9,10-Seco-10-methylestrone

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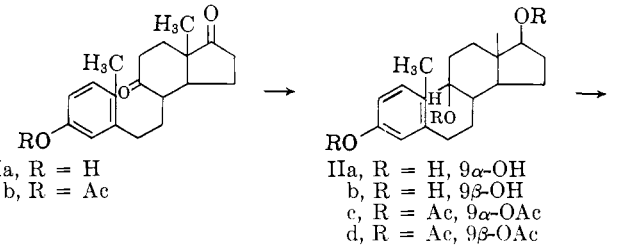
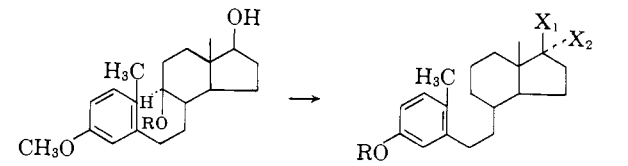
Some years ago we had shown that incubation of 9- or 19-hydroxylated 3-keto Δ^4-steroids with an organism capable of introducing a 1,2-double bond led to the formation of ring A aromatic steroids.[1] Thus, incubation of 19-hydroxy-4-androstene-3,17-dione with a *Pseudomonas* sp. produced estrone,[1] while incubation of 9α-hydroxy-4-androstene-3,17-dione with *Arthrobacter* sp. yielded 3-hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione (Ia).[1,2] Since certain 9,10-seco sterols, the D vitamins, are highly active physiologically, and since the 9,10-seco steroid Ia could be obtained in moderate yield by fermentation of androstenedione with *Nocardia* sp.,[1] it was decided to attempt the synthesis of a 9,10-seco analog of one of the hormones. Consequently, 3-hydroxy-9,10-seco-1,3,5(10)-androstatrien-17-one (9,10-seco-10-methylestrone) (IVe) was prepared and tested.



Ia, R = H
b, R = Ac

IIa, R = H, 9α-OH
b, R = H, 9β-OH
c, R = Ac, 9α-OAc
d, R = Ac, 9β-OAc



IIIa, R = H
b, R = $-O_2SC_6H_5CH_3$-*p*

IVa, R = CH_3; X_1 = OH; X_2 = H
b, R = CH_3; X_1 = $-O_3SC_6H_5CH_3$-*p*; X_2 = H
c, R = CH_3; X_1 = $-O_2CC_6H_5$-$(NO_2)_2$-2,4; X_2 = H
d, R = CH_3; X_1, X_2 = =O
e, R = H; X_1, X_2 = =O

Reduction of 3-acetoxy-9,10-seco-1,3,5(10)-androstatriene-3,17-dione (Ib) with excess sodium borohydride and acetylation of the mixture of isomers so obtained gave the expected 3,9β,17β-triacetoxy-9,10-seco-1,3,5(10)-androstatriene (IId) in 67% yield. Hydrolysis of the material from the crystallization mother liquors of IId gave 9,10-seco-1,3,5(10)-androstatriene-3,9α,17β-triol (IIa). The configurations of the groups at C-8, C-9, and C-17 in these compounds were assigned on the following basis. (1) The alkyl group at C-8 would be expected to retain its equatorial conformation, since in the β configuration, it would lie 1,3 diaxially to the C-18 methyl group. (2) Reduction of the 17-carbonyl group should be from the α side of the molecule. (3) Reduction of the 9-carbonyl group should lead predominantly to the more stable 9β-hydroxy compound IIb (equatorial hydroxyl group).[3] That the above stereochemical conclusions were correct was ascertained by an examination of the n.m.r. spectra of the triacetates IIc and IId. Thus, the n.m.r. spectrum of IIc showed a multiplet at τ 5.31 (17α-H)[4] and an unresolved multiplet at τ 4.79 (9β-H, full width at half-height, W_H = 6.7 c.p.s.). The position of absorption and the relatively small coupling with adjacent protons indicated that the proton at C-9 was equatorial.[5] The n.m.r. spectrum of IId, on the other hand, showed a broad multiplet from τ 5.05 to 5.59 (9α-H and 17α-H).[6]

The 9β-hydroxyl group in IIb was removed by esterification with *p*-toluenesulfonyl chloride and reduction with lithium aluminum hydride. 9,10-Seco-1,3,5(10)-androstatriene-3,9β,17β-triol (IIb) was first converted to the 3-methyl ether IIIa. This was then treated with slightly more than 1 molar equiv. of *p*-toluenesulfonyl chloride.[7] Since the *p*-toluenesulfonate IIIb failed to crystallize, it was reduced directly to 3-methoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol (IVa) with lithium aluminum hydride in tetrahydrofuran.[8] The product proved difficult to crystallize but could be purified by chromatography; some crystalline IVa was obtained directly from the chromatogram. The oily fractions were best purified *via* the more easily crystallizable 3,5-dinitrobenzoate IVc. A small quantity of the corresponding *p*-toluenesulfonate IVb was also isolated from the reaction.

Oxidation of IVa with chromium trioxide in pyridine[9] gave 3-methoxy-9,10-seco-1,3,5(10)-androstatrien-17-one (IVd). This, in turn, was converted to 3-hydroxy-9,10-seco-1,3,5(10)-androstatrien-17-one (IVe) (9,10-seco-10-methylestrone) with pyridine hydrochloride[10]

(1) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4627, 4631 (1961); **80**, 5004, 6148 (1958).

(2) Recently, C. J. Sih has studied both of these microbiological aromatizations in great detail and has developed very efficient syntheses of estrone by the fermentation of 19-oxygenated derivatives of cholesterol: C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *ibid.*, **87**, 1385 (1965); C. J. Sih, K. C. Wang, D. T. Gibson, and W. H. Whitlock, Jr., *ibid.*, **87**, 1386 (1965); C. J. Sih and K. C. Wang, *ibid.*, **87**, 1387 (1965); C. J. Sih, S. S. Lee, Y. Y. Tsong, K. C. Wang, and F. N. Chang, *ibid.*, **87**, 2765 (1965); and preceding papers by C. J. Sih.

(3) W. G. Dauben, G. J. Fonken, and D. S. Noyce, *ibid.*, **78**, 2579 (1956).

(4) The 17α-H of dihydrotestosterone acetate is reported to absorb at τ 5.38. The multiplicity pattern shown for it very closely resembled that obtained from IIc: N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, spectrum 353.

(5) K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).

(6) Our conclusions from the reduction of Ib with $NaBH_4$ differ from those reported by K. C. Wang and C. J. Sih [*Biochemistry*, **2**, 1238 (1963)] for a similar reduction of Ia. They claim to have obtained 3,9α,17β-trihydroxy-9,10-seco-1,3,5(10)-androstatriene, m.p. 147–148.5°, $[\alpha]^{28}D$ −11.1°, as the predominant product from this reduction (reduction of the 9-keto function to the 9α epimer). From its rotation their compound appears to be a 1:1 complex of IIa and IIb. Also, the physical properties of their compound reported to be 3,9α,17β-triacetoxy-9,10-seco-1,3,5(10)-androstatriene agree exceedingly well with those we found for the 9β isomer IId. Our 9α isomer (IIc) was not crystalline. Because of these differences, our separation and characterization of these isomers has been reported in greater detail than usual. It should be noted that we and Drs. Wang and Sih are in basic agreement on the structure and configuration of IIb.

(7) We had previously found that the 17β-hydroxyl group of testosterone reacts very slowly with *p*-toluenesulfonyl chloride in pyridine at room temperature.

(8) N. G. Gaylord, "Reduction with Complex Metal Hydrides," Interscience Publishers, Inc., New York, N. Y., 1956, p. 855.

(9) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

(10) W. S. Johnson, I. A. David, H. C. Dahm, R. J. Highet, E. W. Warnhoff, W. D. Wood, and E. T. Jones, *ibid.*, **80**, 661 (1958).

at 212°. That the oxygenated function at C-9, and not that at C-17, was removed in the lithium aluminum hydride reduction was established by the infrared spectrum of IVd. The carbonyl group of 3-methoxy-9,10-seco-1,3,5(10)-androstatrien-17-one absorbed at 5.77 μ. In addition, in view of the great ease of cyclization of Ib to 1-hydroxy-4-methyl-1,3,5(10),9(11)-estratetraen-17-one,[1] it is highly improbable that a carbonyl group at C-9 (in an isomer of IVd) could survive treatment with pyridine hydrochloride at 212°.

It should be noted that 9,10-seco-10-methylestrone (IVe) was a possible product from the reductive aromatization of the 17-ethylene ketal of 1,4-androstadiene-3,17-dione; its formation, however, could not be demonstrated in that reaction.[11]

Pharmacology—Because of the very diverse structures found in compounds with estrogenic activity, it was felt that the 9,10-seco analogs of the estrogens would have the maximum chance of activity. However, 9,10-seco-10-methylestrone (IVe) failed to increase the uterine weight of an immature mouse when injected subcutaneously (1 mg. in oil, total dose in 3 days). It also failed to inhibit the effects of 0.3 μg. of estrone on the uterus of the immature mouse at the 1-mg. level.

Experimental Section[12]

3,9β,17β-Triacetoxy-9,10-seco-1,3,5(10)-androstatriene (IId).—A solution of 8.07 g. of 3-acetoxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione (Ib) in 680 ml. of methanol and 20 ml. of water was treated at 0–5° with 5.0 g. of $NaBH_4$, with stirring, for 0.5 hr. The reaction mixture was then stirred for an additional 1 hr. at room temperature. The excess methanol was removed by distillation under vacuum; the residue was diluted with water, acidified with aqueous acetic acid, and extracted with ethyl acetate. The residue from the ethyl acetate extract was acetylated by treatment with acetic anhydride (30 ml.) and pyridine (30 ml.) at room temperature for 24 hr. The triacetate was recovered from the acetylation by dilution of the reaction with water and extraction with ethyl acetate. Crystalline IId (6.81 g., 67% yield), m.p. 103–108°, was obtained by trituration of the oily residue from the ethyl acetate extract with ether and petroleum ether (b.p. 60–80°). Two crystallizations of this material from acetone–petroleum ether yielded analytically pure IId: m.p. 110–112°; $[\alpha]D$ +24.9° ($CHCl_3$); n.m.r. ($CDCl_3$) τ 9.11 (singlet, 18-CH_3), 7.94 (singlet, 9- and 17-OAc), 7.75 (singlet) and 7.72 (singlet) (19-CH_3 and 3-OAc), 5.05–5.59 (broad multiplet, axial 9-H and 17-H). Wang and Sih[6] report m.p. 107.5–109°, $[\alpha]^{27}D$ +25.4° ($CHCl_3$), for what they believed to be 3,9α,17β-triacetoxy-9,10-seco-1,3,5(10)-androstatriene.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.68; H, 8.07.

9,10-Seco-1,3,5(10)-androstatriene-3,9β,17β-triol (IIb).—Hydrolysis of the triacetate IId with 85 ml. of 10% aqueous methanolic KOH at room temperature for 12 hr. yielded IIb, m.p. 174–175°, $[\alpha]^{32}D$ +19.9° (95% ethanol); lit.[6] m.p. 171.5–172.5°, $[\alpha]^{28}D$ +19° (95% ethanol).

9,10-Seco-1,3,5(10)-androstatriene-3,9α,17β-triol (IIa).—The oily residue from the ether–petroleum ether trituration mother liquors from three similar preparations of IId weighed 11.9 g. This triacetate was hydrolyzed with 10% aqueous methanolic KOH, as directed above; the methanol was removed by distillation, the mixture was acidified with dilute HCl, and the product was isolated by extraction with ethyl acetate. Crystallization from ethyl acetate yielded 6.47 g. (77% from the crude triacetate) of IIa, m.p. 192–194°. The analytical sample, m.p. 195–197°, $[\alpha]^{32}D$ −44.1° (95% ethanol), was prepared by three crystallizations from acetone; lit.[6] m.p. 192.5–193.5°, m.p. 147–148.5°, $[\alpha]^{28}D$ −11.1° (95% ethanol).

3,9α,17β-Triacetoxy-9,10-seco-1,3,5(10)-androstatriene (IIc). Acetylation of the triol IIa with acetic anhydride and pyridine at room temperature for 42 hr. produced a triacetate which could be obtained as a solid at low temperature but which melted on filtration. The material was purified by chromatography on silica gel before analysis: n.m.r. ($CDCl_3$) τ 9.18 (singlet, 18-CH_3), 7.95 (singlet, 9- and 17-OAc), 7.78 (singlet) and 7.74 (singlet) (19-CH_3 and 3-OAc), 5.31 (multiplet, 17α-H), 4.79 (unresolved multiplet, 9β-H, W_H = 6.7 c.p.s.).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.93; H, 8.26.

3-Methoxy-9,10-seco-1,3,5(10)-androstatriene-9β,17β-diol (IIIa).—To a suspension of 6.00 g. of 9,10-seco-1,3,5(10)-androstatriene-3,9β,17β-triol (IIb) in 180 ml. of water containing 12 ml. of dimethyl sulfate was added, slowly and with stirring at room temperature, 18 g. of NaOH in 150 ml. of water. After 2 hr. the product IIIa (4.87 g., 77.6% yield), m.p. 115–118°, was collected by filtration and purified by crystallization from benzene–ether. The pure IIIa had m.p. 117–119°, $[\alpha]^{30}D$ +23.1° (95% ethanol).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.65; H, 9.60.

Starting material (IIb, 1.12 g., m.p. 166–169°) was recovered by acidification of the alkaline mother liquors.

3-Methoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol (IVa).—To a solution of 2.40 g. (7.55 mmoles) of the dihydroxymethyl ether IIIa in 10 ml. of dry pyridine was added 1.80 g. (9.46 mmoles) of *p*-toluenesulfonyl chloride. The reaction mixture was stirred for 12 hr. at room temperature under nitrogen. Water was added, and the product was extracted with ether. The ether solution was washed with water, dilute HCl, and water; the ether was removed under vacuum. Attempts to crystallize the 3-methoxy-9β-*p*-toluenesulfonoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol (IIIb) were unsuccessful.

To ensure the removal of moisture from the oily tosylate IIIb, benzene was added and then removed by distillation, finally under vacuum. The tosylate IIIb was dissolved in dry tetrahydrofuran (50 ml.) and then added to a suspension of 1.50 g. of $LiAlH_4$ in tetrahydrofuran (50 ml.). The resulting mixture was stirred at room temperature for 36 hr. The excess $LiAlH_4$ was decomposed by the addition of ethyl acetate and then water; the excess tetrahydrofuran was removed by distillation under aspirator vacuum. The reaction mixture was acidified with dilute HCl; the products were extracted with ether and recovered by evaporation of the ether. The residue so obtained was chromatographed on 120 g. of silica gel. Elution of the column with 5% ethyl acetate in benzene gave 0.212 g. (6% yield) of 3-methoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol *p*-toluenesulfonate (IIIb), m.p. 122–125°. Two crystallizations from acetone–petroleum ether gave analytically pure IIIb: m.p. 129–130°; λ_{max}^{Nujol} 6.21, 6.29, 6.37, 7.41, 8.55 μ.

Anal. Calcd. for $C_{27}H_{36}O_4S$: C, 71.02; H, 7.95. Found: C, 70.88; H, 8.07.

Further elution of the column with 10% ethyl acetate in benzene gave 0.402 g. (18% yield) of 3-methoxy-9,10-seco-1,3,5-(10)-androstatrien-17β-ol, m.p. 96–99°. Three crystallizations from ether raised the melting point of IVa to 102–103°; λ_{max}^{Nujol} 3.02 (broad), 6.24, 6.33, 6.68, and 8.04 μ; n.m.r. ($CDCl_3$) τ 9.25 (18-CH_3), 7.78 (19-CH_3), 6.22 (3-OCH_3), 3.27 (17-OH).

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.24; H, 10.27.

Further elution of the column with 20 and 25% ethyl acetate in benzene gave 1.22 g. of an oil that resisted crystallization. Finally, elution of the column with 75% ethyl acetate–25% benzene gave 0.216 g. (9% recovery) of 3-methoxy-9,10-seco-1,3,5-(10)-androstatriene-9β,17β-diol (IIIa), m.p. and m.m.p. 118–120°.

3-Methoxy-9,10-seco-1,3,5(10)-androstatrien-17β-ol 3,5-Dinitrobenzoate (IVc).—The 1.22 g. of oil eluted from the above column with 20 and 25% ethyl acetate in benzene was treated with 2.0 g. of 3,5-dinitrobenzoyl chloride in 20 ml. of pyridine for 4 hr. at room temperature. The product was isolated from the reaction by dilution with aqueous Na_2CO_3 solution. Crystallization of the product from acetone–petroleum ether yielded 1.09 g. of IVc, m.p. 153–155°.

Anal. Calcd. for $C_{27}H_{32}N_2O_7$: C, 65.31; H, 6.50. Found: C, 65.61; H, 6.44.

Hydrolysis of the 3,5-dinitrobenzoate IVc with aqueous methanolic KOH gave 0.503 g. of 3-methoxy-9,10-seco-1,3,5(10)-andros-

(11) H. L. Dryden, G. M. Webber, and J. J. Wieczorek, *J. Am. Chem. Soc.*, **86**, 742 (1964).

(12) Melting points were taken on a carefully calibrated Fisher-Johns melting point apparatus. The n.m.r. spectra were run at 56.4 Mc./sec. We are indebted to Dr. William Schwabacher for determining these spectra for us. Rotations were run at $c \sim 1.0$ and are accurate to $\pm 1°$.

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.

(2) E. H. Eisman, H. A. Lee, Jr., and A. D. Winer, *Biochemistry*, **4**, 606 (1965).

(3) E. D. Bergman, S. Cohen, and I. Shahak, *J. Chem. Soc.*, 3286 (1959).

(4) E. Kun, D. R. Grassetti, D. W. Fanshier, and R. M. Featherstone, *Biochem. Pharm.*, **1**, 207 (1958).

(5) D. R. Grassetti, M. E. Brokke, and J. F. Murray, Jr., *J. Med. Chem.*, **8**, 753 (1965).

(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.