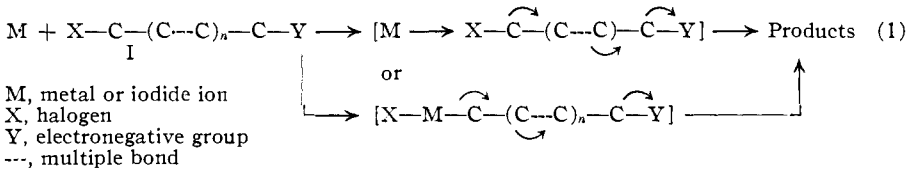
diene from *trans*-1,4-dibromo-2-butene or 1-bromo-4-phenoxy-2-butene plus zinc or magnesium[3a,b]; the reaction of magnesium and *cis*-3,5-dibromocyclopentene to yield cyclopentadiene[4]; the reaction of sodium iodide in acetone on *trans*-1,4-dibromo-2-butene to yield 1,3-butadiene[3b]; and the reaction of zinc with 1,6-dibromo-2,4-hexadiene to yield 1,3,5-hexatriene.[5]



That the group Y in the general reaction 1 in which $n > 0$ can be alkoxy as well as an aryloxy group was shown by the reaction of magnesium with 1-bromo-4-methoxy-2-butene to give 1,3-butadiene in 85% yield. The general reaction was then extended to the action of magnesium in tetrahydrofuran on γ-bromocrotonaldehydediethylacetal. This gave 1-ethoxy-1,3-butadiene in 15% yield of isolated product, identical in boiling point, index of refraction and ultraviolet spectrum with an authentic sample. A considerable portion of the diene was lost by polymerization during its isolation and purification. An ultraviolet spectroscopic analysis of the original solution showed a 78% yield of 1-ethoxy-1,3-butadiene to be present before the isolation procedure was begun.

It also was found that γ-bromocrotonaldehyde diacetate reacted with magnesium in tetrahydrofuran to yield 1-acetoxy-1,3-butadiene. An ultraviolet spectroscopic analysis of the reaction solution showed a 60% yield of the diene to be present. A 37% yield of the pure diene was isolated and found identical with authentic 1-acetoxy-1,3-butadiene.

The elimination reactions forming 1-ethoxy and 1-acetoxy-1,3-butadiene find analogies in some 1,2-elimination reactions brought about by metals (*i.e.*, reaction 1 in which $n = 0$). For example, Wislicenus found that chloroacetaldehyde diethylacetal reacted with sodium to yield vinyl ethyl ether.[6] More recently, Arens and van Dorp observed that the only product resulting from the reaction of magnesium with bromoacetone ethyleneketal followed by hydrolysis was β-hydroxyethylisopropenyl ether.[7]

That the central carbon–carbon bond of compound I need be multiple for the reaction 1 was shown by the failure of either 1,4-dibromobutane or 1-bromo-4-methoxybutane to yield any ethylene on reaction with magnesium.

Experimental

Reaction of Compounds of Type I with Magnesium.—A solution of γ-bromocrotonaldehydediethylacetal[8] (18.3 g., 0.082 mole) in purified tetrahydrofuran (25 ml.) was added dropwise to magnesium turnings (4 g., 0.167 gram atom) in tetrahydrofuran (25 ml.) at such a rate as to maintain gentle reflux. The mixture was heated an additional hour.

The ultraviolet spectrum was taken of an aliquot of the reaction solution diluted with 95% alcohol. The intensity of the single characteristic peak at 237 mμ indicated that a 78% yield of 1-ethoxy-1,3-butadiene was formed. Authentic 1-ethoxy-1,3-butadiene prepared by the method of Flaig[8] showed a single peak in the ultraviolet (in 95% alcohol) at 237 mμ, ε, 19,100. A 1.24-g. (15%) yield of 1-ethoxy-1,3-butadiene, b.p. 35–38° (40 mm.), $n^{25}D$ 1.4582, ϵ_{max} (95% ethanol) 17,900 at 237 mμ, was obtained. The residue of the distillation was a yellow viscous sticky oil (4.67 g.).

The elimination reaction on γ-bromocrotonaldehyde diacetate (12.5 g.) was run similarly except that magnesium amalgam was the metal used and the reaction was initiated with methyl iodide. An ultraviolet spectrum of an aliquot of the reaction solution diluted with ethanol showed a 60% yield of 1-acetoxy-1,3-butadiene to be present. A 37% yield of the diene was isolated by distillation, b.p. 51–52° (31 mm.), $n^{25}D$ 1.4652, ϵ_{max} (broad band) 20,700 at 231 mμ. The properties were in close agreement with those of an authentic sample of 1-acetoxy-1,3-butadiene.[9]

When treated in the same way, γ-methoxycrotyl bromide (15 g.) gave 1,3-butadiene, isolated in 88% yield as the bromination product, *meso*- and *dl*-1,2,3,4-tetrabromobutane.

The reaction of 1-bromo-4-methoxybutane (54 g.) with magnesium in tetrahydrofuran or dibutyl ether gave no ethylene, as evidenced by the failure of the volatile gases entrained in a nitrogen stream to decolorize bromine in carbon tetrachloride. No unsaturated gas was obtained from 1,4-dibromobutane treated in a similar way.

(3) (a) J. Thiele, *Ann.*, **308**, 333 (1899); (b) A. Lüttringhaus, G. V. Saaf and K. Hauschild, *Ber.*, **71**, 1673 (1938).

(4) E. B. Reid and J. Yost, THIS JOURNAL, **72**, 1807 (1950).

(5) E. Farmer, B. Das Loroia, T. Switz and J. Thorpe, *J. Chem. Soc.*, 2937 (1927).

(6) J. Wislicenus, *Ann.*, **192**, 106 (1878).

(7) J. F. Arens and D. A. van Dorp, *Rec. trav. chim.*, **65**, 729 (1946).

(8) W. Flaig, *Ann.*, **568**, 1 (1950).

(9) O. Wichterle and M. Hudlicky, *Coll. Czechoslov. Chem. Communs.*, **12**, 564 (1947).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

Chemistry of Epoxy Compounds. XV.[1] Oxidation of Linoleic Acid with Peracetic and Performic Acid[2]

BY DANIEL SWERN AND GERALDINE BILLEN DICKEL

RECEIVED SEPTEMBER 30, 1953

The oxidation of monounsaturated fatty materials with organic peracids has been studied extensively and is well understood but similar systematic investigation of polyunsaturated analogs has not been carried out. In the few scattered literature reports (for a review of the literature, see references 3 and 4), purity of starting materials is often unknown and open to serious question, yields of products are low and, of major importance, the nature of the main reaction course is not known with certainty. This note describes the oxidation of a linoleic acid concentrate (90–94% *cis,cis*-9,12-octadecadienoic acid) with peracetic and performic acid. The oxidation reactions were followed quantitatively by measuring consumption of peracid with time and reaction products were isolated and identified.

The oxidation of linoleic acid with peracetic and performic acid proceeds normally, contrary to

(1) For paper XIV, see THIS JOURNAL, **74**, 6139 (1952).

(2) Presented at the Spring Meeting of the American Chemical Society, Kansas City, Missouri, March 24–April 1, 1954.

(3) D. Swern, *Chem. Rev.*, **45**, 1 (1949).

(4) D. Swern, "Organic Reactions," Vol. VII, Chapter 7, John Wiley and Sons, Inc., New York, N. Y., 1953.

earlier conclusions.[3,4] With peracetic acid, epoxidation is the predominating reaction. When two moles of peracetic acid is used per mole of linoleic acid, 9,10,12,13-diepoxystearic acid is the main product. With one mole of peracetic acid, monoepoxyoctadecenoic acid is obtained. With performic acid (prepared in advance or *in situ*) in excess formic acid, the expected hydroxyformoxy compounds, resulting from the opening of the oxirane rings of diepoxystearic acid with formic acid, are obtained. Contrary to expectations, however, hydrolysis of the hydroxyformoxy compounds, diepoxystearic acid or the hydroxyacetoxy compounds formed on opening the oxirane rings of diepoxystearic acid with acetic acid results in poor yields of the expected tetrahydroxystearic acids. The failure to obtain good yields of tetrahydroxystearic acid on hydrolysis of these intermediates has been reported by others,[5,6] and differs from the usual experience with monoepoxy compounds or their corresponding hydroxyacyloxy compounds, in which quantitative yields of dihydroxy compounds (α-glycols) can be obtained.

Experimental

Starting Material.—Linoleic acid (composition: 90–94% linoleic, 3–6% oleic, 1% linolenic, 1–3% saturated) was prepared from tobacco seed oil[7] (1100 g.) by mild, rapid hydrolysis,[8] followed by fractional crystallization of the resulting mixture of fatty acids (1030 g.) from acetone (6 ml./g.) at −20° to precipitate saturated acids, and then at −50° (after dilution of the first filtrate to 14 ml./g.) to precipitate oleic acid. These precipitates weighed 125 and 225 g., respectively. Recovery of acetone from the filtrate yielded 660 g. of dark-yellow linoleic acid concentrate, which on distillation through a short Vigreux column yielded 610 g. of almost colorless linoleic acid concentrate, b.p. 145–160° (0.005–0.1 mm.), $n^{24}D$ 1.4673, and iodine number, 171–173. Linoleic acid concentrates of comparable linoleic acid content have also been prepared from corn oil or safflower oil fatty acids by the urea complex precipitation technique, thereby eliminating the low-temperature crystallizations.[9]

Peracetic acid in acetic acid solution was prepared as described earlier.[10] Performic acid was usually prepared and utilized *in situ*.[4]

Epoxidation of Linoleic Acid with Peracetic Acid. (a) 9,10,12,13-Diepoxystearic Acid.—Twenty-eight grams of linoleic acid (iodine number 172; 0.19 mole of double bond) was placed in a 1-l. three-neck flask immersed in an ice-bath. When the contents had cooled to 10–15°, approximately 390 g. of 0.6–0.7 *M* peracetic acid in acetic acid solution was added to supply 0.23–0.27 mole of peracid (20–40% excess over that required for both double bonds). The peracid was added within 2–3 minutes, with efficient stirring, while the reaction temperature was maintained at 20°. Samples were removed every hour and the consumption of peracetic acid was followed quantitatively.[4] After 5 hours approximately 0.19 mole of peracetic acid had been consumed and its rate of consumption had become negligible. (Within 2 hours after the reactants were mixed, 0.17 mole of peracetic acid had been consumed.) The reaction mixture was then poured into several volumes of an ice–water mixture. The white fine granular solid which precipitated was filtered with suction and washed on the funnel with several liters of cold water. After it was dry, the crude reaction product weighed 28.6 g., m.p. 59–67°, oxirane oxygen, 7.51%[11]; neut. equiv. 315; sapon. equiv. 295, iodine number 12–16, ester content about 4%. Its composition was oxirane compounds 80%; hydroxyacyloxy compounds, 4%; unoxidized, 8%; saturated substances originally present in the starting material, 1–3%, unaccounted for, 5–7%.

The crude reaction product was crystallized twice from acetone, the first time at −20° (2 ml./g.) and the second at 0° (5 ml./g.), yielding 10 g. of 9,10,12,13-diepoxystearic acid, m.p. 77.8–78.2° (lit. 79°)[12] oxirane oxygen 9.92%, neut. equiv. 315 (calcd.: oxirane oxygen 10.2%, neut. equiv. 312).

(b) Monoepoxyoctadecenoic Acid.—The epoxidation experiment described above was repeated except that 0.1 mole of peracetic acid (5% excess over that required for one double bond) was employed for 28 g. (0.19 mole of double bond) of linoleic acid. After three hours, consumption of peracid became negligible (0.097 mole consumed) and the reaction mixture was poured into a mixture of ice and water. The oil which separated was dissolved in ether and the ether solution was washed acid-free and dried over calcium sulfate. The non-volatile residue obtained after evaporation of the ether was a straw-colored oil and consisted mainly of monoepoxyoctadecenoic acid, 28 g.; iodine number 85, oxirane oxygen 4.38%, neut. equiv. 305, sapon. equiv. 280 (calculated for monoepoxyoctadecenoic acid: iodine number 86, oxirane oxygen 5.41%, neut. and sapon. equiv. 296).

Crystallization of monoepoxyoctadecenoic acid from acetone (2 ml./g.) at −50° yielded about 10 g. of precipitate (liquid at room temperature). This was recrystallized three times at −20° yielding 0.4 g. of 9,10,12,13-diepoxystearic acid, m.p. 74–77°, and neut. equiv. 317. A mixed melting point with authentic 9,10,12,13-diepoxystearic acid showed no depression.

Hydroxylation of Linoleic Acid with Performic Acid.—Performic acid was prepared by mixing 30.5 g. of 25.2% hydrogen peroxide (0.23 mole) with 275 g. of 98–100% formic acid in a three-neck flask and allowing the solution to stand for one hour at room temperature (26°). The flask and contents were immersed in an ice-bath and 28 g. (0.19 mole of double bond) of linoleic acid was added all at once with efficient agitation (in larger scale runs the linoleic acid was added dropwise). After a short induction period, the reaction became strongly exothermic and the reaction mixture became homogeneous. The temperature was maintained between 25–30°. After two hours, consumption of peracid became negligible and the reaction mixture was poured into several volumes of a mixture of ice and water in a separatory funnel. The oil which separated was dissolved in ether and the ether solution was washed free of acid and dried. Evaporation of the ether yielded 36 g. of a pale-yellow viscous oil consisting predominantly of dihydroxydiformoxystearic acid; neut. equiv. 389, iodine number 3 (calculated: neut. equiv. 404, iodine number 0).

Substantially identical results were obtained when hydrogen peroxide (2.5–20% excess), formic acid and linoleic acid were mixed, and the performic acid prepared and utilized *in situ* at 40°. With small excesses (2.5–5%) of hydrogen peroxide, reaction times were usually about 6 hours. The iodine numbers of the crude reaction products were 3 or less, indicating stoichiometric utilization of hydrogen peroxide, but some hydrolysis of formate ester occurred because of the longer reaction time.

Hydrolysis of the hydroxyformoxy compounds at room temperature for 24 hours or under reflux for 5 minutes to several hours with 1 to 6 *N* aqueous sodium hydroxide followed by acidification at room temperature or 100° with dilute aqueous hydrochloric acid yielded yellow oils with neutralization equivalents in the range of 330–350 (calculated for tetrahydroxystearic acid 348). Attempted solution of these oils in ether yielded a small quantity of insoluble white solid (about 2 g. from 28 g. of oil), identified as 9,10,12,13-tetrahydroxystearic acid, m.p. 145–147° (lit. 148°,[5] 146°[6]) and neut. equiv. 350.

NOTE ADDED IN PROOF.—In the reaction of linolenic (*cis, cis, cis*-9,12,15-octadecatrienoic) acid with excess perbenzoic acid exactly three atoms of active oxygen are consumed per mole of trienoic acid. Ninety per cent. of the consumed oxygen can be shown by analysis to be present in the mole-

(5) A. F. McKay and A. R. Bader, *J. Org. Chem.*, **13**, 75 (1948).

(6) T. G. Green and T. P. Hilditch, *Biochem. J.*, **29**, 1552 (1935).

(7) R. W. Riemenschneider, R. M. Speck and E. G. Beinhart, *Oil & Soap*, **22**, 120 (1945).

(8) D. Swern, H. B. Knight, T. J. Scanlan and W. C. Ault, *ibid.*, **22**, 302 (1945).

(9) D. Swern and W. E. Parker, *J. Am. Oil Chemists' Soc.*, **30**, 5 (1953).

(10) T. W. Findley, D. Swern and J. T. Scanlan, THIS JOURNAL, **67**, 412 (1945).

(11) D. Swern, T. W. Findley, G. N. Billen and J. T. Scanlan, *Anal. Chem.*, **19**, 414 (1947).

(12) W. C. Smit, *Rec. trav. chim.*, **49**, 675 (1930).

cule as oxirane oxygen. Details will be published at some later date.

EASTERN REGIONAL RESEARCH LABORATORY[13]
PHILADELPHIA 18, PENNSYLVANIA

(13) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Service, U. S. Department of Agriculture.

3-Indolecarboxaldehyde Thiosemicarbazone, a New Antitubercular Compound[1]

BY LOWELL E. WELLER, HAROLD M. SELL AND R. Y. GOTTSHALL

RECEIVED JANUARY 11, 1954

The inhibition of growth of *Mycobacterium tuberculosis* by many thiosemicarbazones has been reported.[2a,b,3] 3-Indolecarboxaldehyde thiosemicarbazone has been shown to have high bacteriostatic activity *in vitro* and to suppress tuberculosis in mice after injection of virulent tubercle bacilli. Because of these properties the synthesis of this new thiosemicarbazone is described.

3-Indolecarboxaldehyde Thiosemicarbazone.—Thiosemicarbazide 8.2 g. (0.09 mole), dissolved in 100 ml. of warm 30% acetic acid was added to a solution of 12.3 g. (0.085 mole) of 3-indolecarboxaldehyde[4,5] in 200 ml. of methanol and the resulting mixture refluxed for 2 hours. After cooling, the precipitate was collected by filtration, washed with cold water and air-dried. The crude product was purified by recrystallization from methanol to give light yellow crystals of 3-indolecarboxaldehyde thiosemicarbazone, m.p. 230–232° dec.

Anal. Calcd. for $C_{10}H_{10}N_4S$: N, 25.67. Found: N, 25.48.

Biological Properties.—The following organisms were not inhibited by 100 micrograms of 3-indolecarboxaldehyde thiosemicarbazone: *Diplococcus pneumoniae, Streptococcus hemolyticus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Micrococcus pyogenes* var. *aureus, Klebsiella pneumoniae, Proteus vulgaris, Shigella dysenteriae, Corynebacterium diphtheriae, Aerobacter aerogenes, Mycobacterium phlei, M. smegmatis* and *Mycobacterium* 607.

M. tuberculosis var. *hominis*, strains $H37R_v$, $H37R_a$ and Ts1760 were inhibited by 6.2, 0.8 and 3.2 micrograms of the chemical, respectively.

When injected intraperitoneally as a suspension, 50 mg. was lethal for 20-g. white mice while 25 mg. was tolerated. Four-and-one-half mg. injected daily on seven successive days was tolerated and this dose was used for a preliminary protection test. For this test, 1.0 mg. of *M. tuberculosis*, $H37R_v$ was injected intravenously into eight mice and the following day, 4.5 mg. of the 3-indolecarboxaldehyde was injected subcutaneously. Daily injections of the same dose of chemical were made for 17 days. Nineteen days after the last injection, seven of the eight treated mice were still alive while of the 10 control mice infected at the same time, but which did not receive the chemical, only three survived.

This investigation was supported in part by a research grant E-227(c) from the National Institutes of Health, U. S. Public Health Service.

DEPARTMENT OF AGRICULTURAL CHEMISTRY
MICHIGAN STATE COLLEGE, EAST LANSING, AND
MICHIGAN DEPARTMENT OF HEALTH
LANSING, MICHIGAN

(1) Journal article No. 1515, Michigan Agricultural Experiment Station.

(2) (a) G. Domagk, R. Behnisch, F. Mietzsch and H. Schmidt, *Naturwissenschaften*, **33**, 315 (1946); (b) G. Domagk, *Schweiz. Z. Path. u. Bakt.*, **12**, 575 (1949).

(3) G. Domagk, *Beitr. Klin. Tuberk.*, **102**, 603 (1950).

(4) F. T. Tyson and J. T. Shaw, THIS JOURNAL, **74**, 2273 (1952).

(5) E. Campaigne and W. L. Archer, *ibid.*, **75**, 989 (1953).

The Keto Acids of the Tulip (*Tulipa gesneriana* with Special Reference to the Keto Analog of γ-Methyleneglutamic Acid

BY G. H. N. TOWERS[1] AND F. C. STEWARD

RECEIVED NOVEMBER 11, 1953

Our interest in the keto acids of plants, because of their importance in the understanding of nitrogen metabolism, has prompted the development of a general method for their identification and quantitative determination. This method, which will be published in due course (Towers, Thompson and Steward) depends upon the following procedures.

a. The plant material is killed and the keto compounds fixed with an alcoholic solution of either 2,4-dinitrophenylhydrazine or 1,1-diphenylhydrazine.

b. The keto-acid hydrazones are separated from the amino acids and neutral carbonyl compounds by extraction into ethyl acetate and subsequent extraction of the ethyl acetate solution with dilute sodium carbonate.

c. Specific 2,4-dinitrophenylhydrazones are recognized by chromatography on paper.

d. The hydrazones are converted to the corresponding amino compounds by catalytic hydrogenolysis.

e. The amino compounds so produced are recognized by two-directional chromatography on paper and treatment of the chromatograms with ninhydrin. Thus the keto-acids may be recognized by drawing upon the extensive information, which is now available, on the chromatography of the amino compounds on paper and by utilizing the sensitivity of the ninhydrin method for their detection.

The recent recognition of γ-methyleneglutamine and of γ-methyleneglutamic acid as constituents of the peanut plant[2] and the proof that these substances are identical with nitrogenous compounds earlier recognized in the tulip plant[3,4] gives rise to interesting possibilities.

Therefore, in making the first extensive examination of the ketoacids of the tulip plant, special attention was paid to the recognition and identification of the keto analog of γ-methyleneglutamic acid. Work has been done on the keto compounds present in the tissue of the resting tulip bulb (in which the γ-methyleneglutamine was first recognized) and also of the green foliage leaves in which it is present in larger amount.

The 2,4-dinitrophenylhydrazones from the tulip bulb yielded on hydrogenolysis the following amino compounds: glycine, alanine, aspartic acid, glutamic acid and valine. In addition to these known amino acids, hydrogenolysis also yielded amino compounds whose identity still remains unknown. Therefore, one could infer that the following keto compounds, corresponding to this list of amino acids, are present as constituents of the tulip plant: glyoxylic acid, pyruvic acid, oxaloacetic acid, α-ketoglutaric acid and unknown keto compounds.

(1) Lalor Foundation, Pre-doctoral Fellow at Cornell University.

(2) J. Done and L. Fowden, *Biochem. J.*, **49**, Proc. XX (1951).

(3) F. C. Steward and J. F. Thompson, *Ann. Rev. Plant Physiol.*, **2**, 233 (1950).

(4) R. Zacharias, J. K. Pollard and F. C. Steward, THIS JOURNAL, **76**, 1961 (1954).