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Synthesis of *E*- and *Z*-α-fluorourocanic acids as potential inhibitors of urocanase

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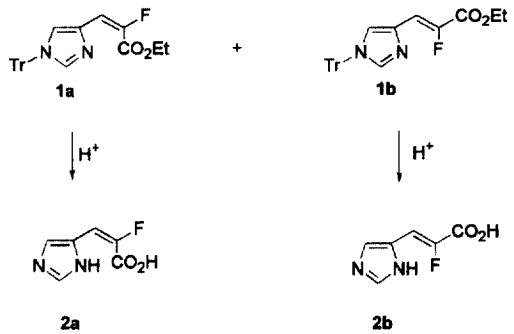
Abstract

Reaction of 1-trityl-4-imidazole carboxaldehyde with triethyl 2-fluoro-2-phosphonoacetate produced an *E*/*Z* mixture of ethyl 2-fluoro-3-(1-triphenylmethylimidazol-4-yl]-2-propenoates (**1a,b**).



After separation of the geometric isomers, acid hydrolysis produced the corresponding urocanic acids (**2a,b**). Neither isomer was a substrate or inhibitor of urocanase. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: *E*- and *Z*-α-fluorourocanic acids; Urocanase; Inhibitor

1. Introduction

Histidine ammonia lyase catalyzes the elimination of ammonia from L-histidine to produce 3-(1*H*-imidazol-4-yl)-2-propenoic acid (urocanic acid) in the first step in the catabolism of histidine. Urocanase catalyzes the hydration-rearrangement of urocanic acid to 4-imidazolone-5-propionic acid which, in turn, is converted to *N*-formamino-L-glutamic acid (Scheme 1) [1]. This route provides one of the sources of one-carbon units for purine biosynthesis and other metabolic and regulatory processes, although serine is considered the more important donor of one-carbon units. As part of our research on the biological properties of ring-fluorinated imidazoles, we previously showed that 2-fluorourocanic acid (**3**) is a potent reversible inhibitor of urocanase, whereas 4-fluorourocanic acid

(**4**) is neither an inhibitor nor a substrate for the enzyme [2]. The biological significance of this, in particular with respect to the biological properties of 2-fluoro-L-histidine, remains unclear.

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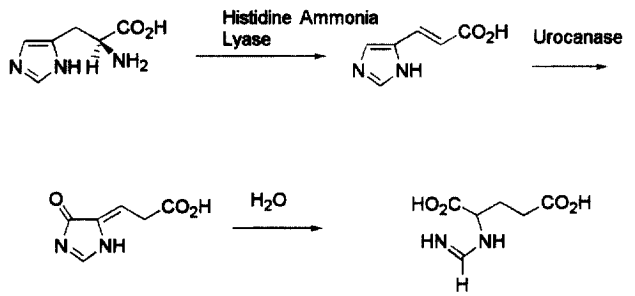
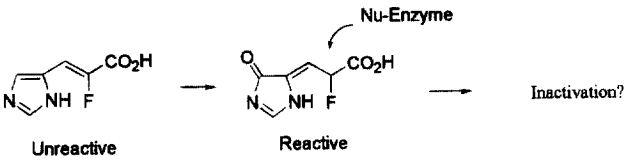


Scheme 1. Metabolism of histidine catalyzed by histidine ammonia lyase and urocanase.



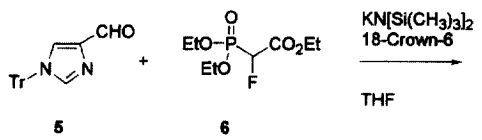
Scheme 2. Rationale for proposing α-fluorourocanic acid as a mechanism-based inhibitor of urocanase.

Unlike the 2-position of the imidazole ring of urocanic acid, the acrylic acid side chain is at the locus of the rearrangement step. In particular, a substituent on the 2-position of the side chain is on a vinylic position in the substrate, but occupies a more reactive allylic position in the product. Further activation presumably would result from the participation of the carboxyl group (see, for example, Ref. [3]). Based on these consideration, we felt that attachment of fluorine at this position could result in a mechanism-based irreversible inhibitor of urocanase (Scheme 2).

Analogues of urocanic acid have additional significance because of the apparent immunosuppressive activity of *cis*-urocanic, formed by the photoisomerization of epidermal *trans*-urocanic acid. *cis*-Urocanic acid is now postulated to be a mediator of UV-induced immune depression, thereby playing a role in the development of skin cancer [4]. The presence of fluorine on the double bond of urocanic acid should influence the wavelength-dependent photoisomerization of urocanic acid.

2. Chemistry

Our initial procedure for the Wadsworth–Emmons condensation of 1-triphenylmethylimidazole-4-carboxaldehyde (**5**) [5]



with triethyl 2-fluoro-2-phosphonoacetate (**6**) [6] was based on formation of the anion with sodium hydride. This produced the *E*- and *Z*-fluoroacrylates **1a,b**, but, consistent with a previous report [6], the modest yield of product mixture was consistently less than 40%. However, crown ethers have been used to enhance reactivities in this olefination procedure [7]. Carrying out the reaction with potassium bis(trimethyl)-amide in the presence of 18-crown-6 gave approximately equal quantities of each isomer, separated by column chromatography, in a total yield of 64%. Acid catalyzed ester hydrolysis with concomitant cleavage of the trityl group produced *E*- and *Z*-α-fluorourocanic acid (**2a,b**), purified as the hydrochloride salts by recrystallization from methanol/ether. During hydrolysis the reaction mixture was protected from light to avoid double bond isomerization.

Assignment of double bond configuration was based on H–F coupling constants. The first compound eluted from the column during separation of the two isomers had an H-F coupling constant of 24.0 Hz and was assigned the *cis*-(*E*) configuration, whereas the second compound eluted had a coupling constant of 36.1 Hz, consistent with the *trans*-(*Z*)-configuration [8]. This assignment was confirmed from ^{1}H and ^{19}F NMR data obtained from the deprotected fluorourocanic acids (see experimental). The assignments were confirmed by UV spectral data. At pH 7.2 *cis*-urocanic acid has a smaller extinction coefficient ($\varepsilon = 13{,}600$ at 277 nm) than *trans*-urocanic acid ($\varepsilon = 18{,}800$ at 277 nm) [9]. The UV spectra (pH 7.4) for *cis*-(*E*)-α-fluorourocanic acid (**2a**) [Λ_{max} (ε) 280 (13,000)] and *trans*-(*Z*)-α-fluorourocanic acid (**2b**) [Λ_{max} (ε) 275 (18,400)] were consistent with these data.

3. Biology

Urocanase was isolated and assayed as described previously [2]. The enzymatic degradation of commercial urocanic acid (Aldrich) was monitored at 277 nm in 0.05 M phosphate buffer (pH 7.4). Under the same assay conditions, urocanase had no effect on *E*- or *Z*-α-fluorourocanic acid. In addition, there was no change in the rate of degradation of urocanic acid in the presence of as much as three-fold higher concentrations of *E*- or *Z*-α-fluorourocanic acid, with or without preincubation of the enzyme with the fluorinated compound. From these data, it was determined that neither compound is a substrate nor an inhibitor of urocanase. The effects of fluorine substitution on the photoreactivity of urocanic acid will be the subject of subsequent research.

4. Experimental details

4.1. General procedures

Capillary melting points are uncorrected. Ultraviolet spectra were measured with a Beckman DU 7 ultraviolet spectrophotometer in 0.05 M phosphate buffer (pH 7.4). ^{1}H NMR were taken on a Varian Unity 500 (500 MHz) or Gemini 300 (300 MHz) spectrometer with TMS as an internal reference.

^{19}F NMR spectra were measured on a JOEL JNM-GX 270 (254 MHz) spectrometer, using $CFCl_3$ as an internal reference. High resolution mass spectra were obtained on a VG Analytical 7070E mass spectrometer. Anhydrous THF, 18-crown-6 and potassium bis(trimethylsily)amide were purchased from Aldrich. Reactions and product purities were monitored by thin layer chromatography on silica gel plates (Analtech).

4.2. Wadsworth–Emmons condensation

To a solution of 355 mg (1.5 mmol) of triethyl 2-fluoro-2-phosphonoacetate (**6**) [6] and 396 mg (1.5 mmol) of 18-crown-6 in 10 ml of anhydrous THF chilled in a dry ice/acetone bath and under a nitrogen atmosphere was added 3.0 ml (1.5 mmol) of 0.5 M potassium bis(trimethylsilyl)amide in toluene and a solution of 340 mg (1 mmol) of 1-tritylimidazole-4-carboxaldehyde (**5**) [5] in 10 ml of THF. The reaction was stirred at −78°C for 30 min and then allowed to warm to ice bath temperature. After stirring for an additional one hour, the reaction was quenched by the addition of 10% ammonium chloride solution. Water was added and the aqueous phase was extracted two times with ethyl acetate. The ethyl acetate layer was washed three times with 10 ml of water, one time with brine and was dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed on silica gel (20% ethyl acetate in petroleum ether) to give two products as white, crystalline solids.

4.3. Ethyl (E)-2-fluoro-3-(1-triphenylmethylimidazol-4-yl]-2-propenoate (1a)

The first product eluted (144 mg, 33%) was recrystallized from ethyl acetate/petroleum ether, to give fine colorless crystals, mp. 135–136°C. ^1H NMR ($CDCl_3$, 500 MHz) δ 1.20 (t, 3H, $J=7.2$ Hz, CH_3), 4.19 (q, 2H, $J=7.1$ Hz, CH_2), 6.96 (d, 1H, $J_{HF}=24.1$ Hz, 3-H), 7.12–7.2 (6H, m, ArH×6), 7.32–7.38 (9H, m, ArH×9), 7.44 (1H, d, J=1.3 Hz, 5′-H), 7.96 (1H, s, 2′-H). ^{19}F NMR ($CDCl_3$, 254 MHz), δ −124.42 (d, $J_{HF}=24.0$ Hz). $C_{27}H_{23}FN_2O_2$ (426.47): calc.: C 76.05, H 5.44, N 6.57; found C 76.07, H 5.45, N 6.49%.

4.4. Ethyl (Z)-2-fluoro-3-(1-triphenylmethylimidazol-4-yl]-2-propenoate (1b)

The second product (130 mg, 31%) was recrystallized from ethyl acetate/petroleum ether to give colorless crystals, mp. 142–143°C. ^1H NMR ($CDCl_3$, 500 MHz) δ 1.33 (t, 3H, $J=7.1$ Hz, CH_3), 4.29 (q, 2H, $J=7.1$ Hz, CH_2), 7.07 (d, 1H, $J_{HF}=36.3$ Hz, 3-H), 7.1–7.17 (6H, m, ArH×6), 7.32–7.38 (10 H, m, ArH×9, 5′-H), 7.49 (1H, s, 2′-H). ^{19}F NMR ($CDCl_3$, 254 MHz), δ −123.92 (d, $J_{HF}=36.1$ Hz: $C_{27}H_{23}FN_2O_2$ (426.47): calc.: C 76.05, H 5.44, N 6.57; found C 76.05, H 5.49, N 6.58%.

4.5. (E)-α-Fluorourocanic acid (2a)

An aluminum foil-wrapped solution of 122 mg (0.29 mmol) of **1a** in 4 ml of 1 N HCl and 0.8 ml of methanol was heated at reflux for 16 h. The solution was cooled and diluted with 5 ml of water. After removal of the triphenylmethanol by filtration, the solution was rotary evaporated to give 49 mg (88%) of **2a-HCl** as a crystalline solid. Dissolution in methanol followed by addition of ether to the point of turbidity and chilling produced fine colorless crystals, mp. 285–300°C (slow and incomplete decomp). UV Λ_{max} (ε) 280 (13,000). ^1H NMR spectrum (DMSO-d_6, 300 MHz) δ 7.04 (d, $J_{HF}=21.4$ Hz, 3-H), 7.80 (s, 5′-H), 8.70 (s, 2′-H). ^{19}F NMR (DMSO-d_6, 254 MHz) δ −110.79 (d, $J_{HF}=21.3$ Hz): MS calc. for $C_6H_5FN_2O_2$: 156.0335; found 156.0332.

4.6. (Z)-α-Fluorourocanic acid (2b)

The procedure used for hydrolysis of **2a** was followed. From 165 mg (0.39 mmol) of **1b** there was obtained 61 mg (81%) of crystalline **2b-HCl**. This was recrystallized as above to give white crystals, mp. 263–270°C (decomp). UV Λ_{max} (ε) 275 (18,400). ^1H NMR (DMSO-d_6, 254 MHz) δ 7.12 (1H, d, $J_{HF}=34.1$ Hz, 3-H), 7.99 (1H, s, 5′-H), 9.16 (1H, s, 2′-H). ^{19}F NMR (DMSO-d_6) δ −115.08 (d, $J_{HF}=34.2$ Hz): MS calc. for $C_6H_5FN_2O_2$: 156.0335; found 156.0329.

Acknowledgements

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References

[1] H. Tabor, A.H. Mehler, J. Biol. Chem. 210 (1965) 559.
[2] C.B. Klee, L.E. La John, K.L. Kirk, L.A. Cohen, Biochem. Biophys. Res. Commun. 75 (1977) 674.
[3] E.S. Gould, Mechanism and Structure in Organic Chemistry, Henry Holt, New York, 1959, p. 563.
[4] K.M. Hanson, B. Li, J.D. Simon, J. Am. Chem. Soc. 119 (1997) 2715.
[5] K.L. Kirk, J. Heterocyclic Chem. 22 (1985) 57.
[6] H. Machleidt, R. Wessendorf, Liebigs Ann. Chem. 674 (1964) 1.
[7] W.C. Still, C. Gennari, Tetrahedron Lett. 24 (1983) 4405.
[8] E.D. Becker, High Resolution NMR, Theory and Chemical Applications, 2nd edn., Academic Press, New York, 1980, p. 100.
[9] D.H. Hug, J.K. Hunter, Photochem. Photobiol. 59 (1994) 303.