NOTE

SYNTHESIS OF MORPHOLINIUM [^{13}C] FORMATE AND ITS APPLICATION IN THE SYNTHESIS OF [8-^{13}C] PURINE BASE

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SUMMARY

Morpholinium [^{13}C]formate is obtained by neutralizing an aqueous solution of equivalent amounts of sodium [^{13}C]formate and hydrochloric acid with morpholine. Morpholinium [^{13}C]formate is isolated in crystalline form and characterized. Treatment of 6-hydroxy-2,4,5-triaminopyrimidine sulfate with morpholinium [^{13}C]formate at 200° affords [8-^{13}C]guanine in 80% yield. There is no necessity to synthesize and isolate [^{13}C]-N-formylmorpholine and the intermediate N^5-[^{13}C]formyl pyrimidine derivative. Mass spectrometric analysis shows that the molar isotopic concentration of ^{13}C label in guanine is identical to that of the morpholinium [^{13}C]formate and there is no isotopic scrambling. The same procedure when used with 4,5,6-triaminopyrimidine sulfate affords adenine in 85% yield.

Key words: Morpholinium formate, carbon-13, guanine, adenine.

INTRODUCTION

In view of increasing interest in DNA adducts in the initiation of carcinogenesis by chemical agents (1-3), synthesis of appropriate nucleic acid model and/or specifically labelled reagents is prerequisite to characterize and understand their interaction at the molecular level. In nucleic acids, the guanine moiety is very susceptible to attack by a variety of chemical agents (4-7). Guanine labelled with ^{13}C or ^{14}C is an essential tool to study the effect of an agent on a treated model compound. Labelling with a stable isotope has great advantage over the conventional methods of analysis of radio-chemical isotopes. Mass spectrometric location of stable isotopes in the purine nucleus is of potential value in studies of purine biosynthesis and metabolism (8). Box et al. (9, 10) have extensively used nmr spectroscopy to characterize alkylated derivatives of guanosine and other nucleosides. They have also shown (11) that measurement of ^{13}C chemical shifts spectra of nucleic acid model before and after treatment by carcinogen identifies the site of action without the necessity of processing such as hydrolysis and chromatography.

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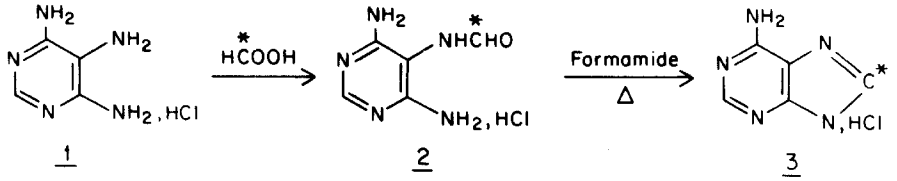
This report describes the synthesis of morpholinium[^{13}C]formate and its application in single step synthesis of C-8 labelled purine base in general with particular reference to [8-^{13}C]guanine. The chemical yield of guanine is 80% with respect to the pyrimidine derivative and the isotopic yield 100% relative to morpholinium[^{13}C]formate

DISCUSSION

Although several isotopic syntheses of purine bases have been reported, the procedure introducing a label specifically at the C-8 position merits particular attention (12-14). The current literature is abundant with reports of isolation and characterization of C-8 guanine adducts from interaction of nucleic acid with a variety of chemical agents. We have already cited a few references (4-7) in the Introduction. The use of such adducts in understanding the mode of reaction between the nucleic acid and the chemical agent is of immense value. From the synthetic point of view, the introduction of label at C-8 position represents the final step in purine synthesis and should therefore give a high yield of labelled material. Cavalieri and Brown (13) described the synthesis of labelled adenine according to the following scheme:

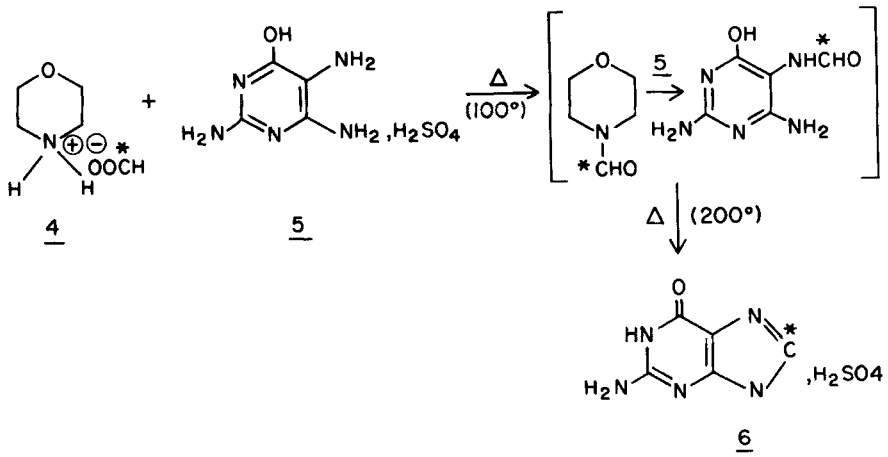


Scheme I

They observed that although the conversion of 1→2 can be affected in fairly good yield, when 2 is heated in formamide to affect the ring closure extensive exchange of label took place with the solvent and the required product 3 contained only 25% of expected isotope concentration. Abrams et al. (14) demonstrated using ^{14}C-labelling that when 4,6-diamino-5-formamido pyrimidine is heated in N-formylmorpholine, the solvent is labelled 50% with ^{14}C.

The demonstration (14) that N-formylmorpholine is 50% labelled with ^{14}C according to Scheme I indicates that N-formylmorpholine is not only a suitable solvent for ring closure step but also a good formylating reagent. The use of labelled N-formylmorpholine should, therefore, prevent the loss of label by isotopic exchange with the unlabelled solvent. Abrams et al. reported the synthesis of adenine-8-^{14}C with no loss of ^{14}C label by cyclizing the pyrimidine derivative with ^{14}C labelled N-formylmorpholine. However their yield of ^{14}C N-formylmorpholine using labelled sodium formate was only 59%. Following their procedure we attempted to synthesize ^{13}C labelled N-formylmorpholine from sodium[^{13}C]formate in less than 30% yield. There was extensive decomposition of labelled product during isolation by fractional distillation even under high vacuum. The general procedure for N-formylmorpholine synthesis required excess formic acid (15); the source of labelled formic acid is ^{13}C or ^{14}C sodium formate. Since sodium[^{13}C]formate is quite expensive, it is important to utilize the enriched formate efficiently. We prepared ^{13}C labelled morpholinium formate in quantitative yield by neutralizing an aqueous solution of [^{13}C]-formic acid, generated from equivalent amounts of sodium[^{13}C]formate and hydrochloric acid, with morpholine. The ^{13}C-labelled salt was isolated in crystalline form and characterized by nmr and mass spectroscopy. The molecular ion at 116 corresponded to ^{13}C labelled N-formylmorpholine. This important result shows that during heating, the morpholinium salt is converted to the formylating agent N-formylmorpholine which then formylates N^5-amino group of 5 and undergoes ring closure at 200° to yield [8-^{13}C]guanine 6 according to Scheme II.

[8-^{13}C]Guanine was obtained in 80% yield. Formylation of N^5-amino group in 5 was complete in half an hour at 100° as checked by tlc. Heating the reaction mixture at 200° for 2 hrs completed the ring closure. The isolation of N^5-formyl (2) derivative as in Scheme I was not necessary. Excess morpholinium ^{13}C formate used in the reaction was recovered by treating the reaction mixture with ethanol. [8-^{13}C]Guanine was filtered from the ethanol solution; evaporation of the filtrate gave the ^{13}C-labelled reagent which was recycled.



Scheme II

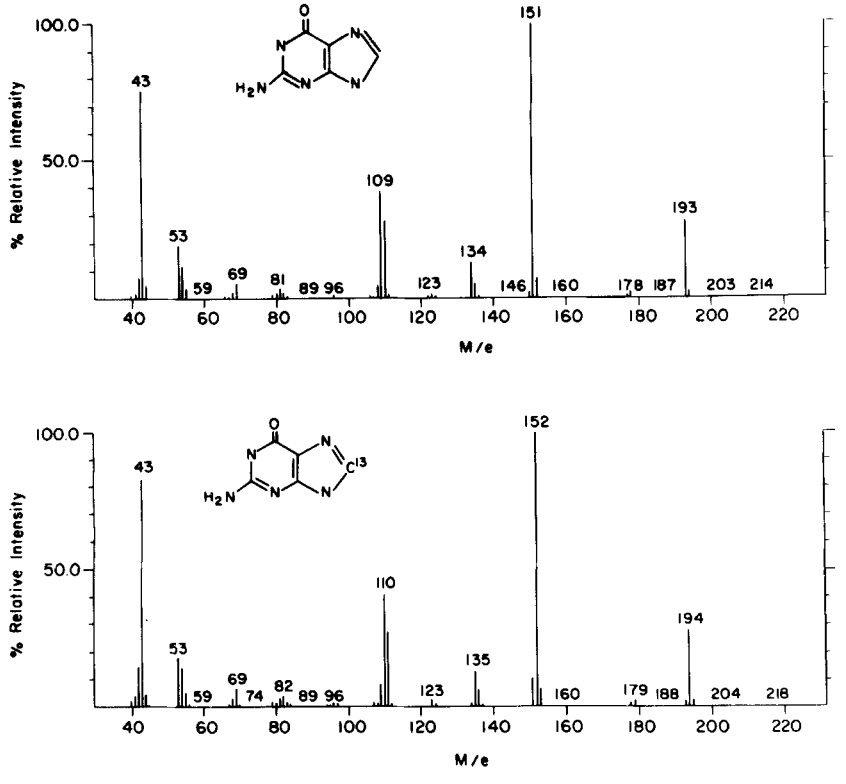


Figure 1. Mass spectrum of guanine acetate and [8-^{13}C] guanine acetate.

The incorporation of ^{13}C label, as calculated by mass spectrometric analysis, was found to be 90% in both 4 and 6 which was the same in commercial sodium [^{13}C]formate. Figure 1 shows the mass spectrum of guanine in acetate form. The molecular ion is observed at m/z 193 in non-labelled guanine acetate. The fragmentation at m/z 151 and below resembles closely that reported for guanine (16). In 8-^{13}C labelled guanine acetate the major fragments result from the same losses observed for the non-labelled compound with +1-shift due to ^{13}C. There is no significant isotopic scrambling.

Reaction of 4,5,6-triaminopyrimidine sulfate with morpholinium formate according to Scheme II gave adenine in 85% yield.

EXPERIMENTAL

^{13}C-labelled sodium formate was purchased from Merck and Co., Inc., Rahway, NJ and 90 atom % ^{13}C enriched.

Morpholinium[^{13}C]formate, 4. ^{13}C-labelled formic acid solution was prepared by dissolving 0.252 g (3.7 mmol) ^{13}C sodium formate in 2.5 ml water followed by addition of 0.37 ml of 12 M hydrochloric acid. Freshly distilled morpholine 0.448 g (4 mmol) was added dropwise to the acid solution at 0° under stirring. The solution was evaporated to dryness under rotary evaporator. Absolute ethanol was added to the solid residue and filtered to separate NaCl. The residue was washed with the same solvent. The filtrate and the washing was combined and evaporated. The residue, a colorless viscous liquid, solidified to crystalline solid at room temperature. Yield 98%; ir. 3077, 1610, 1575 and 1500 cm; nmr ($CDCl_3$) (δ) 3.17 (4H,m), 3.90 (4H,m), 8.55 (1H,s) and 10.45 (2H,s); MS m/e 116 (m^+), 87, 57, 42.

[8-^{13}C]Guanine, 6. 2,4,5-Triamino-6-hydroxypyrimidine sulfate 0.239 g (1 mmol) was mixed with 0.5 g morpholinium[^{13}C]formate and the mixture was heated at 100° in an oil bath for 30 min. The temperature was then raised to 200° and heating continued for 2 hrs. Excess ^{13}C formyl reagent was recovered by treating the reaction mixture with absolute ethanol. ^{13}C-Guanine was filtered from the ethanolic solution (.2 g, 80% yield). U.v. λ_{max} 246, 276, ε_{max} 10,700, 7,600 at pH 7; MS. m/e 152 (m^+), 135, 110, 82, 53 and 43; tlc. R_f 0.58. The

tlc system was isopropanol:water:ammonia (7:2.5:0.5) in silica.

Adenine. 4,5,6-Triaminopyrimidine sulfate when reacted with morpholinium formate under similar condition as above afforded adenine in 85% yield. Uv. λ_{max} 260, ϵ_{max} 13,000 at pH 7.

Crude reaction products are slightly colored but quite pure as indicated by uv. and tlc. with authentic samples of guanine and adenine. Crude products are dissolved in 1-2 N hydrochloric acid and decolorized with charcoal treatment.

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