Synthesis of Some 4-Pyridylpyruvic Acids as Potential Lactate Dehydrogenase Inhibitors

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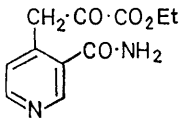
The synthesis is described of a series of (3-substituted 4-pyridyl)pyruvic acids by condensation of the corresponding 4-methylpyridines with diethyl oxalate followed by hydrolysis. Ethyl 3-(3-cyano-4-pyridyl)pyruvate and 3-(3-nitro-4-pyridyl)pyruvic acid are inhibitors of lactate dehydrogenase *in vitro*.

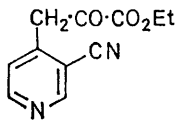
ANAEROBIC organisms such as schistosomes [1] and certain tumour strains [2] probably depend for their energy requirements on the process of glycolysis for which the enzyme lactate dehydrogenase (L-lactate: NAD oxidoreductase, E.C.1.1.1.27) is essential.[3] Mechanistic studies [4] have indicated that the enzymic conversion of lactate into pyruvate involves a hydride transfer from the lactate to the 4-position in the pyridine portion of the coenzyme, nicotinamide adenine dinucleotide (NAD). A molecule containing lactate or pyruvate linked covalently to the 4-position of nicotinamide [*e.g.* (1)] might simulate the normal transition state and therefore selectively inhibit lactate dehydrogenase (LDH) and thus interfere with glycolysis. We have prepared a series of pyridylpyruvates which might be expected to function as LDH inhibitors and therefore be of chemotherapeutic interest.

[1] E. Bueding, *J. Gen. Physiol.*, 1950, **33**, 475.
[2] D. Burk and A. L. Scharde, *Science*, 1956, **12**, 270.
[3] K. F. Gregory, C. W. Ng, and J. F. C. A. Pantebock, *Biochim. Biophys. Acta*, 1966, **130**, 469.
[4] B. Vennesland, *Discuss. Faraday Soc.*, 1955, **20**, 240.

For the synthesis of (3-substituted pyridyl)pyruvates by condensation of a 3-substituted 4-methylpyridine with ethyl oxalate (by analogy with the method of Adams and Miyano[5]) substantial quantities of the appropriate 3-substituted 4-methylpyridines were required. Many of the required compounds were either unknown or had been prepared only on a small scale. The route employed by Bobbitt and Scola[6] for the preparation of 4-methylpyridine-3-carbonitrile was modified, and 3-acetyl-4-methylpyridine was prepared directly from it by treatment with methylmagnesium iodide rather than the four-stage process described in the literature. Convenient syntheses of 4,*NN*-trimethylnicotinamide, 4,*NN*-trimethylpyridine-3-sulphonamide, 3-benzoyl-4-methylpyridine, and 4-methylpyridine-3-thiocarboxamide were also devised (see Experimental section).



(1)



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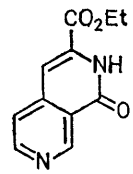
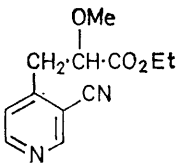


(3)

4-Methylpyridine-3-carbonitrile condensed smoothly with diethyl oxalate in the presence of sodium hydride to give the pyridylpyruvate (2). Mild alkaline hydrolysis afforded the 2,7-naphthyridine (3), presumably by cyclisation of the intermediate nicotinamide (1). The naphthyridine was assigned the cyclic amide structure on the basis of the absence of an OH band in the i.r. and the presence of a carbonyl band at 1653 and an NH band at 3364 cm^{-1}. The latter band is at lower wavenumber than that of the analogous isoquinolone (3411 cm^{-1}),[7] possibly owing to hydrogen bonding with neighbouring ester group in (3). An attempt to prevent



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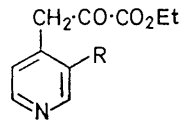
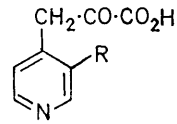
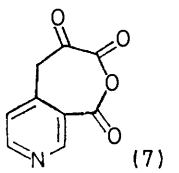


(5) a; R = SO_2·NMe_2
b; R = NO_2
c; R = CO_2Et



(6) a; R = SO_2·NMe_2
b; R = NO_2
c; R = CO_2Et
d; R = SO_3H
e; R = CO_2H



(7)

cyclisation by prior reduction of compound (2) to a lactate derivative with sodium borohydride in methanol gave the methoxy-derivative (4) or the naphthyridine (3) depending on the conditions.

[5] R. Adams and S. Miyano, *J. Amer. Chem. Soc.*, 1954, **76**, 3168.
[6] J. M. Bobbitt and D. A. Scola, *J. Org. Chem.*, 1960, **25**, 560.

The 3-dimethylsulphamoyl-, 3-nitro-, and 3-ethoxycarbonyl-4-methylpyridines gave the pyruvate esters (5a—c) but the required products were not obtained from 4-ethylpyridine-3-carbonitrile and from 3-dimethylcarbamoyl-, 3-thiocarbamoyl-, and 3-benzoyl-4-methylpyridines, although the *N*-oxide of the latter compound reacted normally. Mild acidic hydrolysis of the sulphonamide (5a) gave the puruvic acid (6a), whereas treatment with concentrated acid gave the sulphonic acid (6d). Mild acidic hydrolysis of the nicotinate (5c) gave the pyruvic acid (6c), and strong acid gave the oxepin (7), from which the diacid (6e) was obtained by alkaline hydrolysis. The same acid was obtained by acidic hydrolysis of the nitrile (2).

Compounds (2), (4), (5a—c), and (6a—e) were assayed[8] against rabbit skeletal muscle lactate dehydrogenase (M4 isoenzyme); compounds (2) and (6b) were found to be inhibitors (ID_{50} 7—10 × 10^{-5}M).[9] However the compounds possess no activity *in vitro* against an anaerobic organism, *Clostridium welchii*, and no activity *in vivo* against leukaemia (L1210), Ehrlich ascites tumours, or *S. mansoni*.

EXPERIMENTAL

2,6-Dibromo-4-methylpyridine-3-carbonitrile.— 2,6-Dihydroxy-4-methylpyridine-3-carbonitrile[6] (150 g, 1 mol) and phosphoryl bromide (575 g, 200 ml) were heated at 190° for 2 h. The mixture was cooled and treated with ice to give the crude *dibromopyridine* (260 g, 95%), m.p. 137—140° (from ethanol) (Found: C, 30·8; H, 1·5; Br, 58·0; N, 10·0. $C_7H_4Br_2N_2$ requires C, 30·5; H, 1·5; Br, 57·9; N, 10·2%).

4-Methylpyridine-3-carbonitrile.—The foregoing dibromo-derivative (137·5 g, 0·50 mol), anhydrous sodium acetate (123 g, 1·5 mol), methanol, and palladium chloride (1·5 g) were shaken with hydrogen at 50 lb in^{-2} until no more was absorbed (30 min). The mixture was then filtered and concentrated. The residue was treated with water and sodium carbonate and extracted with ether. Fractional distillation afforded 4-methylpyridine-3-carbonitrile (42 g, 71·5%), b.p. 108—111° at 20 mmHg (lit.,[6] 80° at 3 mmHg).

4,NN-Trimethylpyridine-3-sulphonamide.— 4-Methylpyridine-3-sulphonic acid[10] (17·3 g, 0·1 mol) was treated with potassium hydroxide in methanol to give the potassium salt, which was dried and then heated with chlorosulphonic acid (25 ml) for 40 h at 120°. The mixture was cooled and poured into dimethylamine (200 ml) at −70°. Excess of dimethylamine was evaporated off and the residue was extracted with ethyl acetate. Concentration of the extract followed by sublimation of the residue at 100° and 0·01 mmHg afforded the *dimethylsulphonamide* (4·0 g, 32·4%), m.p. 57—58° (Found: C, 47·7; H, 5·8; N, 14·2; S, 16·0. $C_8H_{12}N_2O_2S$ requires C, 48·0; H, 6·0; N, 14·0; S, 16·0%).

3-Benzoyl-4-methylpyridine.— 4-Methylpyridine-3-carbonitrile (11·8 g, 0·1 mol) in ether (100 ml) was added to phenylmagnesium bromide [from bromobenzene (15·8 g, 0·1 mol), magnesium (2·4 g, 0·1 mol), and ether (200 ml)]

[7] S. F. Mason, *J. Chem. Soc.* (*C*), 1957, 4874.
[8] C. J. Coulson and B. R. Rabin, *F.E.B.S. Letters*, 1969, **3**, 333.
[9] C. J. Coulson, personal communication.
[10] J. Delarge, *Il Farmaco. Ed. Sci.*, 1965, **20**, 629.

during 30 min. After being stirred at room temperature for 24 h, the mixture was treated with excess of dilute hydrochloric acid. The aqueous layer was made alkaline with 2N-ammonium hydroxide and extracted with ether. Fractional distillation of the extract afforded 3-*benzoyl*-4-*methylpyridine* (13·8 g, 70%), b.p. 125—130° at 0·4 mmHg, m.p. 60° (Found: C, 79·1; H, 5·7; N, 6·9. $C_{13}H_{11}NO$ requires C, 79·2; H, 5·6; N, 7·1%); *picrate*, m.p. 168° (Found: C, 53·2; H, 3·4; N, 12·8. $C_{19}H_{14}N_4O_8$ requires C, 53·5; H, 3·3; N, 13·1%).

3-*Acetyl*-4-*methylpyridine*.—Prepared similarly (74%) from 4-methylpyridine-3-carbonitrile and methylmagnesium iodide, this had b.p. 117—120° at 25 mmHg (lit.,[11] 57—58° at 1—2 mmHg).

4-*Methylpyridine*-3-*thiocarboxamide*.—Hydrogen sulphide was passed for 2 h into a solution of 4-methylpyridine-3-carbonitrile (5·9 g, 0·05 mol), and triethylamine (5·05 g, 0·05 mol) in ethanol (250 ml) at 0°. The solution was left overnight at room temperature, then evaporated to give the *thiocarboxamide* (5·5 g, 72·5%), m.p. 102—103° [from ethyl acetate–light petroleum (b.p. 40—60°)] (Found: C, 55·0; H, 5·4; N, 18·3; S, 21·0. $C_7H_8N_2S$ requires C, 55·2; H, 5·3; N, 18·4; S, 21·1%).

Ethyl 3-(3-*Cyano*-4-*pyridyl*)*pyruvate* (2).—Sodium hydride (50% in oil; 2·4 g, 0·5 mol) was added to 4-methylpyridine-3-carbonitrile (5·9 g, 0·05 mol) and diethyl oxalate (25 ml) in benzene (50 ml). The mixture was stirred for 24 h at room temperature and the precipitated sodium salt (8·5 g, 70%) was collected. This was dissolved in water, which was acidified to pH 3 with 2N-hydrochloric acid to give the *cyanopyridylpyruvate ester* (7·5 g, 70%), m.p. 195—196° (from ethanol) (Found: C, 60·1; H, 4·7; N, 12·5. $C_{11}H_{10}N_2O_3$ requires C, 60·5; H, 4·6; N, 12·8%), $\nu_{max.}$ (KBr) 1595, 1630, 1680, 1717, 2250, (CN) 2800, 2900, and 3000 cm^{-1}.

Ethyl 3-(3-*Dimethylsulphamoyl*-4-*pyridyl*)*pyruvate* (5a).—This was prepared similarly (35%), m.p. 183—184° (Found: C, 47·9; H, 5·3; S, 10·9. $C_{12}H_{16}N_2O_5S$ requires C, 48·0; H, 5·4; S, 10·7%), from 4,*NN*-trimethylpyridine-3-sulphonamide.

Ethyl 3-(3-*Benzoyl*-4-*pyridyl*)*pyruvate* N-*Oxide*.—3-Benzoyl-4-methylpyridine (4·92 g, 0·025 mol), 30% hydrogen peroxide (10 ml), and glacial acetic acid (20 ml) were heated on a steam-bath for 2 h. More 30% hydrogen peroxide (10 ml) was added and heating was continued for a further 2 h. Dilution with water and evaporation afforded a light brown oil (6·39 g), which was characterised as its *picrate*, m.p. 109—110° (from ethanol) (Found: C, 51·8; H, 3·38; N, 12·3. $C_{19}H_{14}N_4O_9$ requires C, 51·6; H, 3·2; N, 12·7%). To the crude 3-benzoyl-4-methylpyridine *N*-oxide (6·39 g), diethyl oxalate (25 ml), and dry benzene (100 ml) was added sodium hydride (50% in oil; 1·5 g). After being stirred for 12 h at room temperature, the orange sodium salt was collected and treated with 2N-hydrochloric acid (25 ml) to give *ethyl* 3-(3-*benzoyl*-4-*pyridyl*)*pyruvate* N-*oxide* (6·8 g, 87·5%), m.p. 184—185° (from ethanol) (Found: C, 64·7; H, 4·9; N, 4·22. $C_{17}H_{15}NO_5$ requires C, 65·2; H, 4·8; N, 4·5%).

Ethyl 3-(3-*Nitro*-4-*pyridyl*)*pyruvate* (5b).—This was prepared similarly (34%), m.p. 132—133° (lit.,[12] 129—130°), from 3-nitro-4-methylpyridine.

3-(3-*Dimethylsulphamoyl*-4-*pyridyl*)*pyruvic acid* (6a).—Ethyl 3-(3-dimethylsulphamoyl-4-pyridyl)pyruvate (0·5 g) and 2N-hydrochloric acid (10 ml) were heated at 100° with stirring for 10 min. Evaporation under reduced pressure afforded the *acid* (0·2 g, 44%), m.p. 233—234° (Found: C, 44·3; H, 4·4; N, 10·3; S, 11·7. $C_{10}H_{12}N_2O_5S$ requires C, 44·1; H, 4·4; N, 10·3; S, 11·8%).

3-(3-*Nitro*-4-*pyridyl*)*pyruvic Acid Hydrochloride* (6b).—Prepared similarly (19%), this had m.p. 116° (decomp.) (Found: C, 38·6; H, 3·3; Cl, 14·2; N, 11·6. $C_8H_7ClN_2O_5$ requires C, 39·0; H, 2·9; Cl, 14·4; N, 11·4%).

3-(3-*Sulpho*-4-*pyridyl*)*pyruvic Acid* (6d).—Ethyl 3-(3-dimethylsulphamoyl-4-pyridyl)pyruvate (0·5 g) and concentrated hydrochloric acid (20 ml) were heated under reflux for 2 h. Evaporation afforded the *sulphonic acid* (0·25 g, 58%), m.p. 252—253° (Found: Cl, 4·5; N, 5·6; S, 12·2. $C_8H_7NO_6S$,0·3HCl requires Cl, 4·6; N, 5·5; S, 12·5%).

3-(3-*Ethoxycarbonyl*-4-*pyridyl*)*pyruvic Acid* (6c).—Sodium hydride (50% in oil; 1·92 g, 0·04 mol) was added during 30 min to ethyl 4-methylnicotinate[5] (6·6 g, 0·04 mol) and diethyl oxalate (25 ml) in benzene. After being stirred for 3 days at room temperature the solid sodium salt (4 g) was separated and treated with cold 2N-hydrochloric acid; the solution was then immediately extracted with chloroform. The extract was evaporated to give the *pyruvic acid* (0·2 g), m.p. 71—72° [from ethyl acetate–light petroleum (b.p. 40—60°)] (Found: C, 55·9; H, 4·5; N, 5·89. $C_{11}H_{11}NO_5$ requires C, 55·7; H, 4·7; N, 5·9%).

Oxepino[3,4-c]*pyridine*-1,3,4(5H)-*trione Hydrochloride* (7).—Ethyl 3-(3-ethoxycarbonyl-4-pyridyl)pyruvate sodium salt (2 g) and 2N-hydrochloric acid (20 ml) were heated under reflux for 2 h. Cooling afforded the *oxepinopyridine hydrochloride* (0·3 g, 20%), m.p. 320° (decomp.) (Found: C, 47·4; H, 2·7; Cl, 15·5; N, 6·1. $C_9H_6ClNO_4$ requires C, 47·5; H, 2·7; Cl, 15·6; N, 6·15%), $\nu_{max.}$ (KBr) 1605, 1640, 1718, and 1755 cm^{-1}.

3-(3-*Carboxy*-4-*pyridyl*)*pyruvic Acid* (6e).—(*a*) Ethyl 3-(3-cyano-4-pyridyl)pyruvate (2·18 g) and 2N-hydrochloric acid (10 ml) were heated under reflux for 2 h. Cooling gave the *acid* (1·0 g, 48%), m.p. 319—320° (decomp.) (Found: C, 51·6; H, 3·0; N, 6·7. $C_9H_7NO_5$ requires C, 51·7; H, 3·4; N, 6·7%), $\nu_{max.}$ (KBr) 1618, 1640, and 1700 cm^{-1}.

(*b*) Compound (7) was dissolved in 2N-sodium hydroxide and then acidified to pH 3 with hydrochloric acid. After 10 min the *acid*, m.p. 315—320° (decomp.), was removed [i.r. spectrum identical with that of the product obtained by method (*a*)].

Ethyl 1,2-*Dihydro*-1-*oxo*-2,7-*naphthyridine*-3-*carboxylate* (3).—To ethyl 3-(3-cyano-4-pyridyl)pyruvate (5 g) in 50% aqueous ethanol was added Amberlite IRA 400 resin (basic form; 6 g) and the suspension was heated and stirred under reflux for 3 h. Filtration and evaporation afforded the *naphthyridine* (0·6 g, 10%), m.p. 229—230° (from ethanol) (Found: C, 60·1; H, 4·5; N, 13·3. $C_{11}H_{10}N_2O_3$ requires C, 60·5; H, 4·6; N, 12·8%), $\nu_{max.}$ (KBr) 1595, 1640, 1715, 3000, 3099, and 3175 cm^{-1}.

Ethyl 3-(3-*Cyano*-4-*pyridyl*)-2-*methoxypropionate* (4).—Sodium borohydride (0·185 g, 0·005 mol) in anhydrous methanol (5 ml) was added to ethyl 3-(3-cyano-4-pyridyl)-pyruvate (2·18 g, 0·01 mol) in anhydrous methanol (50 ml) and the mixture was stirred at room temperature for 2 h. The solution was evaporated and the residue was extracted with ether. Concentration of the extract

[11] J. L. Webb and A. H. Corwin, *J. Amer. Chem. Soc.*, 1944, **66**, 1456.

[12] M. H. Fisher and A. R. Matzuk, *J. Heterocyclic Chem.*, 1969, **6**, 775.

afforded the *methoxypropionate* (0·4 g, 17%), m.p. 110—112° (Found: C, 62·5; H, 5·8; N, 11·5; O in OMe, 13·3. $C_{12}H_{14}N_2O_3$ requires C, 61·5; H, 6·0; N, 11·5; O in OMe, 13·7%); *picrate*, m.p. 205—207° (Found: C, 46·7; H, 3·3; N, 15·1. $C_{18}H_{17}N_5O_{10}$ requires C, 46·7; H, 3·7; N, 15·1%).

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