83090-22-6; **34**·HCl, 85151-43-5; **35**, 83090-18-0; **35**·HCl, 85151-44-6; **36**, 83090-15-7; **36**·HCl, 85151-45-7; **37**, 83090-16-8; **37**·HCl, 85151-46-8; **38**, 85151-47-9; **38**·HCl, 85151-48-0; **39**, 85151-49-1; **39**·HCl, 85151-50-4; **40**, 85151-51-5; **40**·HCl, 85151-52-6; **41**, 85151-53-7; **41**·HCl, 85151-54-8; **42**, 85151-55-9; **43**, 85151-56-0; **43**·HCl, 85220-62-8; **44**, 84243-26-5; **44**·HCl, 84269-54-5; **45**, 85151-57-1; **45**·HCl, 84243-27-6; **46**, 85151-58-2; **46**·HCl, 84243-29-8; **47**, 85151-59-3; **47**·HCl, 84243-31-2; **48**, 85151-60-6; **48**·HCl, 84243-30-1; **49**, 85151-61-7; **49**·HCl, 84243-28-7; **20**, 85151-62-8; **21**, 85151-63-9; **51**·HCl, 85151-64-0; **52**, 85151-65-1; **52**·HCl, 85151-66-2; **53**, 85151-67-3; **53**·HCl, 84243-36-7; **54**, 84243-32-3; **54**·HCl, 84243-33-4; **55**, 85151-68-4; **55**·HCl, 84243-34-5; **56**, 85151-69-5; **56**·HCl, 84243-35-6; **57**, 85151-70-8; **58**, 85151-71-9; **58**·HCl, 85151-72-0; **59**, 85151-73-1; **59**·HCl, 85151-74-2; **60**, 85151-75-3; **60**·HCl, 85151-76-4; **61**, 85151-77-5; **61**·HCl, 85151-78-6; **62**, 85151-79-7; **62**·HCl, 85151-80-0; **63**, 85151-81-1; **63**·HCl, 85151-82-2; **64**, 85151-83-3; **64**·HCl, 85151-84-4; **65**, 85151-85-5; **65**·HCl, 84033-74-9; **66**, 84033-72-7; **66**·HCl, 84033-73-8; 2-indolylethyl bromide, 3389-21-7; aniline, 62-53-3; benzoyl chloride, 98-88-4; isonipecotamide, 1453-82-3.

Oxidation of Uric Acid. 4. Synthesis, Structure, and Diabetogenic Action of 5-Imino-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione Salts and Their Alloxan-Like Covalent Adducts[1]

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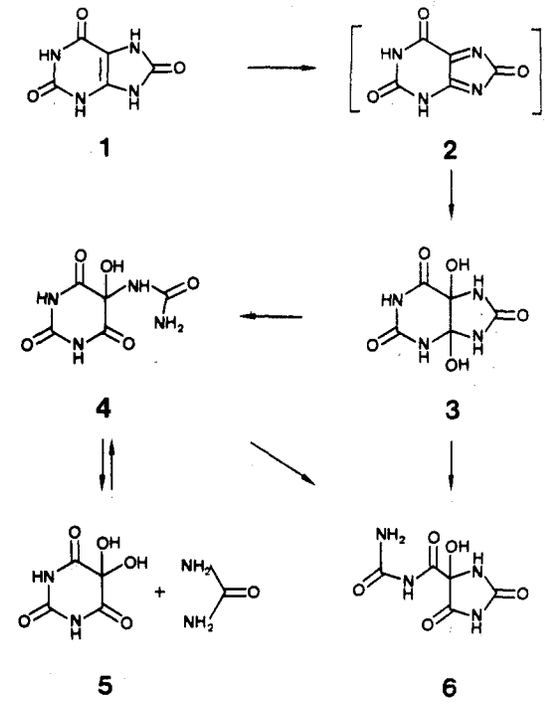
Three synthetic routes to salts of 5-amino-5-hydroxy-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**10**) are described. The key reactions involved acid-catalyzed cleavage of 5-amino-5-ureido-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**7**), conversion of uramil (**8**) to dehydrouramil (**9**) and subsequent hydration, and the condensation of alloxan (**5**) with ammonium salts. The carbinol ammonium salt structure **10a** was unambiguously established by X-ray crystallography. New alloxan-like compounds **7**, **9**, and **10** were evaluated for diabetogenic activity in rats. Compound **7** was inactive, whereas compounds **9** and **10** showed the highest activity comparable to that of streptozotocin (**12**).

The discovery of alloxan diabetes has led to early suggestions that a substance biogenetically related to uric acid (**1**) may have an alloxan-like action on the β cells of islets and, thus, produce diabetes.[2] The question of whether dehydrouric acid (**2**) can be a transient intermediate in uricolysis has aroused recent interest.[3] Mechanistic, as well as practical, considerations make the still unavailable quinonoid system **2** an interesting synthetic target. Although considerable work on the oxidative breakdown of **1** has been described previously by Biltz and his school,[4] the problem of the constitution of intermediates has presented paradoxes that caused much confusion in this field, and little is known about the chemical and biological properties of these intermediates. Our own interest in the oxidation of uric acid (**1**) originated with the intent to explore the biological effects of its alloxan-like derivatives. The structural elucidations of tetrahedral adduct **3** and derivatives **4** and **6**, resulting from regiospecific cleavages of ortho acid aminal array,[5,6] have helped to unravel the chemistry and biological effects of alloxan-like derivatives of **1**. We have examined compounds **3** and **4** to test the concepts of Brückmann and Wertheimer[7] and to determine whether there might be other structural features that correlate with diabetogenic activity. The highly specific diabetogenic action of these compounds has challenged accepted structure–activity relationships.[1] The possible relationship between the quinonoid structure **2**, or adducts derived therefrom, and biological response gave an impetus to further explore the possibility of finding new diabetogenic compounds related to uric acid (**1**).

The essence of our plan germinated from a claim that ammonium salts exerted an apparently specific potentiating action on the diabetogenic effect of alloxan.[7] It appeared therefore, that it would be interesting to study

Scheme I. Oxidative Degradation of Uric Acid (**1**)



alloxan-like systems that incorporate an amino function. To test these ideas, we required a straightforward synthesis

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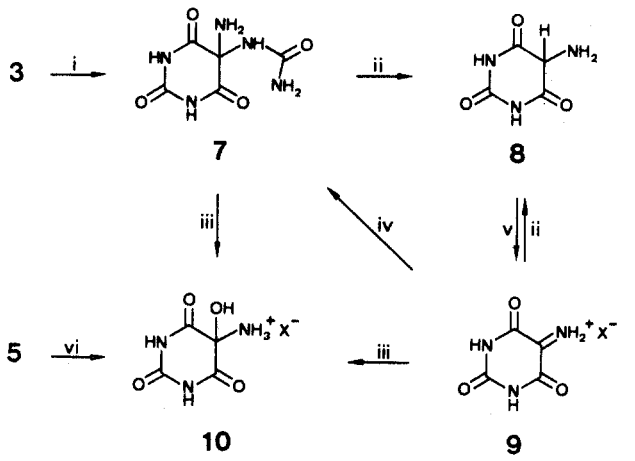
(1) Part 3 of this series: Poje, M.; Ročić, B. *Experientia* **1980**, *36*, 78.
(2) (a) Lazarow, A. *Physiol. Rev.* **1949**, *29*, 48. (b) Griffiths, M. *J. Biol. Chem.* **1950**, *184*, 289, claimed that the administration of **1** in glutathione-depleted rabbits caused diabetes. (c) Reports have been published that a number of enzymes are capable of catalyzing the oxidation of **1** to **5**: Soberon, G.; Cohen, P. P. *Arch. Biochem. Biophys.* **1963**, *103*, 331, and references cited therein.

0022-2623/83/1826-0861$01.50/0

Scheme II[a,b]



[a] a, X = Cl; b, X = Br. [b] i, NH_3/EtOH; ii, HI; iii, HX/H_2O; iv, NH_2CONH_2/$(Me_2N)_2CO$; v, X_2; vi, NH_4X/H_2O.

of the structural type **10**. The reinvestigation of the chemistry of 5-aminopseudouric acid[8,9] and/or the oxidation of uramil (**8**) to dehydrouramil (**9**) promised access to intriguing carbinol ammonium salts **10**. This article presents a complete account of our studies that have implemented this scheme, as well as our results on the evaluation of new compounds of established structures for diabetogenic properties.

In accordance with Biltz's observations,[8] 5-aminopseudouric acid (**7**), mp 146–147 °C dec, is formed best by the action of ethanolic ammonia on dihydroxyuric acid **3**, followed by the decomposition of the intermediate ammonium salt of **7** with dilute acetic acid. The infrared spectrum showed absorptions characteristic of a primary amine, as well as those of a monosubstituted urea. ^1H and ^{13}C NMR spectral patterns (see Experimental Section) were also consistent with the monocyclic structure of 5-amino-5-ureido-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**7**). Urea is split off during the reduction to **8**, as well as by the treatment with hydrochloric acid, which afforded a product $C_4H_2O_4 \cdot NH_4Cl \cdot H_2O$, mp 220–221 °C dec; its formulation, as a molecular complex, seems to have been generally accepted, e.g., in Beilstein's Handbuch. Nevertheless, one may seriously consider the carbinol ammonium chloride structure **10a**, since an identical product can be obtained from alloxan (**5**) and ammonium chloride. The reaction is reminiscent of the formation of **4** from **5** and urea, where a similar controversy has been encountered.[5] Both **4** and **10a** crystallize as monohydrates prone to disproportionation in aqueous solutions but with the added feature of alloxan-ring stability. Attempts to form derivatives led in either case to derivatives of their disproportionation products. Spectral data were not sufficient to support the formulation **10a** as long as no literature analogues were available for a direct comparison. A rigorous proof of structure was therefore undertaken, as shown in Scheme II. Oxidation of **8** with dry chlorine afforded dehydrouramil hydrochloride (**9a**). The analogous reaction with bromine was initially studied by Mulder, who assigned an obviously incorrect bromoamine structure to the product.[10] We found, however, that the action of bromine yields iminium hydrobromide **9b**. It is of special interest that we were able to bring forward a classical chemical demonstration of the iminium salt structures. The addition of urea gave **7**, whereas the reduction with hydriodic acid afforded **8**. The addition of a molecule of water to **9a** led to a crystalline product, which was identical in every respect with the monohydrate of **10a**. The hydration of **9b** gave the corresponding bromide **10b** as the monohydrate. An identical carbinol ammonium bromide was obtained by treatment of **7** with dilute hydrobromic acid and by a reaction of **5** with ammonium hydrobromide.

In order to remove any equivocation about the structural assignment, we undertook a single-crystal X-ray analysis

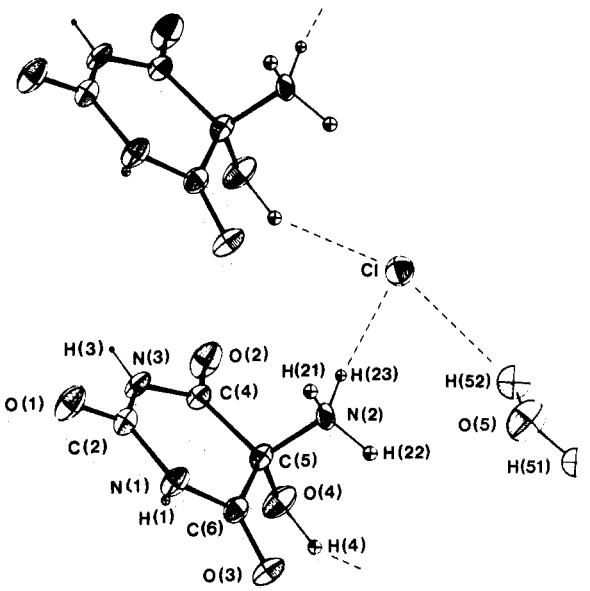


Figure 1. ORTEP drawing of the hydrate **10a**.

Table I. Diabetogenic Activity of Alloxan-Like Compounds in Rats

substance	ED_{50},[a] mmol/kg	rel potency[b] 5	rel potency[b] 12
3	1.06 ip[c]	1.1	
4	0.15[c]	2.1	1.0
5	0.31[c]	1.0	0.5
	1.12 ip[c]	1.0	
7		inactive[d]	
9a	0.14	2.2	1.1
9b	0.16	1.9	0.9
10a	0.14	2.2	1.1
10b	0.15	2.1	1.0
12	0.15[e]	2.1	1.0

[a] Intravenous administration unless otherwise noted. [b] Alloxan (**5**) and streptozotocin (**12**) were used as reference substances. [c] See ref 1. [d] Nondiabetogenic at the dose 2 g/kg ip. [e] A lower ED_{50}, 0.13 mmol/kg was reported by Junod, A.; Lambert, A. E.; Stauffacher, W.; Renold, A. E. *J. Clin. Invest.* **1969**, *48*, 2129.

(3) Recent findings of significantly higher values of blood uric acid in prediabetics, compared with normal and diabetic subjects, have revealed a pattern of changes in the uric acid level that is related to the pathogenesis of diabetes mellitus: Herman, J. B.; Medalie, J. H.; Goldbourt, U. *Diabetologia* **1976**, *12*, 47.
(4) For a review, see Biltz, H. *J. Prakt. Chem.* **1936**, *145*, 65.
(5) Poje, M.; Ročić, B. *Tetrahedron Lett.* **1979**, 4781.
(6) Poje, M.; Paulus, E. F.; Ročić, B. *J. Org. Chem.* **1980**, *45*, 65.
(7) Brückmann, G.; Wertheimer, E. *Nature (London)* **1945**, *155*, 267; *J. Biol. Chem.* **1947**, *168*, 241, have investigated the structural specificity of alloxan homologues in relation to diabetogenic activity. They conclude that an intact pyrimidine nucleus is essential, substitution at one nitrogen atom diminishes activity, and substitution elsewhere abolishes the diabetogenic effect of alloxan.
(8) Biltz, H.; Heyn, M. *Justus Liebigs Ann. Chem.* **1916**, *413*, 7.
(9) Biltz, H.; Klem, W. *Justus Liebigs Ann. Chem.* **1926**, *448*, 134.
(10) Mulder, E. *Chem. Ber.* **1881**, *14*, 1060.

of the hydrate **10a**. The atomic parameters that define the crystal structure, bond lengths and angles, torsion angles, and hydrogen bondings are given as supplementary material. An ORTEP drawing of **10a**, presenting the numbering scheme and hydrogen-bonding pattern, is shown in Figure 1. The crystal structures of alloxan (**5**)[11] and **10a** have many remarkably similar features. There is, however, a fundamental difference in the substituents at position 5. In **5** there is a *gem*-diol grouping, whereas in **10a** there is an entirely unique carbinol ammonium array that was not observed before in the structure of any organic molecule.[12]

With the structure of key compound **10a** firmly established, we proceeded to examine the diabetogenic activity. An effective dose rendering half the animals diabetic (ED_{50}) was determined in the Lewis strain of albino rats and expressed in millimoles per kilogram. A convenient comparison between new compounds and several of the most potent diabetogenic substances, including streptozotocin (**12**), is shown in Table I.

Compounds **9** and **10** were among the most active agents, whereas the conversion of **3** to **7** rendered the compound inactive. With respect to the diabetogenic activity, iminium salts **9** are equipotent or slightly less active than their hydrated derivatives **10**. The ease of conversion of **9** to **10**, however, leaves no doubt that **10** is the actual pancreas-reaching species. The blood sugar response to **10** was remarkably similar to that of **4**[1] in the failure to develop any severe hypoglycemia. Although **5** and **10** differ in potency, the acute metabolic effects and the histological changes in the islets of Langerhans appeared similar. However, **10** produced more extensive damage to the pancreatic β cells, resulting in the reduction of islets that consisted almost entirely of the α cells.

In comparing compounds **5** and **10** it can be seen that there is a twofold difference in the ED_{50} values, being in line with previous observations that the simultaneous injection of **5** with ammonium salts (100 mg/kg) decreased the ED_{50} to about 50%.[7] Moreover, the ED_{50} of **10** corresponds to a dose of **5** that does not in itself cause diabetes; the lowest observed active dose of **5** is 0.19 mmol/kg. Since carbinol ammonium salts **10** are readily formed from alloxan (**5**) and ammonium salts, it seems reasonable to suppose that the potentiating effect could be due to this specific chemical reaction.

Results obtained in this study clearly establish that the central carbonyl in the vicinal tricarbonyl system of alloxan (**5**), previously considered essential for activity,[7] may be replaced by an isosteric iminium function with not only retention of activity but with the added feature of ring stability. The importance of the 5-hydroxy group in tetrahedral adducts is indicated by the lack of activity in **7**. It is, perhaps, rather surprising that fragments of the five-membered ring or uric acid (**1**) may be present in the structure of derivatives **3**, **4**, **9**, and **10** while retaining full or even enhanced activity. Indeed, when the structures of active alloxan-like compounds are considered, the only essential common feature remains a quinonoid system itself. Thus, the active compounds may be regarded as isosteres of dehydrouric acid (**2**) or its hydrated forms. In-depth studies are now underway to further elucidate the exact significance of the structure–activity relationships and the underlying mechanisms of pancreatic β-cell cytotoxicity in this series.

(11) Mootz, D.; Jeffrey, G. A. *Acta Crystallogr.* **1965**, *19*, 717; Singh, C. *Ibid.* **1965**, *19*, 759.

(12) Carbinol ammonium ions have been proposed as transient intermediates in ammonium salt–carbonyl condensation or its reverse iminium salt hydrolysis; Bohme, H.; Haake, M. *Adv. Org. Chem.* **1976**, *9*, 107.

Experimental Section

Melting points were determined with a Kofler microscope and are corrected. Infrared spectra were recorded on a Perkin-Elmer 167 grating infrared spectrometer as KBr disks. ^1H and ^{13}C NMR spectra were measured on either a JEOL JNM-FX-100 or a Bruker WP-80/DS FT NMR spectrometer. Chemical shifts are given in δ units from Me_4Si as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were determined on a Varian MAT CH-7 instrument at 70 eV and 100 μA. Analyses of the elements or functional groups were within $\pm 0.3\%$ of the calculated values, unless otherwise stated.

5-Amino-5-ureido-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (7). A suspension of finely powdered dihydroxyuric acid[5] **3** (2.02 g, 0.01 mol) in ice-cooled 5% ethanolic ammonia (30 mL) was stirred for 30 min. The product was filtered off, washed with absolute ethanol and then thoroughly with ether, and dried in vacuo to yield the ammonium salt of **7** (2.3 g, 97%) as microscopic plates, mp 92–95 °C dec (lit.[8] mp 90–95 °C dec). The salt analyzes for a monohydrate. Anal. ($C_5H_{10}N_6O_4 \cdot H_2O$) C, H, N, NH_3.

Ice-cooled 5% aqueous acetic acid (15 mL) was added to the ammonium salt of **7** and stirred for 5 min. The crystalline product was collected, washed with water and ethanol, and dried under vacuum to give **7** (1.9 g, 87%) as the monohydrate, mp 146–147 °C dec (lit.[8] mp 145–147 °C dec). The product cannot be recrystallized unchanged. The conversion of **7** into the hydrochloride and subsequent decomposition with water afforded a sample with identical melting point and spectral characteristics: IR (KBr) 3500, 3405, 3310, 3250, 3200, 3020, 2820, 1765, 1720, 1670, 1605, 1544, 1400, 1378, 1306, 1250, 1178, 1127, 1058, 1022, 1005, 975, 860, 792, 758 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.87 (s, 2 H, ring NH), 5.82 (br s, 5 H, $NHCONH_2$ and H_2O), 2.73 (br s, 2 H, NH_2). The conversion of basic nitrogen to the positively charged species by adding a few drops of trifluoroacetic acid leads to a simpler pattern: δ 11.37 (s, 2 H, ring NH), 7.28 (t, 3 H, NH_3^+, $J_{NH} = 50.0$ Hz); the interaction of CF_3CO_2H with $NHCONH_2$ and H_2O gives rise to a signal at δ 6.0–11.0, depending on the concentration of the acid; ^{13}C NMR (Me_2SO-d_6) δ 170.1 (s, C_4 and C_6), 157.9 (s, $NHCONH_2$), 150.0 (s, C_2), 66.3 (s, C_5); MS (200 °C), *m/e* (relative intensity) 184 ($M^+ \cdot - NH_3$, 0.9), 156 (0.4), 141 ($M^+ \cdot$ – urea, 0.6), 129 (1.4), 114 (1.9), 113 (2.7), 86 (1.6), 85 (1.1), 70 (7.5), 69 (4.9), 60 (18.0), 55 (4.6), 44 (84.9), 43 (100.0), 42 (56.2). Anal. ($C_5H_7N_5O_4 \cdot H_2O$) C, H, N.

Reduction of 7. Uramil (8). A modified Biltz's procedure[8] was used on a 0.01-mol scale. Finely powdered **7** (2.19 g) was added to a stirred mixture of constantly boiling hydriodic acid (10 mL) and red phosphorus (1 g). The reaction mixture was stirred in a steam bath for 10 min, cooled, and filtered through a sintered-glass funnel. The clear filtrate was diluted with water (30 mL) and cooled to give **8** (1.2 g, 78%), which does not melt below 400 °C. The same product was obtained by reduction of **7** with stannous chloride in hydrochloric acid. The analytical sample was prepared by recrystallization from water. The IR spectrum was identical with the spectrum of an authentical sample. Anal. ($C_4H_5N_3O_3$) C, H, N.

Dehydrouramil Hydrochloride (9a). Dry chlorine was led into an ampule cooled in acetone/dry ice bath containing finely powdered **8** (1.43 g, 0.01 mol). When the substance had been covered with liquid chlorine, the ampule was sealed up and left overnight at room temperature on a mechanical shaker. The chlorine was carefully distilled off to leave an orange residue. Remaining traces of chlorine were removed by high vacuum evacuation to yield **9a** (1.8 g) as an orange powder. The product cannot be recrystallized unchanged, and it is extremely sensitive to moisture and protic solvents: mp 237–240 °C dec. Anal. Calcd for $C_4H_4ClN_3O_3$ (177.56): C, 27.06; H, 2.27; Cl, 19.97; N, 23.67. Found: C, 26.70; H, 2.49; Cl, 20.12; N, 23.36.

Reduction of 9a to Uramil (8). Sodium iodide (3 g) was dissolved in dry acetonitrile (40 mL) and anhydrous *p*-toluenesulfonic acid (1.7 g) was added with stirring. Sodium tosylate precipitation occurred immediately, and the mixture was stirred for an additional 15 min. Then the precipitate was removed by filtration, and finely powdered **9a** (1.78 g, 0.01 mol) was gradually

added. All operations were carried out under nitrogen and exclusion of moisture. After a total time of 1 h, the mixture was poured into saturated $NaHCO_3$ solution (20 mL), and the solid was collected, washed well with water and ethanol, and dried in vacuo to give 8 (0.9 g, 63%).

Reaction of 9a with Urea. 5-Amino-5-ureido-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione (7). Finely powdered **9a** (3.56 g, 0.02 mol) and urea (2 g) were suspended in tetramethylurea (20 mL) under anhydrous conditions and stirred for 5 h at room temperature. The reaction mixture was then diluted with 60% ethanol, and the pink precipitate was collected. The crude product was resuspended in water (15 mL), and the mixture was vigorously stirred for 30 min. The solid was filtered off, washed with ethanol, and dried under high vacuum to give 2.1 g (48%) of white powder, mp 145–147 °C dec, identical in all respects with the hydrate of **7**.

Dehydrouramil Hydrobromide (9b). A modified Mulder's procedure[10] was used on a 0.01-mol scale. Dry bromine (8 g) and finely powdered 8 (1.43 g) were allowed to react in a sealed tube on the shaker for 24 h. Bromine was distilled off, and the product was kept overnight in a vacuum desiccator over KOH. Remaining traces of bromine were removed by high-vacuum evacuation to give **9b** (2.2 g) as an orange powder, mp 200–203 °C dec. Repeated preparations afforded products of similar purity, very sensitive to moisture. Anal. Calcd for $C_4H_4BrN_3O_3$ (222.02): C, 21.64; H, 1.83; Br, 16.00; N, 18.93. Found: C, 21.19; H, 2.17; Br, 36.32; N, 18.48.

Reaction with urea carried out in the manner described above gave **7** (33%), and the reduction with hydrogen iodide in acetonitrile afforded 8 (40%).

Preparations of 5-Amino-5-hydroxy-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione Hydrochloride (10a) and Hydrobromide (10b). A. Hydration of 9a. Dehydrouramil hydrochloride (**9a**; 1.78 g, 0.01 mol) was dissolved in 5% hydrochloric acid at 5 °C, and the resulting pale pink solution was slowly concentrated in a vacuum desiccator over P_2O_5. Colorless prismatic needles that separated were collected, washed with an excess of ethanol and dry ether, and dried in vacuo to give **10a** (1.4 g, 65%): mp 220–221 °C dec; IR (KBr) 3300, 3200, 3110, 3050, 1770, 1743, 1722, 1458, 1417, 1393, 1378, 1240, 1142, 1073, 1000, 812, 795, 779 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.24 (s, 2 H, ring NH), 7.42 (t, 3 H, NH_3^+, J_{NH} = 50.0 Hz), 5.73 (br s, 3 H, OH + H_2O); ^{13}C NMR (Me_2SO-d_6) δ 169.1 (s, C_4 and C_6), 149.9 (s, C_2), 85.0 (s, C_5). Compound **10a** analyses for a monohydrate. Anal. ($C_4H_6ClN_3O_4 \cdot H_2O$) C, H, N.

Hydration of 9b. Dehydrouramil hydrobromide (**9b**; 2.2 g, 0.01 mol) was dissolved in 5% hydrobromic acid (20 mL) at 5 °C, and the reddish solution was stirred with charcoal (1 g) for 2 h and filtered. The pink solution was slowly evaporated in the manner described above. Colorless prisms that separated were collected, washed with ethanol and ether, and dried under vacuum to give **10b** (1.0 g, 39%): mp 198–199 °C dec; IR (KBr) 3200, 3100, 3060, 1765, 1740, 1720, 1460, 1413, 1385, 1375, 1242, 1150, 1142, 1069, 810, 790, 781 cm^{-1}. Anal. ($C_4H_6BrN_3O_4 \cdot H_2O$) C, H, N.

B. Acid-Catalyzed Disproportionation of 7. Finely powdered **7** (4.4 g, 0.02 mol) was stirred in 15% hydrochloric acid (20 mL) until dissolved. The workup according to Biltz's procedure[9] afforded the hydrate **10a** (3.4 g, 80%) identical in all respects with the product obtained by hydration of **9a**. An analogous reaction of **7** (4.4 g, 0.02 mol) with 15% hydrobromic acid (20 mL) under nitrogen afforded the hydrate of **10b** (4.0 g, 78%).

C. Preparation from Alloxan (5) and Ammonium Salts. Alloxan monohydrate (**5**; 3.2 g, 0.02 mol) was dissolved in hot water (15 mL) and admixed to a solution of ammonium chloride (1 g) in 10% hydrochloric acid (20 mL). Slow evaporation of the solvent in a desiccator gave long prisms of the monohydrate **10a** (2.4 g, 56%). The reaction of **5** (3.2 g, 0.02 mol) with ammonium bromide in 10% hydrobromic acid (20 mL) afforded the corresponding hydrate of **10b** (2.5 g, 48%).

Crystallography. Crystal Data: crystals of the monohydrate **10a**, $C_4H_6ClN_3O_4 \cdot H_2O$, M = 213.59, are monoclinic, space group $P2_1/n$; a = 13.677 (5), b = 10.938 (5), c = 5.586 (2) Å, β = 99.84 (3)°, V = 823.4 Å^3, ρ_o = 1.718 g cm^{-3}, ρ_c = 1.722 g cm^{-3} for Z = 4; μ (Cu K_α) = 42.1 cm^{-1}.

Three-dimensional intensity data from a prismatic crystal specimen (0.10 × 0.21 × 0.29 mm) were measured on a Philips PW 1100 four-circle diffractometer (Cu $K\alpha$ radiation, λ = 1.5418 Å, 10° < 2θ < 140°). The intensities of 1431 independent reflections with $I > 3\sigma(I)$, corrected for Lorentz, polarization, and absorption effects, were used in structure determination. The structure was solved by a multiple solution procedure by using MULTAN.[13] Anisotropic thermal parameters were used for nonhydrogen atoms. All hydrogen atoms were identified from difference Fourier maps; H(1) and H(3) were isotropically refined. The protons of water were fixed in the last three cycles, and remaining hydrogens were fixed as found in the difference Fourier map. The atoms H(4) and H(23) were refined by using isotropic factors. However, we preferred to calculate the positions of H(21) and H(22) because it was difficult to locate them correctly. The final reliability factor was R = 0.064 (R_w = 0.077) for 1370 reflections with sin θ/λ < 0.6.

Diabetogenic Activity in Rats. Groups of 24 male Lewis rats (weighing 200–230 g) were used at each dose level. The animals were fasted for 12 h before injection. The substances were adminstered intravenously (tail vein) or, in the case of poor solubility, intraperitoneally as saline suspensions. A rat was considered strongly diabetic if glucosuria (>1%) occurred within 24 h after injection and persisted for 5 days. Additional information was obtained by observation of blood sugar and histological changes of the islets of Langerhans.[14] The dose required to produce diabetes in 50% of animals (ED_{50}) was estimated by graphical method.[15]

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Registry No. **3**, 67708-22-9; **5**, 3237-50-1; **7**, 85066-70-2; **7**·NH_3, 85066-71-3; **8**, 118-78-5; **9a**, 85048-86-8; **9b**, 85048-87-9; **10a**, 85048-88-0; **10b**, 85048-89-1; urea, 57-13-6.

Supplementary Material Available: Positional and thermal parameters, bond lengths and angles with their estimated standard deviations, torsion angles, and intermolecular hydrogen bonding data (3 pages). Ordering information is given on any current masthead page.

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(14) Kikui, Y.; Seguchi, H.; Misoguti, H. *Acta Histochem. Cytochem.* **1977**, *10*, 10. In this differential staining method for α and β cells, granules of β cells are stained deep blue and those of α cells red. Nuclei are violet, and the cytoplasm of the exocrine cells is purplish pink. Collagen fibers are green and elastic fibers blue. Details of hystological studies will be reported elsewhere.

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