(15 min., slightly exothermic). After standing at room temperature for 18 hr. the deep lilac-colored solution was quenched in 800 ml. of water, allowed to stand for 1 hr., and filtered. The washed and dried crude product crystallized from *n*-hexane in long rods, m.p. 139.4–141.2°; $[\alpha]_D^{25}$ −99.4° (1% in $CHCl_3$). The yield was 3.18 g.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 76.30; H, 8.75.

17α-Ethinylandrost-5-ene-3β,17β-diol 3,17-di(3-cyclohexylpropionate). A mixture of 3.14 g. (0.01 mole) of 17α-ethinylandrost-5-ene-3β,17β-diol, 8.9 g. (0.03 mole) of cyclohexylpropionic anhydride and 50 ml. of C.P. pyridine was refluxed for 18 hr. After the usual workup, the product was chromatographed on 250 g. of silica gel. The diester was eluted with 5% ether-pentane and recrystallized from alcohol, m.p. 114.0–115.6°; $[\alpha]_D^{25}$ −61.0° (1% in $CHCl_3$).

Anal. Calcd. for $C_{39}H_{58}O_4$: C, 79.27; H, 9.89. Found: C, 79.47; H, 10.06. The mixed melting point with the 3-monoester was 105–112°.

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Preparation of 2-Nitroisonicotinic Acid Hydrazide and 2-Aminoisonicotinic Acid Hydrazide

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Previous investigators[2] have shown that the introduction of a substituent in the pyridine ring of isonicotinic acid hydrazide usually causes almost complete loss of *in vitro* antituberculous activity. However, the 3-aminoisonicotinic acid hydrazide did show slight activity. For this reason, it seemed worthwhile to prepare and test the isomeric 2-amino derivative for effectiveness.

Another group, the nitro, which has not previously been tested for its effect on antituberculous activity when in the ring, was also introduced into the 2-position and tested.

EXPERIMENTAL[3]

Biological assays. The biological activity of the compounds was determined, using the biologic assay method for isonicotinic acid hydrazide.[4] The inhibitory concentration of 2-nitroisonicotinic acid hydrazide for the standard H37Rv is greater than 10 mcg./ml; that of 2-aminoisonicotinic acid hydrazide is between 2.5 and 5.0 mcg./ml. The standard test organism is inhibited by 0.03 to 0.07 mcg./ml. of isonicotinic acid hydrazide.

2-Amino-4-methylpyridine. Eastman Kodak material recrystallized from hot water was used as the starting material.

2-Nitro-4-methylpyridine. This was prepared by the method of Wiley and Hartman.[5] Oxidation of the 2-amino-4-methylpyridine with persulfuric acid gave a 52% yield of 2-nitro-4-methylpyridine, m.p. 65.5–67° (lit.[5] 61–62°).

2-Nitroisonicotinic acid. This was prepared by the permanganate oxidation of the 2-nitro-4-methylpyridine according to the procedure given by Brown.[6] The yield which was calculated after subtracting the amount of recovered starting material was 26%, m.p. 172.5–173.5° (lit.[6] 175°).

Methyl 2-nitroisonicotinate. One ml. of methanol, 0.4 g. of Victor polyphosphoric acid, and 0.168 g. (0.001 mole) of 2-nitroisonicotinic acid were mixed and heated at reflux for 6 hrs. The methanol was removed *in vacuo* and the acid was neutralized with sodium hydroxide solution. Ether was added to extract the ester. The ether was evaporated to obtain needles, 0.124 g. (68%), m.p. 80–81°. The ester was recrystallized from benzene and washed with petroleum ether before analysis.

Anal. Calcd. for $C_7H_6N_2O_4$: C, 46.16; H, 3.32. Found: C, 46.75; H, 3.27.

2-Nitroisonicotinic acid hydrazide. Methyl 2-nitroisonicotinate (0.036 g., 0.0002 mole) was refluxed with a slight excess of 85% hydrazine hydrate dissolved in 0.6 ml. of ethanol. After 10 min., crystals began to come out of the solution. Heating was continued for 1 hr. The crystals were filtered; yield 0.024 g. (67%), m.p. 181.5–183.5°.

Anal. Calcd. for $C_6H_6N_4O_3$: C, 39.56; H, 3.32. Found: C, 40.01; H, 3.41.

Methyl 2-aminoisonicotinate. The methyl 2-nitroisonicotinate (0.273 g., 0.0018 mole) was reduced by refluxing with excess iron filings in 1 ml. of a solution of 12*N* HCl in methanol (1:5). After 2 hr. the black mixture was filtered and the filtrate neutralized with methanolic sodium hydroxide solution. The solution was evaporated to dryness and extracted with ether. The ether was evaporated to obtain plates, 89 mg. (39%). After recrystallization from benzene, the crystals were pale yellow, m.p. 149.5–151°.

Anal. Calcd. for $C_7H_8N_2O_2$: C, 55.24; H, 5.30. Found: C, 55.90; H, 5.27.

2-Aminoisonicotinic acid hydrazide. Methyl 2-aminoisonicotinate (0.046, 0.0003 mole) was refluxed for 2 hr. with excess 85% hydrazine hydrate in 0.6 ml. of ethanol. On cooling, needles were obtained; yield 14 mg. (31%), m.p. 194.5–195°.

Anal. Calcd. for $C_6H_8N_4O$: C, 47.35; H, 5.30. Found: C, 47.93; H, 5.28.

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Reaction of D-Glucamine with Aromatic Nitro and Halogen Compounds

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Tamm[3] has shown that *N*-glucosides of some

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