of TPNH, in a manner analogous to the competition between DPNH and reduced substrate analogues in binding to horse liver alcohol dehydrogenase [9]. The competition is a reflection of the fact that complexes of the type *enzyme-reduced coenzyme-reduced substrate* are not formed by *iso*citric dehydrogenase, and emphasizes the role of coenzyme in determining the substrate binding properties of the enzyme. The effectiveness of DL-*iso*citric acid in displacing TPNH was markedly increased in the presence of 10^{-4} M Mn++ ions indicating that Mn++ is involved in the binding of *iso*citric acid. Further studies of this system are being carried out and will be reported in a detailed publication.

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Experiments Related to the Preparation of Pyridoxine from Furan

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Pyridoxine (X) has previously been prepared from furan by a six-step reaction [1]. A number of variations of this method have been tried by us (*cf.* Ref. [2]), but have so far not lead to any appreciable improvements of the method. However, in the course of the work eight new compounds were prepared, and it was found worth while to publish directions for their preparation, since they at any time may become of use for synthetic work in this field. The reactions in question are summarized below. Compounds II—VIII and XI are new. Their structures follows from the syntheses and from analyses.

Since 2-(α-acetamidoethyl)-furan (I) is obtained from furan in a 44 % yield [3-5], the overall yield of pyridoxine from furan along the route described here is 10 %, as compared to 23 % of the route published previously.

Experimental. Microanalyses by E. Boss and K. Glens.

2-(α-Acetamidoethyl)-3, 4-dicarbethoxyfuran (IV). 2-(α-Acetamidoethyl)-furan [3] (17.2 g, 0.112 mole) and diethyl acetylenedicarboxylate (20.1 g, 0.118 mole) were mixed and heated to 100° for 3.5 h. After cooling, the reaction mixture was dissolved in acetone (120 ml) and shaken with 10 % palladium on carbon catalyst (0.62 g) at room temperature under 1 atm. of hydrogen until about 2 500 ml of hydrogen had been taken up. After filtration the solvent was removed by distillation and the light-brown residue decomposed in an oil bath (190—200°) under 13 mm during 1 h. The reaction mixture was crystallized from ether. Hereby 19.4 g of IV [white crystals, m. p. 51—53° (Hershberg apparatus, corr.)] was obtained (Found: C 56.9; H 6.4; N 4.7; OC_2H_5 30.5; $COCH_3$ 14.8. Calc. for $C_8H_6O_3N(OC_2H_5)_2(COCH_3)$ (297.3): C 56.6; H 6.4; N 4.7; OC_2H_5

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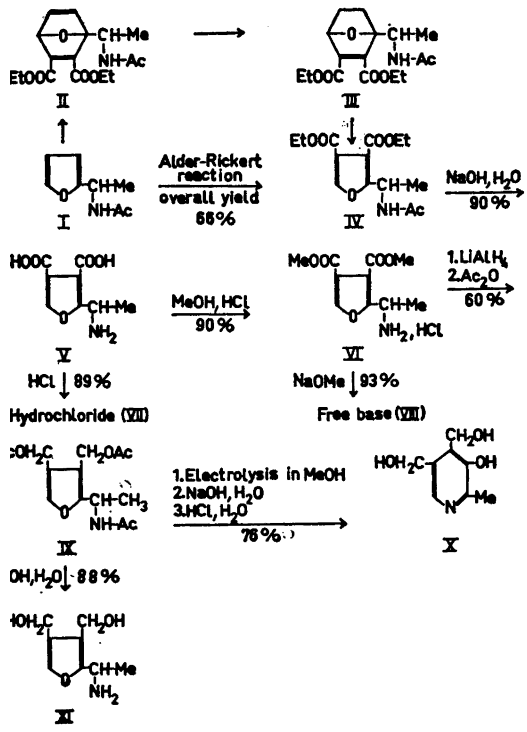

30.3; $COCH_3$ 14.5.) Another 2.6 g of analytically pure IV was isolated by distillation of the mother liquor (b.p. $_{0.1}$ 178—186°) followed by crystallization from ether (m. p. 49—51°), thus bringing the total yield of IV up to 22.0 g (66 %).

In a separate experiment the intermediate compound II was isolated in a 45 % yield; white crystals from ether, m. p. 129—130° (Found: C 59.5; H 6.3; N 4.7; OC_2H_5 27.8; $COCH_3$ 13.8. Calc. for $C_{10}H_9O_3N(OC_2H_5)_2(COCH_3)$ (323.3): C 59.4; H 6.6; N 4.3; OC_2H_5 27.9; $COCH_3$ 13.3.)

In another separate experiment the other intermediate compound (III) was isolated from pure II in a 89 % yield; white crystals from ether, m. p. 109—110°. (Found: C 58.9; H 7.1; N 4.7; OC_2H_5 27.6; $COCH_3$ 13.2. Calc. for $C_{10}H_{10}O_3N(OC_2H_5)_2(COCH_3)$ (325.4): C 59.1; H 7.1; N 4.3; OC_2H_5 27.7; $COCH_3$ 13.2).

2-(α-Aminoethyl)-3,4-dicarboxyfuran (V). IV (10.0 g, 0.034 mole) and sodium hydroxide (3 N, 115 ml, 0.34 mole) were heated under reflux (24 h). After cooling the mixture was brought to pH 8.7 by addition of concentrated hydrochloric acid. A small amount of a voluminous white precipitate was removed by filtration. The filtrate was brought to pH 5.0 by further addition of hydrochloric acid. Hereby 5.42 g of white crystals of V separated. Another 0.56 g of V was obtained by evaporation of the mother liquor to 60 ml. The total yield of V (5.98 g) corresponds to 90 %; m. p. 292—296° after darkening at about 280° (Kofler stage, corr.). (Found: C 48.3; H 4.6; N 7.0. Calc. for $C_8H_9O_5N$ (199.2): C 48.2; H 4.6; N 7.0.)

The hydrochloride of V (VII) was prepared in a 89 % yield in the usual way; white crystals, m. p. 195—200° (decomp.). (Found: C 40.7; H 4.4; N 6.2; Cl 14.5. Calc. for $C_8H_{10}O_5NCl$ (235.6): C 40.8; H 4.3; N 5.9; Cl 15.0.)

2-(α-Aminoethyl)-3,4-dicarbomethoxyfuran hydrochloride (VI). V (5.93 g) and anhydrous methanol (95 ml) were mixed and treated with hydrogen chloride until 4.5 g was absorbed. The pale yellow solution was heated under reflux (10 h). After cooling Drierite was added, the mixture shaken (2 h) and the Drierite removed by filtration. Methanol and excess hydrogen chloride were removed by distillation under vacuum and the residue dried under vacuum in a dessicator over potassium hydroxide. Dissolution in anhydrous methanol (15 ml) and precipitation with anhydrous ether gave 7.10 g (90 %) of VI; white crystals, m. p. 167—169° (decomp.). (Found: C 45.8; H 5.7; N 5.5; Cl 13.6; OCH_3 23.5. Calc. for $C_8H_8O_3NCl(OCH_3)_2$ (263.7): C 45.5; H 5.4; N 5.3; Cl 13.4; OCH_3 23.5.)

The free base of VI (VIII) was prepared from VI and sodium methoxide in methanol in a 93 % yield in the usual way; colorless oil, n_D^{25} 1.4952. (Found: C 52.9; H 5.7; N 6.5; OCH_3 27.1. Calc. for $C_8H_7O_3N(OCH_3)_2$ (227.2): C 52.9; H 5.8; N 6.2; OCH_3 27.3.)

2-(α-Acetamidoethyl)-3,4-bis(acetoxymethyl)-furan (IX). VI (3.27 g, 0.0124 mole) dissolved in anhydrous methanol (12 ml) was transformed into the free base (VIII) as described above. The base was dissolved in anhydrous ether (25 ml) and the solution added with stirring to a mixture of lithium aluminum hydride (0.94 g, 0.025 mole) in ether (40 ml) during 15 min. Stirring was continued for 1 h and the mixture heated under reflux for another hour. After cooling acetic anhydride (40 ml) was added dropwise with stirring at —20°. The ether was evaporated and the residue heated under reflux (30 min). After cooling ether (150 ml) was added, the mixture filtered and distilled from an oil bath. The yield was 2.21 g (60 %) of IX; pale yellow liquid, b. p. $_{0.1}$ 180—190°, n_D^{25} 1.4914; previously found b. p. $_{0.05}$

175–187°, n_D^{25} 1.4905. (Found: C 56.6; H 6.7; N 4.9; $COCH_3$ 42.7. Calc. for $C_8H_{10}O_3N(COCH_3)_3$ (297.3): C 56.6; H 6.4; N 4.7; $COCH_3$ 43.4.) Crystallization from ether gave white crystals, m. p. 89–92°; previously found 95–96° [1].

The transformation of IX into pyridoxine hydrochloride in a 76 % yield has been described previously [1].

2-(α-Aminoethyl) - 3,4-bis(hydroxymethyl)-furan (XI). IX (5.95 g, 0.020 mole) and sodium hydroxide (3 N, 67 ml, 0.20 mole) were mixed and heated under reflux (24 h). After cooling the light brown mixture was continuously extracted with ether (3 days). The etheral extract was distilled from an oil bath. Hereby 3.02 g (88 %) of XI was obtained as a pale yellow oil; b. p. $_{0.05}$ 165–171°, n_D^{25} 1.5304. (Found: C 56.3; H 7.9; N 8.5. Calc. for $C_8H_{13}O_3N$ (171.2): C 56.1; H 7.7; N 8.2.)

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Two Fractions of Melanocyte Stimulating Hormone in Urine

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One of the hormones secreted from the pituitary is the Melanocyte Stimulating Hormone (MSH). Two different melanocyte stimulating substances have been isolated from porcine pituitary (Lerner and Lee [1]; Lee and Lerner [2]), one is alpha-MSH which has an isoelectric point at pH 10.5–11.0, the other is beta-MSH which has an isoelectric point at pH 5.5.

Other investigators found similar substances with isoelectric points around pH 5 but could not find any activity at pH 11. (Porath *et al*[3]; Benfey and Purvis [4]; Geschwind and Li [5]).

MSH is excreted in the urine of normal men and women (Shizume and Lerner [6]; Dahlberg [7]), as well as during pregnancy (Shizume and Lerner [6]; Dahlberg [8]).

Owing to the inadequate methods for extraction of MSH from urine it has previously been difficult to estimate the excretion in the urine of normal subjects.

As MSH is a pituitary hormone a method for determination of the excretion in urine was thought to give information on certain pituitary functions. A biological assay has therefore been elaborated.

For the determination of the hormone hypophysectomized frogs, *Rana Esculenta*, were used. In the method of extraction used an active melanocyte stimulating substance was found at pH 4.0. In recovery experiments 45.4 % ± 6.4 of a known amount of added MSH could be retrieved.

As the degree of accuracy thus seemed rather low it was thought that part of the hormone might remain in solution. In order to investigate this problem extractions were carried out at both pH 4.0 and pH 11.5 on the urine of normal women. The usual activity was found at pH 4.0, *but in the fraction at pH 11.5 activity was also found.* There are thus *two fractions of melanocyte stimulating hormone in the urine of homo.*

Recovery experiments with a known amount of added MSH yielded the following results:

At pH 4.0 = 40–45 %,
at pH 11.5 = 55–60 %

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