

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, THE HEBREW UNIVERSITY]

## Preparation of Monomeric Fructose Nitrates

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The nitration of fructose by means of nitrogen pentoxide-sodium fluoride, yielded a mixture consisting of a crystalline product (I) and sirupy monomeric nitrated products. The isolation, characterization, and elucidation of the structure of I as 1,4,6-tri-*O*-nitro-2,3-anhydrofructofuranose is described.

Unlike the nitration of aldoses, which proved to undergo a smooth *O*-nitration (esterification) reaction by a variety of reagents,<sup>1</sup> the nitration of ketoses attracted considerably less attention. Back in 1898, Will and Lenze<sup>2</sup> studied the nitration of both fructose and sorbose by means of the powerful nitrating agent, nitric acid-sulfuric acid mixture. However, they could not obtain the expected fully nitrated products. In both cases they were able only to isolate products which according to analyses were formulated as the respective anhydroketose-trinitrates. This nitration of fructose has lately been reinvestigated by Schwager and Leibowitz<sup>3</sup> who showed that the crude reaction product consisted exclusively of a mixture of dimeric fructose nitrates. Among the several products isolated, at least two were identified as hexanitrate of the known difructose-dianhydrides.<sup>4</sup>

Since no monomeric fully nitrated fructose has yet been prepared, it was thought desirable to investigate conditions by which such nitrates could be obtained.

Fructose is known to undergo dimerization reactions quite easily under the influence of a variety of protonic acids.<sup>5</sup> In view of this, it seems reasonable to assume that because of the strongly acidic character of the nitrating agent mentioned above, fructose undergoes a rapid dimerization reaction prior to its *O*-nitration. It was therefore suggested that the desired nitration of fructose could successfully be effected by means of nitrating agents of low acidic properties. In this consideration, the use of nitrogen pentoxide in chloroform solution seemed worthy of investigation. This reagent, although a moderate nitrating agent, has successfully been used by the modification of Caesar and Goldfrank,<sup>6</sup> using the mixture  $N_2O_5$ -NaF in chloroform, for nitration of

sugars which are sensitive to acids, or sugars that could not otherwise be successfully nitrated.<sup>7</sup>

As expected, the nitration of fructose under the Caesar and Goldfrank conditions, led to the formation of a mixture of monomeric fructose derivatives. The crude reaction product could be separated into (1) a crystalline compound, and (2) a sirupy fraction. This paper describes the isolation, characterization and elucidation of structure of the crystalline product. The nature of the products of which the sirup consists will be described in a subsequent paper.

The dextrorotatory crystalline product (I), which was isolated from the nitrating mixture through fractional crystallization, was analyzed as  $C_6H_9O_{11}N_3$  and proved to be a non-reducing compound. It shows three characteristic absorption bands at  $1665\text{ cm.}^{-1}$ ,  $1306\text{ cm.}^{-1}$  and  $1280\text{ cm.}^{-1}$  in the  $O\text{-NO}_2$  regions of the infrared spectrum<sup>8</sup> (symmetric and asymmetric covalent nitrate frequencies), but none at the hydroxyl region. This clearly suggests that I is a trinitrate of an anhydrofructose. For the purpose of its characterization, I was first converted into its parent dextrorotatory anhydro-sugar (II), by means of catalytic hydrogenation. This method was chosen not only because of its smoothness but also because it does not involve any configurational changes in the course of reaction.<sup>9</sup> The fact that I could be obtained by renitration of II fully confirms the reliability of the method chosen. Acetylation of II yielded beautiful hexagonal crystals of a triacetate (III), thus further confirming the number of free hydroxyl groups in II. Likewise, III is nonreducing, and does not show any absorption in the hydroxyl region of the infrared spectrum.

II is a non-reducing compound, but undergoes facile acid hydrolysis, yielding fructose quantitatively. It may, therefore, be assumed that the anomeric hydroxyl group at C-2 is not free, but probably is a member of the anhydro-ring.

II could not be identified with any known compound. Seven structural formulas, which do not take into consideration possible inversion of con-

(1) J. Honeyman and J. W. W. Morgan, *Advances in Carbohydrate Chemistry*, **12**, 117 (1957).

(2) W. Will and F. Lenze, *Ber.*, **31**, 68 (1898).

(3) A. Schwager and J. Leibowitz, *Bull. Res. Council of Israel*, **5a**, 266 (1956).

(4) R. F. Jackson and S. M. Goergen, *Bur. Standards J. Research*, **5**, 733 (1930).

(5) (a) A. Pictet and J. Chavan, *Helv. Chim. Acta*, **9**, 809 (1926); (b) H. S. Schlubach and C. Behre, *Ann.*, **508**, 16 (1934); (c) L. Sattler and F. W. Zerban, *Ind. Eng. Chem.*, **37**, 1133 (1945); (d) M. L. Wolfrom, H. W. Hilton, and W. W. Binkley, *J. Am. Chem. Soc.*, **74**, 2867 (1952).

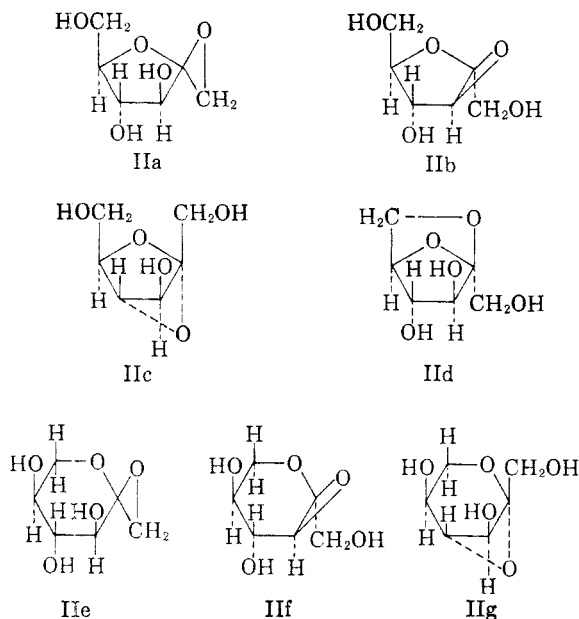
(6) G. V. Caesar and M. Goldfrank, *J. Am. Chem. Soc.*, **68**, 372 (1946).

(7) H. L. Wolfrom and A. Rosenthal, *J. Am. Chem. Soc.*, **75**, 3662 (1953).

(8) L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, John Wiley & Sons, Inc., 1958, p. 301.

(9) L. P. Kuhn, *J. Am. Chem. Soc.*, **68**, 1761 (1946).

figuration in the course of anhydro-ring formation, are possible, as represented in formulas IIa-IIg.



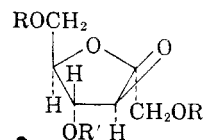
Formulas (IIc) and (IIg), both having the 1,3-propylene oxide ring structure, were ruled out because of the ease of hydrolysis of II in acid solution, as the hydrolytic cleavage of such systems is known to occur with considerable difficulty.<sup>10</sup> It seems probable, therefore, that II contains an epoxy ring which is known to be very easily hydrolyzed. Nevertheless, all five remaining structures were taken into consideration.

Formulas IIa and IIe, containing an 1,2-anhydro-ring were considered next. An anhydride corresponding to formula IIa has already been described,<sup>11</sup> having entirely different properties from those of II. Nevertheless, this formula could not be excluded on the ground that II might be an anomer of that anhydride. The properties recorded for an anhydride having the formula of IIe<sup>5c</sup> are also quite different from those of II. Moreover, serious doubts have been raised regarding the existence of such an anhydride.<sup>12</sup> Therefore this formula could not be excluded. Formulas IIa and IIg were definitely ruled out on the ground that exhaustive methylation of II followed by acid hydrolysis, gave a sirup which did not react with phenylhydrazine.

Since of the remaining three formulas, two, namely, IIId and IIIf, have at least two vicinal hydroxyl groups, the periodate oxidation of II was carried out. II proved to be totally resistant to Malaprade reagent. Thus, only formula IIb conforms with the data presented. Since the resistance to periodate oxidation could not be regarded as conclusive evidence for the lack of vicinal hydroxyl

groups,<sup>13</sup> more direct evidence was sought. Advantage was taken of the fact that only IIb has two primary hydroxyl groups. II formed easily the corresponding ditrityl (IV) and ditosyl (V) derivatives. Since the treatment of V with sodium iodide in acetone afforded two moles of sodium tosylate in quantitative yield, it follows that II contains two primary hydroxyl groups. The latter can therefore be formulated as an 2,3-epoxy-fructofuranose. This formulation is in line with our spectroscopic data. In the ultraviolet absorption spectrum, I shows a band at 220 m $\mu$  which is characteristic for the furanose system.<sup>14</sup> Furthermore, II and all its derivatives show a single band at 1263  $\pm$  2 cm.<sup>-1</sup> in the epoxide region of the infrared spectrum.<sup>15</sup>

From the data presented here, the absolute configuration of II could not be adduced with certainty. Only if the epoxy-ring is oriented above the furanose ring, could the anhydrosugar be regarded as a true anhydrofructofuranose. In the reverse case, it should be formulated as an anhydro-psicofuranose. This point could be clarified by studying the mechanism of formation of I. A clue to this problem could be derived from the observation that I is not formed in the course of the nitration but rather at the washing stage of the crude reaction product. As will be shown in a subsequent paper, I is probably being produced from 1,2,4,6-tetra-*O*-nitrofructofuranose, involving a base-catalyzed trans-elimination of a molecule of nitric acid. This clearly permits the assignment of 2,3 anhydrofructofuranose structure for II:



I. R = R' = NO<sub>2</sub>. II. R = R' = H. III. R = R' = Ac. IV. R = Tr; R' = H. V. R = Ts; R' = H.

A preliminary account of this research has already been published.<sup>16</sup>

#### EXPERIMENTAL<sup>17</sup>

*Preparation of the nitrating mixture.* Nitrogen pentoxide was prepared according to the method of Caesar and Gold-

(13) R. J. Dilmer, H. A. Davis, and G. E. Hilbert, *J. Am. Chem. Soc.*, **68**, 1377 (1946); B. H. Alexander, R. J. Dilmer, and C. L. Mehler, *J. Am. Chem. Soc.*, **73**, 4658 (1951).

(14) H. Bredereck, G. Hoschele, and W. Huber, *Ber.*, **86**, 1271 (1953).

(15) Ref. (8), p. 118.

(16) M. Sarel-Imber and J. Leibowitz, *J. Org. Chem.*, **24**, 141 (1959).

(17) Melting points were taken on a Fisher-Jones apparatus and are uncorrected. Nitrogen determinations were carried out by a micro-Kjeldahl method with an apparatus described by A. J. Kuck, A. Kingsley, D. Dinsey, F. Sheehan, and G. F. Swigert, *Anal. Chem.*, **22**, 604 (1950). Sulfur and iodine analyses by Drs. G. Weiler and F. Strauss, Micro-analytical Laboratory, Oxford. Infrared spectra were taken with a Baird double beam recording spectrophotometer, model B.

(10) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **63**, 2241 (1941).

(11) H. H. Schlubach and H. Elsmer, *Ber.*, **61**, 2358 (1928).

(12) M. L. Wolfrom, W. W. Binkley, W. L. Schilling, and H. W. Hilton, *J. Am. Chem. Soc.*, **73**, 3553 (1951).

frank,<sup>9</sup> using 96% nitric acid ( $d$  1.51) with the exception that the solid nitrogen oxides were first washed with small amounts of ethanol-free chloroform.<sup>18</sup> Chloroform fractions containing more than 10% of low nitrogen oxides were discarded. The rest of the oxides were dissolved in chloroform to a concentration of 20 g. nitrogen pentoxide in 100 ml. solvent, and kept overnight at  $-10^\circ$ .<sup>7</sup>

**Nitration of fructose.** A chloroform solution containing nitrogen pentoxide (73 g., 0.67 mol.) and nitrogen tetroxide (5.3 g.) was mixed with sodium fluoride (12 g.) and cooled to  $-5^\circ$ . To this mixture, finely powdered dry fructose (9 g.) was added slowly with vigorous stirring. The cooling bath was then removed and the stirring was continued for an additional 40 min. while the temperature was not allowed to rise above  $14^\circ$ . After the precipitated inorganic salts were removed by filtration, the filtrate was thoroughly washed with tap water ( $pH$  7.5–8) until washings were free of nitric acid as shown by the diphenylamine reagent. This required numerous washings but it proved to be quite essential, as otherwise the reaction product tended to decompose spontaneously. It was observed that the addition of small quantities of *p*-benzoquinone (0.1 g.) has a remarkable stabilizing effect. The chloroformic solution was dried by sodium sulfate and the solvent was removed by distillation at reduced pressure, leaving behind 15 g. of a viscous sirup with a content of 14–15% nitrogen. The specific optical rotations of different batches ( $[\alpha]_D^{25}$ ) ranged between  $-19$  and  $-24^\circ$ . It is significant to note that these values become less negative as the solution of the crude nitration product was more thoroughly washed.

**2,3-Anhydrofructofuranose trinitrate (I).** When a concentrated methanolic solution of the crude sirupy reaction product was cooled to  $-10^\circ$ , only partial freezing occurred, giving, after decanting of supernatant liquor, a solid material. The supernatant liquor fraction was further concentrated and again fractionated by partial freezing. To the combined solid fractions methanol was added and it was again processed in a like fashion. These operations were repeated several times with each fraction, until the solid fractions became crystalline. Recrystallization from methanol yielded a total of 1.5 g. of white needles melting at  $80.5^\circ$ ;  $[\alpha]_D^{25} +34.5^\circ$  ( $c$  1.1, ethanol),  $+40^\circ$  ( $c$  1.4, methanol);  $\lambda_{max}^{EIOH}$  220  $m\mu$  ( $\log \epsilon$  3.28).

**Anal.** Calcd. for  $C_6H_7O_9N_3$ : N, 14.14; mol. wt. 297. Found: N, 14.02; mol. wt. 300 (benzene). The infrared spectrum shows strong absorption bands ( $cm^{-1}$ ) at 1306, 1280, 1665, ( $-O-NO_2$ ).

It does not reduce Fehling solution. It is readily soluble in methanol, ethanol, and dioxane and insoluble in water. It is stable towards light and strong inorganic acids, but readily decomposes by alkali.

**2,3-Anhydrofructofuranose (II).** Denitration of I could easily be effected by means of catalytic hydrogenation in the presence of 10% palladium over charcoal, at atmospheric pressure. For preparative purposes this reduction was conveniently accomplished by the use of a Parr apparatus. The reduction of I (2 g.) in the presence of the catalyst (8 g.),<sup>9</sup> using ethanol as solvent (100 ml.), was complete within 30 min., at atmospheric pressure. After removal of the catalyst by centrifugation, it was necessary to pass the supernatant alcoholic solution through a Kieselguhr column to get rid of colloidal materials. The solvent was then removed by distillation at reduced pressure and the colorless sirupy residue left in the flask was dried *in vacuo* over phosphorous pentoxide. Yield: 1 g. (92%). It could not be induced to crystallize from the common solvents.  $[\alpha]_D^{25} +79.4^\circ$  ( $c$  0.5, ethanol). The infrared spectrum shows strong absorption bands ( $cm^{-1}$ ) at 3366 (free OH); 1073, 1064, 1027 ( $-C-O-$ ). II does not reduce Fehling solution. It is soluble in methanol, ethanol, and the corresponding

aqueous alcohols, and insoluble in benzene and petroleum-ether. Treatment of II with *N* inorganic acids in the cold, causes hydrolysis within 10 min. with the appearance of reducing power.

**Acid catalyzed conversion of II into fructose.** II (1 g.) was dissolved in 40% aqueous methanol (100 ml.) containing hydrochloric acid (*N*) and heated under reflux for 90 min. The resulting solution was divided into 2 parts, one was used for chromatographic analysis, while the second was employed for the preparation of an osazone after being neutralized with sodium acetate. The osazone thus obtained was identical with an authentic specimen of glucosazone as to melting point, mixed m.p., and crystallographic form under the microscope.

**Chromatographic identification of II and its hydrolysis product.** Paper chromatography was carried out with Whatman paper No. 1, using a mixture of butanol-acetic acid-water in the ratio of 4:1:5 as developer. Spraying was done with a solution of 3% *p*-anisidine hydrochloride in butanol. Paper chromatography of II and of its hydrolysis product gave the following  $R_f$  values: 0.44 for II and 0.23 for the hydrolysis product, identical with the  $R_f$  value of a sample of authentic fructose.

**The denitration of the crude nitration product.** When the catalytic hydrogenation was carried out under 3 atm. pressure, the ratio catalyst/substrate was reduced to 1:1 (w./w.) and the reaction was complete in 20 min. The reaction product was then treated in a manner described for the preparation of II, giving a sirupy product with a negative optical rotation. Paper chromatography of this product as described, showed only 2 spots on the chromatograms, with  $R_f$  values of 0.23 and 0.44, corresponding to fructose and II, respectively. This clearly indicates that the crude nitration product is composed exclusively of monomeric fructose nitrates.

**Chromatographic separation of the denitration product.** The separation of the reaction product into its components was effected by chromatography over a column containing Florex XXX<sup>19</sup>-Celite<sup>20</sup> (5:1 by weight) of 80-mesh.

The ratio substrate/absorbent expressed in w./w. is 1:100. The column was washed first with 95% ethanol and then with 10 ml. of 80% methanol. The product (2.5 g.) was adsorbed on the column from a 50% aqueous methanol solution and was then eluted with 90% methanol and collected in a series of 10 ml. fractions. The first fractions were dextrorotatory, followed by laevorotating ones. The combined dextrorotating fractions, after removal of the solvent, gave a residue which was purified as described. The purified product was obtained as a dry sirup in 30% yield and was shown by specific optical rotation and  $R_f$  value to consist entirely of II. The laevorotatory fractions were identified as pure fructose, both by osazone and  $R_f$  value.

This technique furnishes an alternative route for the isolation of pure 2,3-anhydrofructofuranose from the crude nitration product, and avoids the difficulties sometimes encountered in the isolation of crystalline I from the crude nitration product by fractional crystallization.

**2,3-Anhydrofructofuranose triacetate (III).** A solution of 2 ml. acetic anhydride, 3 ml. pyridine and 200 mg. of II was kept at  $0^\circ$  for 24 hr. Crushed ice was then added with shaking, and the white crystals obtained, after standing a few hours, were separated by centrifugation. Two recrystallizations from ethanol yielded 80 mg. of hexagonal crystals, melting at  $112^\circ$ ,  $[\alpha]_D^{25} +57.4^\circ$  ( $c$  0.68, ethanol).

**Anal.** Calcd. for  $C_{12}H_{16}O_8$ : C, 50.00; H, 5.55; mol. wt., 288. Found: C, 49.93; H, 5.05; mol. wt., 289 (benzene).

The infrared spectrum shows bands ( $cm^{-1}$ ) at 1750 ( $C=O$ ), 1240 ( $-C-O-$ ). The triacetate (III) is soluble in ordinary organic solvents, but slightly soluble in water. It does not reduce Fehling solution.

(18) Purified by a method described by L. F. Fieser, *Experiments in Organic Chemistry*, 2nd ed., D. C. Heath & Co., New York, N. Y., 1941, p. 365.

(19) A fuller's earth type of clay produced by the Floridin Company.

(20) No. 503, Johns-Manville Products.

*Oxidation of II by potassium periodate.* Due to the sensitivity of II towards acids, the Malaprade reaction was carried out by 0.038*M* potassium periodate solution at pH = 6.5. The anhydrofructose (II) was added to the oxidizing system at room temperature, and the amount of the oxidant consumed was estimated by titration of aliquots with arsenic acid and iodine standard solutions. Actually no reaction could be detected, even after 2 days, as was also shown by optical rotation measurements.

*2,3-Anhydrofructofuranose ditrityl ether (IV)* was prepared by the method used for the tritylation of fructose.<sup>21</sup> To a solution of II 0.5 g. (0.003 mol.) in pyridine (5 ml.) was added trityl chloride (1.7 g., 0.006 mol.). The solution was kept at room temperature for 48 hr., then poured into crushed ice and shaken several times during 4 hr. The solid which separated was filtered, dissolved in a small amount of methanol, and reprecipitated by adding water. Several recrystallizations from ethanol yielded 100 mg. of elongated prismatic rods of IV, melting at 165°.

*Anal.* Calcd. for C<sub>44</sub>H<sub>38</sub>O<sub>5</sub>: C, 81.88; H, 5.88. Found: C, 81.74; H, 5.74.

The infrared spectrum shows bands (cm.<sup>-1</sup>) at 3420 (free OH), 3030 (C—H), 1590, 1080 (phenyl).

*2,3-Anhydrofructofuranose ditosylate (V).* A solution of II (1.1 g., 0.007 mol.) in pyridine (8 ml.) was mixed at 0° with a solution containing tosyl chloride (2.6 g., 0.015 mol., m.p. 67°) in pyridine (8 ml.) and allowed to stand at 0° for 3 hr. The temperature was then allowed to rise to 25° and remained so for 20 hr. After the reaction mixture was poured into crushed ice it was allowed to stand overnight, and the brown amorphous precipitate was separated by centrifugation. It was recrystallized first from aqueous methanol and then twice from absolute ethanol, yielding white cubes (500 mg.) of V, melting at 156°.

(21) E. Helferich, *J. prakt. Chem.*, **147**, 60 (1936).

*Anal.* Calcd. for C<sub>20</sub>H<sub>22</sub>O<sub>9</sub>S<sub>2</sub>: C, 51.48; H, 4.68; S, 12.86. Found: C, 51.52; H, 4.86; S, 12.5. The infrared spectrum shows bands (cm.<sup>-1</sup>) at 3546 (free OH), 1184 (O—SO<sub>2</sub>).

Recrystallization of V from methanol afforded microcrystals of m.p. 147° containing one mole of methanol of crystallization which could not be removed easily.

*Anal.* Calcd. for C<sub>20</sub>H<sub>22</sub>O<sub>9</sub>S<sub>2</sub>.CH<sub>3</sub>OH: C, 50.19; H, 5.17; S, 12.74. Found: C, 50.22; H, 4.86; S, 12.75.

This, upon recrystallization from absolute ethanol, again gave the ditosylate of melting point 156°.

Increasing the molar ratio of II/TsCl from 1:2 to 1:3 had no effect either upon the composition or on the yield of the reaction product. V was invariably produced.

*Reaction of V with sodium iodide in acetone.* A solution of V (820 mg.) in 10 ml. of dry acetone was mixed with a solution of sodium iodide (1.7 g.) in dry acetone (10 ml.). The resulting homogeneous mixture was placed in a Pyrex ampule, sealed, and then was heated for 48 hr. at 100°. After a few hours, yellowish crystals separated from the reaction system, the amount of which increased while the reaction progressed. At the end, the precipitate of sodium tosylate was filtered, washed several times with acetone, and its weight determined.

*Anal.* Calcd. for C<sub>7</sub>H<sub>9</sub>O<sub>4</sub>SNa: C, 39.60; H, 4.25. Found: C, 39.46; H, 4.14.

From the filtrate, the solvent was removed by evaporation *in vacuo*, and the residue was dissolved in a chloroform-water mixture, washed with sodium thiosulphate and dried over anhydrous sodium sulphate. After removal of solvent under reduced pressure, the residue was crystallized from benzene, yielding an amorphous product, melting at 126–128°. It contained iodine, but could not be further purified by the usual methods.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

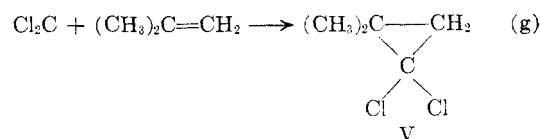
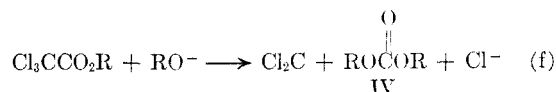
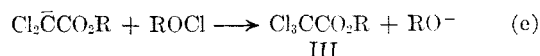
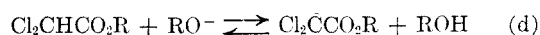
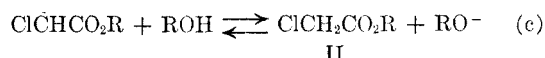
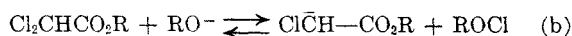
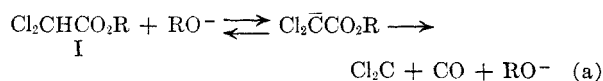
## Mechanism of Carbene Formation from *t*-Butyl Dichloroacetate<sup>1</sup>

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A mechanism is proposed for the formation of dichlorocarbene from the reaction of *t*-butyl dichloroacetate and potassium *t*-butylate, involving a chlorination step with the formation of the intermediate *t*-butyl trichloroacetate.

In a previous communication<sup>2</sup> the reaction of *t*-butyl dichloroacetate(I) and potassium *t*-butylate in the presence of isobutylene was described and reaction (a) was suggested as a possible route for the formation of dichlorocarbene. The final product, 1,1-dichloro-2,2-dimethylcyclopropane(V), was isolated in 13% yield. Further experiments have furnished data which required that the simple mechanism (a) be replaced by the mechanism illustrated by steps (b) to (f), inclusive.



(1) This work was supported by a grant (G-7382) from the National Science Foundation.

(2) W. E. Parham and F. C. Loew, *J. Org. Chem.*, **23**, 1705 (1958).

It is proposed that *t*-butyl dichloroacetate is converted, in the presence of *t*-butylate, into a mixture of *t*-butyl monochloroacetate(II) and *t*-butyl tri-