SYNTHESES WITH STABLE ISOTOPES: THYMINE-2,6-$^{13}C_2$

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SUMMARY

A three-step synthesis of thymine-2,6-$^{13}C_2$ from urea-^{13}C, sodium cyanide-^{13}C, and α-bromopropionic acid is described. The last reaction involves hydrogenation of α-cyano-^{13}C-propionylurea-^{13}C in aqueous acetic acid and produces thymine-2,6-$^{13}C_2$ in 50-60% yield. The mechanism of this reaction is discussed.

Key Words: Carbon-13, Thymine-2,6-$^{13}C_2$, α-Cyano-^{13}C-propionylurea-^{13}C, Hydrogenation

INTRODUCTION

We were interested in preparing thymine labeled with carbon-13 for incorporation into DNA to facilitate nmr studies. Consideration of possible labeling patterns suggested that at least two nonadjacent carbons be labeled. The additional constraint of labeling both a protonated and nonprotonated carbon would allow evaluation of these types of resonances in a macromolecule. Our attention was drawn to a paper by Jezdic (1) that describes the preparation of thymine-2,6-$^{14}C_2$. One disadvantage of this synthesis is the low yield reported for the catalytic hydrogenation of α-cyanopropionylurea to thymine. However, the use of simple precursors and the relatively short synthetic sequence made the method attractive and prompted us to investigate the application of this synthesis to our preparation of thymine-2,6-$^{13}C_2$.

RESULTS AND DISCUSSION

The reaction sequence for the preparation of thymine-2,6-$^{13}C_2$ (1) is shown in Scheme 1. The reaction of sodium cyanide-^{13}C with sodium α-bromopropionate readily afforded an 84% yield of α-cyano-^{13}C-propionic acid (3). Condensation of 3 with urea-^{13}C in the presence of acetic anhydride gave α-cyano-^{13}C-propionylurea-^{13}C (4) in 64% yield. The hydrogenation of α-cyanopropionylurea

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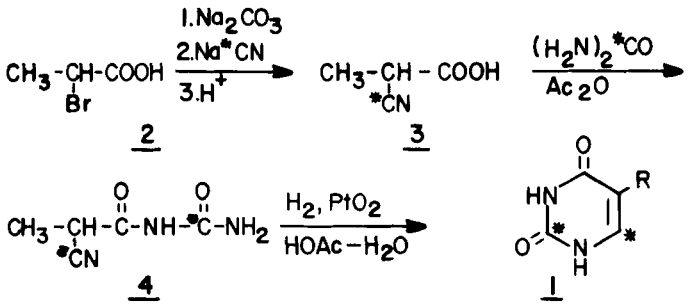
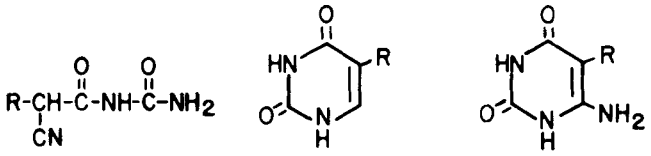
SCHEME 1



in aqueous solution using Adams' catalyst was reported by Jezdic (1) to produce thymine in 20% yield. We have found that the yield of 1 from 4 can be increased to ca. 50-60% by carrying out the hydrogenation in aqueous acetic acid. The mechanism of this last reaction has been the subject of some speculation and, in our opinion, misinterpretation in the literature.

The first example of the hydrogenation of an α-cyanoacylurea to produce the pyrimidine ring system was reported by Rupe et al. (2) who obtained uracil (6a) from the hydrogenation of cyanoacetylurea (5a) in the presence of a nickel catalyst (Scheme 2). Rupe suggested that the conversion proceeded through a

SCHEME 2



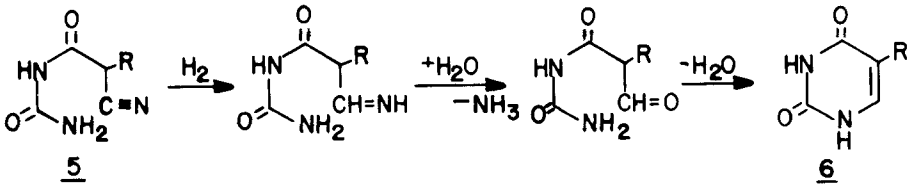
5a, R=H
5b, R=CH3

6a, R=H
6b, R=CH3

7a, R=H
7b, R=CH3

mechanism whereby the cyano group of the α-cyanoacetylurea (5a) was first reduced to an imine that was hydrolyzed to an aldehyde with subsequent cyclization to uracil (6a) with loss of water (Scheme 3). The analogous reduction of α-cyanopropionylurea (5b) to thymine (6b) using platinum black was reported by Bergmann and Johnson (3).

SCHEME 3



Ambelang and Johnson (4) found that the hydrogenation of 6-aminouracil (7a) in water using Adams' catalyst produced uracil (6a). They suggested that one of the mechanisms for the hydrogenation of α-cyanopropionylurea (5b) to thymine (6b) or α-cyanoacetylurea (5a) to uracil (6a) might involve the intermediacy of 6-aminothymine (7b) or 6-aminouracil (7a). This suggestion was reiterated more forcefully by Bendich (5) who termed Rupe's mechanism plausible but lacking support from Ambelang and Johnson's observation. Jezdic (1) also argued that hydrogenation of aminouracil (7a) to uracil (6a) contradicted the reaction mechanism suggested by Rupe *et al*. When Jezdic interrupted the hydrogenation of α-cyanopropionylurea (5b), he found 6-aminothymine (7b) present in the reaction mixture. Citing this evidence, he proposed a different reaction mechanism in which incorporation of one hydrogen atom and subsequent cyclization with the release of hydrogen produce 6-aminothymine that is hydrogenated to thymine and ammonia.

In an attempt to find suitable conditions for converting α-cyano-^{13}C-propionylurea-^{13}C (4) to thymine-2,6-$^{13}C_2$ (1), we conducted several reactions similar to those described above. We found that cyanopropionylurea (5b) was hydrogenated (50 psi, 70-80°C, 2-4 h, water) to thymine (6b) in *ca*. 15% yield. Under similar conditions (50 psi, 70-80°C, 6 h, water), 6-aminothymine (7b) gave only unreacted starting material and a trace of thymine. In contrast, the hydrogenation of 5b (1 atm, 70°C, 8 h) in aqueous acetic acid proceeds smoothly, and yields of 50-60% of thymine can be obtained. Additionally, a reaction carried out in aqueous acetic acid was interrupted before completion, and the cmr spectrum of the reaction mixture showed no 6-aminothymine (7b) present.

These results and a critical evaluation of the literature on α-cyanoacylurea hydrogenations have led us to discount the intermediacy of aminopyrimidines in these processes. To our knowledge, the only experimental evidence to support this mechanism is Jezdic's observation that the hydrogenation of 5b in water at 70°C produces 6b and 7b. However, this cannot be taken as proof that 7b is an actual intermediate in the reaction since it is known that aqueous solutions of 5a produce 7a at elevated temperatures (6). We would suggest that there is no firm experimental evidence for the conversion of 5 to 6 involving 7 as an intermediate and that Rupe's mechanism (Scheme 3) or a process involving cyclization of the imine with subsequent loss of ammonia adequately describes the process.

EXPERIMENTAL

Materials and Methods--Published methods were used to prepare urea-^{13}C (7) and $Na^{13}CN$ (8). Platinum oxide (Adams' catalyst) was obtained from Ventron Corporation (Danvers, Mass.). Cmr spectra of DMSO-d_6 solutions of 2, 3, and 4 were recorded using a Varian Model CFT-20 spectrometer. Chemical shifts were referenced to DMSO-d_6 (39.6 ppm) and are reported relative to TMS. Infrared spectra were recorded on a Perkin-Elmer Model 710 spectrophotometer. The ir spectra of labeled compounds are reported with natural abundance absorptions of peaks showing isotope shifts given in parentheses.

α-Cyano-^{13}C-propionic Acid (3)--Sodium α-bromopropionate was prepared by carefully adding Na_2CO_3 (8.48 g, 0.08 mol) to a solution of α-bromopropionic acid (24.48 g, 0.16 mol) in acetonitrile (100 ml). The mixture was stirred 2 h, although observable evolution of CO_2 ceased after 30 min. The precipitated salt was filtered, washed with ether, and dried. A mixture of this sodium α-bromopropionate, $Na^{13}CN$ (7.99 g, 0.16 mol), and NaOH (0.48 g, 0.012 mol) in water (28 ml) was heated to 50°C and stirred 2.5 h. The cooled reaction mixture was then acidified with 10% HCl (about 55 ml) and the product extracted with ether (3 x 75 ml). The combined ether extracts were dried over $MgSO_4$, filtered, and evaporated to a yellow oil (13.4 g, 84%). This crude product was used without further purification. Cmr 169.3 (COOH), 119.8 (CN), 32.6 (CH), 16.4 ppm (CH_3).

α-Cyano-^{13}C-propionylurea-^{13}C (4)--A mixture of urea-^{13}C (6.84 g, 0.112 mol), acetic anhydride (12.59 g, 0.123 mol), and α-cyano-^{13}C-propionic acid (3, 11.21 g, 0.112 mol) was placed in a flask equipped with a mechanical stirrer and heated. All of the starting material dissolved when the temperature reached 80°C, and a white solid precipitated from the reaction mixture after ca. 15 min at 90°C. Heating was continued for 2 h. The cooled reaction mixture was filtered and the product washed with ether until the yellow color was removed, to give white crystals (10.20 g, 64%), mp 191-192°C [reported (3) 192°C]. Cmr 167.3 (-CONH-), 152.3 (-NHCONH$_2$), 117.7 (CN), 32.1 (CH), 14.5 ppm (CH$_3$); ir (KBr) 3420, 3340, 3240, 2200 (2260), 1715, 1635 (1675 and 1635), 1490, 1380 (1415), 1190, 1090 cm^{-1}.

Thymine-2,6-$^{13}C_2$ (1)--A hydrogenation vessel with sidearm was charged with Adams' catalyst (1.14 g) and water (40 ml), evacuated, and opened to an atmosphere of hydrogen until the catalyst was reduced. After adding a solution of α-cyano-^{13}C-propionylurea-^{13}C (4, 2.86 g, 0.020 mol) in hot glacial acetic acid (40 ml) via the sidearm, the mixture was heated to 70°C and the reaction allowed to proceed until hydrogen uptake ceased. The hot reaction mixture was filtered to remove the catalyst. The filtrate was concentrated under reduced pressure until a white precipitate appeared. The mixture was cooled overnight, and the crystals were filtered and washed with cold water. By further concentration of the filtrate, a total of 1.95 (59%) of thymine-2,6-$^{13}C_2$ was obtained. This process was repeated twice to obtain a total of 1.95 g (59%) of thymine-2,6-$^{13}C_2$. Cmr 165.1 (C-4), 151.7 (C-2), 137.9 (C-6), 107.9 (C-5), 12.0 ppm (CH$_3$); ir (KBr) 3220, 3080, 1685 (1735 and 1675), 1430 (1445 and 1420), 1380, 1240, 1210 cm^{-1}.

ACKNOWLEDGMENT

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