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Absolute Stereochemistry of Alloxanic Acid.

A Configurational Relay for Uricase Reaction

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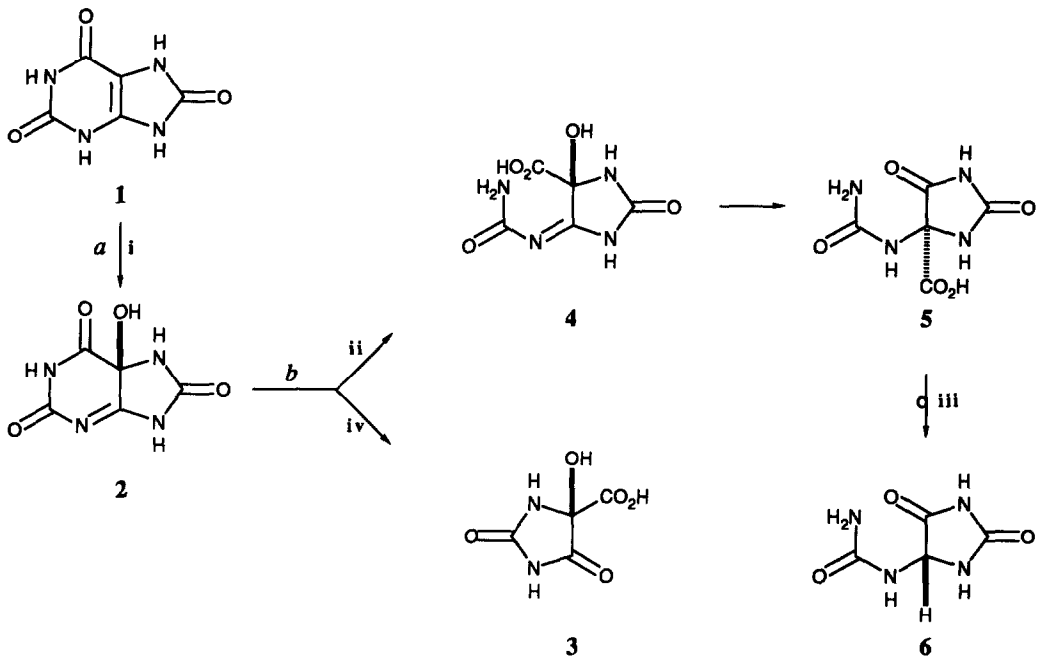
Abstract: Alloxanic acid (**3**) was resolved by means of brucine and converted into its enantiomorphic esters **8** and amides **9**; ethyl (-)-alloxanate whose absolute (*S*)-configuration was established by *X*-ray analysis served as configurational standard for the correlation of (+)-(*R*)-(**3**) and its precursor (-)-(*S*)-5-hydroxyisourate (**2**), the key uricolytic intermediate.

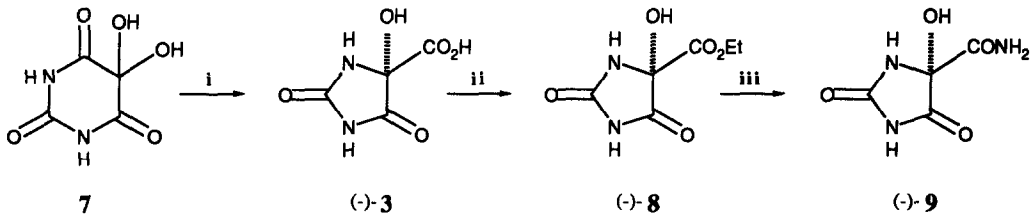
There are two stereochemical ambiguities in the uricolytic pathway to allantoin (**6**).[1] The first problem is met in the facial selectivity of the uricase-mediated formation of the key 5-hydroxyisourate (**2**). The next stereochemical question arises as to whether non-enzymic decarboxylation **5** → **6** proceeds with retention or inversion of configuration.[2] One way of overcoming this limitation might be to take advantage of Vogels' observation that, when borate was present, the uricase yielded (+)-alloxanate (**3**), instead of the natural product (+)-**6**.[3] The transient appearance of a laevorotatory intermediate was detected in both cases. Tracer studies have confirmed that **3** was derived by pyrimidine ring-contraction.[4] The reaction is reminiscent of the alloxan → alloxanate rearrangement, specifically catalysed by borate.[5] This remarkable transformation thus provides a convenient relay for the configurational correlation of **2**, as a necessary precursor with an alloxan-like structure. The configuration of (+)-**6** has already been shown to be (*S*),[6,7] and considerations of conformational asymmetry[8] indicate that (*R*)-**3** should be dextrorotatory (Scheme 1). Accordingly, we report here the resolution of alloxanic acid (**3**), synthesis and characterization of its related optically active esters and amides (Scheme 2), and the *X*-ray determination of the absolute configuration of ethyl (-)-alloxanate (**8**).

Preparation of diastereomeric brucine salts of **3** was achieved in a variety of ways. We found the most favourable method involved refluxing equimolar alloxan (**7**) and brucine in H_2O/MeOH for 30 min, followed by removal of MeOH. This gave brucine salts in 80-85% overall yields. After initial unsuccessful attempts we developed a simple and reliable method for resolution. The best conditions were found to be fractional crystallization from DMSO/EtOH.[9] The hemiaminal functionality at the chiral centre presented a special problem in subsequent work with optically active alloxanic acids and their derivatives. A suspension of finely powdered brucine salt of (-)-**3** (3.3 g) in water (30 ml) was therefore decomposed by stirring it with Dowex 50W resin (15 g). After 1 h, this was filtered and the filtrate passed through a Dowex column. The eluates were lyophilized and dried (10^{-3} Torr, 24 h). This product was dissolved in dry acetone and filtered. The clear filtrate was evaporated *in vacuo* and dried to yield configurationally homogenous (-)-alloxanic acid (**3**, 878 mg, 91%) as a hygroscopic crystalline powder, m.p. 138-140°C dec, $[\alpha]_D^{25}$ -42.1 (*c* 12, EtOH).[10]

Taken in part from the Ph.D. Thesis of N. M., University of Zagreb, 1991.



Scheme 1. Regio- and stereochemical course of uricase reaction.
a) enzyme-mediated step: i, O_2, *b)* non-enzymic decay: ii, OH^-, iii, [$-CO_2$]; iv, borate [-urea].



Scheme 2. i, brucine[H_2O/MeOH], Δ; [DMSO/EtOH]; H^+; ii, EtOH ($SOCl_2$); iii, NH_4OH.[10]

The esterification of (-)-**3** (690 mg) was carried out in dry EtOH (15 ml, 0°C) with thionyl chloride (0.2 ml). After removal of the solvent, the residue was repeatedly treated with dry EtOH and evaporated to dryness *in vacuo*. Recrystallization from acetone/chloroform afforded the (-)-ester **8** (700 mg, 86%) as colourless prisms, m.p. 136-137°C; $[\alpha]_D^{25}$ -50.0 (*c* 6, EtOH). IR (KBr) 1802, 1762, 1743, 1728 cm^{-1}. NMR (DMSO-d_6): δ(^{1}H) 11.00 (*s*, NH), 8.91 (*s*, NH), 7.45 (*bs*, OH), 4.17 (*q*, CH_2), 1.18 (*t*, Me); δ(^{13}C) 171.9 (*s*, C4), 167.1 (*s*, CO), 156.7 (*s*, C2), 84.2 (*s*, C5), 62.3 (*t*, CH_2), 14.2 (*q*, Me).[10] Three recrystallizations gave crystals suitable for *X*-ray analysis. The crystal structure revealing the absolute (*S*)-configuration of the ethyl (-)-alloxanate is presented in Fig. 1.

Crystal data for (-)-**8**: $C_6H_8N_2O_5$, M_r = 188.140, monoclinic, space group $P2_1$ (No. 4), a=7.156(1), b=6.466(1), c=8.419(2) Å, β = 91.69(1)° (from least squares fitting of setting angles for 22 reflections $28.2 \leq \theta \leq 45.9°$), V = 389.4 Å^3, Z=2, D_x=1.605 gcm^{-3}, $\mu(CuK_\alpha)$ = 11.85 cm^{-1}. Data were collected on a CAD-4F diffractometer in ω:2θ scan mode, $0 < 2\theta \leq 72°$ ($-8 \leq h \leq 8$, $-1 \leq k \leq 7$, $-1 \leq l \leq 10$); 2758 reflections measured, 1502 unique (R_{merge} = 0.035) of which 1478 were observed ($I \geq 3\sigma I$). No significant variation in intensity of 3 check reflections was observed. Data were corrected for Lorentz and polarization effects[11] and for absorption (min. and max. corrections of 1.55 and 2.03, respectively).[12]

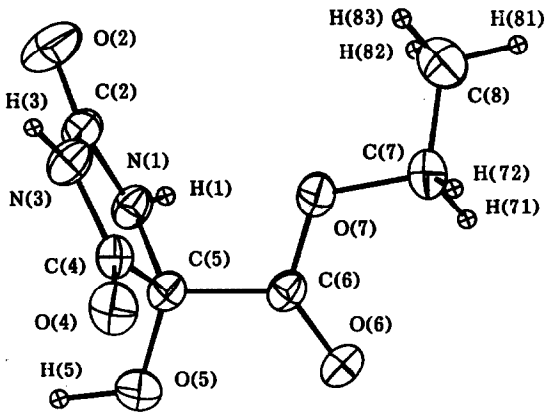


Fig. 1. The molecular structure of (-)-**8**

The structure was solved by direct methods (*SHELX86*).[13] Refinement of the model was undertaken using the *CRYSTALS* program package.[11] Full matrix least-squares refinement of positional and anisotropic thermal parameters for all non-hydrogen atoms was continued until convergence, the hydrogen atom coordinates were geometrically calculated for those attached to carbon and were as observed for those on oxygen and nitrogen. A Flack enantiopole[14] was refined using all Friedel pairs and converged to a value of -0.25(17) consistent with the absolute (*S*)-stereochemistry. An extinction correction was applied and a 3-term Chebychev polynomial weighting scheme was employed. At convergence R = 0.027, R_w = 0.032 for 120 parameters.†

Ethyl (-)-(*S*)-alloxanate (**8**) thus serves as a primary configurational standard for the correlation of other uricolytic products. The (*R*)-amide **9** has been correlated through reaction of (-)-**8** (201 mg, 1.1 mmol) with concentrated ammonia (1.5 ml). After 15 min at room temperature, the solution was evaporated *in vacuo* and the residue passed through a Dowex column. The eluates were lyophilized and recrystallization from EtOH yielded the (-)-amide (**9**, 107 mg, 61%) as colourless prisms, m.p. 174-176°C dec, $[\alpha]_D^{25}$ -18.3 (*c* 4, H_2O).[10,15]

In conclusion, the *X*-ray data have allowed determination of the absolute (*R*)-configuration of the enzymic (+)-alloxanate (**3**). Since the observed labelling pattern[4] places some important constraints on the steric course of the ring contraction, we can now establish the (*S*)-configuration for the elusive (-)-5-hydroxy-isourate (**2**). This is consistent with (5*Si*) facial attack (Scheme 1, *a*), following the uricase-catalysed oxidation of urate (**1**). The complete correlation network for the uricolysis to (+)-(*S*)-allantoin (**6**), must therefore correspond to the ring opening of the common precursor **2**, suprafacial 1,2-shift of carboxylate (*S*)-**4** → (*S*)-**5**, and to inversion of configuration in the final decarboxylation step, as shown in Scheme 1.[1,2] Studies are currently in progress to delineate fully the mechanism(s) of borate-mediated ring transformations of **2**.

Acknowledgement - This work was supported in part by the Ministry of Science of the Republic Croatia.

† Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW.

References and Notes

[1] For mechanistic details, see 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.

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

[3] Bongaerts, G. P. A; Vogels, G. D. *Biochim. Biophys Acta* **1979**, *567*, 295-308. ORD spectra recorded during reaction with uricase from *Bacillus fastidiosus* firmly established the stereoselective course for the conversion into (+)-**3** through a laevorotatory precursor. The published ORD curve for the enzymic product **3** had a positive Cotton effect at the inflection point (λ_o near 235 nm). Similarly, the authentic (+)-acid **3** showed a positive CD peak at 237 nm (0.1*M* borate, *pH* 8).

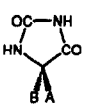
[4] Canellakis, E. S.; Cohen, P. P. *J. Biol. Chem.* **1955**, *213*, 385-395 have identified alloxanate (**3**) and urea as the main products when borate was present in uricase reaction medium and clearly demonstrated regioselective incorporation of the tracer from [2-^{14}C]urate into **3** and from [8-^{14}C]urate into urea.

[5] Kwart, H.; Sarasohn, I. M. *J. Am. Chem. Soc.* **1961**, *83*, 909-919; Kwart, H.; Spayd, R. W.; Collins, C. J. *Ibid.* **1961**, *83*, 2579-2580 have confirmed that the ring contraction **7** → **3** involves a 1,2-shift of nitrogen.

[6] 's-Gravenmade, E. J.; Vogels, G. D.; Van Pelt, C. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 929-939.

[7] Modrić, N.; Drake, A. F.; Poje, M. *Tetrahedron Lett.* **1989**, *37*, 5021-5024; Modrić, N.; Poje, M.; Vicković, I.; Bruvo, M. *Acta Cryst.* **1990**, *C46*, 1336-1338.

[8] Modrić, N. *Ph.D. Thesis*, University of Zagreb, 1991. The representation chosen here is an enlightened guess, based on empirical Brewster's rules (*cf.* Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962; pp. 401-412), that the configuration (i) is dextrorotatory when the polarizability order of other two substituents is A > B; apart from allantoin and hydantoin-5-acetic acid,[6,7] this has now been found to be true for **3**. According to the assumption embodied in (i) the enantiomer **5** (shown in Scheme 1) should be dextrorotatory, whereas **4** has opposite configuration (as an amidine analogue of i) and is therefore laevorotatory.



(i)

[9] Resolution by crystallization from water and alcohol solutions failed because of formation of a double salt. Therefore, dry EtOH (300 ml) was gradually added to a solution of the brucine salt (20 g) in dry DMSO (200 ml) to yield essentially pure brucine salt of (+)-**3** (8.5 g) as long plates. Addition of ether (1200 ml) to the mother liquor gave a diastereomeric mixture (10.2 g), which was separated by careful crystallizations from DMSO/EtOH. The salt of (-)-**3** was obtained by crystallization from DMSO/EtOH/Et_2O as prismatic needles. On treatment with charcoal and recrystallization from water these salts afforded trihydrates: the brucine salt of (+)-**3** as hairlike needles, m.p. 205-208°C dec, $[\alpha]_D^{25}$ -17.4 (*c* 7, H_2O) in an overall 40% yield and the brucine salt of (-)-**3** as flat prisms, m.p. 209-211°C dec, $[\alpha]_D^{25}$ -35.1 (*c* 5, H_2O) in a 38% yield overall.

[10] In a similar fashion we prepared the antipodal acid (+)-**3** and its derived ester **8** and amide **9** (for classical chemistry of **3**, see Biltz, H.; Heyn, M.; Bergius, M. *Liebigs Annln Chem.* **1916**, *413*, 68-77). All compounds gave the expected spectral and analytical (C, H, N ± 0.3%) data. Dowex 50W (H^+-form, 100-200 mesh).

[11] Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. *Crystals User Guide*, Chemical Crystallography Laboratory, Oxford, 1985.

[12] North, A.; Phillips, D. C.; Matthews, D. *Acta Cryst*, **1968**, *A24*, 351-359.

[13] Sheldrick, G. M. *Crystallographic Computing 3*; Oxford University Press: Oxford, 1985.

[14] Flack, H. *Acta Cryst.*, **1983**, *A39*, 876-881.

[15] Amide **9** has been suggested as a possible precursor of urinary mesoxalate: Poje, M.; Palković, A.; Perina, I.; Vicković, I; Bruvo, M. *Tetrahedron* **1985**, *41*, 4681-4684 and references cited therein.

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