05 mole) of phenyldiazomethane[10] in 60 ml. of *n*-hexane, and in the other a solution containing 8.7 g. (0.06 mole) of III in 50 ml. of *n*-hexane. The two solutions were allowed to flow into the flask at about the same rate. A vigorous evolution of nitrogen occurred which at the end amounted to 94% to the theory. The crude mixture was filtered and the solvent removed by distillation from a water-bath at atmospheric pressure. The residue was distilled *in vacuo* from an oil-bath and the fraction boiling at 97.5 to 99.5° at 2.9 mm. was collected and weighed 8.5 g. (82.5%), $n^{25.5}\text{D}$ 1.4977. An intractable nitrogen containing residue weighing about 2 g. remained. Redistillation gave b.p. 97.5–98.5° at 2.75 mm. and $n^{22}\text{D}$ 1.5020.

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.68; H, 8.74; mol. wt., 206. Found: C, 74.58; H, 8.99; mol. wt. (cryoscopically in nitrobenzene), 234.

The H^1 n.m.r. spectrum showed a peak at about −2.0 due to the phenyl group. The spectrum is complex around 3.9 to 4.5 where only methyl and cyclopropyl hydrogens should appear. What appears to have happened is that the ethoxy group resonance has been doubled, the methylene somewhat more than the methyl. It is possible that this could be the result of interaction with the magnetic field about the benzene ring if one of the ethoxy groups spends more time near the ring than the other.[11] This supposition is reasonable in view of the fact that the phenyl group in phenylcyclopropane diethylacetal is *cis* with respect to one and *trans* with respect to the other ethoxy group. The structure of the compound is not definitely established by its n.m.r. spectrum, but in view of the fact that the same substance was obtained by the reaction of diazomethane with V there can be little doubt of its identity.

(b) From V and Diazomethane.—Into an apparatus like that described for the preparation of II were placed 0.25 g. of cuprous bromide, 15 g. (0.078 mole) of V[12] and 10 ml. of *n*-hexane. With stirring provided by a magnetic stirring bar a solution containing 0.057 mole of diazomethane in 125 ml. of *n*-hexane was added dropwise. A vigorous and almost quantitative evolution of nitrogen occurred. The reaction mixture was filtered and the solvent removed by distillation from a water-bath at atmospheric pressure. The residue was distilled from an oil-bath and the fraction boiling at 91.0 to 94.0° at 0.75 mm. was collected. The yield was 2.0 g. (12%), $n^{22}\text{D}$ 1.5020. The H^1 n.m.r. spectrum of this product was virtually identical with that of the product of the reaction of ketene diethylacetal with phenyldiazomethane.

It required six drops of 5% bromine in carbon tetrachloride to produce a permanent color in 0.5 g. of IV; shaking with 5 ml. of 2% permanganate for about five minutes resulted in some loss of color, perhaps due to impurities. Five minutes of shaking 2.0 g. of IV with 10 ml. of 33% sulfuric acid gave no evidence of reaction, and 1.5 g. of unchanged IV was recovered. The aqueous residue exhibited a negative iodoform test.

The Reaction of III with Ethyl Diazoacetate. (a) Catalyzed by Cuprous Bromide in Refluxing *n*-Hexane.—Into a 250-ml. two-neck round-bottom flask, equipped like that described for the preparation of II, were placed 0.15 g. of cuprous bromide, 11.6 g. (0.10 mole) of III and 20 ml. of *n*-hexane. The solution was maintained at reflux temperature by means of an infrared heating lamp while being stirred with a magnetic stirring bar. A solution containing 11.8 g. (0.10 mole) of ethyl diazoacetate,[13] 9.0 g. (0.077 mole) of III, and 20 ml. of *n*-hexane was added dropwise. (Addition of this mixture of the reactants rather than diazoacetic ester alone was for the purpose of minimizing dilution effects). Evolution of nitrogen was vigorous and quantitative. The reaction mixture was filtered and the solvent removed in the usual manner. Vacuum distillation of the residue gave 12.0 g. (59%) of product boiling at 87.5–89.0° at 1.7 mm., $n^{30}\text{D}$ 1.4290. Average yield in six runs was 55%. Infrared peaks at 1040 and 1665 cm.$^{-1}$ indicate a ketene acetal structure,[7] and a band at 1725 cm.$^{-1}$ is assigned to the normal ester carbonyl frequency.[14]

Anal. Calcd. for $C_{10}H_{18}O_4$: C, 59.40; H, 8.91. Found: C, 58.10; H, 9.66.

(b) Catalyzed by Copper Powder in Refluxing Benzene.—In the same set-up of apparatus used in (a) were placed 1.5 g. of 150-mesh copper powder, 14.7 g. (0.127 mole) of III and 25 ml. of anhydrous benzene. To the refluxing solution was added dropwise a solution containing 14.5 (0.127 mole) of ethyl diazoacetate and 7.3 g. (0.063 mole) of III in 25 ml. of anhydrous benzene. Evolution of nitrogen was vigorous and amounted to 97% of theory. Isolation of the product in the manner described in (a) gave 13.0 g. (50.8%) of product boiling at 70.5–74.5°, $n^{22}\text{D}$ 1.4306, identical with that obtained in (a).

(c) Catalyzed by Copper Powder in the Absence of a Solvent.—Experiments similar to those in (a) and (b) in which ethyl diazoacetate was added to refluxing III in the presence of copper powder with no solvent gave, in seven runs, an average yield of product identical with that obtained in (a) of 41%, together with considerable intractable, nitrogen-containing residue and a less than quantitative (*ca.* 75–80% of theory) evolution of nitrogen.

This compound VI reacted rapidly with bromine in carbon tetrachloride and gave an immediate precipitate of

---

(7) E. D. Bergmann and S. Pinchas, *Rec. trav. chim.*, **71**, 161 (1952).

(8) S. M. McElvain and R. E. Starn, THIS JOURNAL, **77**, 4571 (1955).

(9) J. N. Shoolery, private communication to Paul C. Lauterbur, Fellow, Mellon Institute, Pittsburgh, Pa.

(10) R. J. Mohrbacher and N. H. Cromwell, THIS JOURNAL, **79**, 401 (1957).

(11) J. S. Waugh and R. W. Fessenden, *ibid.*, **79**, 846 (1957).

(12) S. M. McElvain and W. R. Davie, *ibid.*, **73**, 1400 (1951).

(13) N. E. Searle, *Org. Syntheses*, **36**, 25 (1956).

(14) L. Bellamy, "The Infra-Red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 31.

manganese dioxide when shaken with 2% potassium permanganate solution, indicative of unsaturation.

About 1.5 g. of VI was shaken with 10 ml. of 5% sulfuric acid. A slight rise in temperature was noted. The aqueous phase was separated and exhibited a strong positive iodoform test. The organic phase was refluxed for two hours with 20 ml. of a 30% potassium hydroxide solution, cooled and acidified with 6 *N* sulfuric acid, extracted with ether and dried over anhydrous sodium sulfate. The ether was evaporated and the residue recrystallized from acetone and identified as succinic acid by a mixed melting point of 184–185° with an authentic sample. These reactions together with the evidence of the infrared spectrum indicate VI to be carbethoxymethylketene diethylacetal.

The Reaction of Bromoform and Potassium *t*-Butoxide with Ketene Diethylacetal.—Into a 250-ml. three-neck round-bottom flask fitted with a reflux condenser protected by a calcium chloride drying tube, a mechanical stirrer, and a constant pressure dropping funnel, were placed 16.7 g. (0.15 mole) of powdered potassium *t* butoxide, 11.6 g. (0.1 mole) of III, 25 ml. of anhydrous *t*-butyl alcohol and 10 ml. of *n*-octane. The flask was immersed in a water-bath maintained at 10°. A solution of 25.2 g. (0.1 mole) of bromoform in 10 ml. of *n*-octane was added dropwise with stirring which was continued for one-half hour after addition was complete. To the reaction mixture was then added 100 ml. of ice-water, the whole shaken and transferred to a separatory funnel. An additional 30 ml. of *n*-octane was added and the phases separated. The organic layer was washed with three 100-ml. portions of ice-water, dried over 10 g. of anhydrous sodium sulfate, and the solvent removed by distillation from a water-bath at 35°. The water-bath was replaced by an oil-bath and the residue distilled *in vacuo* to give 9.2 g. (51%) of ethyl α-bromoacrylate, boiling at 72.0–72.5° at 31 mm. and identified by comparison of its infrared spectrum with that of an authentic sample prepared by the method of Marvel, Dec, Cooke and Cowan.[15] When the crude reaction mixture was heated at higher pressures, *i.e.*, 155 mm. and above, ethyl bromide was condensed in the Dry Ice–acetone trap in the line leading to the pump.

Acknowledgment.—Elemental analyses were by the Clark Microanalytical Laboratory and Mr. Otho E. Harris of the Microanalytical Laboratory of the University of Pittsburgh. The authors acknowledge with thanks the gift of generous quantities of sodium dispersion in mineral oil by the Ethyl Corporation and sodium–potassium alloy by the M. S. A. Research Co. They are indebted to Dr. Foil A. Miller, Dr. Harry M. Nelson and Mr. Gerald L. Carlson of the Spectroscopy Laboratory, Department of Chemical Physics, Mellon Institute of Industrial Research, for interpretation of the infrared spectra, and to Mr. Paul C. Lauterbur and Mr. John J. Burke of the Dow–Corning Multiple Fellowship at the Mellon Institute of Industrial Research for interpretation of the n.m.r. spectra. They are also grateful to Dr. S. M. McElvain of the Department of Chemistry, The University of Wisconsin, for helpful criticism and suggestions.

(15) C. S. Marvel, J. Dec, H. G. Cooke and J. C. Cowan, This Journal, **62**, 3495 (1940).

PITTSBURGH 13, PENNA.

COMMUNICATIONS TO THE EDITOR

STUDIES ON NATURALLY OCCURRING PHOSPHOINOSITIDES[1]

Sir:

The exact structure of the monophosphoinositides (diacyl glycerylphosphorylinositols) has not been established completely. In the present study evidence is presented as to the possible structure and configuration of the glycerylphosphorylinositol core.

Beef liver phosphoinositide[2] (1.02 g.) (P, 3.34; N, <0.08; N/P ~0.05; Inositol/P, 1.05) was dissolved in 70 ml. of water and, after the addition of 18 ml. of 1 *M* methanolic NaOH, hydrolyzed for 1 hr. at 37°.[3] After removal of sodium with resin (IR-120 (H^+)), the mixture was extracted with CCl_4, $CHCl_3$ and petroleum ether. The aqueous layer was neutralized with cyclohexylamine and the water removed by lyophilization. The residue was dissolved in 10 ml. of water and an equal volume of acetone added. After 2 hr. at 0°, the solution was centrifuged at 0° and the precipitate (I) and soluble fraction (II) separated. I represented 7% of the total phosphorus and by paper chromatography contained glycerylphosphoryl inositol (GPI), free inositol and inositol 1- and 2- phosphates.[4] II was concentrated at 10° *in vacuo* to 5 to 7 ml. and then lyophilized. The residue was dissolved in 1.0 ml. of water, 30 ml. of absolute ethanol added and the mixture stored overnight at −30°. The white crystals were collected by filtration and washed with a small volume of cold absolute ethanol. After drying *in vacuo* over P_2O_5 overnight the yield was 302 mg. (63.4%); m.p., 124–126° (uncorr.). Calculated for cyclohexylamine salt of GPI ($C_{15}H_{32}O_{11}NP$) (433.42): P, 7.16; N, 3.23; inositol/P, 1.00; α-substituted glycerol, 21.3; found: P 7.09; N, 3.28; inositol/P, 0.93, 1.1; α-substituted glycerol, 21.1. Optical rotation values (*C*, 2 in water): salt, $[\alpha]^{25}_D$ −13.5° ± 0.3; $[M]^{25}_D$ −58.5° ± 1.3°; free acid[5], $[\alpha]^{25}_D$ −18.7° ± 0.4°; $[M]^{25}_D$ −62.5 ± 1.4°.

Through use of periodic acid (in slight excess of stoichiometric amount) in neutral solution, the glycerol portion of GPI is oxidized quantitatively while the inositol moiety is not attacked. Thus, any contribution by the glycerol to optical activity can be abolished. In a typical experiment: 40.62 mg. cyclohexylamine GPI (94.0 μmoles) was dissolved in 1 ml. of water and over a 15-minute period 0.96 ml. of 0.1 *M* aqueous $NaIO_4$ was added with continuous stirring. After an additional 10 min. the solution was diluted with water to 50 ml., and titrated with thiosulfate. Theoretical

---

(1) Supported by funds from the National Science Foundation.

(2) D. J. Hanahan and J. N. Olley, *J. Biol. Chem.*, **231**, 813 (1959).

(3) R. M. C. Dawson, *Biochim. Biophys. Acta*, **14**, 374 (1954).

(4) F. L. Pizer and C. E. Ballou, This Journal, **81**, 915 (1959). Dr. Ballou very kindly made this manuscript available to us in advance of its publication.

(5) J. N. Hawthorne and G. Hübscher, *Biochem. J.*, **71**, 195 (1959). These authors report an $[\alpha]^{24}_D$ value of −18 ± 2° for GPI isolated by ion exchange chromatography.