Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 7-11 (2001)

Syntheses of ^{2}H-Labelled Dihydropyrimidinediones and their Metabolites

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SUMMARY

Starting with uracil or thymine, [5,5,6,6-$^{2}H_4$]-dihydrouracil and [5,6,6,α,α,α,-$^{2}H_6$]-dihydrothymine were synthesised in high yields by catalytic deuteriation. Subsequent hydrolyses resulted in the corresponding β-ureidoalkanoic acids and β-aminoacids.

Key Words: Pyrimidinones, uracil, thymine, amino acids, catalytic deuteriation

INTRODUCTION

Inherited deficiencies of the enzymes catalysing the metabolic degradation of the biologically important pyrimidine-2,4-diones uracil (1a) and thymine (1b) have been described (1) (Scheme 1).

Syntheses comprising the construction of the pyrimidine ring start with precursors labelled with carbon-13 or nitrogen-15 which is quite expensive. Furthermore, the yields obtained are often very low (2,3). Párkányi and Šorm described the synthesis

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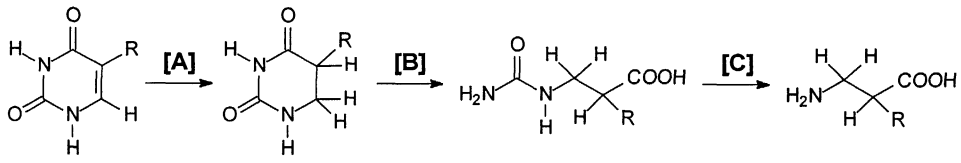
Received 14 July 2000
Revised 30 August 2000
Accepted 31 August 2000

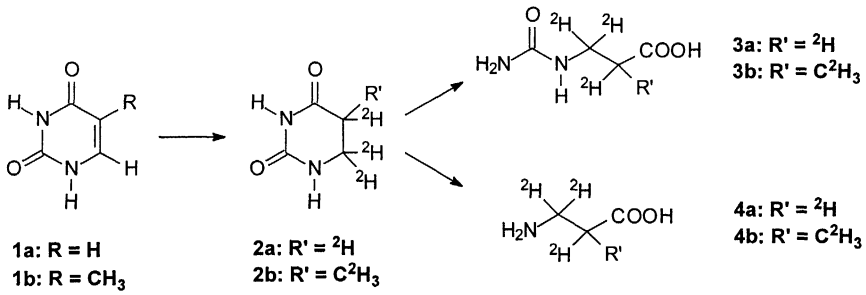


Scheme 1: Biodegradation of 1a and 1b. A: dihydropyrimidine dehydrogenase, B: dihydropyrimidinase, C: β-ureidopropionase

of $^2H_2$-dihydrouracil using a platinum oxide catalyst (4). Green and Cohen obtained unlabelled dihydrouracil and dihydrothymine in high yields by hydrogenation of 1a and 1b using rhodium on alumina as catalyst (5).

RESULTS AND DISCUSSION

For the deuteriation of 1a and 1b we used rhodium on carbon and achieved simultaneous exchange and reduction. Thus, we obtained 2a and 2b in high yields and extensive deuterium labelling. Subsequent hydrolyses under different conditions gave the corresponding β-ureidoalkanoic acids or the respective β-aminoacids with the deuterium label retained (Scheme 2).



Scheme 2:

In their attempt to synthesise $^2H_2$-dihydrouracil via catalytic reduction with $^2H_2$ Párkányi and Šorm obtained a product which contained more than the calculated amount of deuterium. In order to understand the mechanism and the sequence of the reaction, we studied the kinetics of the transformation of 1b into 2b by mass spectrometry. It is obvious from Figure 1 that first the exchange to the intermediate

$^2H_4$-thymine takes place followed by reduction to 2b. This was also confirmed by incubation of dihydrothymine under reaction conditions without any exchange. The results show that the double bond is a prerequisite for hydrogen/deuterium exchange.

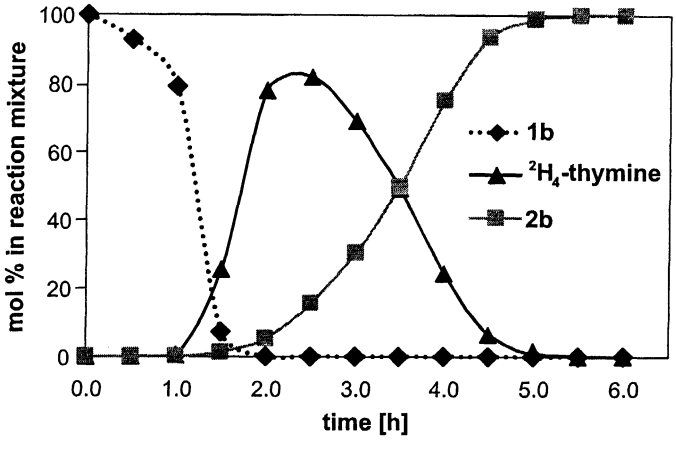


Figure 1: Progress of the H/D exchange and reduction of thymine determined by GC-MS. For 2b the sum of all isotopomeres is given.

CONCLUSION

In summary, we obtained all metabolites of the biodegradation pathway of 1a and 1b in high yields and purity. By introducing 4 or 6 deuterium labels the products were very suitable for internal standard work using mass spectrometry. In addition, isotope labels were stable under both acid and alkaline conditions. This suggests that the products may also be used in *in vivo* studies.

EXPERIMENTAL

[5,5,6,6-$^2H_4$]-dihydrouracil (2a) and [5,6,6,α,α,α,-$^2H_6$]-dihydrothymine (2b). 1a or 1b (100 mg) were dissolved in 40 ml ^2H_2O and treated with $^2H_2$ under atmospheric pressure in the presence of 200 mg 5 % rhodium on carbon (Degussa type G10 NB/W) for 3 or 6 h, respectively. After crystallisation from H_2O 2a and 2b

were obtained in 85 % and 78 % yield. (Anal.: 2a: Calculated for $C_4H_2{}^2H_4N_2O_2$: C, 40.67; H, 1.71; N, 23.71; D, 6.82. Found: C, 40.79; H, 1.74; N, 23.72; D, 6.95. 2b: Calculated for $C_5H_2{}^2H_6N_2O_2$: C, 44.76; H, 1.50; N, 20.88; D, 9.01. Found: C, 44.91; H, 1.51; N, 20.73; D, 9.05). The degree of isotope labelling was determined by mass spectrometry using a Finnigan MAT95 with electron impact ionisation (70 eV). Isotope distribution: 2a: d_4 86.3%, d_3 10.0%, d_2 3.2%, d_1 0.5%, d_0 0%. 2b: d_6 87.6%, d_5 7.9%, d_4 2.9%, d_3 0.9%, d_2 0.6%, d_1 0%, d_0 0%. To determine the mechanism of the formation of 2b samples of the reaction mixture were filtered and evaporated to dryness every 30 min. After derivatisation with N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide, thymine and dihydrothymine isotopomeres were monitored by GC-MS (HP 5890A II and Finnigan TSQ 700; M-57: m/z = 297 - 305). Unlabelled thymine and dihydrothymine was used for calibration.

The stability of the isotope label was confirmed under acid conditions by incubation of 2a and 2b in 1 M HCl for 48 h at room temperature.

[2,2,3,3-$^2H_4$]-3-Ureidopropionic acid (3a) and [2,3,3,α,α,α,-$^2H_6$]-2-Methyl-3-ureidopropionic acid (3b). Alkaline hydrolysis under mild conditions led to ring opening (6). 2a or 2b (1 equivalent) were treated with 1.1 equivalents of NaO^2H in ^2H_2O for 2 h. 3a and 3b were isolated from the reaction mixture using a cationic ion exchange resin (Dowex® 50W-X8-200) in 79 % and 83 % yield, respectively. (Anal.: 3a: Calculated for $C_4H_4{}^2H_4N_2O_3$: C, 35.29; H+D, 5.92; N, 20.58. Found: C, 35.15; H+D, 5.95; N, 20.32. 3b: Calculated for $C_5H_4{}^2H_6N_2O_3$: C, 39.46; H+D, 6.62; N, 18.41. Found: C, 39.39; H+D, 6.68; N, 18.13. Isotope distribution: 3a: d_4 81.1%, d_3 14.9%, d_2 3.6%, d_1 0.4%, d_0 0%. 3b: d_6 91.6%, d_5 6.4%, d_4 1.1%, d_3 0.7%, d_2 0.2%, d_1 0%, d_0 0%.)

[2,2,3,3-$^2H_4$]-3-aminopropionic acid (4a, [2,2,3,3-$^2H_4$]-β-alanine) and [2,3,3,α,α,α-$^2H_6$]-3-amino-2-methylpropionic acid (4b). Under vigorous conditions hydrolysis proceeded to the β-aminoacids (7). 2a or 2b were treated with 1 M NaO^2H for 48 h under reflux. Isolation identical to that of 3a and 3b and

followed by crystallisation from ethanol gave 4a and 4b in 50 % yield. (Anal.: 4a: Calculated for $C_3H_3{}^2H_4NO_2$: C, 38.70; H+D, 7.58; N, 15.04. Found: C, 38.72; H+D, 7.51; N, 14.77; 4b: Calculated for $C_4H_3{}^2H_6NO_2$: C, 44.01; H+D, 8.31; N, 12.83. Found: C, 43.81; H+D, 8.43; N, 12.73. Isotope distribution: 4a: d_4 88.8%, d_3 5.7%, d_2 4.9%, d_1 0.6%, d_0 0%. 4b: d_6 90.7%, d_5 4.2%, d_4 1.9%, d_3 0.5%, d_2 6%, d_1 0%, d_0 0%.)

ACKNOWLEDGEMENT

We thank Mrs. S. Seefried and Mr. F. Schönberger for excellent technical assistance. This work was supported by the Robert Bosch Foundation, Stuttgart and BMBF, Berlin (FKZ 01 GG 9846).

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