# *The Synthesis of Compounds with Potential Anti-folic Acid Activity. Part IV.* 3 : 6-Diaminopyrido(2 : 3)pyrazines.†*

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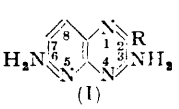
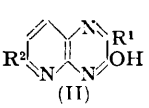
The unequivocal synthesis is reported of 3 : 6-diaminopyrido(2 : 3)pyrazine; † the 2-carboxylic acid and carboxyamide are obtained by the reaction of 2 : 6-diamino-3-nitrosopyridine with cyanoacetic acid and cyanoacetamide respectively. The alkali-hydrolysis product of the amide has been assigned the 3-amino-6-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid structure, since it differed from 6-amino-3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid, synthesised unambiguously from 2 : 6-diamino-3-nitrosopyridine with diethyl malonate. Seventeen aromatic and one heterocyclic derivative of acetonitrile have been treated with 2 : 6-diamino-3-nitrosopyridine, to give 2-substituted 3 : 6-diaminopyrido(2 : 3)pyrazines. Sodium alkoxides were effective catalysts for all the above condensations.

Owing to the close similarity of the pyrido(2 : 3)pyrazine nucleus (cf. I) to that of pteridine it was thought desirable to prepare a number of aminopyrido(2 : 3)pyrazines for biological testing. Only a few such compounds have been described, none of them containing an amino-group in the pyrazine nucleus. Most of those prepared have identical substituents in the 2- and the 3-position, being obtained by reaction of 2 : 3-diaminopyridine or its


(I)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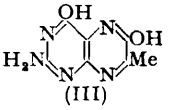
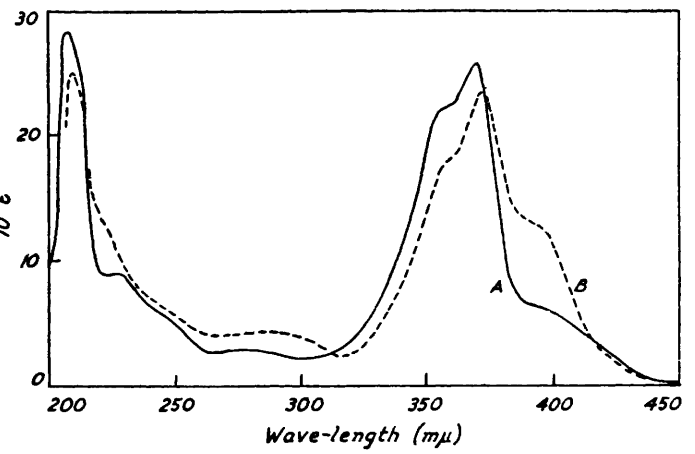

(III)

derivatives with a symmetrical 1 : 2-dicarbonyl compound (Tschitschibabin and Kirsanow, *Ber.*, 1927, **60**, 766; Bernstein, Stearns, Shaw, and Lott, *J. Amer. Chem. Soc.*, 1947, **69**, 1151; Lappin and Slezak, *ibid.*, 1950, **72**, 2806; Petrow and Saper, *J.*, 1948, 1389). Pyrido(2 : 3)-pyrazines unsymmetrically substituted in the 2- and the 3-position were prepared incidentally by Rudy and Majer (*Ber.*, 1938, **71**, 1323; 1939, **72**, 940) who condensed 2 : 3-diaminopyridine and 2-alkylamino-3-amino- and 3-amino-2-arylamino-pyridines with alloxan and claimed to have isolated 2-ureidopyrido(2 : 3)pyrazine derivatives and also 3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid (II; $R^1 = CO_2H$, $R^2 = H$); the only other example is Korte's condensation (*Chem. Ber.*, 1952, **85**, 1012) of 2 : 3-diamino- and 2 : 3 : 6-triamino-pyridine with pyruvic acid, which could yield either the 2-hydroxy-3- or the 3-hydroxy-2-methylpyrido(2 : 3)pyrazine derivative or both; the products were assigned the former structure, on the basis however only of the analogous synthesis of methylxanthopterin (III) from 2 : 5 : 6-triamino-4-hydroxypyrimidine and pyruvic acid (Elion and Hitchings, *J. Amer. Chem. Soc.*, 1947, **69**, 2553). By extending the reactions of *o*-aminonitroso-compounds with cyanoacetic acid and amide (Osdene and Timmis, *Chem. and Ind.*, 1954, 405; and preceding paper) and with phenylacetonitrile derivatives (Spickett and Timmis, *J.*, 1954, 2887) to 2 : 6-diamino-3-nitrosopyridine we have synthesised a number of unambiguously orientated 3 : 6-diaminopyrido(2 : 3)pyrazine derivatives substituted in the 2-position by carbamyl and carboxyl groups and by aromatic and heterocyclic substituents.

2 : 6-Diaminopyridine was nitrosated in dilute acetic acid to give 2 : 6-diamino-3-nitrosopyridine (cf. Tschitschibabin and Seide, *J. Russ. Phys. Chem.*, 1918, **50**, 522) which was selected as the only readily available *o*-aminonitrosopyridine derivative which would yield products closely analogous to the biologically important pteridines containing a 2-amino-group in the pyrimidine ring. 2 : 6-Diamino-3-nitrosopyridine and cyanoacetic acid in

* Part III, preceding paper. † Named and numbered according to Ring Index No. 968.

2-ethoxyethanol containing 2 mols. of sodium 2-ethoxyethoxide gave sodium 3 : 6-diaminopyrido(2 : 3)pyrazine-2-carboxylate (I; R = $CO_2Na$). The acid was readily decarboxylated in boiling quinoline containing a little copper bronze, to yield 3 : 6-diaminopyrido(2 : 3)pyrazine (I; R = H) which was purified by sublimation and characterised also as the picrate and the 3 : 6-diacetamido-derivative. Similar reaction of the nitroso-compound with cyanoacetamide yielded 3 : 6-diaminopyrido(2 : 3)pyrazine-2-carboxyamide (I; R = $CO{\cdot}NH_2$). This amide with ethyl orthoformate in the presence of acetic anhydride (cf. Albert, Brown, and Cheeseman, *J.*, 1951, 474) or with ethyl chloroformate alone (cf. Gowenlock, Newbold, and Spring, *J.*, 1948, 517) yielded yellow insoluble products which resisted purification. Alkaline hydrolysis of the amide (I; R = $CO{\cdot}NH_2$) with boiling N-sodium hydroxide for 24 hours yielded a product, soluble in aqueous sodium




carbonate solution, whose analysis indicated its being either 3-amino-6- or 6-amino-3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid. This question was resolved by unambiguous synthesis of the latter acid (II; $R^1 = CO_2H$, $R^2 = NH_2$) from 2 : 6-diamino-3-nitrosopyridine and diethyl malonate.* The absorption spectrum of this acid in N-hydrochloric acid is shown as the curve *A*. The hydrolysis product of the amide (I; R = $CO{\cdot}NH_2$) in N-hydrochloric acid gave the spectrum shown as curve *B*. Since the two curves are not identical, although the maxima and minima occur at very similar wavelengths the structure 3-amino-6-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid has been assigned to the hydrolysis product of the amide. Decarboxylation of the acid (II; $R^1 = CO_2H$, $R^2 = NH_2$) obtained from malonic ester yielded 6-amino-3-hydroxypyrido(2 : 3)pyrazine (II; $R^1 = H$, $R^2 = NH_2$), characterised as the acetyl derivative.

TABLE 1. *Absorption spectra of* 3 : 6-*diaminopyrido*(2 : 3)*pyrazines* (I) *in* N-*hydrochloric acid.*

| R | | (Approx.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H | $\lambda_{max.}$ (mμ) | 208 | 249 | 295 | 364 | 382 | $\lambda_{min.}$ (mμ) | 238 | 269 | 313 | 371 |
| | $10^{-3}\varepsilon$ | 30·4 | 5·7 | 3·04 | 25·3 | 28·3 | $10^{-3}\varepsilon$ | 5·2 | 1·6 | 1·0 | 22·8 |
| $CO_2H$ | $\lambda_{max.}$ (mμ) | 222 | 253·5 | 282 | 300 | 401 | $\lambda_{min.}$ (mμ) | 250 | 268 | 290 | 328 |
| | $10^{-3}\varepsilon$ | 18·1 | 5·84 | 5·2 | 5·45 | 22·4 | $10^{-3}\varepsilon$ | 5·77 | 4·6 | 5·0 | 8·0 |
| $CO{\cdot}NH_2$ | $\lambda_{max.}$ (mμ) | 213 | 255 | 280 * | 303 | 400 | $\lambda_{min.}$ (mμ) | 247 | 288 | 328 | — |
| | $10^{-3}\varepsilon$ | 27·4 | 8·2 | 5·6 | 6·6 | 27·7 | $10^{-3}\varepsilon$ | 7·8 | 5·3 | 2·5 | — |

* Shoulder.

In the preparation of the 2-aryl derivatives, 2 : 6-diamino-3-nitrosopyridine was condensed with 17 different substituted phenylacetonitriles and with 2-thienylacetonitrile

* *Note added in Proof.*—After the submission of this paper, Leese and Rydon (*J.*, 1955, 304) published details of the preparation of 6-amino-3-hydroxypyrido(2 : 3)pyrazine and its 2-carboxylic acid by essentially the same method as that used by us.

in ethanol in the presence of sodium ethoxide to give 2-aryl-3 : 6-diaminopyrido(2 : 3)-pyrazines. The compounds which all had sharp melting points, were highly crystalline, and exhibited intense fluorescence in daylight and in ultraviolet light.

Ultraviolet absorption spectra are shown in Tables 1 and 2. Owing to the insolubility of most of these compounds in water and organic solvents, for determination of spectra the compounds were dissolved in 10N-hydrochloric acid and then diluted.

TABLE 2. *Absorption spectra of* 3 : 6-*diaminopyrido*(2 : 3)*pyrazines* (I) *in* N-*hydrochloric acid.*

| R | | (Approx.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *p*-F·$C_6H_4$ | $\lambda_{max.}$ (mμ) | 208·5 | 258 | 301 | 389 | $\lambda_{min.}$ (mμ) | 246 | 282 | 321 |
| | $10^{-3}$ ε | 33·1 | 6·72 | 3·48 | 28·88 | $10^{-3}$ ε | 5·95 | 2·8 | 2·2 |
| *o*-MeO·$C_6H_4$ | $\lambda_{max.}$ (mμ) | 210 | 258 | 300 * | 388 | $\lambda_{min.}$ (mμ) | 246 | — | 320 |
| | $10^{-3}$ ε | 39·5 | 6·51 | 3·0 | 28·94 | $10^{-3}$ ε | 5·6 | — | 2·0 |
| *m*-MeO·$C_6H_4$ | $\lambda_{max.}$ (mμ) | 212 | 260 | 300 | 390 | $\lambda_{min.}$ (mμ) | 248 | 286 | 322 |
| | $10^{-3}$ ε | 42·2 | 6·90 | 3·67 | 28·00 | $10^{-3}$ ε | 5·8 | 3·3 | 2·4 |
| *p*-MeO·$C_6H_4$ | $\lambda_{max.}$ (mμ) | 207 | 256 * | 306 | 392 | $\lambda_{min.}$ (mμ) | — | 294 | 326 |
| | $10^{-3}$ ε | 39·0 | 9·92 | 4·05 | 28·63 | $10^{-3}$ ε | — | 4·0 | 3·2 |
| *p*-$NO_2$·$C_6H_4$ | $\lambda_{max.}$ (mμ) | 207·5 | 258 | — | 393 | $\lambda_{min.}$ (mμ) | 249 | — | 314 |
| | $10^{-3}$ ε | 33·6 | 12·2 | — | 33·9 | $10^{-3}$ ε | 11·6 | — | 4·0 |
| Ph | $\lambda_{max.}$ (mμ) | 210 | 259 | 301 | 389·5 | $\lambda_{min.}$ (mμ) | 247 | 282 | 322 |
| | $10^{-3}$ ε | 33·1 | 6·48 | 3·52 | 28·64 | $10^{-3}$ ε | 5·6 | 2·9 | 2·25 |
| 2-Thienyl | $\lambda_{max.}$ (mμ) | 215 | 267 | 315 | 398·5 | $\lambda_{min.}$ (mμ) | 259 | 293 | 335 |
| | $10^{-3}$ ε | 25·3 | 7·8 | 4·6 | 27·8 | $10^{-3}$ ε | 7·4 | 3·6 | 2·9 |

* Shoulder.

## EXPERIMENTAL

M. p.s were determined in an electrically heated copper block. Absorption spectra were measured with Beckman and Uvispek spectrophotometers. Analyses were by Mr. P. R. W. Baker of Beckenham and Mr. F. Oliver of Imperial College.

3 : 6-*Diaminopyrido*(2 : 3)*pyrazine*-2-*carboxylic Acid*.—2 : 6-Diamino-3-nitrosopyridine (5·52 g.) and cyanoacetic acid (3·75 g.) were added to a solution of sodium (1·9 g.) in 2-ethoxyethanol (200 ml.) and the mixture was boiled under reflux for 30 min. The yellowish-brown precipitate was removed and purified by dissolution in boiling N-hydrochloric acid followed by neutralisation with dilute aqueous ammonia. Recrystallisation from water yielded 3 : 6-*diaminopyrido*(2 : 3)-*pyrazine*-2-*carboxylic acid* (4·4 g.) as yellow rods, m. p. 284° (decomp.) (Found, in material dried at 180° : C, 47·2; H, 3·1; N, 33·7. $C_8H_7O_2N_5$ requires C, 46·8; H, 3·4; N, 34·1%).

3 : 6-*Diaminopyrido*(2 : 3)*pyrazine*.—Finely ground 3 : 6-diaminopyrido(2 : 3)pyrazine-2-carboxylic acid (3·0 g.) was boiled under an air-condenser with quinoline (50 ml.) containing a trace of copper bronze. The clear solution obtained after 15 min. was filtered and concentrated. Treatment with ether and light petroleum (b. p. 40—60°) yielded a buff powder (2·34 g.). Sublimation at 220°/1 mm. yielded 3 : 6-*diaminopyrido*(2 : 3)*pyrazine* as a bright yellow powder, m. p. 238° (Found : C, 52·5; H, 4·7; N, 43·3. $C_7H_7N_5$ requires C, 52·2; H, 4·4; N, 43·5%).

Alcoholic picric acid solution yielded the *picrate*, as orange irregular prisms, m. p. 260° (decomp.) (from aqueous ethanol) (Found : C, 40·5; H, 3·0; N, 28·8. $C_{13}H_{10}O_7N_8$ requires C, 40·0; H, 2·6; N, 28·7%).

Treatment of the diamine with hot acetic anhydride yielded 3 : 6-*diacetamidopyrido*(2 : 3)-*pyrazine* as silky needles (from water), m. p. 300° (Found : C, 54·2; H, 4·9; N, 28·6. $C_{11}H_{11}O_2N_5$ requires C, 53·9; H, 4·5; N, 28·6%).

3 : 6-*Diaminopyrido*(2 : 3)*pyrazine*-2-*carboxyamide*.—2 : 6-Diamino-3-nitrosopyridine (5·6 g.) and cyanoacetamide (3·7 g.) were boiled with a solution of sodium (0·6 g.) in ethanol (200 ml.) for 2 hr. The thick yellow precipitate obtained was purified by acid- and alkali-treatment and recrystallised from a large volume of water to yield 3 : 6-*diaminopyrido*(2 : 3)*pyrazine*-2-*carboxy-amide* (4·2 g.) as yellow needles, m. p. >300° (Found : C, 46·9; H, 4·16; N, 41·3. $C_8H_8ON_6$ requires C, 47·05; H, 3·95; N, 41·2%).

*Hydrolysis*.—The amide (1 g.) was boiled with N-sodium hydroxide (50 ml.) for 22 hr. The clear solution was filtered and acidified with acetic acid. The yellow precipitate obtained was dissolved in 2N-sodium carbonate and reprecipitated with acid. Recrystallisation from 80% formic acid yielded 3-*amino*-6-*hydroxypyrido*(2 : 3)*pyrazine*-2-*carboxylic acid* as clusters of yellow needles (Found : C, 46·6; H, 3·25; N, 27·5. $C_8H_6O_3N_4$ requires C, 46·6; H, 2·9; N, 27·2%).

*6-Amino-3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic Acid.*—2 : 6-Diamino-3-nitrosopyridine (4·14 g.) and diethyl malonate (5·3 g.) were boiled under reflux for 8 min. with a solution of sodium (1·3 g.) in 2-ethoxyethanol. The precipitate was removed, dissolved in boiling water, and recovered by hydrochloric acid. The yellow precipitate obtained was purified by several treatments with sodium carbonate or sodium hydroxide solution, followed by acidification, to yield *6-amino-3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid* as a yellow powder, m. p. >300° (Found, in material dried at 180°: C, 46·6; H, 3·1; N, 27·3. $C_8H_6O_3N_4$ requires C, 46·6; H, 2·9; N, 27·2%).

*6-Amino-3-hydroxypyrido(2 : 3)pyrazine.*—6-Amino-3-hydroxypyrido(2 : 3)pyrazine-2-carboxylic acid (0·8 g) was boiled with redistilled quinoline (10 ml.) for 1 hr., then was cooled and diluted with light petroleum (b. p. 40—60°), and the brown precipitate (0·64 g.) was removed and purified by dissolution in dilute sodium hydroxide solution (charcoal) followed by acidification with hydrochloric acid. The flocculent precipitate was dissolved by further addition of boiling water, and on cooling yielded a crystalline precipitate. Two recrystallisations from water yielded *6-amino-3-hydroxypyrido(2 : 3)pyrazine*, as very long pale yellow needles, m. p. >300° (Found, in material dried at 180°: C, 51·7; H, 3·6; N, 34·7. $C_7H_6ON_4$ requires C, 51·85; H, 3·7; N, 34·6%).

Treatment with hot acetic anhydride for 1 hr. afforded the *6-acetyl* derivative, pale yellow small needles (from *n*-butanol), m. p. >300° (Found, in material dried at 180°: C, 52·6; H, 4·2; N, 27·35. $C_9H_8O_2N_4$ requires C, 52·9; H, 3·95; N, 27·4%).

*Preparation of 3 : 6-Diamino-2-arylpyrido(2 : 3)pyrazines.*—Sodium (0·01 g.-atom) was dissolved in dry ethanol (100 ml.), and 2 : 6-diamino-3-nitrosopyridine (1·4 g.) and the appropriate arylacetonitrile (0·012 mol.) were added to the hot solution which was then refluxed for 1—4 hr. In some cases at this stage the pyrido(2 : 3)pyrazine was precipitated and the mixture was then

TABLE 3. *3 : 6-Diamino-2-arylpyrido(2 : 3)pyrazines* (I).

| R | Form * | M. p. | Formula | Found (%) § C | H | N | Required (%) C | H | N |
|---|---|---|---|---|---|---|---|---|---|
| Ph | Plates C | 245° | $C_{13}H_{11}N_5$ | 65·5 | 4·7 | 29·6 | 65·8 | 4·7 | 29·5 |
| *p*-F·$C_6H_4$ | Needles A | 284—285 | $C_{13}H_{10}N_5F$ | 61·4 | 3·9 | 27·1 | 61·2 | 3·9 | 27·4 |
| *p*-$NO_2$·$C_6H_4$ | Rods † B | 370 ‡ | $C_{13}H_{10}O_2N_6$ | 55·3 | 3·8 | 30·0 | 55·3 | 3·6 | 29·8 |
| *o*-Cl·$C_6H_4$ | Prisms B | 340 | $C_{13}H_{10}N_5Cl$ | 57·5 | 3·9 | 25·4 | 57·5 | 3·7 | 25·8 |
| *m*-Cl·$C_6H_4$ | Prisms C | 275—276 | $C_{13}H_{10}N_5Cl$ | 57·4 | 3·8 | 26·1 | 57·5 | 3·7 | 25·8 |
| *p*-Cl·$C_6H_4$ | Rods C | 274 | $C_{13}H_{10}N_5Cl$ | 57·3 | 3·4 | 25·8 | 57·5 | 3·7 | 25·8 |
| *o*-MeO·$C_6H_4$ | Rods A | 273 | $C_{14}H_{13}ON_5$ | 62·8 | 5·0 | 25·9 | 62·9 | 4·9 | 26·2 |
| *m*-MeO·$C_6H_4$ | Plates A | 205 | $C_{14}H_{13}ON_5$ | 62·9 | 4·9 | 26·1 | 62·9 | 4·9 | 26·2 |
| *p*-MeO·$C_6H_4$ | Plates A | 275 | $C_{14}H_{13}ON_5$ | 62·8 | 5·1 | 26·4 | 62·9 | 4·9 | 26·2 |
| *o*-EtO·$C_6H_4$ | Prisms AC | 290 | $C_{15}H_{15}ON_5$ | 64·0 | 5·3 | 25·2 | 64·0 | 5·4 | 24·9 |
| *m*-EtO·$C_6H_4$ | Rods C | 170 | $C_{15}H_{15}ON_5$ | 64·7 | 5·4 | 25·0 | 64·0 | 5·4 | 24·9 |
| *p*-EtO·$C_6H_4$ | Plates C | 256 | $C_{15}H_{15}ON_5$ | 63·8 | 5·3 | 24·8 | 64·0 | 5·4 | 24·9 |
| *o*-Me·$C_6H_4$ | Rods B | 332 | $C_{14}H_{13}N_5$ | 67·2 | 5·4 | 27·6 | 66·9 | 5·2 | 27·9 |
| α-$C_{10}H_7$ | Plates D | 259 | $C_{17}H_{13}N_5$ | 70·2 | 4·95 | 24·4 | 71·1 | 4·6 | 24·4 |
| β-$C_{10}H_7$ | Rods C | 256 | $C_{17}H_{13}N_5$ | 70·95 | 4·2 | 24·5 | 71·1 | 4·6 | 24·4 |
| 3 : 4-$(MeO)_2C_6H_2$ | Rods B | 264 | $C_{15}H_{15}O_2N_5$ | 61·1 | 4·8 | 23·45 | 60·6 | 5·1 | 23·6 |
| 3 : 4-$Cl_2C_6H_3$ | Rods B | 243 | $C_{13}H_9N_5Cl_2$ | 50·5 | 3·1 | 23·0 | 51·0 | 3·0 | 22·9 |
| 2-Thienyl | Rods A | 212—213 | $C_{11}H_9N_5S$ | 54·0 | 3·7 | 28·6 | 54·3 | 3·7 | 28·8 |

* Letters refer to solvents: A, water; B, *n*-butanol; C, ethanol; D, ethyl acetate and light petroleum. All products were yellow except † orange.
‡ With decomp. § After drying at 110° *in vacuo*.

cooled and filtered and the product recrystallised from the appropriate solvent. Alternatively the ethanol solution was reduced to small bulk, and the residue was diluted with water to precipitate the crude product which was then recrystallised. The *products* are reported in Table 3.

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