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KEY INTERMEDIATES IN THE CAFFOLIDE PATHWAY FOR DEGRADATION OF URIC ACID. X-RAY STRUCTURE OF METHYLAMMONIUM 1-METHYLCAFFOLIDE

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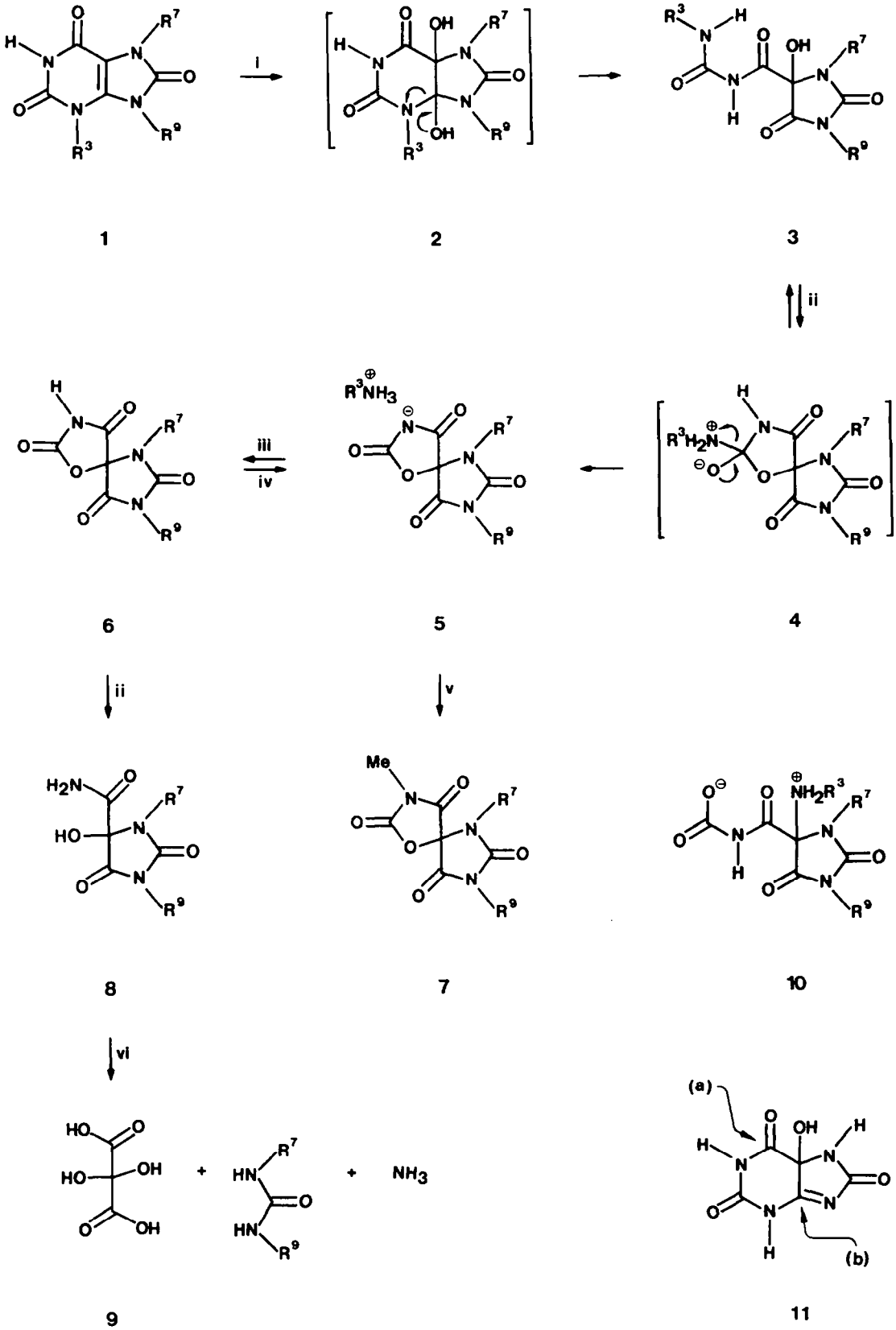
Abstract—Alloxanic acid ureides (3) formed by oxidative ring fission at the 3,4-bond of uric acids (1), undergo a facile rearrangement to isomeric products whose ammonium caffolide structure 5 was established by synthesis and confirmed by X-ray analysis of methylamine salt of 1-methylcaffolide (5b).

Recent studies on electrochemical oxidation of purines have provided valuable insights and guidance into chemical aspects of the redox mechanisms of enzyme processes.[1] The intermediates and products in peroxidase-catalysed oxidation of uric acid are virtually identical to those noted upon electrooxidation. Identification of allantoin and alloxanic acid amide, as the principal products at physiological pH values,[2] has reactualized the question of an alternative uricolytic pathway. It is of interest in this connection that a report has appeared describing the occurrence of an unidentified urinary excretion product in animals and man, which on alkaline hydrolysis yielded mesoxalic acid.[3] The unravelling of the alternative metabolic pathway presupposes, however, a detailed knowledge of oxidative transformations of purines which, eschewing β-cytotoxic alloxan-like intermediates, lead to alloxanic acid amide as a likely candidate for the precursor of mesoxalate. Although Biltz and his school[4] had determined the structure of caffolides (6)[5] and shown that they could be easily converted into alloxanic acid amides (8) and mesoxalic acid (9), the problem of the constitution of intermediates in the ring transformation 1 → 6 presented paradoxes which caused much confusion in this field, and little is known about chemical properties and biological role of these compounds.

We draw attention to the oxidative ring fission of uric acids (1) to alloxanic acid ureides (3)[6] which provides a biogenetically patterned access to three-carbon units. In this paper we extend the scope of this ring-modifying reaction, assigning the formula 3b to Clemm's oxy-3,7-dimethyluric acid,[7] and establishing the structure of iso-oxy-3,7-dimethyluric acid and related constitutional isomers of ureides 3. The isomerization of 3 was effected by simple heating with water, and the same, apparently untenable, structure was originally attributed to the products.[8] The acidic work-up gave caffolides (6),[9] and the obvious inference that the compounds are ammonium salts 5 has now been confirmed by their resynthesis from 6 and ammonia or methylamine. Products 5 were identical to those prepared by Biltz's methods,[8] as judged by general physical and chemical properties, mixed melting-point determinations, and spectral characteristics (Experimental); the infrared spectra support the assigned structure as salts 5 lacked absorption in the 1600-1550 cm^{-1} region typical of the zwitterionic alternative 10. The reported decomposition into alloxanic amides (8) or mesoxalate (9) and the immediate and quantitative liberation of ammonia or methylamine on addition of a strong base is no longer surprising with the revised constitution, and salts 5 are converted into trimethylcaffolide (7) and trimethylamine by reaction with an excess of ethereal diazomethane.

In order to remove any equivocation about the structural assignment we took advantage of the propensity which the compound 5b showed to produce well formed crystals and carried out a single-



Scheme. a: $R^3 = R^7 = R^9 = H$; b: $R^3 = R^7 = Me$, $R^9 = H$; c: $R^3 = H$, $R^7 = R^9 = Me$
i, $Cl_2/H_2O,\Delta$; ii, H_2O,Δ; iii, H^+; iv, $R^3NH_2/EtOH$; v, CH_2N_2/Et_2O; vi, OH^-.

crystal X-ray analysis (Figure 1).†

The revised constitution of the key intermediates requires a modification of the mechanism for the ring transformation 1 → 6. The precise nature of the initial oxidation step is still an open question, but it seems likely that allantoin and alloxanic acid ureide (3a) are formed *via* incipient quinonoid species, dehydro-uric acid; the branching pathways result from different ring opening modes at the 5-hydroxy-isouric acid (11) stage[10]. Accordingly, the ring fission at the 3,4-bond of 2, initiated by attack at the 4-position of 11, occurs after the proton transfer. The smooth isomerisation of alloxanic ureides (3) into caffolide salts 5 represents a new example of the cyclization of α-hydroxy acylureas and is presumably due to a proximity effect; the formation and decomposition of tetrahedral orthocarbonate-type intermediate 4 is rate determining. The ring opening and decarboxylation of caffolides 5 and 6 occurs under relatively mild conditions to give alloxanic acid amides (8). The biomimetic sequence 3 → 8 (Scheme) could, therefore, nicely account for the formation of the three-carbon precursors of urinary mesoxalate, providing a basis for further investigations of an alternative uricolytic pathway *in vivo*.

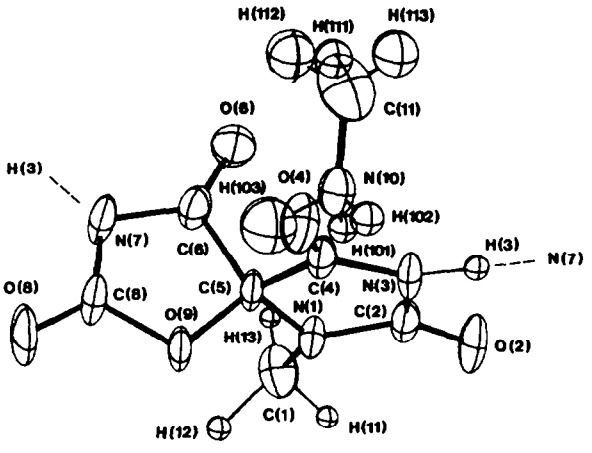


Figure 1. ORTEP view of the molecule 5b.†
H-bond: N(3)-H(3)...N(7) 1.81(1) Å, 170.0(2)°

EXPERIMENTAL

M. ps. were determined on a Tottoli apparatus (Büchi) and are corrected. IR spectra were recorded for KBr disks on a Perkin-Elmer 257 instrument. NMR spectra were measured on a JEOL FX-100 spectrometer in DMSO-d_6 solns. Chemical shifts are given in ppm relative to internal TMS, and coupling constants are expressed in Hz. Elemental analyses (C, H, N) were within ±0.3% of calculated values. Alloxanic acid ureides 3a and 3c were prepared as described previously;[6] an analogous structure was assigned to the compound obtained by chlorination of theobromine or 3,7-dimethyluric acid (1b) according to original procedures;[7] 3b, m.p.203-4° decomp (H_2O). IR 3315,3130,3050,1790,1740,1730,1715,1690,1540 cm^{-1}. ^1H NMR, δ 11.23(s,1H,NH), 9.52(s,1H,NH), 7.91(q,1H,NHMe,J=4.7), 6.80(b,1H,OH), 2.73(d,3H,NHMe,J=4.7), 2.70(s,3H,NMe). ^{13}C NMR, δ 170.4(s), 166.2(s), 155.5(s), 151.6(s), 86.6(s), 26.2(q), 24.1(q).

Preparation of Caffolide Salts 5. To a stirred solution of caffolides 6a-c (0.01mol) in the minimum of dry ethanol 1*N* ethanolic ammonia or methylamine (10ml) was gradually added and the crystalline precipitate was collected. *Ammonium caffolide* (5a,1.8g,90%), m.p. 211° decomp alone and 207-8° when mixed with a sample prepared from 3a (lit.[8a] m.p. 204-6° decomp). IR 3300-3000,1800sh,1780,1745, 1635 cm^{-1}. ^1HNMR, δ 6.90(b) was shifted to δ 7.38(t,NH_4^+,J_{NH}=51) by addition of trifluoroacetic acid. *Methylammonium 1-methylcaffolide* (5b,2.0g,87%), m.p. 203-4° decomp (H_2O) alone and when mixed with a sample prepared from 3b (lit.[8b] m.p. 203-4° decomp). IR 3240,3180,2910,2690,1800sh,1755,1735,1670 cm^{-1}. ^1H NMR, δ 7.53(b,4H), 2.63(s,3H,NMe), 2.43(s,3H,$MeNH_3^+$); on addition of trifluoroacetic acid the signals appeared at δ 7.77(b,3H,$MeNH_3^+$), 2.43(q,3H,$MeNH_3^+$,J=6), and 2.88(s,6b-NMe). ^{13}C NMR, δ 180.2(s), 169.0(s), 167.1(s), 155.4(s), 91.1(s), 24.3(q), 24.2(q). *Ammonium 1,3-dimethylcaffolide* (5c,1.9g,83%), m.p. 216° decomp (H_2O) alone and when mixed with a sample prepared from 3c (lit.[8c] m.p. 216° decomp). IR 3220,3110,1800,1788,1726,1650 cm^{-1}. ^1H NMR, δ 6.86(b,4H,NH_4^+), 2.92(s,3H,NMe), 2.67(s,3H,NMe); on addition of trifluoroacetic acid the signals appeared at δ 7.37(t,4H,NH_4^+,J_{NH}=51) and 3.00s, 2.89s (6c-NMe). ^{13}C NMR, δ 180.0(s), 167.7(s), 167.1(s), 155.3(s), 90.3(s), 24.8(q), 24.6(q).

Conversion of 5 *into Caffolides* (6). Aqueous solutions of 5a-c (0.01mol) were passed through an Amberlite IR-120 column (H^+-form,30ml), which was, in turn, washed with water (100ml). Evaporation of eluates under reduced pressure gave the corresponding caffolides in virtually quantitative yields. *Caffolide* (6a) was obtained as needles from Et_2O/hexane, m.p. 220-1° decomp (lit.[9] m.p. 220° decomp); *1-Methylcaffolide* (6b) crystallized as prisms (H_2O), m.p. 215-6° decomp (lit.[8b] m.p. 215-6° decomp); *1,3-Dimethylcaffolide* (6c), plates ($CHCl_3$), m.p. 163-4° (lit.[8c] m.p. 163-4°).

Reaction of 5 *with diazomethane*. Finely powdered 5a-c (0.002mol) and an excess of ethereal diazomethane (150ml) were allowed to react for 48h. From the ethereal distillate trimethylamine was isolated as its hydrochloride (0.1g), m.p. 277-9° decomp, and the oily residue was crystallized from glacial acetic acid to give 1,3,7-trimethylcaffolide (7, 0.32-0.36g, 70-80%), m.p. 204-5° (lit.[5] m.p. 205°). IR 1838,1800,1765,1735 cm^{-1}. ^1H NMR, δ 3.10s, 3.00s, 2.90s (NMe).

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

Crystallography. A parallelepiped-shaped crystal of 5b (0.17 x 0.15 x 0.05 mm), prepared by isomerisation of 3b according to Biltz and Topp's method,[8a] was used for single-crystal X-ray analysis. *Crystal data*. Crystals of 5b, $C_7H_{10}N_4O_5$, $M = 230.18$, are monoclinic, space group $P2_1/n$, with $a = 12.385(4)$, $b = 10.190(4)$, $c = 8.104(3)$ Å, $\beta = 96.89(3)^\circ$, $V = 1015.36$ Å^3, $D_c = 1.506$ gcm^{-3}, $Z = 4$, $\mu(CuK_\alpha) = 9.41$cm^{-1}. The data were collected on a Philips PW 1100 diffractometer with a graphite monochromator (CuK_α radiation, $\lambda = 1.5418$ Å, $11 \leq \theta \leq 140^\circ$). The intensities of 1064 independent reflections with $I > 3\sigma(I)$, corrected for Lorentz and polarization effects, were used in structure determination. The structure was solved by direct methods[11] and refined by full-matrix least-squares procedure to give a final $R = 0.049$ ($R_w = 0.057$). The structure of 5b consists of discrete $C_6H_4N_3O_5^-$ and $CH_3NH_3^+$ ions connected by hydrogen bonds (Figure 1).[12]

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