SYNTHESIS OF CARBON-13 LABELLED URACIL, 6,7-DIMETHYLLUMAZINE, AND LUMICHROME, VIA A COMMON INTERMEDIATE: CYANOACETYLUREA.

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Received October 29, 1976
Revised November 29, 1976

SUMMARY

Uracil, 6,7-dimethyllumazine, and lumichrome have been labelled with carbon-13 at positions 6, 8a, and 10a, respectively. This was accomplished via a common intermediate, cyanoacetylurea-6-^{13}C. Lumazine and lumichrome were condensation products of 5,6-diaminouracil and either 2,3-butanedione. or a condensate of it. Uracil was produced by a Raney Nickel reduction followed by acid catalyzed cyclization. This method is also applicable to labelling these compounds at other carbons within the molecules.

Key Words: Uracil, 6,7-Dimethyllumazine, Lumichrome, Carbon-13, Cyanoacetylurea.

INTRODUCTION

Nuclear magnetic resonance spectrometers with multinuclear capabilities are proving to be powerful tools in bioorganic chemistry. Instruments, employing ^{13}C probes have been utilized in studies of such complex systems as RNA (1) (using uracil labelled with ^{13}C at carbon-2), and in the investigation of the fluidity of the fatty acids within biological membranes (2) (by feeding ^{13}C labelled acetate). These examples clearly demonstrate the adaptability of NMR to the study of complex biochemical systems when isotopically labelled compounds are utilized.

We are involved in mechanistic studies of systems which include both the pyrmidine and the alloxazine/isoalloxazine ring systems. In this work it be-

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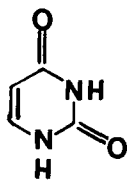
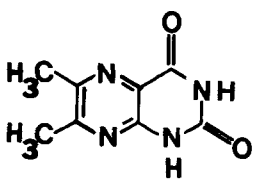
0362-4803/78/0114-0035$01.00/0

came necessary to obtain both ring systems specifically labelled with ^{13}C at various positions. We therefore devised a synthetic pathway for each compound, all employing a common intermediate which was both isolable, and stable for storage. The compounds we needed were uracil (I), labelled in positions 5 or 6; 6,7-dimethy-lumazine (II) labelled in positions 4a or 8a; and lumichrome (III) labelled on positions 4a or 10a.



(I)



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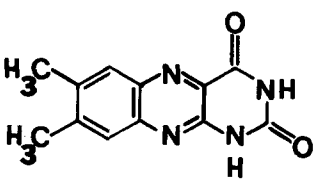


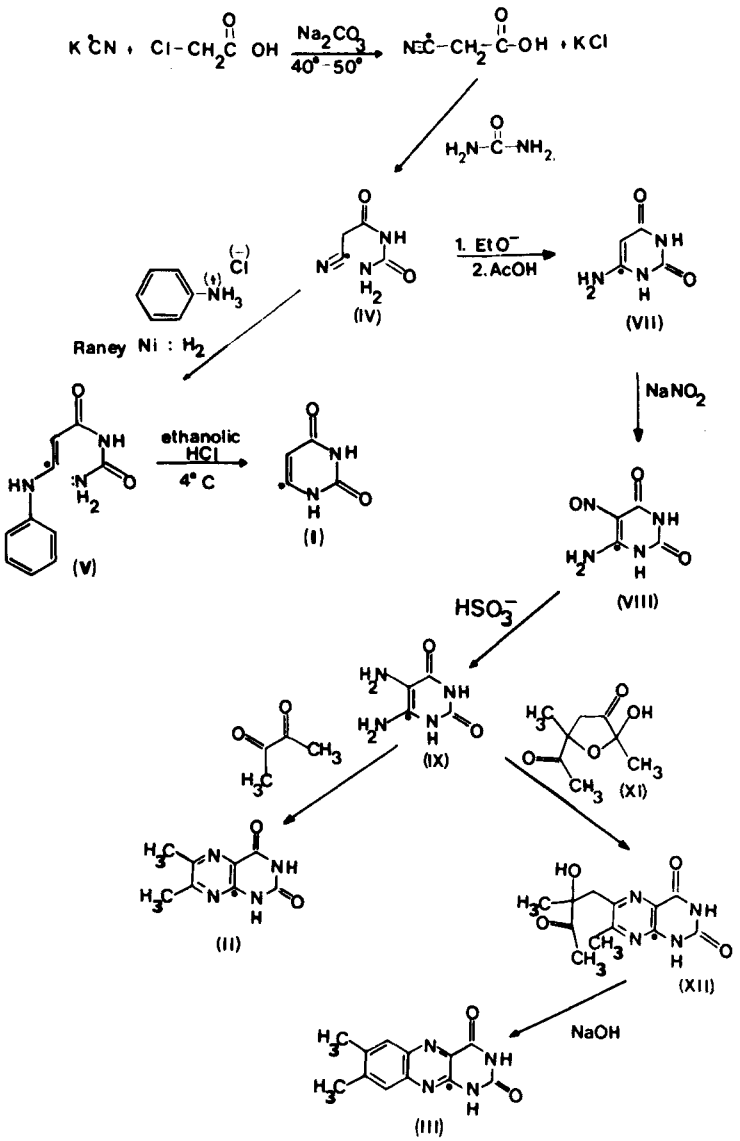
(III)

These compounds will be used in the assignment of the ^{13}C NMR spectra of the covalent adducts which result from nucleophilic attack of bisulfite anion (3,4). Although alternate methods for the synthesis of uracil have appeared in the literature (5), we have chosen this approach as the most attractive, primarily because there is potential for utilization of the common intermediate, cyano-acetylurea (IV), from which we can rapidly generate either uracil, or the alloxazine/isoalloxazine system as required.

The synthetic approach (scheme I), starts with commercially available potassium cyanide-^{13}C* and proceeds to the common intermediate cyanoactylurea (IV). This compound can be utilized to form 6-aminouracil a precursor to lumazine (II) and lumichrome (III), or can, by means of a Raney Nickel reduction and subsequent cyclization, be converted to uracil (I).

*(if cyanoacetic acid, labelled with ^{13}C in the methlene carbon is used as the starting material the label will appear in the carbon adjacent to the one shown in the schemes).

SCHEME I



EXPERIMENTAL

The ^{13}C enriched potassium cyanide used in these synthetic procedures was obtained from Stohler Isotope Chemicals and contained carbon-13 in 90% abundance. This was diluted with natural abundance potassium cyanide (Aldrich Chemical Company, Inc.) to an abundance of 5.45%. This level of enrichment was maintained throughout the synthesis and was monitored by intensity data in the ^{13}C-

NMR. Carbon-13 NMR spectra were obtained using a Varian Associates CFT-20 spectrometer; ^{1}H-NMR spectra were obtained using a Varian Associates EM-360 spectrometer, infrared spectra were obtained using a Perkin-Elmer 567, and ultraviolet spectra were obtained using a Carey-15 spectrometer.

Cyanoacetylurea (6-^{13}C): (IV)

The preparation of cyanoacetylurea (6-^{13}C) (IV) was a modification of the methods of Rupe (6), Traube (7), and Fisher (8), as described by Bergmann and Johnson (9). Chloracetic acid (3.46g, 36.6 mmoles) was dissolved in a minimum amount of water (5-7 ml) and neutralized by the addition of anhydrous sodium carbonate (1.95g, 18.4 mmoles). In a separate flask, potassium cyanide (^{13}C) (1.97g, 30.3 mmoles) was dissolved in water (3 ml) by heating to 60°C. The KCN solution was added to the sodium chloracetate solution, and the temperature allowed to rise to 60°C. However, further increases in temperature were avoided by emersion in cool water. The reaction was allowed to continue until the temperature ceased to rise (about 45 minutes). Then the mixture was allowed to stand for twelve hours at room temperature. The resultant pale yellow solution was acidified (3 ml conc. HCl) to free the cyanoacetic acid (10), excess HCN, water, and HCl were removed under reduced pressure at 50°C. The syrupy residue was solubilized in alcohol (15 ml) and separated from the salt by filtration. After washing the salt with alcohol the filtrates were combined and the solvent removed by evaporation under reduced pressure (rotary evaporator at 45°C). The residue was taken up in absolute alcohol, and the solvent removed. This process was repeated several times to assure removal of water (note: the temperature was kept below 50°C). A suspension of urea (1.86g, 31.0 mmoles) in freshly distilled acetic anhydride (5 ml) was added to the dried residue, and the mixture heated at 100°C for 30 minutes. Water (20 ml) was added to the solution and upon cooling a crystalline solid was isolated which proved to be cyanoacetylurea, in 76% yield from KCN (2.92g, 23.0 mmoles). The i.r. of the product was identical with that of an authentic sample (Aldrich Chemical Co., Inc.). The melting point of the product (211-2°C) matched the literature (16), and the mixed melting point showed no depression.

β-phenylaminoacryloylurea-(6-^{13}C): (V)

The method of Safonova and Nesterov (11) was used in the preparation of β-phenylaminoacryloylurea (6-^{13}C) (V). Cyanoactylurea (6-^{13}C) (IV) (3.60g, 28.3 mmoles) and activated Raney Nickel (approximately 1.0g) were suspended in water (125 ml) containing aniline HCl (4.04g, 31.2 mmoles). The suspension was placed in a 500 ml Parr bottle, exposed to hydrogen gas (35 psi) and agitated for ten hours at room temperature. The resultant paste was extracted with alcohol several times (until the catalyst regained the black color). The filtrates were combined and the volume reduced on a rotary evaporator. Upon cooling a crystalline solid was isolated and identified as the desired product (V) (2.30g, 11.2 mmoles, 40% yield). The melting point, 208°C decomp., was consistent with the literature value. Elemental analysis (found: C=58.79%, H= 5.42%, N= 20.36%) matched that calculated for $C_{10}H_{11}N_3O_2$ (C= 58.54%, H= 5.37%, N= 20.49%).

Uracil (6-^{13}C): (I)

In a continuation of the method of Safonova and Nesterov (11) β-phenylaminoacryloylurea (6-^{13}C) (V) (1.5g, 7.3 mmoles) was dissolved in absolute alcohol which had been saturated with HCl gas at room temperature. The suspension was stored at 4°C for four days, filtered, and the solid washed with cold ethanol (3 ml). The solid was washed with NH_4OH, filtered, and the filtrates combined. The pH was adjusted to 3 with concentrated H_2SO_4. The crystalline powder that resulted was identified as uracil (I) (0.390g, 3.48 mmoles, 48%yield). The i.r. of the product (I) was identical with that of an authentic sample (Aldrich Chemical Co.). The ^{13}C-NMR was identical with the authentic sample, and the published spectra (16). With the singular exception of the anticipated increase in intensity for the signal from carbon-6.

6-Aminouracil (6-^{13}C): (VII)

Cyanoacetylurea (6-^{13}C) (IV) (2.9g, 22.8 mmoles) was refluxed in sodium ethoxide (2g sodium in 30 ml alcohol) for one hour. Water (30 ml) was added and the mixture stirred until solution was effected. The solution was heated

*(Note: Upon reduction, the suspension foamed and filled the bottle, and was forced into line during hydrogen evacuation. To prevent contamination of the hydrogenation apparatus we utilized a 3 way stopcock to divert the suspension into an appropriate vessel.)

at 80°C for fifteen minutes, then acidified to pH 5 with glacial acetic acid The solution was allowed to cool and a white solid was isolated. This proved to be 6-aminouracil (6-^{13}C) (2.68g, 21.1 mmoles, 92% yield). The i.r. of the product (VII) was identical with that of an authentic sample (Sigma Chemical Co.).

5,6-Diaminouracil (6-^{13}C) hemisulfate: (X)

The preparation of 5,6 diaminouracil (6-^{13}C) (IX) was accomplished by the method of Sherman and Taylor (12), 6-aminouracil (6-^{13}C) (2.68g, 21.1 mmoles) was suspended in water (20 ml), and acidified with glacial acetic acid. Sodium nitrite (1.78g, 25.8 mmoles) was added to the resultant suspension and heat applied (80°C for 15 min). This resulted in a rose colored precipitate [5-nitroso-6-aminouracil (12)] which was not purified for identification, but used in total in the next synthetic step. Sodium hydrosulfite (excess) was added to the stirred heated (80°C) suspension until the rose color was completely bleached. The suspension was heated for an additional 15 min to assure completion of reaction. Upon cooling a buff colored solid precipitated and was isolated by filtration. The dry weight of the product IX was 2.80g (19.7 mmoles; a 93% yield). To purify the product it was converted to its hemisulfate (X) (17) by dissolving in dilute NaOH (5 ml) and pouring the resultant solution into boiling H_2SO_4 (dilute). The crystalline hemisulfate which precipitated upon cooling, was found to be identical with an authentic sample (Sigma Chemical Co.) by both uv and ir spectroscopy.

6,7-Dimethyllumazine (8a-^{13}C): (II)

The preparation of 6,7-dimethyllumazine (8a-^{13}C) (II) was by the method of Weijlard and Erickson (13). 5-6-diaminouracil (6-^{13}C) hemisulfate (5.0g, 26.15 mmoles) was heated (100°C) in water (30 ml) with diacetyl (2.25g, 26.1 mmoles) for fifteen minutes, and cooled. A crystalline solid was isolated (3.34g, 17.4 mmoles, a yield of 67%). This was shown to be identical with an authentic sample (Aldrich Chemical Co.) of 6,7-dimethyllumazine by ir, uv, and ^{13}C-NMR. The increased peak intensity for the signal of carbon-8a indicated the presence of the label as well as its position within the molecule.

2,4-Dihydroxy-7-(2-hydroxy-2methyl-3-oxobutyl)-6methylpteridine (8a-^{13}C): (XII)

The method of Birch and Moye (15) was used to prepare the 2,4-dihydrox-6-

(2-hydrox-2-methyl-3-oxobutyl)-6-methyl-pteridine (8a-^{13}C) (XII). 5,6-Diamino-uracil (6-^{13}C) hemi-sulfate (X) (0.80g, 4.16 mmoles) was added to water (15 ml); 5-acetyltetrachydro-2-hydroxy-2,5-dimethyl-3-oxofuran (14) (XI) (0.716g, 4.16 mmoles was added; and the suspension heated (100°C) for one hour. The suspension was filtered, and upon cooling pale yellow crystals (XII) were isolated. A yield was not determined as the product was used in its entirety in the next step, the formation of lumichrome (10a-^{13}C) (III).

Lumichrome (10a-^{13}C): (III)

The synthesis of lumichrome (10a-^{13}C) (III) was accomplished by a continuation of the method of Birch and Moye (15). 2,4-Dihydroxy-6-(2-hydroxy-2-methyl-3-oxobutyl)-6-methylpteridine (8a-^{13}C) (XII) was dissolved in sodium hydroxide (2N, 20 ml) and heated on a steam bath for one hour. Upon cooling a bright yellow precipitate (485 mg, 1.84 mmoles) was isolated. This was shown to be the sodium salt of lumichrome, by u.v., ir, and ^{13}C-NMR. This represented a 44.1% yield. The retention and position of the carbon-13 label was demonstrated by intensity data in the ^{13}C-NMR spectra.

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