*Tetrahedron* Vol 47, No 2, pp 317-322, 1991
Printed in Great Britain

0040-4020/91 $3 00+00


# SYNTHESIS AND STRUCTURE OF DEHYDRO-4-IMINOALLANTOIN AND ITS COVALENT ADDUCTS

T.POPOVIĆ, LEA SOKOLIĆ, NEVENKA MODRIĆ, A.PALKOVIĆ, and M.POJE*

Laboratory of Organic Chemistry, Faculty of Science, University of Zagreb, P.O.Box 153, 41001 Zagreb, Croatia, Yugoslavia

(*Received in UK 24 September* 1990)

**Abstract** – Representations of two consecutive products of the Denicke reaction were shown to be incorrect. Oxidation of uric acid (**1a**) by ferricyanide in aqueous ammonia gives 5-amino-4-iminoallantoin (**4**) and 1,5-diamino-3,7-dioxo-2,4,6,8-tetraazabicyclo[3.3.0]octane (**5a**), while methylated uric acids **1b-g** afford only the corresponding end products **5**. The key dehydro-4-iminoallantoin (**6**) arose on exposure of the primary adduct **4** to dilute acetic acid; a direct route to **6** was provided by oxidation of 4-iminoallantoin (**2**) with iodine. Both dehydro-allantoin (**8**) and its 4-imino analogue **6** form covalent adducts (e.g. with MeOH) at the 5-position. A brief rationale which focusses upon the stepwise nature and regiochemical course of the reaction is presented.

The accumulated evidence adds a new dimension to the biological significance of uric acid as a primary antioxidant which replaces some of the functions of ascorbate.[1,2] Recent studies of the uricolytic pathway to allantoin leave little doubt that the quinonoid dehydro-uric acid is a requisite intermediate.[3,4] These experiments define the ring-opening of the key 5-hydroxy adduct at the 1,6-bond and conclusively argue against the dogma of an initial ring-contraction. Oxidation of uric acid (**1a**) to 4-imino analogues **2** of allantoin, in the presence of ammonia[5] and primary or secondary amines,[6] eliminates any possible ambiguity concerning the incipient nucleophilic addition. The significance of these products lies not only in their intrinsic interest but also in the fact that they are likely to occur under physiological conditions. A particularly intricate set of rearrangements accompanies the oxidation of **1a** by ferricyanide[7] in aqueous ammonia. Denicke in 1906 reported the isolation of an unusual product, supposed to have the structure **3**.[5] Under the reaction conditions, this readily loses a molecule of water to give a secondary product, formulated as **4**. Most striking was the subsequent finding by Grohmann[8] that both **1c** and **1d** afforded an identical product, which was thought to be a methyl homologue of **3**. This result is rather surprising since it would mean that the Denicke transformation has an entirely different regiochemical course from that established for allantoin and dehydroallantoin.[3] Consideration of this problem led us to speculate that the Denicke rearrangement may have been misconstrued twice and that Grohmann's product might, in fact, be a hydrate of the secondary product.

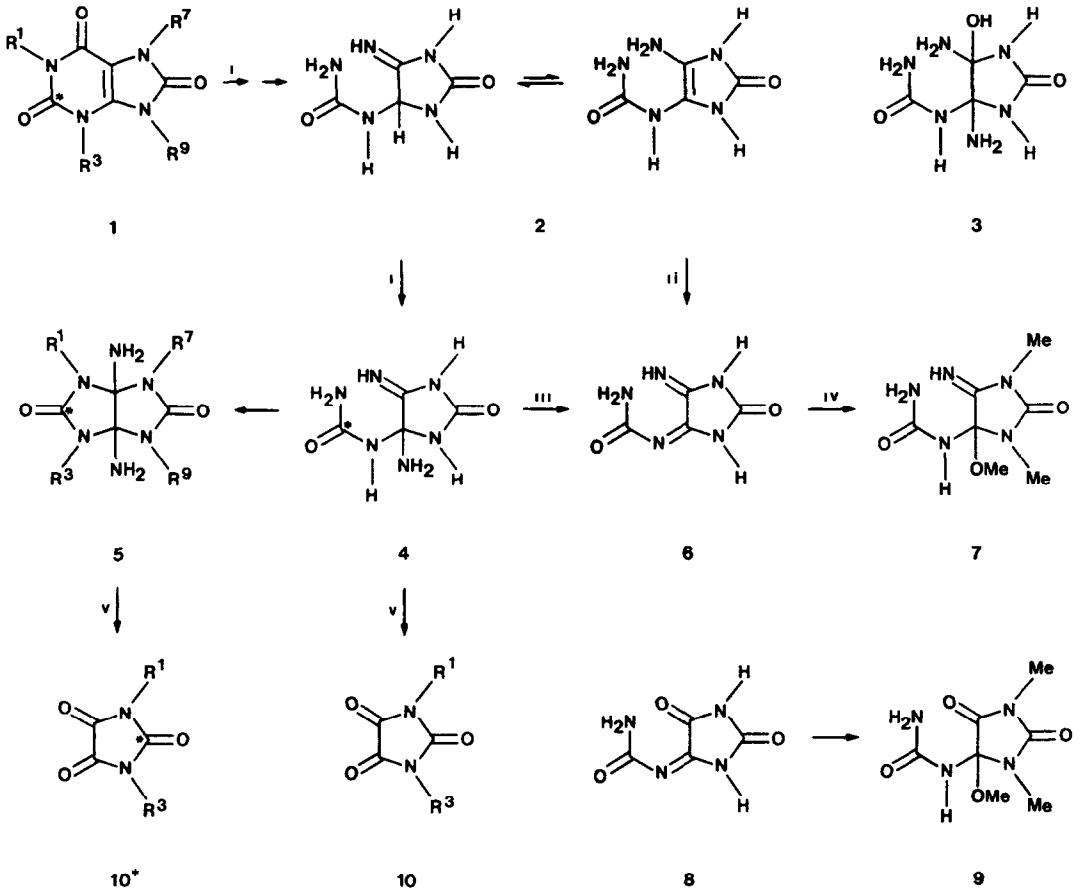
The first clue to the chemical nature of these products arose from degradative studies and IR spectral characteristics.[9] The labile primary product was rapidly transformed to dehydro-4-iminoallantoin (**6**) with dilute acetic acid. An identical compound was obtained by oxidation of 4-iminoallantoin (**2**) with iodine. Unambiguous evidence for the structure **6** derives from the reaction with ethereal diazomethane in the presence of methanol which yielded a well defined

crystalline adduct **7**, suitable for detailed spectroscopic characterization. Significantly, an analogous transformation occurred in conversion of dehydro-allantoin (**8**)[3] into 5-methoxy-1,3-dimethylallantoin (**9**). There remains then the question of the position of attachment of elements of $NH_3$ and $H_2O$ to the basic skeleton of **6**. In accordance with Denicke's observations, the primary oxidation product shows the presence of one molecule of water which is lost at 105°. On the other hand, it exhibits an IR absorption band at 1617 $cm^{-1}$, characteristic of C=N, which rules out the covalent hydrate array such as **3**, leaving two possible structures for the primary adduct with ammonia.[10] The type of structure which best fits the circumstances is the 5-amino-4-iminoallantoin (**4**) monohydrate, by analogy with adducts **7** and **9**. There are various forms of tautomerism which could operate in the different 4-iminoimidazolidin-2-one species. Imine-amine tautomerism in **2**, **4**, and **6** and the related lactam-lactim tautomerism as in **8** are not supported by IR evidence and seem to be of little importance.[11] It is possible and probably very likely that ring-chain tautomeric equilibria of the allantoin-bicyclol type are occurring in imine analogues.[4]

The secondary oxidation product, however, did not afford **6**. In addition, the nitrogen atom cannot be present as an amidine function, for the IR spectrum did not exhibit a band in the 1630-1600 $cm^{-1}$ region. Most of the regiochemical ambiguity was dispelled by experiments using isotopically labelled compounds derived from [2-$^{14}$C]uric acid. Parabanic acid (**10a**) obtained from hydrolysis of $^{14}$C-labelled **4** was non-radioactive, while the resultant urea had the same isotope content as the starting material. However, in the case of the secondary $^{14}$C-labelled oxidation product, hydrolysis proceeded with a nearly equal distribution of the label between urea and **10a**, a result which at once eliminates a non-symmetrical structure. Taken in conjunction with the molecular formula, this means that the secondary oxidation product must be a bicyclic diureide **5a**. The final proof for the structure **5** was provided by preliminary X-ray data (Fig. 1),[12] representing a new structural type among oxidation products of uric acid - a unique example of a bicyclic diureide of orthooxamide.

The reported properties for Grohmann's compound, however, were not in line with those expected for a homologue of the primary product, because it was unaffected by aqueous ammonia. The IR spectrum was not typical of structure **4**, but rather suggestive of the bicyclic structure **5**. A further confirmation was provided by conversion of both **1b** and **1e** into the same product **5b**. The alkylation sites exert a steering effect on the Denicke reaction, the failure of monosubstituted **1b-e** to give a homologue of **4** may be attributed to the relative efficiencies of ureide side-chain in performing the neighbouring group function.[4] Dimethyluric acids **1f** and **1g** afforded the corresponding end products **5f** and **5g** in high yields, when two of the three redox-active sites of uric acid at N(3), N(7), and N(9) were blocked, the reaction path was altered (Table 1). This can be rationalized by an effect of N-substitution on the stability of the obligatory urate anion radical intermediate. An interesting parallel exists with respect to the reactivity of uric acids (**1**) toward 2,2-diphenyl-1-picrylhydrazyl free radical.[13] It has also been demonstrated that urate radical formation occurs in some chemical and enzymic oxidations and that the radical site resides in the imidazolone subnucleus of the purine system.[14]

Regardless of the precise nature of the electron-abstracting process, the formation of the products by ring-opening at the 1,6-position of the quinonoid dehydro-uric acid precursor and the absence of an initial ring-contraction are thus a characteristic, self-consistent, and invariant pattern of behaviour in alkaline oxidations of uric acid.[15]



Scheme 1. Oxidation of uric acids (1) by ferricyanide in aqueous ammonia. **a** $R^1=R^3=R^7=R^9=H$, **b** $R^1$=Me; **c** $R^3$=Me, **d** $R^7$=Me, **e** $R^9$=Me, **f** $R^1=R^3$=Me, **g** $R^1=R^9$=Me, **h** $R^3=R^7$=Me, **i** $R^3=R^9$=Me, **j** $R^7=R^9$=Me, (*) designates $^{14}$C-labelled atom. I, $K_3Fe(CN)_6/NH_4OH$, II, $J_2$/AcOH,$NH_4OAc$, III, 10%AcOH, IV, $CH_2N_2/Et_2O$,MeOH, V, 10%HCl.

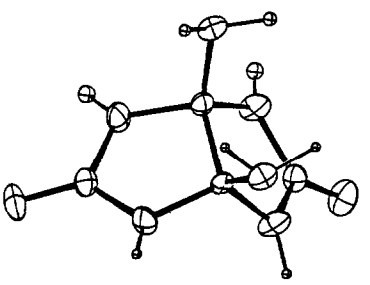


Figure 1. X-Ray structure of **5a** (R = 0.09) [12]

Table 1 Oxidation of methylated uric acids (1)

| 1 | Product | m p [c] | Composition [d] | ν(C=O) |
|---|---|---|---|---|
| b | **5b**(38%)[a] | 184-5° | $C_5H_{10}N_6O_2\,H_2O$ | 1730,1690 |
| c | **5b**(20%)[a] | 183-5° | ref 8 | 1730,1690 |
| d | **5b**(25%)[a] | 183-5° | ref 8 | 1730,1690 |
| e | **5b**(32%)[a] | 183-5° | $C_5H_{10}N_6O_2\,H_2O$ | 1730,1690 |
| f | **5f**(65%)[b] | 188° | $C_6H_{12}N_6O_2\,H_2O$ | 1725,1690 |
| g | **5g**(62%)[a] | 220-1° | $C_6H_{12}N_6O_2{\cdot}H_2O$ | 1687 |
| h-j | no crystalline product | | | |

[a] Prisms, [b] Needles, [c] Decomposition, [d] Satisfactory analytical data (±0.4%) were obtained, all compounds contained a molecule of water of crystallization which was lost at 60° in vacuum (0.001mm)

## EXPERIMENTAL

M.ps. were determined on a Tottoli apparatus (Buchi) and are uncorrected. IR spectra were recorded for KBr disks on a Perkin-Elmer 257 grating instrument. $^{1}H$- and $^{13}C$-NMR spectra were measured for DMSO-$d_6$ solns on a JEOL FX-100 spectrometer. Chemical shifts are given in $\delta$ units (ppm) relative to internal TMS. Coupling constants are expressed in Hz (s,singlet; d,doublet, t,triplet, q,quartet, mc, multiplet centre). Mass spectra were determined on a Varian MAT CH-7 instrument (70eV), m/e values are given with relative intensities (%) in parentheses. [2-$^{14}C$]uric acid (The Radiochemical Centre, Amersham, U.K.) was used for isotope distribution studies, specific activities were measured in Aquasol on a Beckmann LS-100 liquid scintillation counter (90% efficacy). Methylated uric acids **1b–j** were prepared according to the literature.[16]

*5-Amino-4-iminoallantoin* (**4**) *and 1,5-diamino-3,7-dioxo-2,4,6,8-tetraazabicyclo[3.3.0]octane* (**5a**)

The procedure of Denicke[5] was used with slight modifications. To a stirred suspension of finely powdered uric acid (**1a**, 16.8g, 0.1mol) in water (30ml) aqueous ammonia (200ml, 8%) was added. Powdered potassium ferricyanide (132g, 0.4mol) was gradually added to precooled (−2°) mixture and the temperature kept below 3° for 1hr. After stirring for further 30 min at room temperature, the reaction mixture was cooled to 0°, stirred for additional 1hr, and the precipitate was filtered off. Ferrocyanide was removed by dissolving in a minimal amount of cold water, and repeated treatment afforded the microcrystalline product **4** (8.55g, 45%). This product cannot be recrystallized unchanged, it looses water of crystallization at 105°; m.p. 295°dec. (Found C, 24.97, H, 5.53, N, 44.02. $C_4H_{10}N_6O_3$ Requires C, 25.27; H, 5.30, N, 44.19%). IR. 3540($H_2O$), 3455, 3360($CONH_2$), 3380sh, 3280($NH_2$), 3240–3160, 3130–3060(NH), 1725, 1695, 1670, 1650(C=O), 1617(C=N) $cm^{-1}$.

The crystalline product **5a** (5.16g, 30%), which slowly separated from the mother liquor (6–8days), was collected, washed with water, EtOH, and ether and dried. Recrystallization from water, accompanied by partial decomposition, gave the analytical sample, m p. >300°(browning >200°). (Found C, 27.73, H, 4.79, N, 49.00. $C_4H_8N_6O_2$ Requires C, 27.91, H, 4.68, N, 48.82%). IR 3380, 3350, 3300, 3280sh($NH_2$), 3260–3200, 3190–3130, 3110–3050(NH), 1735, 1690(C=O) $cm^{-1}$.

The yields of consecutive products **4** and **5a** depend on the reaction conditions, at higher temperature and concentration of aqueous ammonia only **5a** (50–60%) could be isolated. Significantly, high yields of **5a** (70–80%) were obtained on stirring **4** with conc aqueous ammonia for 5 h at room temperature.

*$^{14}C$-labelled* **4** *and* **5a**

The foregoing procedure was repeated with a suspension of [2-$^{14}C$]uric acid (420mg, 59.4 μCimmol$^{-1}$) in aqueous ammonia (8ml) and potassium ferricyanide (3.8g), to yield $^{14}C$-labelled **4** (161mg, 34%, specific activity 57.6 μCimmol$^{-1}$) and **5a** (142mg, 33%, specific activity 59.2 μCimmol$^{-1}$).

*Oxidation of N-methyluric acids* **1b–j** *by ammoniacal ferricyanide. General procedure*

Slightly modified Grohmann's procedure[8] was used on a 0.01 molar scale. To a stirred suspension of powdered methyluric acid (**1b–j**) in conc aqueous ammonia (40ml), finely ground potassium ferricyanide (13.2g, 0.04mol), was gradually added during 20 min. Stirring was continued for 1 hr, and then the mixture was cooled to 0°. Ferrocyanide which separated was filtered off and the filtrate was concentrated in a vacuum desiccator over CaCl. Colourless crystals that separated after standing for 1–2 days, were collected and washed with cold water. Recrystallization from water gave the analytical samples of **5**. Yields and analytical data are given in Table 1

*Dehydro-4-iminoallantoin* (**6**)

**Method A** A suspension of powdered **4** (3.8g, 0.02mol) in cold aqueous acetic acid (30ml, 10%) was stirred for 10 min. The resultant solid was filtered off, washed with cold water (6x5ml), EtOH, and ether and dried in vacuo ($10^{-3}$Torr, 48h) to yield **6** (3.0g, 97%) as a white powder, m.p.>300°. (Found C,30.87, H,3.43; N,44.84. $C_4H_5N_5O_2$ Requires: C,30.97; H,3.25, N,45.15%). IR 3460,3405($CONH_2$),3260-3200,3190-3130,3090sh(NH),1755,1680sh,1660(C=O),1635-1610(C=N) It is noteworthy that **6** forms an unstable compound with ammonia which is spectroscopically different from the initial adduct **4**; dehydro-4-iminoallantoin (**6**, 1.55g, 0.01mol), however, on stirring in conc aqueous ammonia, yielded colourless prisms (1.1g, 64%), identical with **5a**.[10]

**Method B** To a stirred suspension of 4-iminoallantoin[5] (**2**, 3.14g, 0.02mol) in a mixture of $NH_4OAc$ (6g) and acetic acid (20ml, 10%), powdered iodine (5.1g, 0.02mol) was gradually added After 15-20 min, the precipitate was filtered off, thoroughly washed with cold water, EtOH, and ether and dried. The resultant product (**6**, 1.3g, 42%) was identical with that prepared by the method A. On standing, an unsoluble substance becomes to separate from the mother liquor, this was identified as oxalyldiurea (**11**, 0.7g, 20%) by comparison with an authentic sample.[3]

*5-Methoxy-1,3-dimethyl-4-iminoallantoin* (**7**)

A suspension of finely powdered **6** (3.1g, 0.02mol) in methanol (40ml) and an excess of ethereal diazomethane (150ml) were allowed to react for 24 h. The product was filtered off, washed with MeOH, ether and dried. Recrystallization from MeOH/ether gave **7** (3.0g, 70%) as colourless prisms, m.p. 151°dec (Found· C,38.94, H,6.11, N,32.48. $C_7H_{13}N_5O_3$ Requires C, 39.07, H,6.09; N,32.54%). MS 215($M^{+}$,4),200(1),185(28),183(43),167(17),166(25),156(12),155 (20),141(32),140(100),83(77),72(12),71(15),70(23),58(26),57(61),56(65),55(26),43(23),42(17). IR: 3393,3280($CONH_2$),3290-3210,3200-3120(NH),1757,1690,1672(C=O),1600(C=N)$cm^{-1}$. $^1$H-NMR 7.94(s,1H,=NH),7.11(s,1H,NH),5.78(s,2H,$NH_2$),3 03(s,3H,OMe),2 95(s,3H,NMe),2.65(s,3H, NMe). $^{13}$C-NMR 161.0(C=N),157 0($C_7$),155.5($C_2$),93.6($C_5$),49.1(OMe),24.7(NMe),23.0(NMe).

*Conversion of dehydro-allantoin* (**8**) *into 5-methoxy-1,3-dimethylallantoin* (**9**)

The foregoing reaction with ethereal diazomethane (100ml) was repeated with dehydro-allanoin[3] (**8**, 1.56g, 0.01mol) in methanol (40ml). After 24 h, the resultant solution was filtered and evaporated to dryness The oily residue was crystallized from MeOH/$Et_2O$/light petroleum to yield **9** (2.14g, 99%). Recrystallization from $CH_2Cl_2$/$CCl_4$ gave the analytical sample, m p. 145-146°dec (Found C,38.63, H,5.74, N,25 70 $C_7H_{12}N_4O_4$ Requires C,38.89, H,5.59, N, 25.92%). IR 3460,3340($CONH_2$),3280-3180(NH),1782,1720,1690(C=O),1615(C=N)$cm^{-1}$ $^1$H-NMR 7.28(s,1H,NH),5 84(s,2H,$NH_2$),3.13(s,3H,OMe),2 92(s,3H,NMe),2.68(s,3H,NMe).

Analogous 5-ethoxy adduct was obtained in the presence of ethanol m.p.151-152°dec. $^1$H-NMR 7.28(s,NH),5.79(s,$NH_2$),3.33(qq,$OCH_2$,J=7.0),2.90(s,NMe),2.67(s,NMe),1.13(t,Me, J=7.0). These adducts were also obtained by dissolving dehydro-1,3-dimethylallantoin[3] in boiling methanol or ethanol and evaporation of the solvent.

*Acidic hydrolysis of* $^{14}$*C-labelled* **4** *and* **5a**

$^{14}$C-Labelled **4** (380mg, 10.3 μCimmol$^{-1}$) was heated in HCl (6ml, 10%) until dissolved. The soln was evaporated to dryness; repeated recrystallizations from water afforded non-radioactive parabanic acid (**10a**, 66mg, 29%) The mother liquors were passed through an Amberlite IR-45 ($OH^-$) column (10ml) which was eluted with water (50ml). Evaporation of the eluate gave [$^{14}$C]-urea (72mg, 60%), specific activity 10.6 μCimmol$^{-1}$. Hydrolysis of $^{14}$C-labelled **5a** (344mg, 8.8 μCimmol$^{-1}$) was carried out as described for **4** to yield $^{14}$C-labelled **10a** (68mg, 30%), specific activity 4.3 μCimmol$^{-1}$ and [$^{14}$C]urea (59mg, 49%), specific activity 4.0 μCimmol$^{-1}$.

*Acknowledgement* — We thank to the Research Council of Croatia for support of this work

## REFERENCES AND NOTES

[1] B.N.Ames, R Cathcart, E.Schwiers, and P.Hochstein, *Proc.Natl Acad.Sci.USA* **78**, 6858(1981), K.J.A.Davies, A.Sevanian, S.F.Muakkassah-Kelly, and P.Hochstein, *Biochem.J.* **235**, 747(1986).

[2] Uric acid (**1a**) has been shown to be oxidized at physiological pH by a number of haem-containing proteins, and allantoin was usually among the oxidation products The potential role of cytochrome-*c* mediated oxidation of **1a** in the aetiology of Reye's syndrome has received considerable attention in recent years *cf.* M.E.Martens, B.T Storey, and C.P Lee, *Arch.Biochem.Biophys.* **252**, 91 (1987).

[3] Evidence is overwhelming that, under alkaline conditions, nucleophilic addition to quinonoid dehydro-uric acid, opening of the pyrimidine ring at the 1,6-bond, and decarboxylative rearrangement occur with retention of geometrical configuration, and the initial product is thus never symmetrical *cf.* M.Poje and L.Sokolić-Maravić, *Tetrahedron* **42**, 747(1986), **44**, 6723(1988).

[4] N.Modrić, A.F.Drake, and M.Poje, *Tetrahedron Lett.* **30**, 5021 (1989). Circumstantial evidence indicates that N-substituents determine the position of ring-chain tautomeric equilibria between allantoins and their bicyclol forms, the (-)-menthyl ether of the bicyclol tautomer of 1,3-dimethylallantoin has been prepared and characterized

[5] G.Denicke, *Liebigs Annln Chem.* **349**, 269 (1906), both permanganate and ferricyanide produced 4-iminoallantoin (**2**) in ammoniacal solutions, however, an interesting difference between these two reagents was the formation of products beyond the 4-iminoallantoin stage in the case of ferricyanide, irrespective of stoichiometric ratios to uric acid (**1a**).

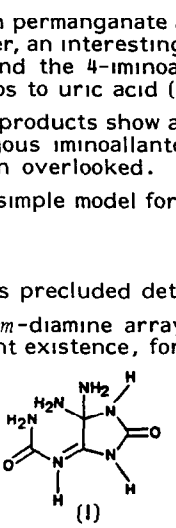
[6] P.H.Stahl, *Biochemistry* **8**, 733 (1969). These labile products show an increased UV absorption at about 250 nm, the potential formation of analogous iminoallantoins under biological conditions has frequently been misinterpreted or even overlooked.

[7] The ferro/ferricyanide couple can be thought as a simple model for the ferro/ferricytochrome system.

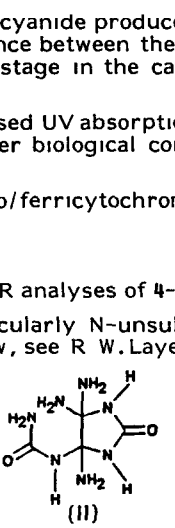
[8] O.Grohmann, *Liebigs Annln Chem.* **382**, 62 (1911).

[9] The instability and low solubility in organic solvents precluded detailed NMR analyses of **4-6**

[10] It is notorious that tetrahedral carbinolamine or *gem*-diamine arrays, particularly N-unsubstituted ones, are not stable and have only transient existence, for a review, see R W.Layer, *Chem.Rev.* **63**, 489 (1963). The equilibrium between the imine and *gem*-diamine in ammoniacal solution is so readily reversible that an isomeric *gem*-diamine (I) cannot be regarded as a probable alternative for the structure of primary product. However, the metastable *gem*-diamines (I) and/or (II) could well be intermediates in cyclizationa **4** → **5a** and **6** → **5a**, the fact that the conversion **4** → **5a** fails in the absence of ammonia would be consistent with such a view. The formation of tetrahedral arrays, such as the ester aminal in **7** and **9** or the ortho-amide arrays in **5**, points to an interesting stabilization by geminal diureide groupings.



(I)



(II)

[11] Although the imine-enamine tautomerism could explain different reactivities of allantoin (*cf.* ref.3) and its 4-imino analogue **2** toward the oxidation with iodine, IR evidence indicates that **2** exists in the imine rather than enamine form in the solid state 3420, 3340 ($CONH_2$), 3290-3250, 3200-3140, 3090-3010(NH), 2880(CH), 1723, 1660(C=O), 1610(C=N) $cm^{-1}$. 4-(Phenylimino)allantoin did not show any evidence for the presence of amine forms in DMSO soln, $^1$H-NMR 10,20(s,1H,$N_3$H), 7.92(d,1H,$N_1$H, J=0.9), 7.40(mc,5H,Ph), 6 97(d,1H,NH, J=8.9), 6 76(s,2H,$NH_2$), 5 80(dd,1H,CH,J=8 9,0.9) The available evidence suggests that the imine form predominates when there is a hydrogen on the amidine nitrogen *cf.* ref 6.

[12] Further refinement is in progress, complete X-ray data will be submitted to *Acta Cryst (C)*.

[13] R C Smith, J Reeves, M L McKee, and H.Daron, *Free Radicals Biol.Med.* **3**, 251 (1987).

[14] R Maples and R.P.Mason, *J.Biol Chem.* **263**, 1709(1988).

[15] Related to the course of formation of the symmetrical end product **5a**, is that of oxalyldiurea (**11**), a non-cyclic equivalent of **5a**, which results from the ring opening of **8** *cf.* ref 3.

[16] W.Pfleiderer, *Liebigs Annln Chem* 2030 (1974) and refs cited therein.