Studies of Organic Fluorine Compounds. Part IV. Synthesis of Esters of Fluoro-oxaloacetic and of Fluoropyruvic Acid.*

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Esters of fluoroacetic acid condense with alkyl oxalates in presence of alkali alkoxides to the alkali enolates of the esters of fluoro-oxaloacetic acid. These esters show a very low (8·9%) enol content. Hydrolysis of the esters to the free acid failed, the main reaction being fission to oxalic and fluoroacetic acid. Under suitable conditions, fluoropyruvic acid could be prepared in small yield by the fission. Both diethyl fluoro-oxaloacetate and fluoropyruvic acid are very much less toxic than the fluoroacetate.

The infrared spectra of the new compounds have been determined.

MUCH research has recently been devoted to the biological effects of fluoroacetate and related compounds (Martius, *Annalen*, 1949, **561**, 237; Peters, *Proc. Roy. Soc.*, 1952, **139**, *B*, 143; *Brit. Med. J.*, 1952, 1165). It has been shown that fluoroacetic acid is metabolised in the organism to fluorocitric acid, which eventually blocks the tricarboxylic acid cycle. It seemed, therefore, of interest to prepare the monofluoro-derivatives of the members of the citric acid cycle, to study their biological behaviour, and, in particular, to prepare diethyl fluoro-oxaloacetate as a prospective starting material for fluoropyruvic acid and a number of other substances. We have published short communications on the synthesis and biological properties of diethyl fluoro-oxaloacetate and fluoropyruvic acid (*Bull. Res. Council Israel*, 1953, **3**, 101; see also Mager and Blank, *Nature*, 1954, **173**, 126; Blank and Mager, *Experientia*, 1954, **10**, 77), and a synthesis of diethyl fluoro-oxaloacetate by essentially the same method has been described by Rivett (*J.*, 1953, 3710).

Wislicenus (*Ber.*, 1910, **43**, 3552) showed that diethyl oxalate condenses with ethyl chloroacetate, but not with ethyl bromoacetate, without loss of the halogen atom. Since the C–F bond is known to be the most stable of the halogen–carbon bonds (Swarts, *Bull. Soc. chim. France*, 1896, **15**, 1134; Glockler, in "Fluorine Chemistry," Academic Press Inc., New York, 1950, Vol. I, p. 314), it was expected, and has now been confirmed, that ethyl fluoroacetate would lend itself easily to Claisen condensation. Condensing ethyl fluoroacetate with diethyl oxalate in the presence of alcohol-free sodium ethoxide gave yields of about 80% of pure diethyl sodiofluoro-oxaloacetate, from which the free ester was easily

* Part I, *J.*, 1953, 3786; Parts II and III, *Proc. k. ned. Akad. Wetenschap.*, c, 1953, **56**, 423, 427. (The last two papers were erroneously numbered Parts I and II.)

prepared. Attempts, however, to prepare the free fluoro-oxaloacetic acid by hydrolysis with cold concentrated hydrochloric acid failed under conditions under which the fluorine-free parent compound yields oxaloacetic acid—albeit only in 5—8% yield—and pyruvic acid (as the main product) (cf. Wislicenus, *Annalen*, 1888, **246**, 327; see also "Biochemical Preparations," 1953, Vol. III, p. 59) ("ketonic fission" of oxaloacetic acid). From diethyl fluoro-oxaloacetate, only fluoropyruvic acid and large quantities of oxalic acid could be obtained. It seems that fluoro-oxaloacetic acid, being even less stable than oxaloacetic acid, decomposes immediately on formation. However, the main reaction is "acid fission," leading to oxalic and fluoroacetic acid: this reaction is brought about for the parent substance only by alkali (Wislicenus, *loc. cit.*).

In this respect, diethyl chloro-oxaloacetate occupies an intermediate position, since it yields much smaller amounts of oxalic acid on treatment with cold concentrated hydrochloric acid. (Dilute sulphuric acid gives oxalic and chloroacetic acid; Peratoner, *Gazzetta*, 1892, **22**, II, 38.) "Acid fission" of fluoro-oxaloacetic acid could not be appreciably prevented even under the very mild conditions adopted by Heidelberger and Hurlbert (*J. Amer. Chem. Soc.*, 1950, **72**, 4704) for the preparation of oxaloacetic acid, *viz.*, pyrolysis of its di-*tert.*-butyl ester. In this case, too, large amounts of oxalic acid, together with fluoropyruvic acid, were formed, and only minor quantities of the desired fluoro-oxaloacetic acid could be identified.

Di-*tert.*-butyl fluoro-oxaloacetate was prepared by the Claisen condensation of di-*tert.*-butyl oxalate with *tert.*-butyl fluoroacetate, dry potassium *tert.*-butoxide being the condensing agent. Both diethyl and di-*tert.*-butyl fluoro-oxaloacetate give a characteristic bluish-brown colour reaction with alcoholic ferric chloride solution, whereas the colour given by the non-fluorinated analogue is brown-red. This is in keeping with Henecka's findings (*Ber.*, 1948, **81**, 179) concerning the effect of halogen substitution on the colour of the ferric complex of β-diketones. The intensity of the colour is also much weaker than that obtained with equivalent amounts of the parent substance, owing to the low degree of enolisation characteristic of fluoro-oxaloacetates. The keto–enol equilibrium (enol %) was determined by Meyer's indirect method for the following diethyl esters in *n*-hexane: Oxaloacetate, 72—79; chloro-oxaloacetate, 24—27; fluoro-oxaloacetate, 8—9. According to Meyer (*Ber.*, 1912, **45**, 2860), dimethyl oxaloacetate occurs in the solid state and in light petroleum almost wholly as the enol.

Replacement of the hydrogen in the α-position of the oxaloacetate by a halogen atom decreases the enolisation, more so for fluorine than for chlorine. Similar results have been obtained in the acetoacetate series. Ethyl acetoacetate was found to contain 7·4% of enol in accordance with previous data (Meyer *et al.*, *Annalen*, 1911, **380**, 222; *Ber.*, 1911, **44**, 2720; 1914, **47**, 841), and the αγ-difluoro- and -dichloro-acetoacetate showed 5·2 and 5·4%, respectively. Although α-halogen atoms decrease the tendency to enolisation (ethyl α-bromoacetoacetate 4% enol; Meyer, *Annalen*, 1911, **380**, 241), halogen in the γ-position increases it; *e.g.*, Arndt, Loewe, and Capuano (*Rev. Fac. Sci. Istanbul*, 1943, **8**, *A*, 122) found for ethyl γ-chloro- and γγγ-trichloro-acetoacetate 10·9 and 40—50% enol, respectively. The pronounced ketonic character of diethyl fluoro-oxaloacetate makes it understandable that it undergoes the Reformatzky reaction with ethyl bromoacetate and zinc in the normal manner (Rivett, *loc. cit.*).

For the preparation of fluoropyruvic acid, we first attempted to hydrolyse fluoropyruvonitrile in a manner similar to that described by Tschelinzeff and Schmidt (*Ber.*, 1929, **62**, 2210), but always obtained complete decomposition. Fluoropyruvic acid was prepared, however, by treatment of diethyl fluoro-oxaloacetate with hot dilute hydrochloric acid, which led to concurrent hydrolysis and decarboxylation. Although the main reaction under these conditions is the "acid fission," giving a mixture of oxalic, fluoroacetic, and fluoropyruvic acid, the method described on p. 2193 gives pure fluoropyruvic acid as a colourless liquid crystallising at low temperature, but in only 9% yield; it is characterised by a well-crystallised semicarbazone. The methyl ester was prepared easily by means of diazomethane or of hydrogen chloride in anhydrous methanol at room temperature.

The toxicity of diethyl sodiofluoro-oxaloacetate and fluoropyruvic acid was tested on rats and mice. The former proved to be practically non-toxic (LD_{50} 750 mg./kg.); the

latter (as well as its methyl ester) was lethal at doses of about 80 mg./kg. The biological aspects of this study will be described elsewhere.

In the infrared, the carbonyl group of diethyl fluoro-oxaloacetate, fluoropyruvic acid, and methyl fluoropyruvate absorbs at 1738—1750 (broad band), 1723, and 1743 cm.$^{-1}$, respectively. The fluorine atom is responsible for peaks at 1094, 1035, and 1046 cm.$^{-1}$, respectively. These variations, and the observation that the C–F absorption in the semicarbazone of fluoropyruvic acid is shifted as far as 980 cm.$^{-1}$, will form the subject of a forthcoming publication. In fluoropyruvonitrile, the C≡N frequency was found at 2250 cm.$^{-1}$ and the carbonyl band at 1730 cm.$^{-1}$.

Experimental

Carbon and hydrogen determinations were carried out by Bodenheimer and Goldstein's method (*Bull. Res. Council Israel*, 1953, **3**, 53), and fluorine analyses by that of Eger and Yarden (*ibid.*, 1954, **4**, 305).

Diethyl Fluoro-oxaloacetate.—To a suspension of alcohol-free sodium ethoxide, prepared from metallic sodium (23 g.) in anhydrous ether (200 ml.), freshly distilled diethyl oxalate (146 g.) and after a few minutes ethyl fluoroacetate (106 g.) were added dropwise. The mixture was left overnight at room temperature, and the solid yellow enolate was filtered off, washed several times with ether until the filtrate was colourless, and dried (yield 180 g., 79%). The enolate is a cream-coloured, hygroscopic powder which becomes yellow on storage, is soluble in water and alcohol, insoluble in ether and hydrocarbons, and gives a bluish-brown colour reaction with alcoholic ferric chloride solution.

A solution of the enolate (100 g.) in cold water (50 ml.) was acidified with dilute sulphuric acid to a pH of 1—2 in the cold and extracted repeatedly with ether. The extract was dried (Na_2SO_4), and the solvent evaporated. The free diethyl fluoro-oxaloacetate (60 g., 67%) distilled at 120—122°/9 mm. as a colourless oil, soluble in alcohol and ether, insoluble in water and hydrocarbons. With alcoholic ferric chloride, it gives a deep bluish-brown colour; it has d_4^{20} 1·261, n_D^{20} 1·42, $[M]_R$ 41·44 (Calc.: 42·27) (Found: C, 46·7; H, 5·7. Calc. for $C_8H_{11}O_5F$: C, 46·6; H, 5·3%) (Rivett, *loc. cit.*, gives b. p. 99°/3 mm., n_D^{25} 1·4203). The 2 : 4-dinitrophenylhydrazone, recrystallised from alcohol, had m. p. 142° (Found: C, 44·0; H, 4·0; N, 13·9. Calc. for $C_{14}H_{15}O_8N_4F$: C, 43·5; H, 3·9; N, 14·5%) (Rivett, *loc. cit.*, gives m. p. 145°).

Acid Hydrolysis of Diethyl Fluoro-oxaloacetate and Chloro-oxaloacetate.—The fluoro-ester (50 g.) was shaken vigorously with concentrated hydrochloric acid (100 ml.) for 2 hr. and set aside at −15°. After 5 days, a white precipitate had been formed, and was filtered off and dried in a desiccator (yield *ca.* 4 g.). After recrystallisation from ethyl methyl ketone, it did not give a colour with ferric chloride or liberate carbon dioxide on treatment with aniline citrate in the Warburg apparatus. It gave a strong colour reaction for oxalic acid with diphenylamine (Feigl, " Qualitative Analysis by Spot Tests," Elsevier Publ. Co. Inc., New York, 1947), and was quantitatively precipitated from its aqueous solution by calcium acetate. Titration with potassium permanganate gave the value expected for anhydrous oxalic acid. In the mother-liquor left after the separation of this acid, the presence of fluoroacetic acid was indicated by the lanthanum nitrate reaction (Hutchens and Kass, *J. Biol. Chem.*, 1949, **177**, 571).

Oxalic acid was also detected in the hydrolysis products of diethyl chloro-oxaloacetate, but in much smaller quantity than from the equivalent amount of the fluoro-oxaloacetate.

tert.-*Butyl Fluoroacetate.*—This, prepared by a method similar to that of Westheimer and Shookhoff (*J. Amer. Chem. Soc.*, 1940, **62**, 269) for the chloroacetate, had b. p. 129·5—131°, d_4^{20} 0·9904, n_4^{20} 1·386, $[M]_R$ 32·04 (Calc.: 31·42) (Found: C, 54·2; H, 8·2. $C_6H_{11}O_2F$ requires C, 53·8; H, 8·2%).

Di-tert.-*butyl Fluoro-oxaloacetate.*—Dry potassium *tert.*-butoxide, from potassium (2·4 g.) and anhydrous *tert.*-butanol (75 ml.), was suspended in ether (50 ml.), and di-*tert.*-butyl oxalate (12·8 g.) (Backer and Homan, *Rec. Trav. chim.*, 1939, **58**, 1048) and *tert.*-butyl fluoroacetate (8 g.) were added successively, with shaking. After several minutes, a voluminous precipitate formed. After being kept overnight at room temperature, the di-*tert.*-butyl potassiofluoro-oxaloacetate was isolated as a white, very hygroscopic powder, soluble in water and alcohol, insoluble in hydrocarbons. The free ester, prepared from the enolate as for the diethyl compound (above), solidified at about 15°, and was soluble in benzene, chloroform, and ethyl acetate, insoluble in non-polar solvents. With alcoholic ferric chloride solution, it gives a very faint violet-red colour reaction. The 2 : 4-*dinitrophenylhydrazone*, recrystallised from alcohol,

formed needles, m. p. 137—138° (Found: C, 48·5; H, 5·4; N, 12·1. $C_{16}H_{23}O_8N_4F$ requires C, 48·9; H, 5·2; N, 12·7%).

*Hydrolysis of Di-*tert.*-butyl Fluoro-oxaloacetate.*—A solution of the ester (3 g.) in dry benzene (150 ml.) was subjected to azeotropic distillation until no more traces of water appeared in the distillate. Then a small quantity of toluene-*p*-sulphonic acid (0·2 g.) was added and the substance heated until gas evolution ceased (*ca.* 1·5 hr.). The solution was decanted from a small amount of amorphous yellowish deposit on the walls of the reaction vessel and cooled at 4° for 48 hr. The precipitate so obtained (300 mg.) was easily soluble in water and consisted mainly of oxalic acid. However, the aqueous solution of the crystals also gave a violet colour with ferric chloride and a precipitate with 2 : 4-dinitrophenylhydrazine. That these reactions are due to the presence, in the crystals, of some fluoro-oxaloacetic acid is demonstrated by the following observation: when the aqueous solution is heated on the water-bath for several minutes, the reaction with ferric chloride becomes negative, but the solution still gives a reaction with 2 : 4-dinitrophenylhydrazine, leading to the 2 : 4-dinitrophenylhydrazone of fluoropyruvic acid.

It was not possible to isolate pure fluoro-oxaloacetic acid from its mixture with an excess of oxalic acid.

Fluoropyruvonitrile.—Fluoroacetyl bromide. Phosphorus tribromide (95 g.) was added dropwise to fluoroacetic acid (70 g.) cooled in ice–water, and the mixture refluxed for 3 hr. and distilled. The fraction distilling at 85—95° was collected; on redistillation, it boiled at 87—88° (yield 60 g., 47%).

In a three-necked flask, cuprous cyanide (75 g.), dried at 110° for 3 hr., was added portionwise to fluoroacetyl bromide (90 g.), and the mixture heated in an oil-bath (120°) for 6 hr. Unchanged fluoroacetyl bromide was removed, and the residue distilled *in vacuo*; the product, b. p. 88—93°/20 mm. (yield 25%), did not give satisfactory analytical figures, but the infrared spectrum indicated that the desired nitrile had been formed.

Fluoropyruvic acid. A solution of diethyl sodiofluoro-oxaloacetate (200 g.) in a mixture of hydrochloric acid (*d* 1·12; 400 ml.) and water (600 ml.) was heated at 130°, with stirring, during 6 hr. The solution was concentrated *in vacuo* to 200 ml., cooled, and filtered, and the filtrate extracted with five 100-ml. portions of ether. The ether residue was fractionated; the acid distilled at 98°/5 mm. as a colourless liquid which solidified to a cream-white solid. It was freed from the last traces of oxalic acid by redissolving it in water, extracting the solution several times with ether, and renewed distillation, then having m. p. 50° (with slight previous sintering) (9·5 g., 9%) (Found: C, 34·0; H, 3·1%; equiv., 106. $C_3H_3O_3F$ requires C, 34·0; H, 2·8%; equiv., 106). *Fluoropyruvic acid* is very soluble in water, insoluble in hydrocarbons. It readily gives a *semicarbazone*, m. p. 205° (decomp.) (from *n*-butanol) (Found: C, 29·2; H, 3·8; N, 25·3. $C_4H_6O_3N_3F$ requires C, 29·4; H, 3·7; N, 25·7%). The purity of the acid was also assayed by Silverman and Werkman's manometric method (*J. Biol. Chem.*, 1941, **138**, 35): a solution of fluoropyruvic acid (1·36 mg., 12·8 μmole) was treated in a Warburg vessel with a saturated solution (0·5 ml.) of ceric sulphate in 2N-sulphuric acid at 37°. The amount of carbon dioxide evolved was 290 mm.^{3}, exactly equivalent to 12·8 μmole.

Methyl fluoropyruvate. (*a*) To fluoropyruvic acid (8 g.) in dry ether (50 ml.), cooled in ice–salt, an excess of ethereal diazomethane was added. The ethereal solution was dried (Na_2SO_4) for 24 hr. and distilled (b. p. 85°/14 mm.; 5·5 g., 60%). (*b*) A current of dry hydrogen chloride was passed for 2 hr. through a solution of fluoropyruvic acid (18 g.) in anhydrous methanol (50 ml.). The excess of the methanol was distilled off, and the residue dissolved in ether (100 ml.). This solution was washed with water, sodium hydrogen carbonate solution, and again with water, and dried (Na_2SO_4). The fraction distilling at 85°/14 mm. was collected (yield 50%). *Methyl fluoropyruvate* is a colourless liquid, soluble in hydrocarbons, insoluble in water; n_D^{20} 1·423, d_{20}^{20} 1·510, $[M]_R$ 22·80 (Calc.: 22·25) (Found: F, 15·7. $C_4H_5O_2F$ requires F, 15·8%).

The infrared spectra were determined in CCl_4 solution, except that the semicarbazone of fluoropyruvic acid was used as a suspension in paraffin oil and fluoropyruvonitrile was investigated in chloroform solution.

The infrared spectra were kindly measured by Dr. S. Pinchas, Weizmann Institute of Science, Rehovoth.

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