tatrien-17β-ol (IVa), m.p. 95–98°. The total yield of IVa from this sequence of reactions was 0.905 g. (40%).

3-Methoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol *p*-Toluenesulfonate (IVb).—Treatment of 3-methoxy-9,10-seco-1,3,5-(10)-androstatrien-17β-ol (IVa) with *p*-toluenesulfonyl chloride in pyridine gave IVb, m.p. 128–130°, identical with the *p*-toluenesulfonate (IVb) obtained above.

3-Methoxy-9,10-seco-1,3,5(10)-androstatrien-17-one (IVd).—To a slurry of chromium trioxide (0.200 g.) in pyridine (2 ml.) at 0° was added a solution of the alcohol IVa (0.200 g.) dissolved in dry pyridine (2 ml.). The reaction mixture was stirred at room temperature for 12 hr., diluted with a saturated NaCl solution, and extracted with ether. Emulsions were broken by filtration, and both the residue and filtrate were extracted with ether. The combined ether extracts were washed with water, dried, and evaporated. On trituration of the residual oil with ether–petroleum ether, IVd, m.p. 103–104° (0.134 g., 67.5%), crystallized. Two crystallizations from ether–petroleum ether gave analytically pure IVd: m.p. 106–107°; $[\alpha]^{30}D$ +52.4° (95% ethanol); λ_{max}^{Nujol} 5.77 (five-membered ring C=O), 6.21, 6.35, 6.68, 11.48, 12.66 μ.

3-Hydroxy-9,10-seco-1,3,5(10)-androstatrien-17-one (9,10-Seco-10-methylestrone) (IVe).—A mixture of 0.134 g. of 3-methoxy-9,10-seco-1,3,5(10)-androstatrien-17-one (IVd) and 3.0 g. of dry pyridine hydrochloride was heated in a Wood's metal bath at 212–214° for 40 min. It was then cooled, diluted with 5% HCl in water (50 ml.), and repeatedly extracted with ether. The ether solution was washed with water and extracted with a cold, 10% aqueous NaOH solution. The alkaline extract was acidified and the product was again extracted into ether. The ether solution was washed with water, dried (Na_2SO_4), and evaporated. On trituration with ether–petroleum ether, the residue yielded a solid, m.p. 106–110° (0.082 g., 65% yield). Two crystallizations from ether–petroleum ether gave IVe, m.p. 115–117°, $[\alpha]^{30}D$ +58.8° (95% ethanol).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 8.15. Found: C, 79.90; H, 9.17.

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Synthesis of Some Homologs of Fluoropyruvic Acid and Their Effect on the Carbohydrate Metabolism of Ehrlich Ascites Tumor and on Lactate Dehydrogenase[1]

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Recent interest in the biochemical properties of fluoropyruvic acid[2] prompts us to report the results of our work in this field. We have prepared three homologs of fluoropyruvic acid and studied their effect (a) on the carbohydrate metabolism of Ehrlich ascites tumor using Warburg's manometric technique and (b) on the reduction of pyruvate by reduced diphosphopyridine nucleotide (DPNH) catalyzed by rabbit muscle lactate dehydrogenase. The effect of the known fluoropyruvic acid has been studied, along with that of its homologs.

β-Substituted fluoropyruvic acids were prepared by oxalylation of the appropriate carboxylic ester, which gave the β-keto ester; this was fluorinated with perchloryl fluoride, and the resulting fluoro ester was hydrolyzed and decarboxylated by refluxing with hydrochloric acid.

The intermediate fluoro keto esters were characterized by infrared spectra, elemental analyses, and the preparation and analysis of the corresponding 2,4-dinitrophenylhydrazones. It was found advantageous to carry out the fluorination in methanol; only partial fluorination could be achieved in ethanol. Similar difficulties have been encountered with other fluorinated esters.[3]

As has been previously noted,[4] acids of this type tend to be strongly hydrated. The two lower homologs of fluoropyruvic acid (III and VI) were obtained as distillable liquids, containing up to 0.5 mole of water. On exposure to air, they both became crystalline solids containing 1.5 moles of water (IIIa and VIa). Examination of the infrared spectra of these compounds showed that the crystalline form had only one carbonyl band (at 5.75 μ), whereas the liquid VI had peaks at 5.5 and 5.75 μ (carbonyl and carboxyl), indicating that the carbonyl is hydrated in the solid form. The hydrated acid IIIa, upon heating in air at 70°, absorbed more water, giving a crystalline material which gave a correct analysis for the trihydrate. The properties of the compounds prepared are reported in Table I.

The effect of the fluoro keto acids on the metabolism of Ehrlich ascites tumor was studied manometrically, as described previously.[5] The results are summarized in Table II. The figures reported are the average of the results of three to five experiments. Compounds III, VI, and fluoropyruvate cause a slight inhibition of oxygen uptake by ascites cells and a considerably stronger inhibition of lactate production. Anaerobic glycolysis is inhibited to a lesser degree than aerobic glycolysis. The effect of compound IX is almost negligible. The significance of the lack of correlation between the per cent inhibition of $Q_L^{N_2}$ and $Q_{CO_2}^{N}$, which is consistently observed in the presence of the compounds studied, is at present obscure.

The effect of fluoropyruvate and its homologs was next studied on rabbit muscle lactate dehydrogenase. The results are summarized in Table III. It was found that fluoropyruvate is a good substrate for this enzyme, reacting at about half the rate of pyruvate.[6] The methyl homolog, III, is also a substrate, with a rate of about one-fifth that of pyruvate. Compounds VI and IX do not function as substrates for this enzyme.

The last column of Table III shows the effect of the same compounds on the reduction of pyruvate by DPNH, catalyzed by lactate dehydrogenase from rabbit muscle. At a concentration of 1×10^{-2} *M* pyruvate, the activity of lactate dehydrogenase was increased about 13% over the value obtained for the activity of this enzyme at a concentration of 1×10^{-3} *M* pyruvate. Concentrations of pyruvate above 1×10^{-2} *M* did not appreciably affect the rate. Therefore, the concentration of 1×10^{-2} *M* pyruvate was used in the

(1) This investigation was supported by U. S. Public Health Service Research Grant CA 07296, from the National Cancer Institute.

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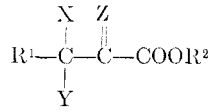
(6) E. H. Eisman, *et al.*,[2] reported that fluoropyruvate is a substrate for bovine heart lactate dehydrogenase, with a rate one-third that of pyruvate.

TABLE I

HOMOLOGS OF FLUOROPYRUVIC ACID AND THEIR DERIVATIVES



Compd.	R^1	X	Y	Z	R^2	Prepn. method	M.p. or b.p. (mm.), °C.	Crystn. solvent	Yield, %
I	CH_3	$COOC_2H_5$	H	O	C_2H_5	A[a]	103 (1.75)	...	38.5
II	CH_3	$COOC_2H_5$	F	O	C_2H_5	B	55–56 (0.1)	...	32
IIa	CH_3	$COOC_2H_5$	F	DNP[b]	C_2H_5	*c*	159–161	Bz	55
III	CH_3	H	F	O	H	C	70–75 (1)	...	54
IIIa	CH_3	H	F	O	H	C	58–59	...	100
IIIb	CH_3	H	F	O	H	C	60–61	...	100
IV	C_2H_5	$COOC_2H_5$	H	O	C_2H_5	A[d]	105–110 (25)	...	31.5
V	C_2H_5	$COOC_2H_5$	F	O	C_2H_5	B	77 (0.25)	...	67
Va	C_2H_5	$COOC_2H_5$	F	DNP[b]	C_2H_5	*c*	150–151	EtOH	80
VI	C_2H_5	H	F	O	H	C	54–62 (0.05)	...	42
VIa	C_2H_5	H	F	O	H	C	56–57	...	100
VII	$C_6H_5CH_2$	$COOC_2H_5$	H	O	C_2H_5	A[e]	*f*	...	72
VIII	$C_6H_5CH_2$	$COOC_2H_5$	F	O	C_2H_5	B	*g*	...	38
VIIIa	$C_6H_5CH_2$	$COOC_2H_5$	F	DNP[b]	C_2H_5	*c*	193–194	*i*-PrOH	82
IX	$C_6H_5CH_2$	H	F	O	H	C	76–77	Bz	64

[a] Previously reported by R. F. B. Cox and S. M. McElvain, *Org. Syn.*, **17**, 54 (1937). [b] DNP = N–NH–$C_6H_3(NO_2)_2$-2,4. [c] Prepared by the procedure of R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171. [d] See ref. 9. [e] Previously reported by Charles Weizmann, U. S. Patent 2,474,175 (June

TABLE II

EFFECT OF COMPOUNDS ON METABOLISM OF EHRLICH ASCITES TUMOR

Compound	Concn., *M*	% change[a] Q_{O_2}[b]	Q_{O_2}(G)[c]	Q_L^{air}[d]	$Q_L^{N_2}$[e]	$Q_{CO_2}^{N_2}$[f]
$FCH_2\overset{\overset{O}{\parallel}}{C}COOH$	10^{-3}	−25	0	−63	−50	−28
$CH_3CHF\overset{\overset{O}{\parallel}}{C}COOH$ (III)	10^{-3}	−20	0	−59	−28	−15
$CH_3CH_2CHF\overset{\overset{O}{\parallel}}{C}COOH$ (VI)	10^{-3}	−23	0	−41	−25	−9
$C_6H_5CH_2CHF\overset{\overset{O}{\parallel}}{C}COOH$ (IX)	10^{-3}	−13	0	−19	0	+4

[a] Experiments were carried out at 37°. [b] Rate of oxygen uptake in air. [c] Rate of oxygen uptake in air in the presence of added glucose (0.05 *M*). [d] Rate of formation of lactate in air in the presence of added glucose (0.05 *M*). [e] Rate of formation of lactate in 95% N_2 and 5% CO_2 in the presence of added glucose (0.01 *M*) measured by direct determination of lactate, according to S. B. Barker and W. H. Summerson, *J. Biol. Chem.*, **138**, 535 (1941). [f] Rate of CO_2 evolution in the presence of added glucose (0.01 *M*) in Krebs–Ringer bicarbonate buffer: W. W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," Burgess Publishing Co., Minneapolis, Minn., 1957, p. 149.

subsequent studies on the effect of the fluorinated acids on the activity of lactate dehydrogenase.

Three experiments were performed with each compound, and the results presented in Table III are the average of these closely agreeing values. The results clearly indicate that the compounds studied do not inhibit lactate dehydrogenase. Additional experiments demonstrated that even when the concentration of pyruvate was 3.3×10^{-4} *M*, addition of any of the fluoro acids at that same concentration (3.3×10^{-4} *M*) did not inhibit the activity of lactate dehydrogenase.

Experimental Section

Fluoropyruvic acid was prepared from fluorooxalacetic ester[7] by the method of Nair and Busch.[8] It was stored in the cold, and small samples were sublimed (80° and 0.1 mm.) prior to use.

Procedure A.—Oxalylations were carried out by the method described by Adickes and Andresen.[9]

Procedure B.—Fluorinations were carried out as described by Inman, *et al.*,[10] using perchloryl fluoride in methanol. When fluorination of compound IV was attempted in ethanol under the same conditions, a mixture was obtained which contained half the theoretical amount of fluorine. This was considered to be an equimolar adduct of β-keto ester and fluoro β-keto ester; b.p. 74–75° (0.275 mm.).

Anal. Calcd. for $C_{10}H_{15}FO_5 \cdot C_{10}H_{16}O_5$: C, 53.4; H, 6.89; F, 4.22. Found: C, 53.27; H, 7.01; F, 4.07.

Procedure C.—The appropriate β-keto ester was suspended in 5 times its weight of concentrated HCl, and the resulting mixture was heated under reflux for 2 hr. After evaporation of the excess HCl under reduced pressure, the residue was crystallized (compound IX) or distilled under reduced pressure (compounds III

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(10) C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, *J. Am. Chem. Soc.*, **80**, 6533 (1958).

Formula	Calcd., % C	H	N	F	Neut. equiv.	Found, % C	H	N	F	Neut. equiv.
$C_9H_{14}O_5$	...	...	...	...	...	...	...	...	...	...
$C_9H_{13}FO_5 \cdot H_2O$	45.38	6.35	...	...	...	45.26	5.92	...	...	...
$C_{15}H_{17}FN_4O_8$	45.00	4.28	14.00	4.75	...	44.43	3.68	13.93	5.37	...
$C_4H_5FO_3 \cdot 0.5H_2O$	37.2	4.68	...	...	...	37.98	4.72	...	...	...
$C_4H_5FO_3 \cdot 1.5H_2O$	...	...	...	12.9	147.0	...	...	...	12.36	146.6
$C_4H_5FO_3 \cdot 3H_2O$	27.6	6.38	...	10.92	...	27.8	6.54	...	11.10	...
$C_{10}H_{16}O_5$	...	...	...	...	...	...	...	...	...	...
$C_{10}H_{15}FO_5$	51.28	6.46	...	8.11	...	50.50	6.23	...	8.31	...
$C_{16}H_{19}FN_4O_8$	46.38	4.62	13.52	4.59	...	45.91	4.49	13.15	5.36	...
$C_5H_7FO_3$	44.78	5.26	...	14.17	134.1	44.10	5.58	...	13.51	139.6
$C_5H_7FO_3 \cdot 1.5H_2O$	37.27	6.26	...	11.79	161.1	38.00	5.75	...	11.01	159.0
$C_{15}H_{18}O_5$	...	...	...	...	...	...	...	...	...	...
$C_{15}H_{17}FO_5$	60.80	5.78	...	6.41	...	61.46	5.84	...	6.03	...
$C_{21}H_{21}FN_4O_8$	52.94	4.44	...	3.99	...	53.26	4.44	...	4.38	...
$C_{10}H_9FO_3 \cdot H_2O$	56.07	5.18	...	8.87	214.2	56.13	5.14	...	8.79	218.2

21, 1949); *Chem. Abstr.*, **44**, 3025*b* (1950). [f] Nondistillable liquid. [g] Nondistillable liquid. The analytical sample was prepared by passing a solution [4 g. in a mixture of 50 ml. of ether and 50 ml. of petroleum ether (b.p. 30–60°)] through a 6 × 150 mm. column of alumina (Matheson Coleman and Bell chromatographic grade, 80–200 mesh).

TABLE III

EFFECT OF COMPOUNDS ON RABBIT MUSCLE LACTATE DEHYDROGENASE (RATES OF REDUCTION BY DPNH)[a]

Compound	Concn., *M*	Rate relative to pyruvate: Compd. alone	Compd. plus pyruvate
$CH_3COCOOH$	1×10^{-2}	1.00[b]	...
$FCH_2COCOOH$	1×10^{-3}	0.48	0.97
$CH_3CHFCOCOOH$ (III)	1×10^{-3}	0.19	1.03
$CH_3CH_2CHFCOCOOH$ (VI)	1×10^{-3}	0.02	1.05
$C_6H_5CH_2CHFCOCOOH$ (IX)	1×10^{-3}	0.00	0.97

[a] Rates were determined by following the decrease in optical density at 340 mμ with a Beckman DU spectrophotometer. Each cuvette contained 2.00 ml. of triethanolamine buffer (0.4 *M*, adjusted to pH 7.4 with HCl, prepared according to H. U. Bergmeyer, "Methods of Enzymatic Analysis," Academic Press Inc., New York, N. Y., 1963, p. 254); 0.40 ml. of DPNH, 5×10^{-4} *M*; 0.60 ml. of substrate and/or inhibitor; and 0.002 ml. of lactate dehydrogenase (0.1 mg./ml.). DPNH and lactate dehydrogenase from rabbit muscle were obtained from Sigma Chemical Co., St. Louis, Mo. The reaction was started by the addition of enzyme and the optical density change was measured against a blank containing all the components listed above except DPNH. [b] This rate (40.8 μmoles of DPN formed/min.) was arbitrarily taken as 1.00.

and VI). In the case of compounds III and VI, the anhydrous acid was converted to the sesquihydrates IIIa and VIa by allowing it to stand exposed to the air for a few hours. Upon heating (about 70°) in a beaker covered with a watch glass, IIIa melted and evaporated; the condensate on the watch glass soon solidified, giving the trihydrate IIIb.

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Preparation of Some ω-(Pyridylalkyl)benzamide and Benzoate Derivatives

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In the present communication we report on the preparation of some pyridylalkyl esters and amides of benzoic acid and its nuclear-substituted derivatives and their quaternary salts. The appropriate acid chloride was treated in benzene solution with 2-(β-aminoethyl)pyridine, 2-aminopyridine, and γ-hydroxypropylpyridine. On boiling the mixture for several hours the base of the corresponding acid amide was obtained from the benzene filtrate. Acid esters were isolated as hydrochlorides after evaporation of the filtrate, dissolving the residue in ethanol, and acidification by hydrochloric acid. The hydrochlorides and methiodides of the acid amide derivatives were also prepared. The compounds thus prepared are listed in Table I.

According to Dew's method the compounds showed a sedative activity against the excitant effect of deoxyephedrine (DOE).[1] For comparison, Trioxazine (3,4,5-trimethoxybenzoylmorpholine), a new Hungarian drug, was chosen.[2] Some biological properties of the most active compounds are shown in Table II.

(1) P. B. Dews, *Brit. J. Pharmacol.*, **8**, 46 (1953).
(2) J. Borsy, *Arch. Intern. Pharmacodyn.*, **127**, 426 (1960).