Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 14, pp. 1685-1686, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00216-9

The Structure of a $C_5H_4N_4O_4$ Species Trapped by Silylation in Peroxidase Mediated Uricolysis.

A Reactive Ring-Contraction to Spirodihydantoin

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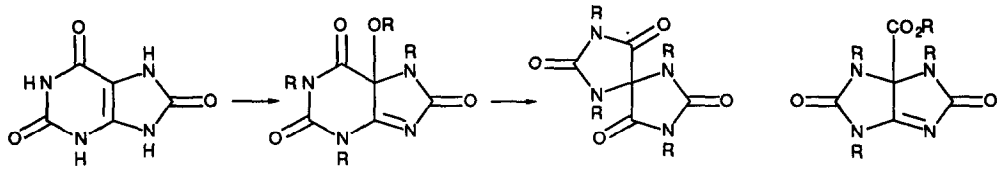
Abstract: Evidence for the species trapped by trimethylsilylation in peroxidase mediated oxidation of urate (**1**) having the tetrakis(trimethylsilyl)spirodihydantoin (**4b**) structure is provided by its hydrolysis, reaction with diazomethane, and actual isolation and identification of tetramethylspirodihydantoin (**4c**).

Following the first report on the stepwise nature of the uricase reaction, a transient UV-absorbing species has been detected sufficiently often in chemical and enzymic oxidations of uric acid (**1**) to establish it as being of general validity as the initial uricolytic intermediate.[1] This intermediate has hitherto defied all attempts at direct identification owing to its high chemical reactivity. Hence, the assumption, chiefly based on inferential evidence,[2] that **2a** is the key uricolytic intermediate has long been awaiting an experimental confirmation.[3] A problematic ring contraction process **2a**→**3a** is often encountered in mechanistic interpretations of the pathway to allantoin.[4] The announcement that **3a** can be trapped as **3b** by silylation during the initial stage of electrochemical or peroxidase-catalysed oxidation of **1**[5,6] is therefore sufficiently provocative that it can hardly avoid drawing critical attention to the evidence on which it is based. In the absence of a detailed structural information this evidence consists of the mass spectrum of a tetrasilylated species, exhibiting a molecular ion at m/e 472, the most intense peak at m/e 357, and prominent fragments at 457, 342, 329 and 314. In fact, the reported mass spectral data have a suspicious resemblance to those of the tetrasilylated derivative **4b**[7,8] of spirodihydantoin (**4a**).[9]



1 **2** **4** **3**

a: R = H; **b**: R = $SiMe_3$; **c**: R = Me

To decide whether the silylated species lies on the pathway leading to allantoin or it is merely an alternative end-product of the silylation reaction, the oxidation of **1** with peroxidase [EC 1.11.1.7] was quenched and the UV-absorbing intermediate trapped according to the published procedure,[6] and the resultant silylation mixture was evaporated *in vacuo*. The residual solid was dissolved in hot water (10 ml), evaporated, and dried (10^{-4} Torr, 48 h over P_2O_5). This was treated with ethereal diazomethane (2×10 ml); after 48 h ether was removed and the product (*Rf* 0.16) isolated by preparative TLC (0.5 mm silica gel PF_{254}, $CHCl_3$). Sublimation (220°C, 10^{-3} Torr)[10] yielded the pure product (*ca.* 3 mg) identical with the authentic tetramethylspirodihydantoin (**4c**).[11]

This simple experiment has been used successfully to demonstrate the stable nature of the basic skeleton of the species trapped by trimethylsilylation. It is obvious that the parent spirodihydantoin (**4a**) cannot be a uricolytic intermediate, but the spiro-contraction does provide support for **2a** as the real intermediate since it is most unlikely that **3a** could undergo the sequence which ultimately leads to actual silylated **4b**.

In the explanation of the ring-modifying reaction of **2a** under silylation conditions, two general types of mechanism may be discerned. The reaction could clearly involve either [6→4]ring-contraction by shift of carbon or [4→5]ring-contraction by shift of nitrogen, *via* a hydration equilibrium. No detailed information is available in order to allow a clear distinction between these regiochemical alternatives, which are otherwise reminiscent of ring-contraction processes in flavin pseudobases.[12] Therefore, all the available experimental evidence now seems consistent only with the intermediacy of **2a** without recourse to **3a**; a coherent uricolytic pathway to allantoin involves ring opening at the 1,6-bond, migration of carboxylate, and decarboxylation as salient steps.[2,3]

References and Notes

1 For reviews, see Mahler, H. R., "Uricase", in The Enzymes, 2nd ed., Vol. 8, eds. P. D. Boyer *et al.*, Academic Press, N. Y., 1963, pp. 285-296; Vogels, G. D.; Van der Drift, C. *Bacteriol. Rev.* **1976**, *40*, 403-468.

2 Poje, M.; Sokolić-Maravić, L. *Tetrahedron* **1986**, *42*, 747-751; *Ibid.* **1988**, *44*, 6723-6728; Sokolić, L.; Modrić, N.; Poje, M. *Tetrahedron Lett.* **1991**, *32*, 7477-7480; Modrić, N.; Poje, M.; Watkin, D. J.; Edwards, A. J. *Ibid.* **1993**, *34*, 4679-4682.

3 Modrić, N.; Derome, A. E.; Ashcroft, S. J. H.; Poje, M. *Tetrahedron Lett.* **1992**, *33*, 6691-6694.

4 The tetraazabicyclo[3.3.0]octene structure **3a** for the incipient intermediate (λ_{max} 305nm) was first proposed by Bentley, R.; Neuberger, A. *Biochem. J.* **1952**, *52*, 694-699; Dalgliesh, C. E.; Neuberger, A. *J. Chem. Soc.* **1954**, 3407-3414. *Cf.* also ref. 1.

5 Goyal, R. N.; Brajter-Toth, A.; Dryhurst, G. *J. Electroanal. Chem.* **1982**, *131*, 181-202. In earlier papers the trapped species had been regarded as **2b**: *cf.* Brajter-Toth, A.; Dryhurst, G. *Ibid.* **1981**, *122*, 205-213. If, instead of quenching the reaction, the initial UV-absorbing intermediate has been allowed to decay away and the resulting solution is lyophilized and then silylated, a pentasilylated derivative of allantoin was detected by its mass spectrum.

6 Goyal, R. N.; Brajter-Toth, A.; Dryhurst, G.; Nguyen, N. T. *Bioelectrochem. Bioenerg.* **1982**, *9*, 39-60. The procedure adopted by us was following: reaction of **1** (3m*M*, 4ml) with peroxidase (*Sigma*, type XII, 80 units/ml, 3ml) in 0.5*M* NaCl and 5m*M* phosphate (*pH* 7.5) was quenched by freezing the mixture in a thin layer (liquid N_2) 100 s after addition of H_2O_2 (10m*M*, 3ml). This was lyophilized (<0°C, 4 10^{-4} Torr), and the dry residue heated (125°C, 30 min) with bis(trimethylsilyl)acetamide (3ml) in pyridine (10ml). The procedure was repeated ten times, and the mixtures were combined and used in the next step.

7 Tetrakis(trimethylsilyl)spirodihydantoin (**4b**), m. p. 128-130°C, was prepared in up to 88% isolated yield by heating (130°C, 30 min) powdered **4a** (552 mg, 3 mmol) and bis(trimethylsilyl)acetamide (*Merck*, 10ml) in dry pyridine (10ml); after removal of solvent the residue was kugelrohr-distilled (trimethylsilylacetamide: 95°C/10^{-2} Torr, and **4b**: 190°C/10^{-2} Torr). MS, m/e 474(1), 473(2), 472(4, *M*+), 459(3), 458(6), 457(14), 359(14), 358(29), 357(100), 344(2), 343(3), 342(8), 331(2), 330(3), 329(8), 316(2), 315(4), 314(9), 301(1), 286(2), 285(5), 270(1), 269(1), 242(2), 241(1), 199(5), 188(3), 174(1), 173(2), 172(2), 171(6), 147(7), 127 (3), 100(10), 73(43). IR (KBr) 1780, 1710 (CO) cm^{-1}. NMR ($CDCl_3$), δ^1H: 0.45 (s, $SiMe_3$), 0.32 (s, $SiMe_3$); δ^{13}C 174.5 (s, C-4), 162.3 (s, C-2), 83.6 (s, C-5), -0.9 (q, $SiMe_3$), -1.1 (q, $SiMe_3$).

8 M. ps. are corrected. IR spectra: Perkin-Elmer FT-IR 1725X; NMR spectra: Varian Gemini-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz; in δ units from internal TMS or $CDCl_3$). Mass spectra: Varian MAT CH-7, 70 eV, 100 μA, 3 kV.

9 For classical chemistry of spirodihydantoins (**4**), see Biltz, H. *J. Prakt. Chem.* **1936**, *145*, 65-228 (*cf.* pp. 220-228); it should be emphasized that the bicyclic uric acid glycol structure, originally assigned to the synthetic precursor of **4a**, was subsequently revised and shown to be a 3,4-ring-opened isomer: Poje, M.; Paulus, E. F.; Ročić, B. *J. Org. Chem.* **1980**, *45*, 65-68.

10 The substance was transferred into a tube (6×1 cm) with hot methanol and concentrated at its lowest part by slow evaporation of the solvent. A simple sublimation device, dealing with minute amounts of material, consists of a stout capillary, acting as a receiver, made by drawing out the tube *ca.* 3 cm above the sample.

11 **4c**, m. p. 228-229°C, was prepared by reaction of **4a** with an excess of ethereal diazomethane (*cf.* ref. 9). MS, m/e 240(40), 225 (2), 211(35), 183 (18), 168(4), 155(66), 154(14), 140(9), 127(2), 126(4), 98(7), 83(14), 70(100). IR (KBr) 1776, 1718 (CO) cm^{-1}. NMR (DMSO-d_6), δ^1H: 2.99 (s, NMe), 2.77 (s, NMe); δ^{13}C: 165.4 (s, C-4), 155.2 (s, C-2), 80.0 (C-5), 25.5 (s, 2NMe).

12 Mechanistic precedents are found in formation of their isomeric 10a- and 4a-spirohydantoins: Smith, S. H.; Bruice, T. C. *J. Am. Chem. Soc.* **1975**, *97*, 2875-2881; Iwata, M.; Bruice, T. C.; Carrell, H. L.; Glusker, J. P. *Ibid.* **1980**, *102*, 5036-5099.

(*Received in Belgium* 5 *April* 1994; *accepted* 25 *May* 1994)