Z Lebensm Unters Forsch (1995) 201: 381–386

Zeitschrift für

Original paper

Reaction of 3-deoxypentosulose with *N*-methyl- and *N*,*N*-dimethylguanidine as model reagents for protein-bound arginine and for creatine

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Received: 29 July 1994/Revised version: 1 December 1994

Abstract. Deoxyosones are established key-intermediates in Maillard processes. Due to their dicarbonyl structure, they undergo condensation to form heterocyclic compounds with guanidine derivatives. In biological systems, guanidino functions are present in protein-bound arginine moieties as well as in creatine. The reactivity of such structures towards 3-deoxypentosulose is investigated with *N*-methyl- and *N*,*N*-dimethylguanidine as model substrates. Two diastereoisomers each are isolated from both reactions; they have been characterized unequivocally, respectively, as 4-(2,3-dihydroxypropyl)-2-*N*-methylamino-2-imidazolin-5-one and 4-hydroxy-5-(2,3-dihydroxypropyl)-2-(*N*,*N*-dimethylamino)-5*H*-imidazole. In aqueous medium as well as in the crystalline state, both diastereoisomer pairs exist in different tautomeric forms.

Introduction

A number of potential carbohydrate substrates are present in foodstuffs for the reaction with amino acids or protein-bound amino acid moieties, which is known as the Maillard reaction or "nonenzymatic browning". Modification of amino acids is frequently observed when proteins are heated in the presence of reducing carbohydrates; especially lysine and arginine undergo extensive derivatization in the course of nonenzymatic browning [1, 2]. Guanidino functions, however, have been reported not to initiate Maillard reactions [3]. If glucose, for instance, is heated together with N^{α}-acetylarginine, no browning of the solution is observed, i.e. the sugar molecule remains more or less unchanged [1, 2]. The guanidino function of an arginine moiety, incorporated in a protein, may be supposed, therefore, to react only with the more reactive Maillard intermediates, such as deoxyosones.

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The deoxyosones (compounds 1–3), formed in the course of the Maillard process by conversion of reducing pentoses and hexoses, respectively, may be characterized as intramolecular disproportionation products (see Fig. 1). They are much more reactive than the native carbohydrates.

From the reaction of a guanidino group with the α-dicarbonyl function of the 3-deoxyosones (compounds 1a, b), formation of imidazolinones may be envisaged, for which various tautomeric forms can be formulated (compounds 4–8; Fig. 2).

Likewise, an equilibrium is possible between the α- and β-dicarbonyl tautomeric form of the 1-deoxydiketoses (compounds 3a, b; Fig. 1), and thence formation of pyrimidine derivatives (compounds 9a, b; Fig. 3) [4].

Maillard reactions in food, as well as in vivo, are investigated predominantly with model substrates where undesirable reaction pathways are blocked, and isolation, purification and identification procedures are much simplified. The results thus obtained can be extrapolated to reactions in food and in the human organism.

In biological systems, guanidino groups are present in both the amino acid arginine (compound 10) and in the characteristic meat constituent creatine (compound 11; Fig. 4). We now report on products formed from the reaction of 3-deoxypentosulose (compound 1a) with *N*-methyl- and *N*,*N*-dimethylguanidine (compounds 12 and 13, respectively) as model substrates for protein-bound arginine and for creatine (Fig. 4).

Materials and methods

General methods

Spectra. IR spectra (KBr discs) were measured with Pye Unicam SP 1100 (Cambridge, UK) and Perkin Elmer 283 (Überlingen, Germany) spectrometers, UV spectra with a Perkin Elmer Lambda 2; [^{1}H]NMR and [^{13}C]NMR spectra were recorded on a Bruker (Karlsruhe, Germany) AC-250 spectrometer at 250 MHz and 63 MHz nominal frequency, respectively; for liquid-secondary-ion mass spectrometry (analogous to FAB-MS) a Finnigan MAT 95 (Bremen, Germany) was employed.

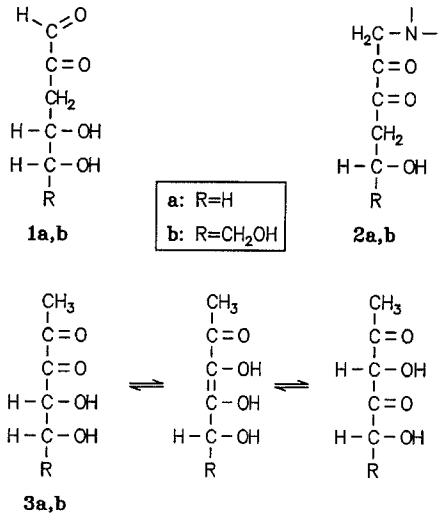


Fig. 1. Chemical structure of compounds 1–3: 3-deoxypentosulose (compound 1a); 3-deoxyhexosulose (compound 1b); *N*-substituted-1-amino-1,4-dideoxypentodiulose (compound 2a); *N*-substituted-1-amino-1,4-dideoxyhexodiulose (compound 2b); 1-deoxypentodiulose (α-diketo, reductone, β-diketo structure) (compound 3a); 1-deoxyhexodiulose (α-diketo, reductone, β-diketo structure) (compound 3b)

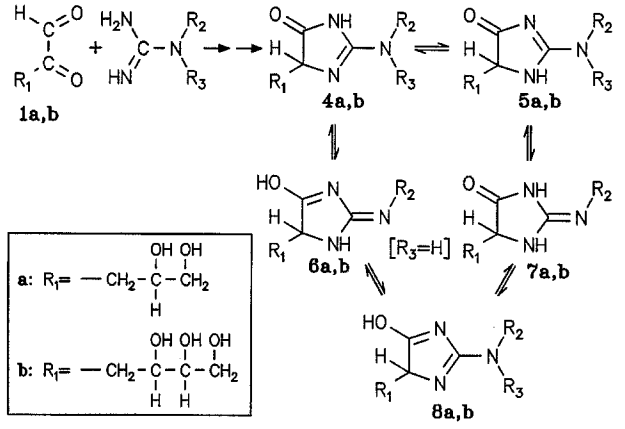


Fig. 2. Tautomeric forms (compounds 4–8) for the imidazolinones, formed by reaction of compound 1a, b with guanidine derivatives: 2-(di)alkylamino-4-(2,3-dihydroxypropyl)-2-imidazolin-5-one (compound 4a); 2-(di)alkylamino-4-(2,3,4-trihydroxybutyl)-2-imidazolin-5-one (compound 4b); 2-(di)alkylamino-5-(2,3-dihydroxypropyl)-2-imidazolin-4-one (compound 5a); 2-(di)alkylamino-5-(2,3,4-trihydroxybutyl)-2-imidazolin-4-one (compound 5b); 2-alkylimino-4-hydroxy-5-(2,3-dihydroxypropyl)-3-imidazoline (compound 6a); 2-alkylimino-4-hydoxy-5-(2,3,4-trihydroxybutyl)-3-imidazoline (compound 6b); 2-alkylimino-5-(2,3-dihydroxypropyl)imidazolidin-4-one (compound 7a); 2-alkylimino-5-(2,3,4-trihydroxybutyl)imidazolidin-4-one (compound 7b); 2-(di)alkylamino-4-hydroxy-5-(2,3-dihydroxypropyl)-5*H*-imidazole (compound 8a); 2-(di)alkylamino-4-hydroxy-5-(2,3,4-trihydroxybutyl)-5*H*-imidazole (compound 8b)

Semipreparative HPLC purification. A Merck Hitachi 655A-11 liquid chromatograph (Merck, Darmstadt, Germany) was used, combined with a 655A variable wavelength detector and a Bischoff (Leonberg, Germany) HPLC column (SNC + SPC Lichrosorb RP 18 – 5 μm; 8 × 250 mm): flow rate 2 ml·min^{-1}, detection at 220 nm.

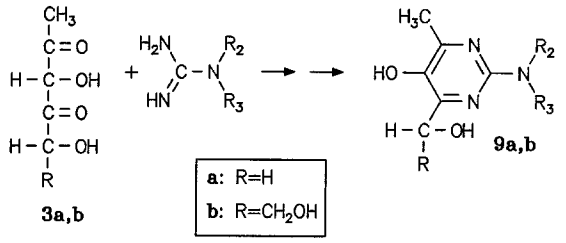


Fig. 3. Postulated formation of 2-(di)alkylamino-5-hydroxy-4-(2,3-dihydroxypropyl)-6-methylpyrimidine (compound 9a) and 2-(di)alkylamino-5-hydroxy-4-(2,3,4-trihydroxybutyl)-6-methylpyrimidine (compound 9b), respectively, from compound 3a, b and guanidine derivatives

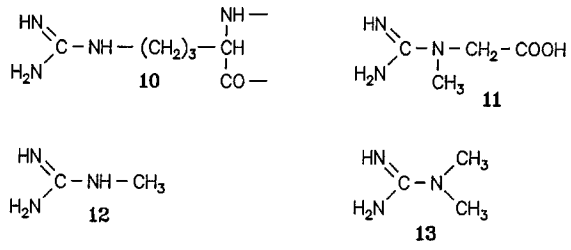


Fig. 4. Chemical structures of: protein-bound arginine (compound 10); creatine (compound 11); *N*-methylguanidine (compound 12); *N,N*-dimethylguanidine (compound 13)

Preparative HPLC purification. A Knauer (Berlin, Germany) 64 liquid chromatograph was employed, combined with an A0293 variable wavelength detector and a Kronlab (Sinsheim, Germany) HPLC column (guard column 20 × 50 mm, column 20 × 250 mm; Nucleosil RP 18 – 7 μm): flow rate 10 ml·min^{-1}, detection at 220 nm.

Capillary gas chromatograms. These were run on a Perkin-Elmer 8600 instrument with a flame-ionization detector (FID); quartz capillary column (length 25 m, ID = 0.32 mm, PVMS 54, film thickness = 1 μm, carrier gas = He, initial pressure = 80 kPa, 33 cm·s^{-1}); the temperature of the injection and detection ports was 270 °C; temperature programme was such that temperature rose at 8 °C·min^{-1} from 100 °C to 270 °C at which temperature it was held for 15 min.

Gas chromatography – mass spectrometry analysis. GC/MS was performed on a Finnigan MAT Ion Trap 800, EI and pos. CH_3OH–CI mode, coupled to a Perkin-Elmer 8420 gas chromatograph: quartz capillary column (length = 25 m, ID = 0.25 mm, PVMS 54, film thickness = 0.3 μm, carrier gas = He, initial pressure = 80 kPa, 21 cm·sec^{-1}); injection port temperature was 270 °C; the temperature programme was as mentioned above.

Chromatography. Silica gel 60 F_{254} Merck, 5554 and 5717 (Darmstadt, Germany) was used for thin-layer chromatography (TLC).

Lyophilization. A Leybold-Heraeus (Cologne, Germany) Lyovac GT 2 was applied.

Trimethylsilyl derivatives. These were obtained by treating 1 mg of the respective substrate with 100 μl *N,O*-bis(trimethylsilyl)acetamide (BSA) in 100 μl pyridine for 60 min at room temperature.

Materials

N-Methylguanidine·HCl (no. 22,240-2) and *N,N*-dimethylguanidine-sulphate (no. 27,666-9) were obtained from Aldrich

(Milwaukee, Wis., USA); *N,O*-bis(trimethylsilyl)acetamide (no. 15241) from Fluka (Neu-Ulm, Germany).

Synthetic procedures

Synthesis of 4-(2,3-dihydroxypropyl)-2-N-methylamino-2-imidazolin-5-ones (compounds 17a, b). A total of 250 mg (1.89 mmol) of 3-deoxypentosulose (compound 1a), synthesized according to [5], and 207 mg (1.89 mmol) *N*-methylguanidine·HCl were dissolved in 5 ml water, and the pH adjusted to 7.0 with 0.01 N NaOH. The solution was heated under reflux conditions for 4.5 h and the disappearance of compound 1a in the course of the reaction was monitored by TLC (eluent: ethylacetate:methanol 19:1; detection with 2,4-dinitrophenylhydrazine). After filtration (membrane filter 0.45 µm), the two diastereoisomers, compounds 17a and b, were separated by preparative HPLC (eluent: 0.01 M NH_4HCOO-buffer, pH = 5.0: CH_3CN 99.5:0.5; 6 injections, 1 ml each). The fraction with t_R (prep. HPLC) 8.5 and t_R 10.3 yielded, after lyophilization, 45 mg (0.24 mmol; 12.7%) of compound 17a and 35 mg (0.19 mmol; 9.9%) of compound 17b, respectively; GC t_R values of the silyl derivatives were 21.9 for compound 17a and 20.8 for compound 17b. According to the capillary gas chromatograms, fraction t_R 8.5 contains both diastereoisomers in a 3:1 ratio (17a:17b). Fraction t_R 10.3 contains only the diastereoisomer compound 17b.

UV absorbance, IR characteristics, and FAB-MS data of compounds 17a and b are identical:

1. UV (H_2O): maximum absorption wavelength (λ max) lg molar absorption coefficient (lg ε) = 192 nm (3.98); sh ≈ 215.
2. IR (KBr disc): peaks were measured at: 3200 (broad), 1710, 1615, 1410, 1355, 1115, 1070 cm^{-1}
3. FAB-MS (*m*-nitrobenzyl alcohol): 188 (M + H).

For compound 17a, the following characteristics apply:

1. CI-GC/MS [tetrakis(trimethylsilyl) derivative]: m/z 476(M + H; 100) 460(26) 404(4) 257(26) 73(14).
2. [^{1}H]NMR (^2H_2O): (chemical shifts (δ, ppm) 2.01 [ddd, 1H, J = 4.5, 10.8, (−)14.8 Hz] δ 2.17 [ddd, 1H, J = 3.2, 6.0, (−)14.8 Hz] δ 3.18 (s, 3H) δ 3.49 [dd, 1H, J = 6.3, (−)11.8 Hz] δ 3.57 [dd, 1H, J = 4.2, (−)11.8 Hz] δ 3.90 (dddd, 1H, J = 3.2, 4.2, 6.3, 10.8 Hz) δ 4.55 (dd, 1H, J = 4.5, 6.0 Hz), where J is the coupling constant (Hz), S = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet.
3. [^{13}C]NMR (^2H_2O): δ 28.6, 35.7, 59.2, 68.1, 70.9, 161.6, 178.6.

For compound 17b the following characteristics apply:

1. CI-GC/MS [tris(trimethylsilyl) derivative]: m/z 404(M + H; 100) 388(12) 198(7) 73(49).
2. [^{1}H]NMR (^2H_2O): δ 1.89 [ddd, 1H, J = 3.6, 9.8, (−)14.6 Hz] δ 2.03 [ddd, 1H, J = 3.7, 9.7, (−)14.6 Hz] δ 3.19 (s, 3H) δ 3.54 [dd, 1H, J = 6.2, (−)11.7 Hz] δ 3.60 [dd, 1H, J = 4.4, (−)11.7 Hz] δ 3.86 (dddd, 1H, J = 3.6, 4.4, 6.2, 9.7 Hz] δ 4.58 (dd, 1H, J = 3.7, 9.8 Hz).
3. [^{13}C]NMR (^2H_2O): δ 28.6, 36.7, 58.8, 68.1, 70.8, 161.7, 178.4.

Synthesis of 4-Hydroxy-5-(2,3-dihydroxypropyl)-2-(N,N-dimethylamino)-5H-imidazoles (compounds 18a, b). Following the above described procedure, 250 mg (1.89 mmol) 3-deoxypentosulose (compound 1a) and 350 mg (1.89 mmol) *N,N*-dimethylguanidine·H_2SO_4 were heated under reflux conditions for 3 h. The membrane filtrate was separated by semipreparative HPLC (eluent: 0.01 M NH_4HCOO-buffer pH = 5.0:CH_3CN 99:1; 25 injections, 50 µl each). The fraction with t_R (semiprep. HPLC) 14.3 and t_R 18.8 yielded, after lyophilization, 14 mg crude compound 18a and 7 mg of compound 18b, respectively. The purity of both fractions could not be analysed by GLC; the peak shapes indicated decomposition of the silyl derivatives. Further analysis by semipreparative HPLC showed that fraction t_R 18.8 contained pure compound 18b. Fraction t_R 14.3, in contrast, contains both diastereoisomers in a 2:1 ratio (18a:18b). The lyophilized fraction t_R 14.3 was dissolved in 1 ml water and once again subjected to semipreparative HPLC to obtain pure compound 18a (eluent as mentioned above; 10 injections, 100 µl each). Fractions t_R 14.3 and 18.8 were collected and lyophilized, yielding 8.5 mg (0.042 mmol; 8.9%) compound 18a and 10 mg (0.05 mmol; 10.6%) compound 18b.

UV absorbance, IR characteristics, and FAB-MS data of compounds 18a and b are identical:

1. UV (H_2O): λ max(lg ε) = 225 nm (4.15).
2. IR (KBr disc) peaks were measured at: 3360, 3110, 1615, 1440, 1390, 1318, 1290, 1080 cm^{-1}
3. FAB-MS (*m*-nitrobenzyl alcohol): 202 (M + H).

For compound 18a, the following characteristics apply:

1. [^{1}H]NMR (^2H_2O): δ 1.84 [ddd, 1H, J = 6.0, 9.5, (−)14.7 Hz] δ 2.06 [ddd, 1H, J = 4.0, 5.5, (−)14.7 Hz] δ 3.09 (s, 3H) 3.16 (s, 3H) δ 3.52 [dd, 1H, J = 6.5, (−)11.8 Hz] δ 3.61 [dd, 1H, J = 4.1, (−)11.8 Hz] δ 3.92 (ddt, 1H, J = 4.0, 6.5, 9.5 Hz) δ 4.32 (dd, 1H, J = 5.5, 6.0 Hz).
2. [^{13}C]NMR (^2H_2O): δ 37.1, 39.4, 41.1, 62.2, 68.3, 71.8, 170.2, 193.3.

For compound 18b, the following characteristics apply:

1. [^{1}H]NMR (^2H_2O): δ 1.72 [ddd, 1H, J = 3.2, 9.4, (−)14.5 Hz] δ 1.93 [ddd, 1H, J = 3.7, 9.8, (−)14.5 Hz] δ 3.07 (s, 3H) 3.15 (s, 3H) δ 3.53 [dd, 1H, J = 6.5, (−)11.7 Hz] δ 3.60 [dd, 1H, J = 4.2, (−)11.7 Hz] δ 3.84 (dddd, 1H, J = 3.2, 4.2, 6.5, 9.8 Hz) δ 4.34 (dd, 1H, J = 3.7, 9.4 Hz).
2. [^{13}C]NMR (^2H_2O): δ 38.1, 39.4, 41.1, 62.1, 68.5, 71.7, 172.0, 195.4.

Results and discussion

For 3-deoxypentosulose (compound 1a), an equilibrium may be envisaged between the various cyclic isomers (compounds 14–16; Fig. 5), with the open-chain form, compound 1a, as only a minor constituent. Since for the intramolecular hemiacetal/ketal structures 14–16 much lower α-dicarbonyl activity is expected, cyclization reactions with guanidine derivatives may be retarded significantly relative to those of proper α-dicarbonyl compounds, such as benzil or methylglyoxal.

We heated equimolar aqueous solutions of compound 1a and *N*-methylguanidine·HCl (compound 12) or *N,N*-dimethylguanidine·H_2SO_4 (compound 13) under reflux conditions for 3–4.5 h to simulate cooking conditions. The pH, adjusted to 7.0 at the outset of the reaction, decreases to 3.5–4 during the heating period, and the reaction mixtures turn dark brown. The pH decrease may be due to carboxylic acids being formed by degradation of

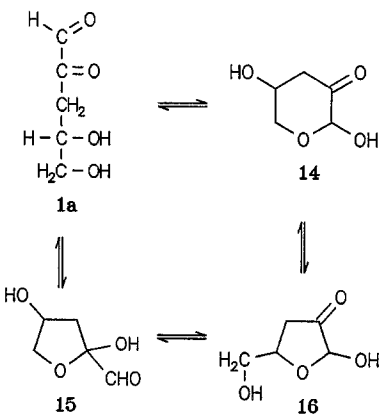


Fig. 5. Hemiacetals/ketals of compound 1a: 2,5-dihydroxytetrahydro-3-pyranone (compound 14); 2,4-dihydroxytetrahydrofuran-2-carbaldehyde (compound 15); 2-hydroxy-5-hydroxymethyl-4,5-dihydro-3(2*H*)-furanone (compound 16)

compound 1a. However, the decidedly lower basicity of the imidazolinone products, relative to that of the starting guanidine derivatives, may play an even more important role.

After membrane filtration, the solutions are subjected directly to either preparative or semipreparative HPLC. Two diastereoisomers each are isolated from both the reactions with compounds 12 and 13. Spectroscopic data (see below) unequivocally prove the formation of the diastereoisomeric 4-(2,3-dihydroxypropyl)-2-*N*-methylamino-2-imidazolin-5-ones (compounds 17a, b) from the reaction with *N*-methylguanidine (compound 12), and of the diastereoisomeric 4-hydroxy-5-(2,3-dihydroxypropyl)-2-(*N*,*N*-dimethylamino)-5*H*-imidazoles (compounds 18a, b) from reaction with *N*,*N*-dimethylguanidine (compound 13). A plausible reaction mechanism for formation of compounds 17a, b and compounds 18a, b is outlined in Fig. 6. An intramolecular Cannizzaro reaction may safely be excluded under Maillard conditions, i.e. pH 4–7. The proton at C4 in compound 17a, b, and at C5 in compound 18a, b, therefore must be solvent-derived, and does not stem from a 1,2-hydride shift.

The yield of compounds 17a, b and 18a, b from the reaction of 3-deoxypentosulose (compound 1a) with the respective guanidine derivative is about 20% for each diastereoisomer pair. Formation of these compounds thus constitutes a major reaction pathway for 3-deoxypentosulose (compound 1a) derivatization in the presence of guanidino moieties. Investigations are now in progress to study formation of compunds 17a, b and 18a,.b under physiological conditions. Oxidation of such structures would give very reactive imidazolinone systems.

There are several reports in the literature about the reaction of α-dicarbonyl compounds with guanidine derivatives. Nishimura and Kitajima [6] describe 4-hydroxy-2-dimethylamino-4,5-diphenyl-4*H*-imidazole (compound 19) being formed from benzil and *N*,*N*-dimethylguanidine (compound 13) at room temperature (Fig. 7). If heated in either *N*,*N*-dimethylformamide (DMF), ethanol, or in neat form, compound 19 is transformed quantitatively into an imidazolinone (compounds 20 or 21). Mainly on the basis of the C=O stretching frequency, the authors favour the tautomeric form, compound 20, in the solid state.

Duerksen-Hughes et al. [7] have studied the reaction of guanidine derivatives with 4-(oxoacetyl)phenoxyacetic acid. Reaction (37 °C, basic conditions) of *N*-methylguanidine (compound 12) with 4-(oxoacetyl)phenoxyacetic acid yields 4-(2-methylimino-4-imidazolidinon-5-yl)phenoxyacetic acid (compound 22; Fig. 7). Structure 22, with an exocyclic imino group, was assigned from [^{1}H]NMR and [^{13}C]NMR spectra interpretation.

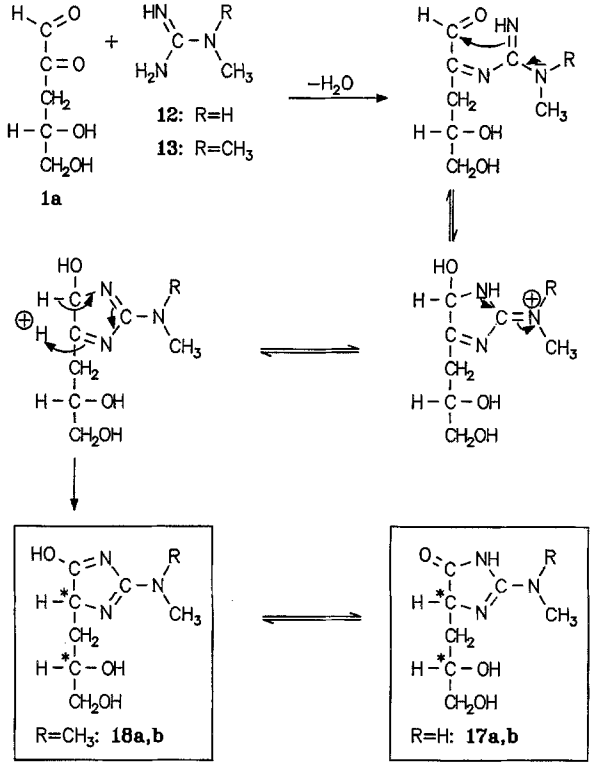


Fig. 6. Hypothetical reaction mechanism for the formation of the two diastereoisomeric 4-(2,3-dihydroxypropyl)-2-*N*-methylamino-2-imidazolin-5-ones (compounds 17a, b) and 4-hydroxy-5-(2,3-dihydroxypropyl)-2-(*N*,*N*-dimethylamino)-5*H*-imidazoles (compounds 18a, b)

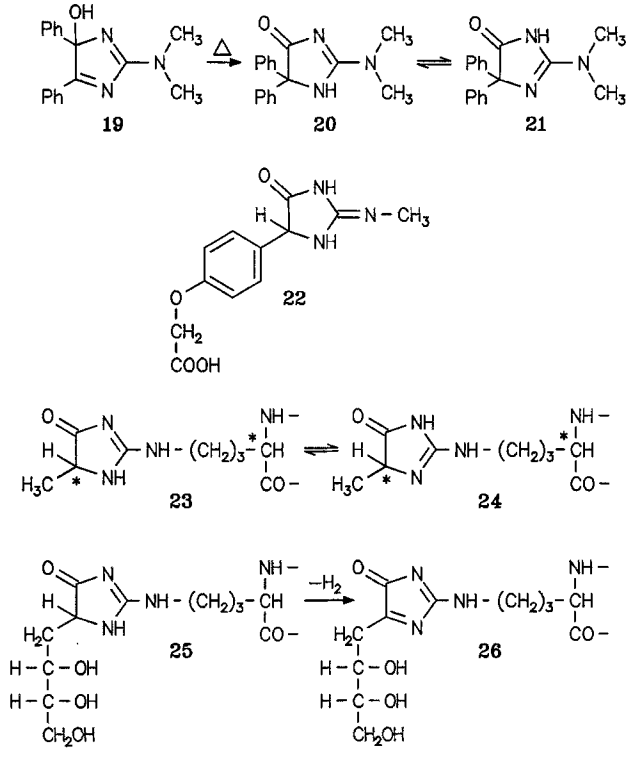


Fig. 7. Diazole structures from reactions of α-dicarbonyl compounds and guanidine derivatives as described in the literature: 2-*N*,*N*-dimethylamino-4-hydroxy-4,5-diphenyl-4*H*-imidazole (compound 19); 2-*N*,*N*-dimethylamino-5,5-diphenyl-2-imidazolin-4-one (compound 20), 2-*N*,*N*-dimethylamino-4,4-diphenyl-2-imidazolin-5-one (compound 21), 4-(2-methylimino-4-imidazolidinon-5-yl)phenoxyacetic acid (compound 22), 5-methyl-2-(δ-*N*-ornithyl)-2-imidazolin-4-one (compound 23), 4-methyl-2-(δ-*N*-ornithyl)-2-imidazolin-5-one (compound 24), 5-(2,3,4-trihydroxybutyl)-2-(δ-*N*-ornithyl)-2-imidazolin-4-one (compound 25)5-(2,3,4-trihydroxybutyl)-2-(δ-*N*-ornithyl)-4-imidazolone (compound 26)

Thornalley [8] has postulated that 5-methyl-2-(δ-*N*-ornithyl)-2-imidazolin-4-one (compound 23) is a fluorescent reaction product formed from methylglyoxal and protein-bound arginine (compound 10; Fig. 7). Henle et al. [9] have isolated compound 23 and the tautomeric 4-methyl-2-(δ-*N*-ornithyl)-2-imidazolin-5-one (compound 24) from a food sample, and subsequently synthesized this structure independently. One diastereoisomer only was detected for each tautomer from both the foodstuff and the independent chemical reaction. Structural assignment of compounds 23 and 24 is based on the [^{1}H]NMR, [^{13}C]NMR, and FAB-MS data.

Hayase et al. [10, 11] reported on the formation of 5-(2,3,4-trihydroxybutyl)-2-(δ-*N*-ornithyl)-2-imidazolin-4-one (compound 25) and its oxidation product 5-(2,3,4-trihydroxybutyl)-2-(δ-*N*-ornithyl)-4-imidazolone (compound 26) from 3-deoxyhexosulose (compound 1b) and protein-bound arginine (compound 10; Fig. 7); both products were characterized, after acid hydrolysis of the protein and chromatographic purification, by [^{1}H]NMR, [^{13}C]NMR, and FAB-MS.

Formation of 2-alkylamino-imidazolinone-type structures from α-dicarbonyl compounds and guanidine derivatives thus may be considered to be firmly established. Which tautomeric form is present in each case, though, is discussed controversially in the literature references cited above – despite the fact that the individual structures will differ significantly in their UV, IR, and NMR characteristics. Therefore, we have put special efforts into an unequivocal structural assignment for our compounds 17a, b and 18a, b.

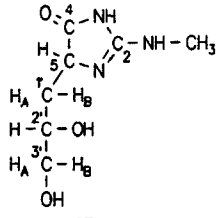
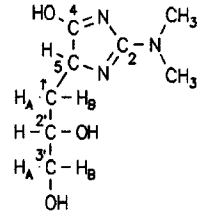
Structural assignment

The differentiation between the two tautomeric forms of the *N*-methyl- and *N*,*N*-dimethyl derivatives, compounds 17a, b and 18a, b respectively, rests on a number of individual spectroscopic arguments. Each of these, if taken alone, provides only circumstantial evidence; taken together, however, the two structures may be considered as firmly established.

[^{1}H] and [^{13}C] chemical shifts (δ) and coupling constants (J) or multiplicities are given in Table 1. The chemical shifts for carbons C4 and C2 differ strikingly between compounds 17a, b and 18a, b. In compounds 17a, b, the C4 shift (178.6/178.4 ppm) is in the range expected for a cyclic amide. This is confirmed by a 1710 cm^{-1} C=O stretching vibration, characteristic for a γ-lactam. The C2 resonances in compounds 17a, b (161.6/161.7 ppm) appear only slightly shifted to lower field compared to that of the starting alkyl guanidine (158 ppm); C2, therefore, cannot be conjugated with the carbonyl function. The UV absorption maximum (192 nm; ε ≈ 9500) likewise excludes extended conjugation, being characteristic, rather, for the $\pi \rightarrow \pi^*$ transition of a structurally isolated imine group. The CH_3 carbon shift (28.6 ppm) for compounds 17a, b clearly proves the imine to be endocyclic; for an imine-bound CH_3 carbon, a chemical shift of about 39 ppm would be expected.

The C4 and C2 resonances for compounds 18a, b (193.3/195.4 ppm and 170.2/172.0 ppm, respectively) are shifted by 10–15 ppm to low field relative to those of compounds 17a, b. Such shifts are reasonable for a C=N conjugated enaminole structure. This hypothesis is supported by the IR spectrum which shows no proper C=O stretching vibration. The intensive band at 1615 cm^{-1} can be assigned to the coupled C=N stretching modes for the cyclic conjugated C=N functions. Such a diene structure for compounds 18a, b is also confirmed by the UV absorption maximum (225 nm; ε ≈ 14000).

Table 1. [^{1}H]NMR and [^{13}C]NMR spectroscopic data of compounds 17a, b and 18a, b in ^2H_2O (for a direct comparison between the two diazole structures, the same numbering is used for compounds 17a, b and 18a, b)

Compound structures				
				
17a	**17b**	**18a**	**18b**	
[^{1}H]NMR	δ (ppm)			
5-H	4.55	4.58	4.32	4.34
1′-H_A	2.01	1.89	1.84	1.72
1′-H_B	2.17	2.03	2.06	1.93
2′-H	3.90	3.86	3.92	3.84
3′-H_A	3.49	3.54	3.52	3.53
3′-H_B	3.57	3.60	3.61	3.60
CH_3	3.18	3.19	3.16	3.15
			3.09	3.07
	J (Hz)			
^2J(1′-H_A; 1′-H_B)	(−) 14.8	(−) 14.6	(−) 14.7	(−) 14.5
^2J(3′-H_A; 3′-H_B)	(−) 11.8	(−) 11.7	(−) 11.8	(−) 11.7
^3J(5-H; 1′-H_A)	4.5	9.8	6.0	9.4
^3J(5-H; 1′-H_B)	6.0	3.7	5.5	3.7
^3J(1′-H_A; 2′-H)	10.8	3.6	9.5	3.2
^3J(1′-H_B; 2′-H)	3.2	9.7	4.0	9.8
^3J(2′-H; 3′-H_A)	6.3	6.2	6.5	6.5
^3J(2′-H; 3′-H_B)	4.2	4.4	4.1	4.2
[^{13}C]NMR	δ (ppm)			
2C	161.6 (4)	161.7 (4)	170.2 (4)	172.0 (4)
4C	178.6 (4)	178.4 (4)	193.3 (4)	195.4 (4)
5C	59.2 (3)	58.8 (3)	62.2 (3)	62.1 (3)
1′C	35.7 (2)	36.7 (2)	37.1 (2)	38.1 (2)
2′C	70.9 (3)	70.8 (3)	71.8 (3)	71.7 (3)
3′C	68.1 (2)	68.1 (2)	68.3 (2)	68.5 (2)
CH_3	28.6 (1)	28.6 (1)	39.4 (1)	39.4 (1)
			41.1 (1)	41.1 (1)

δ, Chemical shift for the indicated bond; J coupling constant between the indicated bonds; (1), (2), (3), (4) denotes a primary, secondary, tertiary, or quaternary carbon atom, respectively, as indicated by the polarization in the [^{13}C]-DEPT NMR spectrum

Acknowledgement. The work reported here is taken in part from Sopio R (1994), Engeneer thesis, Naturwissenschaftlich-Technische Akademie, D-88316 Isny, Germany. We thank Priv.-Doz. Dr. Peter

Fischer, Institut für Organische Chemie, Universität Stuttgart, for valuable help with NMR and IR spectra interpretation and for helpful discussions.

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