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Reactions of sodium hypochlorite with some compounds having reactive methylene groups

B. Vickery and F. Kaberia

Chemistry Department, Kenyatta University College, P.O. Box 43844, Nairobi (Kenya), 10 July 1978

Summary Alkaline sodium hypochlorite solution oxidises a number of compounds having reactive methylene groups to the corresponding gem diols, but in some cases only decarboxylation occurs at room temperature.

During investigations into the use of sodium hypochlorite as a halogenating reagent in organic chemistry, the reaction between this compound and malonic acid was studied. In acidic solution, low yields of chloro- and dichloroacetic acids, and chloro- and dichloromalonic acids were obtained. In alkaline solution, the product, in 22% yield, was 2,2-dihydroxypropan-1,3-dioic acid, disodium salt (sodium mesoxalate). Previous syntheses of this compound had either been via diethyl 2-oxopropan-1,3-dioate[1]; or 2,2-dibromopropandioate[2].

It seems possible that the reaction producing the sodium mesoxalate is as follows:

$$CH_2(CO_2Na)_2 \xrightarrow[OH^-,\,25°]{NaOCl} Cl_2C(CO_2Na)_2 \xrightarrow[25°]{OH^-} (HO)_2C(CO_2Na)_2$$

We were able to show by gas chromatography that small amounts of dichloromalonate were present in the reaction mixture. Also, synthesis of sodium dichloromalonate[2] and reaction of the latter with sodium hydroxide yielded sodium mesoxalate.

In an attempt to determine the scope of the reaction, we also carried out the following reactions:

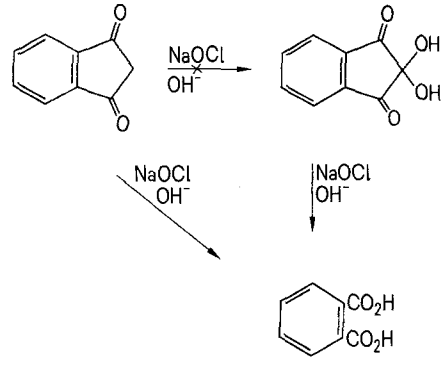
$$CH_3COCH_2COCH_3 \xrightarrow[OH^-,\,25°]{OCl^-} CH_3COC(OH)_2COCH_3$$

$$HOOCCH_2COCH_2CO_2H \xrightarrow[OH^-,\,25°]{OCl^-} CH_3COCH_3$$

$$HOOCCOCH_2CO_2H \xrightarrow[OH^-,\,25°]{OCl^-} CH_3COCO_2H$$

3,3-dihydroxypentan-2,4-dione for reference purposes was synthesised by the method of Calvin and Wood[3].

We were hopeful that indan-1,3-dione could be converted to 2,2-dihydroxyindan-1,3-dione (ninhydrin) with alkaline hypochlorite. In the event we obtained an almost quantitative yield of phthalic acid. We found, however, that ninhydrin was also converted almost quantitatively to phthalic acid with alkaline hypochlorite:



All products have been characterized by comparison of melting points and spectra (UV, IR and NMR) with those obtained from authentic material.

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A possible biomimetic synthesis of fluoroacetic acid

B. Vickery, Margaret L. Vickery and F. Kaberia

Chemistry Department, Kenyatta University College, P.O. Box 43844, Nairobi (Kenya), 10 July 1978

Summary. We propose that fluoroacetate may be formed in plants by fluorodecarboxylation of malonic acid.

Fluoroacetate and a number of related organofluorine compounds are biosynthesised in plants either naturally[1] or as the result of fluoride pollution[2]. Fluorination of organic molecules generally takes place under fairly drastic conditions in the laboratory, and the fluorinating agent is normally hydrogen fluoride or some other inorganic fluoride, or elemental fluorine[3]. Since these conditions are not likely to obtain in a living plant cell, the precise method by which fluorine is introduced into organofluorine compounds in plants has so far remained unknown. A number of specula-

tions have been made[4-6], but until now no experiment has been performed which might be analogous to the natural synthesis of fluoroacetate.

Our approach to this synthesis arose from consideration of 2 facts: only fluoroacetate, or compounds to which fluoroacetate might be readily converted in vivo, for example, fluoroacetone[7], fluorocitrate[8] or long chain ω-fluorofatty acids[9], have been characterized from plants. Secondly, chlorine may be introduced into organic molecules in cultures of *Caldariomyces fumago* by chlorodecarboxylation thus[10]:

$$\mathrm{HOOCCH_2CH_2COCH_2CO_2H} \xrightarrow[\text{chloro-peroxidase}]{\mathrm{Cl^-},\ \mathrm{H_2O_2}} \mathrm{HOOCCH_2CH_2COCH_2Cl}$$

In order to obtain fluoroacetic acid in a similar way, it is necessary that malonic acid - which is a universal constituent of plants - be fluoro-decarboxylated.

$$\mathrm{HO_2CCH_2CO_2H} \xrightarrow[[O]]{\mathrm{F^-}} \mathrm{HO_2CCH_2F} + \mathrm{CO_2}$$

Our first unsuccessful attempts to carry out this reaction involved using horseradish peroxidase and hydrogen peroxide. Subsequently we used sodium fluoride and malonic acid with either sodium hypochlorite or sodium peroxodisulphate as oxidising agents. In both cases after 4 days at 25 °C, the formation of fluoroacetate in small yield was readily detected by gas chromatography after conversion of the acid to the methyl ester. The methyl fluoroacetate formed had the same retention time and co-chromatographed with an authentic sample of methyl fluoroacetate on columns having apiezonal, methylsilicone gum, and polyethylene glycol adipate as stationary phases.

While it seems unlikely that either sodium hypochlorite or sodium peroxodisulphate is present in plants, our experiments demonstrate that fluoroacetate is readily synthesized, at ordinary temperature, from components, fluoride and malonic acid, which are present in plants which accumulate fluoride. Perhaps plants which biosynthesize fluoroacetate have evolved a fluoroperoxidase, analogous to the chloroperoxidase of *Caldariomyces fumago*.

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Insensitivity of the ferritin iron core to heat treatment[1]

M.L. Bertrand and D.C. Harris

Department of Chemistry, University of California, Davis (California 95616, USA), 18 July 1978

Summary. To test whether the reactivity of ferritin iron is affected by the heat treatment used in ferritin isolation, we prepared ferritin from the same horse spleen with or without heating. Both samples exhibited similar reactivity upon reduction or chelation of iron.

Colloidal aggregates of inorganic iron with sizes and properties similar to those of the ferritin core may be prepared by raising the pH or heating solutions of Fe(III)[2-6]. Over a period of 2 days at 25 °C, such synthetic iron polymers undergo a 'hardening' process in which they become denser, more colored, and more resistant to acid hydrolysis[7]. Current procedures for the isolation of ferritin call for heating tissue homogenate to 70–80 °C for 10 min to precipitate other proteins[8]. It is possible to prepare ferritin without heating[9], but there has been little reason to avoid the heat step. The observation of hardening of colloidal iron prompted the suggestion[7] that if ferritin were isolated without heating, it might be more reactive. The present work was undertaken to test this suggestion.

Methods. Horse spleen ferritin was isolated by 5 methods. The spleens were obtained on the day the animal died and were stored at 4 °C for 1 day before use. All operations were done at 4 °C. Method A. Spleen was ground in a meat grinder and homogenized for 30 sec in a Waring blender with 1.5 g H_2O/g spleen. The mixture was warmed to 70 °C over a period of ~6 min in a preheated vessel. It was maintained at 70 °C for 10 min and then cooled on ice. Following centrifugation and filtration to remove debris, ferritin was precipitated by addition of 300 g of ammonium sulfate per l while maintaining the pH at 7.0. The precipitate was isolated by centrifugation and dissolved in a minimal volume of water. Following centrifugation to remove insoluble material, and dialysis against 0.1 M Tris, pH 7.0, the supernatant was adsorbed onto a column of Bio-Rad Cellex-D anion exchange cellulose equilibrated with 0.1 M Tris, pH 7.0. The adsorbed material was eluted with 0.5 M KCl. After dialysis against 0.1 M Tris, pH 7.0, the red solution was centrifuged at 23,000 × g for 1 h and the supernatant recentrifuged at 100,000 × g for 3 h in a 5.5 cm long tube to pack $\gtrsim$ 95% of the ferritin iron. Pellets were dissolved in 0.1 M Tris, pH 7.0, and passed through an ascending column of Sepharose 6B (2.6 × 60 cm) eluted with the same buffer. The center of the single symmetrical protein peak was retained and dialyzed against 0.1 M KCl. Method B was the same as method A, except that the heating to 70 °C was omitted. Method C was the same as method A through the ammonium sulfate precipitation.