THE REACTION OF PENTA-*O*-NICOTINOYL-α-D-GLUCOPYRANOSE WITH AQUEOUS AMMONIA

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ABSTRACT

Penta-*O*-nicotinoyl-α-D-glucopyranose reacts with aqueous ammonia to yield *N*-nicotinoyl-D-glucofuranosylamine, 1-deoxy-1,1-bis(nicotinamido)-D-glucitol, and 2-(D-*arabino*-tetrahydroxybutyl)-6-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine. The structure of these compounds was ascertained by use of oxidation techniques and spectroscopic data. The formation of the pyrazine compound is discussed.

INTRODUCTION

The reaction of penta-*O*-nicotinoyl-α-D-glucopyranose[1] (**1**) with aqueous ammonia afforded *N*-nicotinoyl-D-glucofuranosylamine (**2**; 16%), 1-deoxy-1,1-bis(nicotinamido)-D-glucitol (**3**; 3.8%), and 2-(D-*arabino*-tetrahydroxybutyl)-6-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine (**4**; 7.6%).

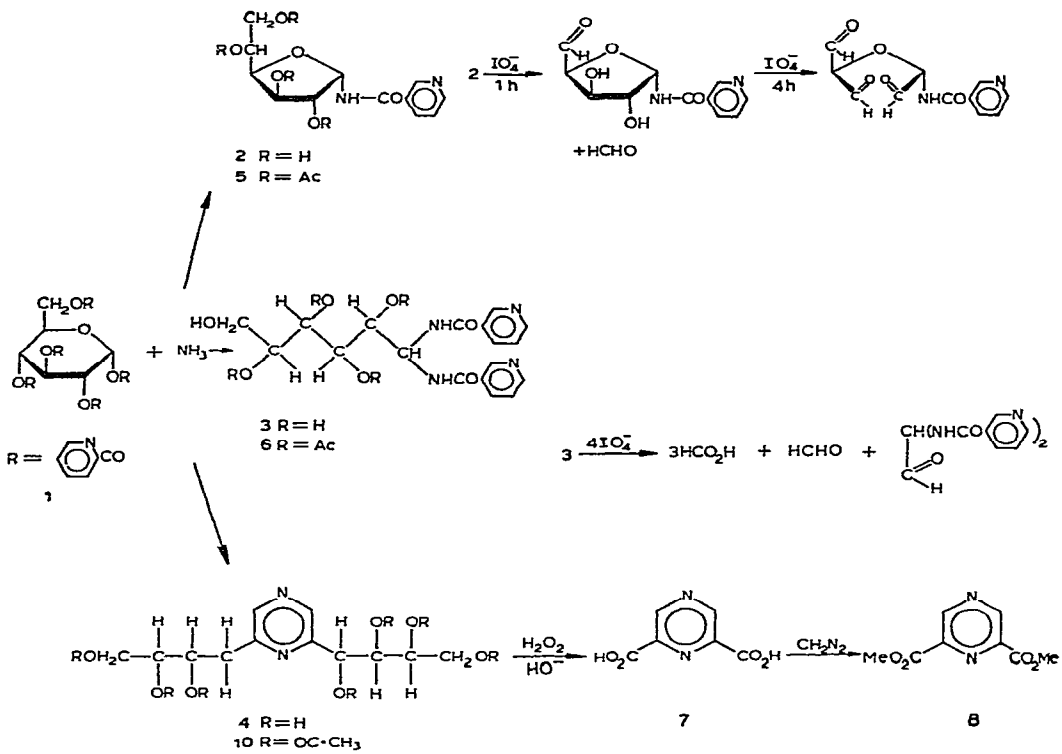
The furanose structure of **2** was demonstrated by periodate oxidation (see Scheme I). The C-5–C-6 bond was the first to be oxidized, as the uptake of the first mole of periodate per mole was simultaneous with the formation of one mole of formaldehyde. After four hours, a second mole of periodate was consumed, without appearance of significant amounts of formic acid, indicating oxidation of the C-2–C-3 bond in a furanose ring. Overoxidation began after 19 hours. Compound **2** gave a crystalline acetate (**5**).

Although we have not yet experimentally determined the anomeric configuration of **2**, known precedents in an ammonolysis reaction are the formation of *N*-acetyl-D-*gluco*- and *N*-acetyl-D-*xylo*-furanosylamines, both having the α-D configuration. As previously discussed[2], the assumption of this configuration by sugars possessing the D-*xylo* configuration is probably ascribable to a more balanced interaction of bulky groups, especially considering that an *alpha* substituent on C-1 is in *trans* relationship to the bulky group on C-4. In compound **2**, it would be reasonable to

presume that the *alpha* anomeric configuration would be energetically favored, as this would take cognizance of the bulky nicotinamido group.

Compound **3** was oxidized with periodate, with an uptake of four moles of oxidant per mole and production of three moles of formic acid and one mole of formaldehyde. These data indicated an open chain structure for **3**. A crystalline acetate (**6**) was obtained from this compound.

The structure of compound **4** was established on the basis of the molecular formulas calculated from the elemental analysis of **4** and its acetate (**10**), and from the molecular weight of the latter; these data suggested that **4** results from the condensation of two monosaccharide units. The u.v. spectrum of **4** showed λ_{max} at 274 nm, corresponding to a known transition in pyrazines[3] and coincident with the absorption shown by "deoxy-D-fructosazine"* and "D-fructosazine" (Ref. 5).



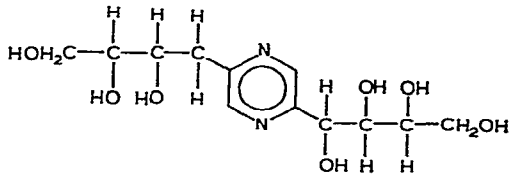
Scheme I

To ascertain the position of the substituents on the pyrazine ring, compound **4** was oxidized with hydrogen peroxide in alkaline medium, and the previously described 2,6-pyrazinedicarboxylic acid[6] (**7**) was obtained; it had the same u.v. absorption as **4**, and reacted with ferrous ion to give the purple color characteristic of pyrazinedicarboxylic acids[7]. Methylation of **7** with diazomethane in ether gave

*Synthesized according to Kuhn *et al.*[4].

2,6-bis(methoxycarbonyl)pyrazine (**8**), whose melting point was higher than that recorded in the literature[6]. However, the substitution is undoubtedly at C-2 and C-6, as the 2,3- and 2,5-bis(methoxycarbonyl) pyrazines have melting points far removed from that of **8**.

The n.m.r. spectrum of **4** agrees almost exactly with that of its isomer, "deoxy-D-fructosazine" (**9**), whose structure was proved by Kuhn and co-workers[4].



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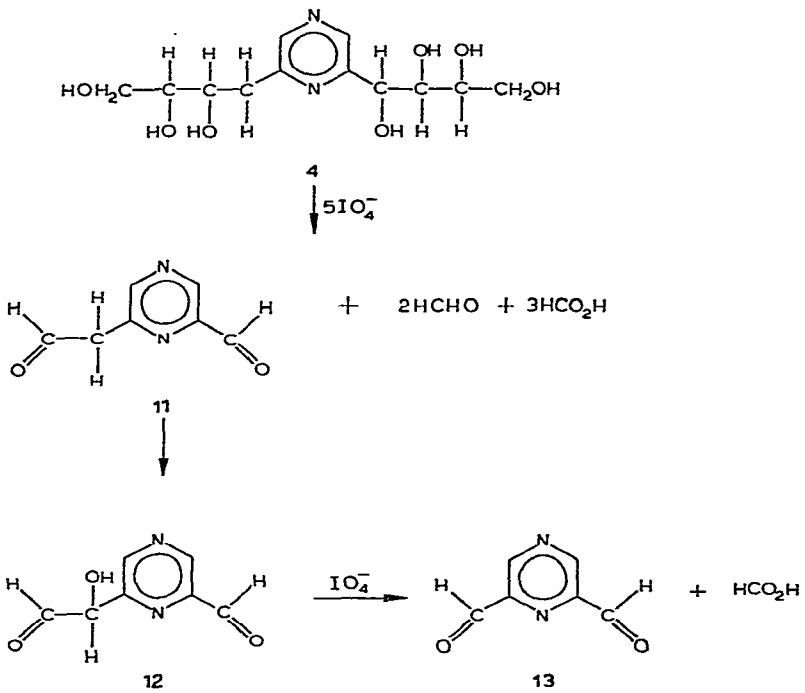
Acetylation of **4** afforded a heptaacetate (**10**), whose molecular weight (Rast method) was 620 ±40, supporting the molecular formula proposed. Its n.m.r. spectrum gave values for the protons of the side-chain agreeing with those published for the corresponding protons of the acetates of "D-fructosazine" (Ref. 5) and 2-(tetrahydroxybutyl)quinoxaline[8], the only exception being a doublet for the methylene protons on C-1″ (τ 6.8) linked to the aromatic ring.

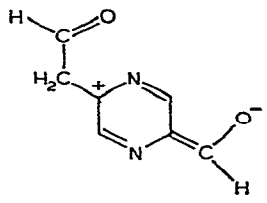
To confirm the results obtained from the n.m.r. spectra with regard to the location of hydroxyl and methylene groups, compound **4** was oxidized with periodate.



Scheme II

The uptake of 5 moles of periodate per mole, with production of three moles of formic acid and two moles of formaldehyde, agrees with the presence of two primary alcohol groups, and two secondary hydroxyl groups in one side-chain and three secondary in the other. The uptake, afterwards, of two more moles of periodate and the production of one mole of formaldehyde can be explained by the hydroxylation of the activated methylene group in **11** to give the hydroxyaldehyde **12**, whose subsequent rupture would lead to the dialdehyde **13** (see Scheme II). Previous studies[9] on model compounds support this proposed pattern of overoxidation.

Comparable oxidation of "deoxy-D-fructosazine" (**9**) gave the same total values for the periodate uptake (7 moles per mole), formaldehyde (2 moles), and formic acid (4 moles), but the time required was one fourth. The increased rate of oxidation may be attributable to an enhanced reactivity of the methylene group in the hydroxylation stage, ascribable to the contributing structure **14**, which is not possible for compound **11**.



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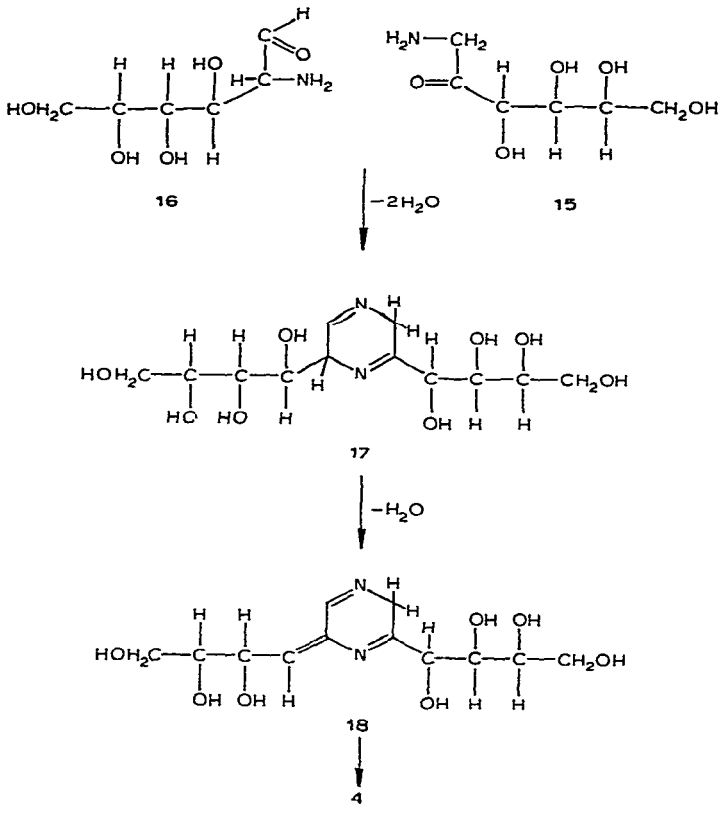
The formation of compound **4** in this reaction can be rationalized on the basis that free D-glucose, obtained by removal of all nicotinoyl groups by ammonolysis, would be in equilibrium with D-fructose by a Lobry de Bruyn–Alberda van Ekenstein rearrangement. These sugars, in the ammoniacal medium, would give D-glucosylamine and D-fructosylamine, respectively. The former compound, by an Amadori rearrangement, would give 1-amino-1-deoxy-D-fructose (**15**), whereas D-fructosylamine, by a Heyns rearrangement, would be transformed into 2-amino-2-deoxy-D-glucose (**16**). The condensation of **15** with **16** would lead to **4**, but, in the first stage, a dihydropyrazine (**17**), followed by its dehydration product (**18**), would be produced (see Scheme III).

This interpretation is based on the mechanism proposed by Jezo and Luzak[10] to explain the formation of 2,6-dimethylpyrazine by ammonolysis of sucrose. The formation of D-glucosylamine in the ammonolysis of acylated esters of D-glucose had already been reported by Deulofeu and Deferrari[11] as an intermediate for the di-D-glucosylamine produced in the ammonolysis of penta-*O*-acetyl-β-D-glucopyranose.

The low yield of compound **3** (3.8%) obtained in this reaction, compared with that of 1,1-bis(benzamido)-1-deoxy-D-glucitol (19.5%) obtained by ammonolysis of penta-*O*-benzoyl-D-glucopyranose[11], indicated a definite influence of the structure of the nicotinoyl groups on the course of the reaction. Since it implies an acyl–oxygen rupture, any substituent that diminishes the electronic density at the



Scheme III

carbonyl carbon atom will enhance the rate of ammonolysis. This effect is produced by the nitrogen atom of the nicotinoyl residues. Falkner and Harrison[12] found that the hydrolysis of ethyl nicotinate is forty times faster than that of ethyl benzoate.

The higher rate of ammonolysis of nicotinoyl groups would explain the low tendency to form a bis(acylamido) compound by a migration reaction, allowing operation of the influence of other structural factors, previously discussed for monosaccharide acetates[13], that favor formation of *N*-acylglycosylamines. This is noteworthy, because the ammonolysis of sugar benzoates has always been conducted in alcoholic media, whereas that of nicotinates was carried out in aqueous ammonia, in which the yields of bis(acylamido) derivatives are higher[14].

EXPERIMENTAL

General procedures. — Melting points are not corrected. Paper chromatography was conducted on Whatman No. 1 paper with the following eluants, also used in cellulose-column chromatography (conducted on S. & S. cellulose No. 123): (*A*) 5:1:4 (v/v, top layer) butyl alcohol–ethanol–water; (*B*) 10:1:2 (v/v) butyl alcohol–ethanol–water. The spray reagent was silver nitrate–sodium methoxide[15]. The

fractions from the chromatographic columns were separately evaporated under diminished pressure below 60°, and suitably combined after comparison by paper chromatography. The periodate oxidations (see Table I) were conducted at 20°, unless otherwise stated, by the following general technique: to 7 ml of 100 mM sodium metaperiodate was added a solution of the sugar (15–20 mg) in water (3 ml). The solution was made to 25 ml with water, and kept at 20° during the oxidation. The uptake of sodium metaperiodate was measured[16] at 222.5 nm with a Beckman DU spectrophotometer, and the formaldehyde[17] at 570 nm; formic acid was titrated with 10 mM sodium hydroxide. N.m.r. spectra were recorded at 20–25° at 60 MHz with a Varian A-60 spectrometer; tetramethylsylane (τ 10.00) was used as the standard. The following abbreviations are used: d, doublet; m, multiplet; s, singlet.

TABLE I

OXIDATIONS WITH SODIUM METAPERIODATE

Time (*h*)	$NaIO_4$ (*moles*)	*Formic acid* (*moles*)	*Formaldehyde* (*moles*)
***N*-Nicotinoyl-D-glucofuranosylamine (2)**			
0.25	0.9		1.0
1	1.0		1.0
2	1.2	0.06	1.0
5	1.9	0.30	1.0
19	3.4	1.2	1.0
29	4.0	1.8	1.0
50	5.0	3.0	1.0
1-Deoxy-1,1-bis(nicotinamido)-D-glucitol (3)			
0.5	3.8	2.9	1.0
1	4.0	2.9	1.0
2	4.0	2.9	1.0
2-(D-*arabino*-Tetrahydroxybutyl)-6-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine (4)			
0.5[a]	4.9	2.7	2.0
1[a]	4.9	2.7	2.0
2[a]	5.2	2.8	2.0
5[a]	5.2	2.8	2.0
9	6.0	3.1	2.0
26	6.5	3.2	2.0
57	7.0	3.9	2.0
77	7.0	3.9	2.0
2-(D-*arabino*-Tetrahydroxybutyl)-5-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine (9)			
1[a]	5.9	3.3	2.0
2[a]	6.3	3.4	2.0
3.5	6.7	3.8	2.0
5	6.8	3.8	2.0
19	7.0	4.0	2.0

[a]Conducted at 0°.

N-*Nicotinoyl*-D-*glucofuranosylamine* (**2**). — Penta-*O*-nicotinoyl-α-D-glucopyranose[1] (m.p. 187–189°; 82 g)was suspended in 25% aqueous ammonia (2 liters) and dissolved by shaking for 6 h. After 24 h at room temperature, the solution was evaporated to dryness. The syrup obtained was dried in a vacuum desiccator, and then extracted with cold (2 × 150 ml) and hot (8 × 250 ml) ethyl acetate, to remove nicotinamide. The residual syrup (57 g) was dissolved in methanol (300 ml), and compound **2** (5.3 g, 16%) crystallized out. After recrystallization from 8:1 methanol–water, it gave rectangular plates, m.p. 176–177°, $[\alpha]_D^{25}$ +38° (*c* 0.5, water). N.m.r. spectrum: H-1 (d) at τ 3.93, $J_{1,2}$ 3.5 Hz. The rest of the protons appeared as three multiplets, not amenable of analysis.

Anal. Calc. for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.85. Found: C, 50.30; H, 5.40; N, 9.60.

1-Deoxy-1,1-bis(*nicotinamido*)-D-*glucitol* (**3**). — The mother liquors from **2** were combined and evaporated, and the resulting syrup (47 g) was chromatographed on a column (4.5 × 90 cm) of cellulose; elution was performed with solvent *A*, and 30 fractions (100 ml each) were collected. From fractions 7–12, nicotinamide (1.2 g, m.p. and mixed m.p. 130–132°) crystallized. Fractions 13–22 gave nicotinic acid (8.3 g, m.p. and mixed m.p. 230°).

The mother liquors from the nicotinic acid were combined and evaporated to dryness, and the syrup obtained (23 g) gave, on paper chromatography with solvent *B*, two spots (R_F 0.25 and 0.44), and nicotinamide. The syrup was rechromatographed on a column (4.5 × 90 cm) of cellulose; elution was performed with solvent *B*, and 36 fractions (100 ml each) were collected. Fractions 22–25 were evaporated, and, by treatment with ethanol, gave compound **3**, m.p. 185–187° (1.8 g, 3.8%), that, after recrystallization from ethanol, afforded needles, m.p. 188–189°, $[\alpha]_D^{25}$ −3° (*c* 0.6, water); R_F in solvent *B*, 0.25.

Anal. Calc. for $C_{18}H_{22}N_4O_7$: C, 53.19; H, 5.46; N, 13.79. Found: C, 53.06; H, 5.25; N, 14.16.

2-(D-arabino-*Tetrahydroxybutyl*)-*6*-(D-erythro-*2,3,4-trihydroxybutyl*)*pyrazine* (**4**). — Fractions 23–27 from the first chromatography on a column of cellulose (solvent *A*) afforded material having m.p. 155–160° (1.85 g), that, by paper chromatography (solvent *A*), consisted of two compounds, R_F 0.14 and 0.08. This mixture was rechromatographed on a column (2.6 × 59 cm) of cellulose, by using solvent *A*, and 60 fractions (25 ml each) were collected. Fractions 36–42 gave, from ethanol, compound **4** as needles (1.26 g, 7.6%), m.p. 166–167°, $[\alpha]_D^{25}$ −110° (*c* 1.0, water); R_F 0.14 in solvent *A*.

Anal. Calc. for $C_{12}H_{20}N_2O_7$: C, 47.36; H, 6.63; N, 9.21. Found: C, 47.51; H, 6.50; N, 9.20.

Acetates of compounds **2**, **3**, *and* **4**. — The following general procedure was employed for acetylation: the compound (200 mg) was suspended in 1:1 (v/v) acetic anhydride–pyridine (2 ml) and the mixture was heated until dissolution occurred (20 min). After 24 h at room temperature, the solution was evaporated in a vacuum

desiccator, and the residue was recrystallized from water. The following properties were observed for the respective acetates.

2,3,5,6-Tetra-*O*-acetyl-*N*-nicotinoyl-D-glucofuranosylamine (**5**), m.p. 129–130°, $[\alpha]_D^{25}$ +39.0° (*c* 0.7, chloroform).

Anal. Calc. for $C_{20}H_{28}N_2O_{10}$: C, 53.09; H, 5.35; N, 6.19. Found: C, 52.88; H, 5.17; N, 6.38.

2,3,4,5,6-Penta-*O*-acetyl-1-deoxy-1,1-bis(nicotinamido)-D-glucitol (**6**), m.p. 164–165°, $[\alpha]_D^{25}$ −20.0° (*c* 0.9, chloroform).

Anal. Calc. for: $C_{28}H_{32}N_4O_{12}$: C, 54.54; H, 5.23; N, 9.09. Found: C, 54.54; H, 5.50; N, 9.30.

2-(D-*arabino*-Tetraacetoxybutyl)-6-(D-erythro-2,3,4-triacetoxybutyl)pyrazine (**10**), m.p. 141–142°, $[\alpha]_D^{25}$ −8.0° (*c* 0.73, chloroform). N.m.r. spectrum ($CDCl_3$): H-3 and H-5 at τ 1.58 (s); H-1′ at τ 3.83 (d); C-4′ and C-4″ methylenes at τ 5.67 (m); C-1″ methylene at τ 6.80 (d); acetate methyl groups (six singlets) between τ 7.75 and 8.04.

Anal. Calc. for $C_{26}H_{34}N_2O_{14}$: C, 52.17; H, 5.73; N, 4.68. Found: C, 52.48; H, 5.62; N, 4.58.

Structure of compound **4**. — The u.v. spectrum of compound **4** showed λ_{max} 274 nm (ε 8,900); lit. λ_{max} 274 nm for "D-fructosazine" (Ref. 5) and "D-tagatosazine" (Ref. 18). N.m.r. spectrum (D_2O): H-3, pyrazine ring, at τ 1.33 (s); H-5, pyrazine ring, at τ 1.48 (s); H-1′ at τ 4.80 (s); H-2′, H-2″, H-3′, H-3″, and C-4′, C-4″ methylenes (eight protons) at τ 6.18 (m); C-1″ methylene at τ 6.89 (m). For periodate oxidation of **4**, see Table I.

For comparison with compound **4**, "deoxy-D-fructosazine" (**9**) was synthesized according to Kuhn *et al.*[4]. This compound showed the following properties: u.v. spectrum: λ_{max} 276 nm (ε 8,800); n.m.r. spectrum (D_2O): H-3, pyrazine ring, at τ 1.20 (s); H-6, pyrazine ring, at τ 1.38 (s); H-1′ at τ 4.78 (s); H-2′, H-2″, H-3′, H-3″, C-4′ and C-4″ methylenes (eight protons) at τ 6.15 (m); C-1″ methylene at τ 6.90 (m). For periodate oxidation of **9**, see Table I.

2,6-Pyrazinedicarboxylic acid (**7**). — Sodium hydroxide (2 g) was added to a solution of compound **4** (1.2 g) in 6% hydrogen peroxide (50 ml). After 45 min at room temperature, the solution was slowly heated to 80° in a water bath, and 100% hydrogen peroxide (3 ml) was added. The solution was kept at 80° until it gave a negative reaction with Fehling solution (1 h), and, after being cooled to room temperature, the solution was acidified to pH 1 with hydrochloric acid. By cooling and keeping at 0°, compound **7** (208 mg, 31%) was obtained, m.p. 224–225°; lit.[6] m.p. 224–225°; λ_{max} 274 nm (ε 9,200). On reaction with ferrous ion, a red-violet color developed, characteristic of pyrazinecarboxylic acids[7].

Anal. Calc. for $C_6H_4N_2O_4$: C, 42.86; H, 2.40; N, 16.67. Found: C, 42.66; H, 2.43; N, 16.75.

2,6-Bis(methoxycarbonyl)pyrazine (**8**). — To compound **7** (31 mg) in ethyl ether (5 ml) at 0° was added a solution of diazomethane (180 mg) in ether (8 ml). After 24 h at 0°, the solution was evaporated, and the residue was sublimed at

90°/0.01 torr. Compound **8** (29.6 mg, 91%) was obtained; after recrystallization from water, it had m.p. 126–127°; lit.[6] m.p. 119–120°. 2,3-Bis(methoxycarbonyl)pyrazine[19] has m.p. 56°, and 2,5-bis(methoxycarbonyl)pyrazine[20] has m.p. 169–170°.

Anal. Calc. for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.83; H, 4.00; N, 14.45.

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