SYNTHESIS OF NITROGEN-15 LABELLED URACIL AND ITS 1-DEUTEROMETHYL 3-DEUTEROMETHYL, AND 1,3-DIDEUTEROMETHYL DERIVATIVES (1)

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SUMMARY

A procedure is described for synthesizing uracil-1,3-$^{15}N_2$ in 77% yield by the condensation of urea-$^{15}N_2$ with propiolic acid. The uracil-1,3-$^{15}N_2$ was employed subsequently in a random alkylation with dimethyl sulfate-d_6 to give 1-methyl-d_3-uracil-1,3-$^{15}N_2$, 3-methyl-d_3-uracil-1,3-$^{15}N_2$, and 1,3-dimethyl-d_6-uracil-1,3-$^{15}N_2$. Purification of the products resulting from both these reactions was achieved on an ion exchange column. The ^{15}N and ^{1}H NMR spectra obtained are consistent with these structures.

Key Words: Uracil-1,3-$^{15}N_2$, 1-methyl-d_3-uracil-1,3-$^{15}N_2$, 3-methyl-d_3-uracil-1,3-$^{15}N_2$, 1,3-dimethyl-d_6-uracil-1,3-$^{15}N_2$, ^{15}N NMR, ^{1}H NMR.

INTRODUCTION

The preparation of biological molecules with 99% enrichment in ^{15}N substantially increases the ^{15}N NMR detection sensitivity, and allows for the convenient measurement of ^{1}H-^{15}N and ^{13}C-^{15}N coupling constants from the corresponding proton and carbon-13 spectra (2). Such selective isotopic enrichment would permit facile detection and identification of a relatively small number of NMR signals in an otherwise complex molecule.

We synthesized the title $^{15}N_2$-enriched compounds (Scheme I) to test the applicability of these NMR parameters in determining the uracil monoanion tautomeric equilibrium (5). The deuteromethyl rather than methyl substituent reduced the chemical shift range of observed NMR spectral transitions, and provided increased precision in our Fourier Transform measurements of the ^{1}H and ^{13}C (6) NMR spectra.

Uracil-1,3-$^{15}N_2$ (3) has been prepared previously through the condensation of urea-$^{15}N_2$ with malic acid in the presence of fuming sulfuric acid (4,7) in 22.5-24.7% yield based upon the urea-$^{15}N_2$, as described by Davidson and Baudisch (8) for making the nonlabelled compound.

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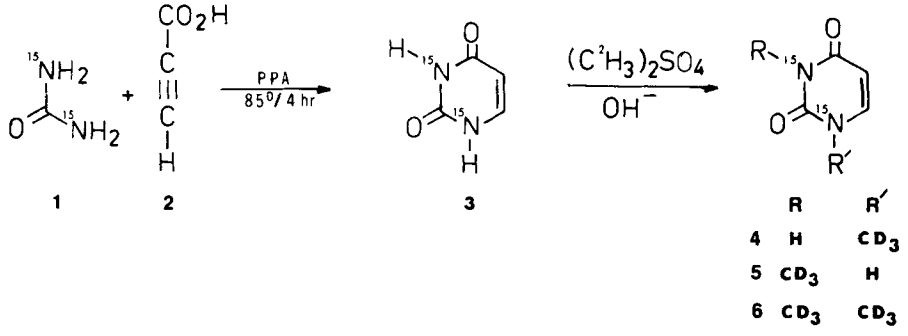
0362-4803/80/0217-0247$01.00

Received March 5, 1979
Revised April 11, 1979

SCHEME I



1 2 3

	R	R′
4	H	CD_3
5	CD_3	H
6	CD_3	CD_3

Of the several other methods for the synthesis of uracil (9-16), that of Harada and Suzuki (17) involving the condensation of urea and propiolic acid, in practically equimolar quantities, in polyphosphoric acid (17) proved highly satisfactory, providing an overall yield of 77% uracil-1,3-$^{15}N_2$. We employed a random methylation providing all three products 4, 5 and 6 in a single step rather than separate synthesis of each of these compounds (8,18,19). The reaction of uracil-1,3-$^{15}N_2$ with one equivalent of dimethyl sulfate-d_6 in the presence of one equivalent of aqueous sodium hydroxide proved successful. The three products and unreacted starting material were separated on an ion exchange column. The unreacted starting material could be recycled after recovery.

EXPERIMENTAL

The ^1H and ^{13}C FT NMR spectra were obtained on a JEOL-PFT-100 Spectrometer. The ^{15}N NMR spectra were obtained on a highly modified Bruker HX-90 Spectrometer operating at 9.12 MHz (20). The UV spectra were recorded using a Varian Superscan 3 Spectrophotometer. In all chromatographic separations, water, which was first deionized and then glass distilled was used as an eluent, and delivered by means of a Cole Parmer Masterflex peristaltic pump. The eluate was monitored with an ISCO UA-4 detector at 254 nm, and fractions were collected

on an ISCO Model 273 Fraction Collector. The UV absorbing, chromatographically homogeneous fractions were combined and evaporated to dryness in tared flasks in vacuo with a Buchler flash evaporator. The structures of the isolated materials were deduced from their characteristic UV spectra obtained under neutral and basic conditions when compared to similar spectra of authentic samples of the corresponding nonenriched compounds.

Uracil-1,3-$^{15}N_2$ (3).

A 775 mg (12.5 mmoles) portion of urea-$^{15}N_2$, 99.6% ^{15}N (1) (KOR Isotopes, Inc., Cambridge, Mass.), dried overnight in vacuo at 78°, was introduced into a flask containing 20 g of syrupy polyphosphoric acid (BHD Laboratory Reagents, Poole, England). A 0.85 ml (0.96 g, 13.8 mmoles, 10% excess) portion of propiolic acid (2) (Aldrich, 98%) was then added. The flask was capped with a drying tube (Drierite), and the mixture was heated in an oil bath at 85° for 4 hr with magnetic stirring (21,22). The mixture was allowed to remain overnight at room temperature, and afterwards 40 ml of water was added to the yellow-orange oil and swirled until a uniform solution was produced. White crystalline product began to separate out from the mother liquors after about 10 min. The resulting suspension was allowed to stir for ~20 min in an ice bath, and filtered through a sintered glass funnel. The collected crystalline solid was redissolved in hot water and applied to a 2.5 x 90 cm column packed with AG-50W-X8 resin, 200-400 mesh, H^+ form (Bio-Rad Laboratories), and eluted with water. The uracil-1,3-$^{15}N_2$ fractions were combined and taken to dryness to yield 728 mg of pure white crystalline product. Similar treatment of the above mother liquors afforded an additional 373 mg of crystalline product to give a total of 1,101 mg of 3, 77.3% yield. The proton NMR spectrum of the material recorded at high sensitivity reveals no detectable impurities.

1-Methyl-d_3-uracil-1,3-$^{15}N_2$ (4), 3-Methyl-d_3-uracil-1,3-$^{15}N_2$ (5) and 1,3-Dimethyl-d_6-uracil-1,3-$^{15}N_2$ (6).

A 787 mg (6.90 mmole) sample of recrystallized 3 was dissolved in 175 ml of water and 6.9 ml of 1.0 N sodium hydroxide (6.90 mmoles). A 0.68 ml (0.91 g, 0.90 mmoles) volume of freshly opened dimethyl-d_6 sulfate (Aldrich, 99% d) was

then added from a glass syringe. The mixture was magnetically stirred overnight at room temperature, concentrated to a small volume, and applied in two equal portions to a 2.5 x 90 cm AG-50W-X8, 200-400 mesh, H^+ form (Bio-Rad Laboratories) ion-exchange column. The products were eluted with water in the order 3, 4, 5, and 6. Those overlapping fractions containing small amounts of 3, 4, and 5 not completely resolved in the primary separation were combined, concentrated, and rechromatographed. The total yields of each of 4, 5, and 6 were 155 mg (17.2%), 166 mg (18.4%), and 105 mg (10.3%), respectively. These amounts added to the 395 mg (50.2%) of recovered unreacted starting material, 3, represents a total recovery of 96.1%. Unreacted 3 could be recycled, if desired, to obtain additional quantities of the deuteromethyl products.

NMR Measurements

The NMR parameters δH_5, δH_6, and J_{H_5,H_6} derived from the measurement of the proton spectra of 3, 4, and 6 in DMSO-d_6 are consistent with those of Stolarski et al. (23) and Roberts et al. (7) (Tables I and II) for the corresponding non-^{15}N-enriched compounds. The values for $J_{H6,N1}$, $J_{H5,N1}$, and J_{H_5,N_3} for 3 are consistent with those reported previously by Roberts et al. (7). Since the values of these corresponding ^{15}N-^{1}H couplings for 4, 5, and 6 are approximately the same as those for 3, this measurement provides strong evidence for structure 4, 5, and 6. Compound 5 is soluble in $CDCl_3$, and its spectrum in this solvent (Figure 1) reveals appreciable long range couplings of the H_1 nucleus to H_5, H_6, and N_3 that were obscured in DMSO-d_6 because of the more rapid prototopic exchange of H_1 in the latter solvent.

We have also obtained the ^{15}N NMR spectrum of uracil-1,3-$^{15}N_2$ (Figure 2) and that of its mono-N-deuteromethyl derivatives. The chemical shifts (Table III) found are consistent with those reported in the literature for similar compounds (24-26), and these results provide additional support for structures 3, 4, and 5. In the case of 3, we have resolved a splitting of 2.6 Hz for each nitrogen (Figure 2), which we assign to the two bond ^{15}N-^{15}N coupling, and which compares favorably with that value (2.2 Hz) reported by Büchner et al. for ^{15}N-enriched uridine-3'-monophosphate (24).

FIGURE 1. Proton NMR Spectrum of 3-Methyl-d_3-uracil-1,3-$^{15}N_2$ (5) in CDCl

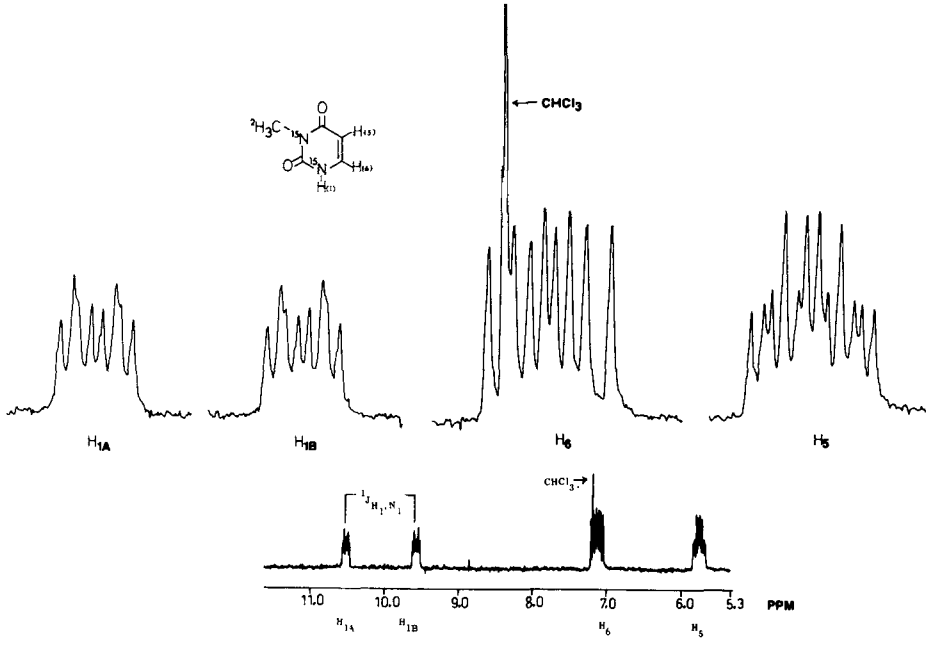
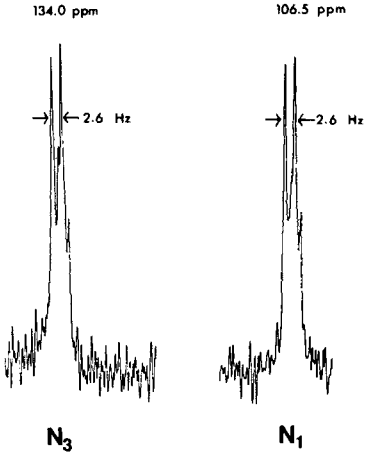


FIGURE 2. Nitrogen-15 NMR Spectrum of uracil-1,3-$^{15}N_2$ (3) in DMSO-d_6



Acknowledgments. We would like to express our appreciation to Mr. Marvin Olsen for recording the 100 MHz pmr spectra, and Dr. George B. Brown for his continuing interest and encouragement. We also wish to thank Prof. David Cowburn and Prof. David H. Live for helpful discussions and for making available for our use the modified Bruker HX-90 NMR spectrometer at Rockefeller University for the ^{15}N measurements. We are indebted to Mr. William M. Wittbold, Jr. for his expert assistance in the operation of the Bruker instrument.

TABLE I

Proton NMR Chemical Shifts for Uracil-1,3-$^{15}N_2$ and its Deuteromethyl Derivatives (ppm downfield from internal TMS)[a]

Compound	Solvent	δH_5	δH_6	δH_1	δH_3
Uracil-1,3-$^{15}N_2$ (3)	DMSO-d_6	5.451	7.393	10.83[d]	11.02
		(5.46)[b]	(7.39)[b]		
		(5.50)[c]	(7.38)[c]	(10.78)[c]	(10.96)[c]
1-Methyl-d_3-uracil-1,3-$^{15}N_2$ (4)	DMSO-d_6	5.515	7.609	-	11.22
		(5.52)[b]	(7.61)[b]	-	-
3-Methyl-d_3-uracil-1,3-$^{15}N_2$ (5)	DMSO-d_6	5.584	7.431	11.15	-
	$CDCl_3$	5.803	7.205	10.18	-
1,3-Dimethyl-d_6-uracil-1,3-$^{15}N_2$ (6)	DMSO-d_6	5.657	7.665	-	-
		(5.66)[b]	(7.66)[b]	-	-

a) At ambient probe temperature, 22°C. b) For the non-^{15}N-enriched compounds, from Stolarski et al. (23). c) For the ^{15}N-enriched compound, from Roberts et al. (7). d) For non-^{15}N-enriched compound, this work.

TABLE II

Proton-Nitrogen-15 and Proton-Proton Coupling Constants for Uracil-1,3-$^{15}N_2$ and its Deuteromethyl Derivatives (In Hz)[a]

	Compounds					
	3		4	5		6
Parameter	DMSO-d_6		DMSO-d_6	DMSO-d_6	$CDCl_3$[b]	DMSO-d_6	
J_{H_1,N_1}	(91)[c]		-	-	96.13	-	
J_{H_1,N_3}	-		-	-	2.42	-	
J_{H_1,H_5}	-		-	-	1.77	-	
J_{H_1,H_6}	(5.8)[c]		-	-	5.74	-	
J_{H_3,N_3}	90.0	(97)[c]	86.1	-	-	-	
J_{H_5,N_1}	4.27	(4.4)[c,d]	4.66[d]	4.35[d]	4.64[d]	4.88[d]	
J_{H_5,N_3}	2.44	(2.5)[c,d]	2.52[d]	2.82[d]	2.75[d]	2.90[d]	
J_{H_5,H_6}	7.63	(7.70)[e]	7.79	(7.82)[e]	7.63	7.63	7.78 (7.78)[e]
J_{H_6,N_1}	3.44	(3.5)[c]	2.45	3.51	3.45	2.52	
J_{H_5,H_3}	1.98[f]		2.29[f]	-	-	-	

a) At ambient probe temperature, 22°C; nominal spectral resolution from 1.25 KHz sweep width and 16 K data points is 0.15 Hz; the numerical values were extracted from the averages of the corresponding repeated spacings, since $J/\Delta\nu \ll 0.1$ in all such cases, allowing a first order treatment (27). b) Under the conditions of slow prototopic exchange in this solvent, the long range NH couplings are observed; the poor solubility of 3 and 4 in this solvent prevented making similar measurements for these compounds. c) From Roberts et al. (7). d) The two couplings J_{H_5,N_1} and J_{H_5,N_3} could not be assigned unambiguously by Roberts et al. (7). We have now assigned them on the basis of changes in their magnitudes for 4 and 5 related to the transformation of these molecules from the neutral to monoanionic species in D_2O solution(5).

e) For the non-^{15}N-enriched compound, from Stolarski *et al.* (22). f) Obtained from the non-^{15}N-enriched compound, this work; nominal resolution is 0.30 Hz in this case.

TABLE III

Nitrogen-15 Chemical Shifts of 3, 4, and 5, and Other Uracil Derivatives

(ppm downfield from external 5 M $^{15}NH_4NO_3$ in 2 M NNO_3)

Compound	Solvent	δN_1	δN_3	Reference
Uracil-1,3-$^{15}N_2$	DMSO	112.6[b]	140.1[b]	This work
1-Methyl-d_3-uracil-1,3-$^{15}N_2$	DMSO	-	138.9[b]	This work
	H_2O	-	139.5	This work
3-Methyl-d-uracil-1,3-$^{15}N_2$	H_2O	114.1[b]	-	This work
Uridine	DMSO	123.5	137.5	Hawkes *et al.* (25)
	DMSO	123.4[c]	137.5[c]	Markowski *et al.* (26)
	D_2O/H_2O	125.0	137.3	Hawkes *et al.* (25)
Uridine-5'-monophosphate	H_2O	125.0[c]	138.5[c]	Markowski *et al.* (26)
Uridine-3'-monophosphate	H_2O	124.7[d]	137.4[d]	Buchner *et al.* (24)

a) Data for this work obtained at ambient probe temperature, 26°C; 2.5 KHz sweep width and 2 K data points; for uracil-1,3-$^{15}N_2$, $J_{N_1N_3}$ = 2.6 Hz measured at 0.5 KHz sweep width; due to the rapid pulsing and small number of accumulations employed, no nitrogen signal was observed for the methylated nitrogen from 1-methyl-d_3-uracil-1,3-$^{15}N_2$ and 3-methyl-d_3-uracil-1,3-$^{15}N_2$. b) Measured in ppm downfield from external saturated aqueous $^{15}NH_4Cl$, and corrected according to the equation, $\delta(^{15}NH_4NO_3) = \delta(^{15}NH_4Cl) + 6.1$ (28). c) Measured in ppm upfield from external 0.1 M $D^{15}NO_3$ in D_2O, and corrected according to the equation $\delta(^{15}NH_4NO_3) = 355.0 - \delta(D^{15}NO_3)$ (25). d) Measured in ppm upfield from 4 M $^{15}NH_4NO_3$ in 2 M HNO_3 and corrected according to the equation, $\delta(^{15}NH_4NO_3)^* - 0.1$ (28).

REFERENCES AND NOTES

1. This investigation was supported in part by Grant Number CA 08748 awarded by the National Cancer Institute and a Biomedical Research Grant No. 5 S07 RR 0534-04 awarded by the National Institutes of Health, DHEW, to R.L.L.

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