Experiments on the Synthesis of Pyrazine Nucleosides

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Pyrazine nucleosides were prepared by condensation of acylglycosyl halides with trimethylsilyloxypyrazines in benzene in the presence of silver perchlorate. Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1) with 2,3-bis(trimethylsilyloxy)pyrazine (2) gave 1,4-dihydro-1,4-bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)pyrazine-2,3-dione (3). Similar reactions of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (4) with the pyrazine (2) and 1-benzyl-3-trimethylsilyloxypyrazine-2(1*H*)-one (14) afforded 1,4-dihydro-1,4-bis-(β-D-ribofuranosyl)pyrazine-2,3-dione (6) and 1-benzyl-1,4-dihydro-4-(β-D-ribofuranosyl)pyrazine-2,3-dione (16), respectively, *via* their 2',3',5'-tri-*O*-benzoates [(5) and (15)]. 1-Benzyl-1,4-dihydropyrazine-2,3-dione (12) was prepared by acid-catalysed cyclization of *N*-benzyl-*N'*-(2,2-dimethoxyethyl)oxamide (9), which was obtained by condensation of ethyl *N*-(2,2-dimethoxyethyl)oxamate (7) with benzylamine.

PYRAZINE nucleosides are analogues of the naturally occurring pyrimidine nucleosides. Under certain conditions unnatural nucleosides may be incorporated into RNA which will block cell division and growth. Such nucleosides are potential anti-tumour agents since tumour cells metabolize faster than normal cells and tend to incorporate antagonistic compounds preferentially.[1] In attempts to synthesize a pyrazine analogue (17) of uridine, we have isolated the pyrazine nucleosides (6) and (16), which gave negative results when tested against Ridgeway osteogenic sarcoma and L.1210.[2]

Wagner and Frenzel [3] and also Reisser and Pfleiderer [4] showed that direct glucosidation of the silver or mercury salt of pyrazine-2(1*H*)-one furnished the *O*-glucoside and only traces of the *N*-glucoside. We have investigated the use of trimethylsilyloxy-derivatives for pyrazine nucleoside synthesis.[5] This constitutes a special application of the Hilbert–Johnson reaction in which alkoxy-derivatives of nitrogen heterocycles are employed for *N*-glycosylation.[6] While our work was in progress Bloch and his colleagues [7] reported the preparation of *N*-ribosides from the trimethylsilyloxy-derivatives of pyrazin-2(1*H*)-one and its 4-oxide.

2,3-Bis(trimethylsilyloxy)pyrazine (2) was prepared by reaction of 1,4-dihydropyrazine-2,3-dione [8] (11) (Scheme 1) with hexamethyldisilazane in the presence of ammonium sulphate. The product was obtained crystalline after vacuum distillation, but was used directly for nucleoside synthesis without characterization. The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide [9] (1) with equimolar quantities of the trimethyl-

[1] A. Block, *Ann. Reports Medicin. Chem.*, 1974, **9**, 139, Academic Press.

[2] Personal communications from the Chester Beatty Institute for Cancer Research, London and the Sloan-Kettering Cancer Research Institute, New York.

[3] G. Wagner and H. Frenzel, *Arch. Pharm.*, 1967, **300**, 421.

[4] F. Reisser and W. Pfleiderer, *Chem. Ber.*, 1966, **99**, 542.

[5] W. W. Zorbach, *Synthesis*, 1970, 329; U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**. 3668 and preceding papers.

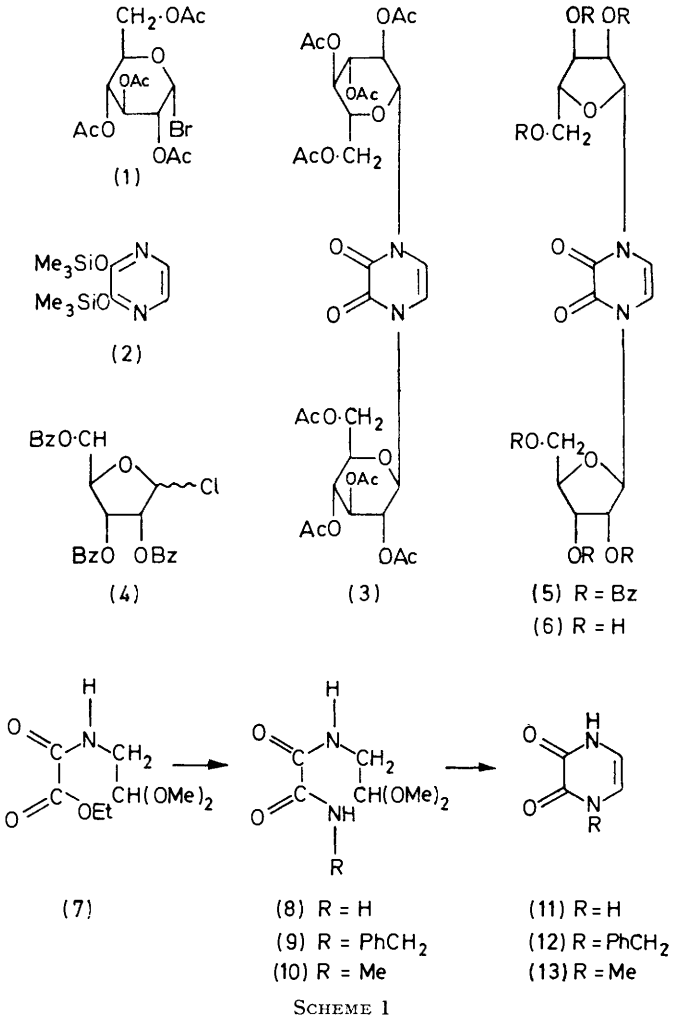
[6] J. Priml and M. Prystas, *Adv. Heterocyclic Chem.*, 1967, **8**, 115.

[7] M. Bobek and A. Bloch, *J. Medicin. Chem.*, 1972, **15**, 164; P. T. Berkowitz, T. T. Bardos, and A. Bloch, *ibid.*, 1973, **16**, 183.

[8] G. Palamidessi and L. Panizzi, Fr.Pat. 1,372,807 (*Chem. Abs.*, 1965, **62**. 1674c).

[9] A. J. Freestone, L. Hough, and A. C. Richardson. *Carbohydrate Res.*, 1973, **28**. 378.

silyloxy-derivative (2) and silver perchlorate afforded the bis(glycosyl)pyrazine (3), albeit in only 33% yield. The 100 MHz ^{1}H n.m.r. spectrum of (3) showed $J_{1',2'}$



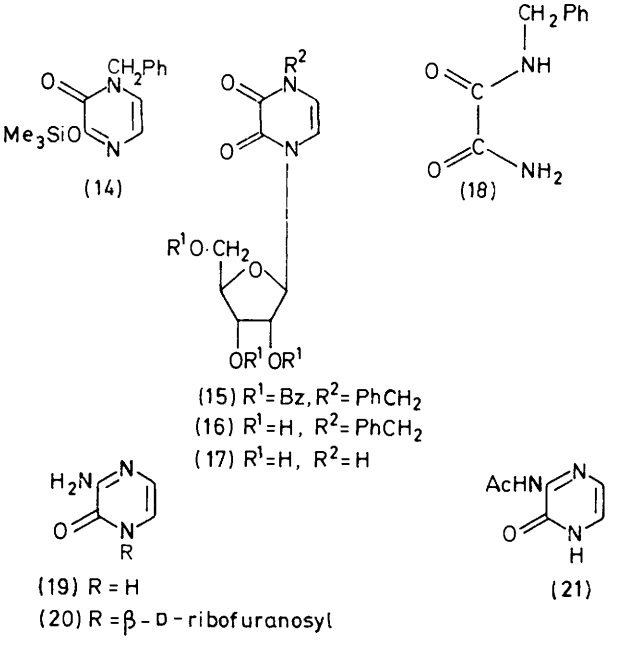
SCHEME 1

9 Hz, characteristic of β-D-glucopyranosides predominantly in the $^4C_1$ conformation in which there is a *trans*-diaxial orientation of H-1′ and H-2′.

Analogous condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[10] (4) and 2,3-bis(trimethylsilyloxy)-pyrazine (2) also gave a bis(glycosyl)pyrazine (5). The free nucleoside (6) was obtained by *O*-debenzoylation with methanolic sodium methoxide. The ^{1}H n.m.r. spectra of compounds (5) and (6) contained doublets which could be assigned to H-1′, with $J_{1',2'}$ 5 and 4 Hz, respectively. However, this does not enable the stereochemical relationship of H-1′ and H-2′ to be determined because of the greater flexibility of furanose than of pyranose rings. The anomeric configuration of compounds (5) and (6) was tentatively assigned as β in view of the Tipson–Baker *trans* rule.[11]

The products (3) and (5) differ from naturally occurring nucleosides in that two glycosyl residues are linked to the heterocycle. A monoglycosylpyrazine was obtained from 1-benzyl-1,4-dihydropyrazine-2,3-dione (12), which was prepared by application of a standard procedure[8] (Scheme 1). Dropwise addition of 2,2-dimethoxyethylamine to an equimolar quantity of ethyl oxalate afforded ethyl *N*-(2,2-dimethoxyethyl)oxamate[8] (7). Reaction of the latter (7) with benzylamine gave *N*-benzyl-*N*′-(2,2-dimethoxyethyl)oxamide (9), which was cyclized [to (12)] by refluxing with acetic acid containing hydrogen chloride. The 1-methyl analogue[12] (13) was prepared similarly (Scheme 1) by using methylamine in place of benzylamine.

The 1-benzylpyrazine (12) was converted into 1-benzyl-3-trimethylsilyloxypyrazin-2(1*H*)-one (14) by treatment with hexamethyldisilazane. The crude product (14) reacted with equimolar quantities of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[10] (4) and silver perchlorate to give the monoglycosylpyrazine (15) in 83% yield. The anomeric configuration was tentatively assigned as β according to the Tipson–Baker *trans* rule.[11] *O*-Debenzoylation of (15) with methanolic sodium methoxide afforded the free nucleoside (16). Although the 1-benzylpyrazine derivative (12) could be *N*-debenzylated with sodium in liquid ammonia, various attempts at *N*-debenzylation of the 1-benzylpyrazine riboside (16) to give the uridine analogue (17) were unsuccessful.



In view of the importance of 5-substituted pyrimidines, *e.g.* thymine and 5-fluorouracil, the preparation of analogous pyrazine derivatives would be of interest.

[10] E. F. Reckondo and H. Rinderknecht, *Helv. Chim. Acta*, 1959, **42**, 1117.

[11] R. S. Tipson, *J. Biol. Chem.*, 1939, **130**, 55; B. R. Baker, CIBA Foundation Symposium on the Chemistry and Biology of Purines, 1957, p. 120.

[12] G. W. H. Cheeseman and E. S. G. Törzs, *J. Chem. Soc.*, 1965, 6681.

However, successive treatment of the 1-benzylpyrazine (12) with aqueous bromine and alkali, under conditions that converted uridine into 5-hydroxyuridine,[13] unexpectedly afforded *N*-benzyloxamide (18).

Attempts to prepare a pyrazine analogue (20) of cytidine have not so far been successful. A trimethylsilyl derivative of 3-aminopyrazin-2(1*H*)-one [14] (19) was readily prepared but it gave a complex mixture on reaction with the acylglycosyl halide (4). *N*-Acetylation of (19) gave 3-acetamidopyrazin-2(1*H*)-one (21).

EXPERIMENTAL

Evaporations were carried out under reduced pressure. M.p.s were determined on a Reichert hot-stage or Gallenkamp apparatus. Optical rotations were determined with a Perkin-Elmer 141 automatic polarimeter (1 dm tube). T.l.c. was performed on plates coated with Merck Kieselgel G. Spots were located by spraying with ethanolic 5% sulphuric acid and heating for 1—2 min at 200 °C. I.r. spectra were recorded for Nujol mulls on a Pye Unicam SP 200 spectrophotometer. ^{1}H N.m.r. spectra were measured routinely on a Perkin-Elmer R-10 spectrometer at 60 MHz. The spectra of nucleosides, for which first-order parameters are given, were determined at 100 MHz on a Varian HA-100 spectrometer with tetramethylsilane as internal standard.

2,3-*Bis*(*trimethylsilyloxy*)*pyrazine* (2).—A suspension of 1,4-dihydropyrazine-2,3-dione [8] (11) (4.0 g) and a few crystals of ammonium sulphate in hexamethyldisilazane (25 ml) was refluxed for 24 h under anhydrous conditions. The excess of hexamethyldisilazane was evaporated at a water pump (bath temp. 100 °C) and the residual oil was distilled (oil pump) to give 2,3-*bis*(*trimethylsilyloxy*)*pyrazine* (2) (7.4 g, 80%) as an oil (b.p. 70° at 5 mmHg) which slowly deposited white needles. The product (2) was employed directly for the following reactions.

1,4-*Dihydro*-1,4-*bis*-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*pyrazine*-2,3-*dione* (3).—A solution of 2,3-bis(trimethylsilyloxy)pyrazine (2) (5.8 g) in anhydrous reagent grade benzene (80 ml) was added to 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide [9] (1) (9.35 g, 1.0 mol), and the mixture was cooled to 0 °C. A solution of silver perchlorate (4.7 g, 1.0 mol) in benzene (300 ml) was added dropwise over 1 h with stirring and exclusion of atmospheric moisture. Precipitation occurred during the addition, after which stirring was continued overnight at room temperature. The mixture was filtered; t.l.c. (ether) showed that the filtrate contained only a small quantity of fast-moving material. The residue from the filtration was extracted with dichloromethane (Soxhlet). Evaporation of the extract afforded the crude product as a syrup which crystallized (2.9 g, 33%) on trituration with ethanol. Recrystallization from ethanolic dichloromethane gave needles of the *diglycosylpyrazine* (3), m.p. 277—279° (decomp.), $[\alpha]_D^{22}$ +10° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ 1 750 and 1 660 cm^{-1}; τ (C_5D_5N; 60 °C) 3.13 (2 H, s, H-5 and H-6), 3.60 (2 H, d, $J_{1',2'}$ 9.0 Hz, H-1'), 4.16—4.31 (4 H, complex m, H-2' and H-3'?), 4.57br (2 H, t, *J* *ca.* 9 Hz, H-4'?), 5.5—5.9 (6 H, complex m, H-5' and H_2-6'), and 8.00, 8.04, 8.06, and 8.18 (24 H, s, acetyls) (Found: C, 49.2; H, 5.4; N, 3.3. $C_{32}H_{40}N_2O_{20}$ requires C, 49.8; H, 5.2; N, 3.6%).

[13] D. W. Visser, 'Synthetic Procedures in Nucleic Acid Chemistry,' ed. W. W. Zorbach and R. S. Tipson, Interscience, London, 1968, vol. 1 p. 429.

1,4-*Dihydro*-1,4-*bis*-(2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*pyrazine*-2,3-*dione* (5).—A solution of 2,3-bis(trimethylsilyloxy)pyrazine (2) (2.56 g) in anhydrous benzene (15 ml) was added to syrupy 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (4) [*ca.* 1 mol. equiv; obtained by treating 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5.04 g) with ethereal hydrogen chloride [9]]. A solution of silver perchlorate (2.07 g, 1.0 mol) in benzene (120 ml) was added under the conditions described for (3). Methanol (2 ml) and chloroform (200 ml) were added and the precipitated silver chloride was filtered off. The filtrate was washed successively with water (30 ml), saturated aqueous sodium hydrogen carbonate (50 ml), and water (20 ml), dried (Na_2SO_4), and evaporated, and the residue was triturated with acetone to give the crystalline *diribosylpyrazine* (5) (4.6 g, 46%), m.p. 262—264°, $[\alpha]_D^{25}$ −5.9° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ 1 710 cm^{-1}; τ ($CDCl_3$) 1.80—2.20 (12 H, complex m, benzoyl *ortho*-protons), 2.35—2.80 (18 H, complex m, benzoyl *meta*- and *para*-protons), 3.45 (2 H, d, $J_{1',2'}$ 5.0 Hz, H-1'), 3.60 (2 H, s, H-5 and H-6), 4.00—4.40 (4 H, complex m, H-2' and H-3'), and 5.24br (6 H, s, H-4' and H_2-5') (Found: C, 66.7; H, 4.5; N, 2.6. $C_{56}H_{44}N_2O_{16}$ requires C, 67.2; H, 4.4; N, 2.8%).

1,4-*Dihydro*-1,4-*bis*-(β-D-*ribofuranosyl*)*pyrazine*-2,3-*dione* (6).—A solution of the benzoylated nucleoside (5) (2.3 g) and sodium methoxide (1.0 g) in methanol (30 ml) was refluxed for 30 min, then adjusted to pH 6 with ion-exchange resin [Amberlite IR 200 (H^+)]. The resulting suspension was decanted from the resin and filtered. Recrystallization of the crude product from water gave needles of the free *nucleoside* (6) (0.6 g, 80%), m.p. 269—270° (decomp.), $[\alpha]_D^{25}$ +59.4° (*c* 0.50 in H_2O); $\nu_{max.}$ 3 350—3 450 and 1 680 cm^{-1}; τ [$(CD_3)_2SO$] 3.10 (2 H, s, H-5 and H-6), 4.10 (2 H, d, $J_{1',2'}$ 4.0 Hz, H-1'), 4.40—5.30 (6 H, m, 6 × OH), and 5.85—6.25 (10 H, complex m, H-2', H-3', H-4', and H_2-5') (Found: C, 44.9; H, 5.2; N, 7.2. $C_{14}H_{20}N_2O_{10}$ requires C, 44.7; H, 5.4; N, 7.4%).

1-*Benzyl*-1,4-*dihydropyrazine*-2,3-*dione* (12).—Ethyl *N*-(2,2-dimethoxyethyl)oxamate [8] (7) (10.0 g) was dissolved in ethanol (10 ml) and the solution was cooled to 0 °C. Benzylamine (5.2 g, 1.0 mol) was added, the mixture was stirred for 15 min, and the resulting white precipitate was filtered off, dried, and recrystallized from methanol to give *N*-*benzyl*-N'-(2,2-*dimethoxyethyl*)*oxamide* (9) (12.0 g, 92%), m.p. 129—130° (Found: C, 58.5; H, 7.0; N, 10.6. $C_{13}H_{18}N_2O_4$ requires C, 58.6; H, 6.8; N, 10.5%).

The *N*-benzyl amide (9) (11.8 g) was added to acetic acid (70 ml) containing concentrated hydrochloric acid (0.5 ml) at *ca.* 90 °C and the mixture was refluxed for 30 min. The solution was concentrated to *ca.* 10 ml and the material which crystallized was filtered off and washed with ether. Recrystallization from methanol afforded the *pyrazinedione* (12) (6.0 g, 67%), m.p. 226—228°; τ [$(CD_3)_2SO$] 2.80 (5 H, s, Ph), 3.50 (1 H, d, $J_{5,6}$ 6.0 Hz, H-5), 3.70 (1 H, d, $J_{5,6}$ 6.0 Hz, H-6), and 5.13 (2 H, s, PhCH_2) (Found: C, 65.3; H, 5.1; N, 13.5. $C_{11}H_{10}N_2O_2$ requires C, 65.3; H, 5.0; N, 13.9%).

1,4-*Dihydro*-1-*methylpyrazine*-2,3-*dione* [12] (13).—Ethyl *N*-(2,2-dimethoxyethyl)oxamate [8] (7) (10.0 g) was dissolved in ethanol (10 ml) and a solution of methylamine hydrochloride (5.0 g) and sodium hydroxide (3.0 g) in water (10 ml) was added. The white precipitate was filtered off, dried, and recrystallized from methanol to give N-(2,2-

[14] F. L. Muehlmann and A. R. Day, *J. Amer. Chem. Soc.*, 1956, **78**, 242.

dimethoxyethyl)-N'-*methyloxamide* (10) (4.7 g, 51%), m.p. 131—132° (Found: C, 44.3; H, 7.5; N, 14.5. $C_7H_{14}N_2O_4$ requires C, 44.2; H, 7.4; N, 14.7%).

The *N*-methyl amide (10) (9.4 g) was cyclized under the conditions described for cyclization of the *N*-benzyl amide (9) to give the 1-methylpyrazine (13) (5.1 g, 82%), m.p. 210—211° [lit.,[12] 233—234° (ethanol)], identical (i.r. spectrum) with an authentic sample.[12]

1-*Benzyl*-1,4-*dihydro*-4-(2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*pyrazine*-2,3-*dione* (15).—A suspension of 1-benzyl-1,4-dihydropyrazine-2,3-dione (12) (2.0 g) and a few crystals of ammonium sulphate in hexamethyldisilazane (10 ml) was refluxed for 4 h. The solution was evaporated to give crude 1-*benzyl*-3-*trimethylsilyloxypyrazin*-2(1H)-*one* (14). The crude solid (14) was dissolved in anhydrous benzene (30 ml) and treated with 2,3,4-tri-*O*-benzoyl-D-ribofuranosyl chloride (4) as described for the diribosylpyrazine (5). The crude product was crystallized from acetone to give white needles of the *diribosylpyrazine* (15) (5.4 g, 83%), m.p. 205°, $[\alpha]_D^{25}$ −4.4° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ 1 720 cm^{-1}; τ ($CDCl_3$) 1.90—2.20 (6 H, complex m, benzoyl *ortho*-protons), 2.40—2.90 (14 H, complex m, benzoyl *meta*- and *para*-protons and Ph), 3.55 (2 H, t, H-5 and H-6, $J_{5,6}$ *ca.* 6 Hz), 3.95 (1 H, d, $J_{1',2'}$ 6.0 Hz, H-1'), 4.05—4.35 (2 H, complex m, H-2' and H-3'), and 5.00—5.40 (5 H, complex m, PhCH_2, H-4' and H_2-5') (Found: C, 68.7; H, 4.6; N, 4.2. $C_{37}H_{30}N_2O_9$ requires C, 68.7; H, 4.7; N, 4.3%).

1-*Benzyl*-1,4-*dihydro*-4-(β-D-*ribofuranosyl*)*pyrazine*-2,3-*dione* (16).—A solution of the benzoylated nucleoside (15) (2.5 g) was *O*-debenzoylated with sodium methoxide (0.5 g) in methanol (50 ml) as described for the diribosylpyrazine (6). After deionization, the solution was filtered and evaporated to a gum which was triturated with ether to remove methyl benzoate. The crude product was crystallized from water to give needles of the free *nucleoside* (16) (1.2 g, 88%), m.p. 141—142°, $[\alpha]_D^{25}$ +10.8° (*c* 1.0 in MeOH); τ [$(CD_3)_2SO$] 2.70 (5 H, s, Ph), 3.12 (1 H, d, $J_{5,6}$ 6.0 Hz, H-5?), 3.36 (1 H, d, $J_{5,6}$ 6.0 Hz, H-6?), 4.10 (1 H, d, $J_{1',2'}$ 4.0 Hz, H-1'), 4.4—5.5 (3 H, m, 3 × OH), 5.10 (2 H, s, PhCH_2), and 5.95—6.50 (5 H, complex m, H-2', H-3', H-4', and H_2-5') (Found: C, 56.1; H, 5.4; N, 8.0. $C_{16}H_{18}N_2O_6$ requires C, 57.6; H, 5.4; N, 8.4%).

N-*Debenzylation of* 1-*Benzyl*-1,4-*dihydropyrazine*-2,3-*dione* (12).—The 1-benzylpyrazine (12) (0.6 g) was suspended in liquid ammonia (40 ml) and small pieces of sodium were added until the solution retained a blue colour. The colour was discharged by addition of ammonium chloride and the ammonia was allowed to boil off at room temperature. The residue was dissolved in water (10 ml) and the solution was neutralized with dilute hydrochloric acid. Storage of the solution at 0 °C afforded crystalline 1,4-dihydropyrazine-2,3-dione (11), which was filtered off, washed with a few ml of ethanol and dried (0.2 g, 60%); m.p. >300° (lit.,[8] 370—380°), identical (i.r. spectrum) with an authentic sample.[8]

Conversion of 1-*Benzyl*-1,4-*dihydropyrazine*-2,3-*dione* (12) *into* N-*Benzyloxamide* (18).—An aqueous suspension (10 ml) of the 1-benzylpyrazine (12) (1.0 g) was warmed to 90 °C and treated dropwise with bromine until a pale yellow solution was obtained. The solution was partially evaporated to remove bromine, was made slightly alkaline (pH 8) with aqueous sodium hydroxide, and was heated for 10 min on a water-bath. The solution was cooled and filtered to give the product (0.55 g, 62%). Recrystallization from acetone afforded pure *N*-benzyloxamide, m.p. 222—223° (lit.,[15] 223°); $\nu_{max.}$ 3 400, 3 350, 3 200, and 1 660 cm^{-1}; τ [$(CD_3)_2SO$] 0.60—0.95br (1 H, t, NH, *J* *ca.* 6 Hz), 1.80—2.00br (1 H, s, N'H_a), 2.05—2.30br (1 H, s, N'H_b), 2.70 (5 H, s, Ph), and 5.60 (2 H, d, *J* 6.0, PhCH_2); after addition of a few drops of D_2O, τ [$(CD_3)_2SO$] 2.65 (5 H, s, Ph), 5.60 (2 H, s, PhCH_2), and 6.30 (s, HOD) (Found: C, 60.6; H, 5.8; N, 15.9. Calc. for $C_9H_{10}N_2O_2$: C, 60.6; H, 5.7; N, 15.7%).

3-*Acetamidopyrazin*-2(1H)-*one* (21).—A solution of 3-aminopyrazin-2(1*H*)-one [14] (19) (9.0 g) in acetic anhydride (25 ml) was refluxed for 3 h, then evaporated, and the residue was dissolved in boiling ethanol. After decolourization (charcoal) the solution was filtered and cooled to give 3-*acetamidopyrazin*-2(1H)-*one* (21) (9.8 g, 89%), m.p. 215—217° (Found: C, 46.7; H, 4.7; N, 27.6. $C_6H_7N_3O_2$ requires C, 47.1; H, 4.6; N, 27.4%).

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[15] J. Thiele, *Annalen*, 1910, **376**, 239.