SYNTHESIS OF SEVEN DEUTEROMETHYL-CAFFEINE ANALOGUES OBSERVATION OF DEUTERIUM ISOTOPE EFFECTS ON CMR ANALYSIS

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SUMMARY

This article describes synthesis of all 7 N-trideuteromethyl isotopomers of caffeine by reaction of trideuteromethyl iodide (C^2H_3I) with the appropriate xanthine molecules.
The use of proton, deuterium and carbon-13-NMR as a first step in purity assessment revealed ^{13}C-NMR deuterium isotope effects on the resonance of per-deuteromethyl carbons.

Key Words : caffeine, deuterium, isotope effects, NMR.

INTRODUCTION

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione), an alkaloid well known for its many actions on the central nervous system (alteration of psychomotor coordination, EEG spectra, sleep, mood and cognition) induces numerous other pharmacodynamic effects (1,2).

Despite its long use in alimentation and for therapeutic purposes, its intimate actions on biological systems have been uncovered recently (3-9). Its metabolic pathways (10-12) and pharmacokinetics (13-16) have also been precised.

Though speculation has been growing concerning caffeine toxicity (1,17) ; one generally lacks evidence for it (1).

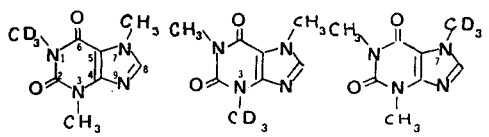
Moreover, new interest has emerged as to its potential effectiveness in the treatment of atopic dermatitis (1,18) and neonatal apnea (1,19-21).

In the course of stable-isotope studies of methyl xanthines metabolism and pharmacokinetics (22,23), we found of interest to search for possible isotope effects due to deuterium labelling of caffeine. So all 7 N-trideuteromethyl

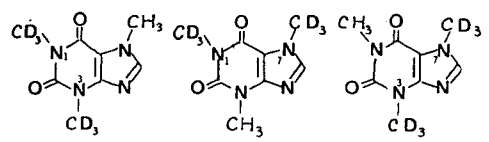
0362-4803/86/030267-10$05.00

Received July 18, 1985

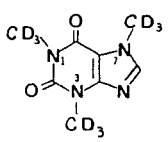
analogues of caffeine (Fig. 1) were prepared via alkylation of the appropriate xanthine molecules using $[^2H]_3$-iodomethane.



1- CD_3 CAFFEINE (1) 3- CD_3 CAFFEINE (2) 7- CD_3 CAFFEINE (3)



1,3 $(CD_3)_2$ CAFFEINE (4) 1,7 $(CD_3)_2$ CAFFEINE (5) 3,7 $(CD_3)_2$ CAFFEINE (6)



1,3,7 $(CD_3)_3$ CAFFEINE (7)

Figure 1 - Structure of the trideuteromethyl-caffeine molecules
(For clearness of presentation ^2H was noted D)

The present paper is intended to describe the method used for the synthesis and present the results of NMR analysis.

EXPERIMENTAL

SYNTHESIS

. The mono-trideuteromethyl caffeine-molecules = 1-trideuteromethyl-3,7-dimethylxanthine (1), 3-trideuteromethyl-1,7-dimethylxanthine (2) and 7-trideuteromethyl-1,3-dimethylxanthine (3) were synthesized from theobromine (3,7-dimethylxanthine, Sigma Chemical Co), paraxanthine (1,7-dimethylxanthine, Fluka) and theophylline (1,3-dimethylxanthine, Sigma Chemical Co) respectively.

. The di-trideuteromethyl caffeine-molecules = 1,3-di-trideuteromethyl-7-methylxanthine (4) ; 1,7-di-trideuteromethyl-3-methylxanthine (5) and 3,7-di-trideuteromethyl-1-methylxanthine (6) were synthesized from 7-, 3- and 1- methylxanthines (Fluka) respectively.

. Tri-trideuteromethyl caffeine (7) was synthesized from xanthine (Sigma Chemical Co).

In all cases, the method we used was derived from Horning and al (24) according

to the following procedure. To a stirred solution of 500 mg xanthine (or mono- or dimethylxanthine) in 25 ml acetone : water (1:1) were added successively 25 ml of 0.5 N sodium hydroxide solution and 750 ul (or 500 ul or 250 ul for synthesis of di- and mono-trideuteromethyl caffeine-molecules respectively) C^2H_3I (CEA, Saclay, France, isotopic enrichment : 99.25 %).

Additional aliquots of $[^2H]_3$ methyl iodide (750 - 500 or 250 ul) were added after 1 and 2 hours.

After a 2-day standing at room temperature, acetone was removed under a stream of nitrogen and 50 ml of water added.

The rough product was extracted from the aqueous solution using $CHCl_3$ (3 x 50 ml). The solvent was removed in a rotary evaporator (55°C).

PURIFICATION

The white solid residue obtained was redissolved in chloroform and purified via preparative plate chromatography (home-made 20 x 40 cm glass-plates coated with 0.8 mm HF-254 Merck silica gel).

Before use, the plates were activated by heating at 100°C for 3 hours.

All chromatographic separations were conducted in tanks saturated with solvent system ethyl acetate-methanol-25 % ammonia (80:20:10 ; V/V).

After migration, trideuteromethyl caffeine spots were located on the plates, the silica was scraped out and the compounds were extracted using chloroform (4 x 50 ml).

Solvent evaporation (rotary evaporator) yielded the 7 purified products. Overall yields ranged from 15 to 60 % ; however no valuable conclusion may be drawn from this variability, considering the several steps involved in preparation.

NMR ANALYSIS

- Proton Magnetic Resonance (P.M.R.) studies [0,15 ppm] used a Bruker WP-80-FT high-resolution spectrometer and $CDCl_3$ as solvent (internal lock).
The following conditions were used for all spectra : 8 scans, pulse angle : 30° ; acquisition time : 2.73 s. ; pulse delay : 0 s.

- Deuterium Magnetic Resonance (D.M.R.) studies ([-275, + 100 Hz], reference $CDCL_3$) were carried out with $CHCl_3$ as solvent (same spectrometer).
The following conditions were used : 16,32 or 48 scans ; pulse angle : 30°,

acquisition time : 2.73 s. ; pulse delay : 0 s.

- Carbon-13 Magnetic Resonance-Proton decoupled ($\{^1H\}$ CMR) spectra were obtained using $CDCl_3$ as solvent and lock.
Reference was TMS.
A Varian XL - 100 - FT (25.2 MHz) high-resolution spectrometer was used in all cases (acquisition time : 1 s. ; pulse angle : 35° ; pulse delay : 0 s.) except for the magnified spectra of 1, 4, 5 and 7 which were obtained on a Cameca 350-FT (88 MHz) high-resolution spectrometer (acquisition time : 0.475 s. ; pulse angle : 38° ; pulse delay : 6×10^{-3} s.).

All spectra were recorded at room-temperature.

C^2H_3I isotopic purity was assessed by GC/electron-impact mass-spectrometry (70 eV) using a HP 5970 B mass-spectrometer equipped with a HP 59970 A Workstation (Hewlett Packard, Evry, France).

RESULTS AND DISCUSSION

PROTON MAGNETIC RESONANCE (P.M.R.) signals of natural and synthetic caffeine analogues are gathered in following table :

Position of caffeine protons (For atom numbering, see Fig.1)	Corresponding δ (ppm)	Caffeine analogues concerned
$N_1 - CH_3$	3.40	Caffeine, 2, 3, 6
$N_3 - CH_3$	3.58	Caffeine, 1, 3, 5
$N_7 - CH_3$	3.99	Caffeine, 1, 2, 4
C_8	7.51	all analogues

Table 1 : PMR signals of caffeine and trideuteromethyl caffeine-molecules

Deuterium labelling is of particular interest here, permitting unequivocal assignment of the three N-methyl proton groups : each time one position is labelled, disparition of natural caffeine corresponding signal ensues.

Moreover no offset signal could be observed in the spectra of the 8 analogues investigated (absence of N-H signal from partially methylated xanthines used for synthesis).

To finish with, P.M.R. reveals no isotope effect on the chemical shifts of N-methyl protons at other positions, as well as on that of H on C-8.

DEUTERIUM MAGNETIC RESONANCE (D.M.R.)

D.M.R. permits monitoring of the labelling process from another standpoint, namely emergence of isolated or combined resonance signals each time one or more N-trideuteromethyl groups are introduced into xanthine molecules (table 2).

Position of trideuteromethyl substitution	Corresponding δ (ppm)	Caffeine analogues concerned
N - 1	- 0.60	1, 4, 5, 7
N - 3	- 0.57	2, 4, 6, 7
N - 7	- 0.51	3, 5, 6, 7

Table 2 : DMR signals of trideuteromethyl caffeine-molecules

As expected, in the case of multiple labelling, the relative intensities of N-methyl deuteron signals are in proportion 1:1 to each other.

No D.M.R. signal could be observed apart from those corresponding to the three N-trideuteromethyl positions.

PROTON-DECOUPLED CARBON-13 MAGNETIC RESONANCE ($\{^1H\}$CMR)

Deuterium labelling results in the splitting into seven lines of N-methyl carbons signals.

Chemical shifts of carbon atoms common to all 8 analogues of caffeine are :

δ = 151,5 ppm for C-2 δ = 155,2 ppm for C-6
δ = 148,4 ppm for C-4 δ = 141,1 ppm for C-8
δ = 107,5 ppm for C-5

Table 3 gathers the resonance data of non-splitted N-methyl signals.

Position of methyl group	δ (ppm)	Caffeine analogues concerned
N 1	27.9	Caffeine, 2, 3, 6
N 3	29.7	Caffeine, 1, 3, 5
N 7	33.6	Caffeine, 1, 2, 4

Table 3 : $\{^1H\}$ CMR signals of perhydrogeno-methyl carbons in caffeine and deuterated analogues

The introduction of trideuteromethyl groups in caffeine does not appear to affect the chemical shifts of other carbon atoms.

To get further insight into possible deuterium isotope effects on ^{13}C chemical shifts and measure $J\ ^{13}C\text{-}^2H$; the regions corresponding to methyl-carbon-resonance were magnified in four instances, namely spectra of 1, 4, 5 and 7 (Figures 2 A - 2 D).

From these spectra, three types of data can be extracted :

- Zooming of the baseline zone [25 ; 35 ppm] reveals ^{13}C splitted signals (7 lines each time one methyl group is labelled), thus allowing determination of $J_{^{13}C\text{-}^2H}$ = 21.2 Hz in all cases.

- Chemical shifts of trideuteromethyl carbons can also be measured, therefore revealing isotope shifts on ^{13}C chemical shifts on deuterium labelling: $^1H/^2H$ replacement is known to induce upfield shifts on the resonance signals of carbons bearing the substituted hydrogen(s) (27 - 30).

These diamagnetic shifts, attributed to an increase in magnetic shielding at isotopically substituted atoms (31), were also observed here, with a magnitude of 0.6 - 0.7 ppm (isotope effect at C on N_7 being nearer the 0.7 ppm mark).

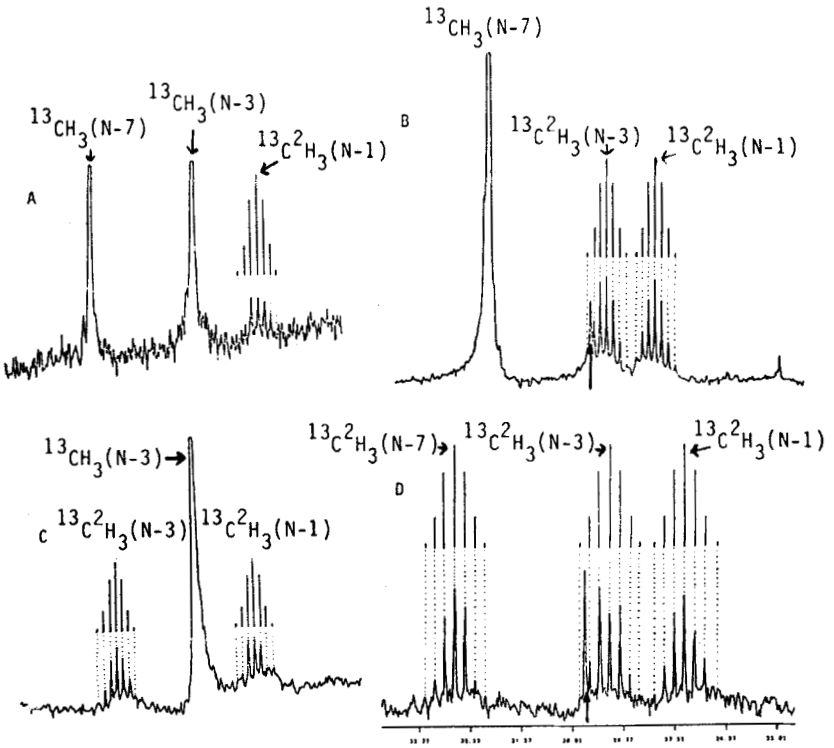


Figure 2 = Fine {^{1}H} CMR patterns of methyl carbons for 1(A) ; 4(B) ; 5(C) and 7(D) with mention of theoretical splitting of $^{13}C^2H_3$ signals ($J_{^{13}C-^2H}$ = 21.2 Hz)

Position of isotopic substitution	^{2}H-isotope shifts (ppm) = $\delta_{^{13}C}$ (caffeine) - $\delta_{^{13}C}$ (deuterocaffeine)			
	1	4	5	7
N_1 substituting carbon	+ 0.6	+ 0.6	+ 0.6	+ 0.6
N_3 substituting carbon	-	+ 0.6	-	+ 0.6
N_7 substituting carbon	-	-	+ 0.7	+ 0.7

Table 4 : {^{1}H} CMR deuterium isotope shifts on signals of N-methyl carbons in 4 caffeine analogues.

- As concerns the spectra of 4 and 7, intense parasite peaks occur at δ = 29.7 ppm (see arrows in fig. 2 B and 2 D) ; corresponding to the resonance signal of a perhydrogenomethyl carbon at position N-3 (Table 3).

This suggests :

a) Isotopic purity C^2H_3I used for methylation is less than 99.25 %.

In order to test for this hypothesis, SIM mass spectrometric analysis was conducted on C^2H_3I, permitting quantitation of the most likely impurity in C^2H_3I, namely CH^2H_2I (m/z = 144).

Effectively a relative-abundance value of 4.6 % was found for ion $(M-1)^+$; which is well over the predictable value of 2.2 % corresponding to 99.25 % isotopic purity.

On that basis, it may be infered that, while replacement of CH_3 by C^2H_3 groups in caffeine induces an upfield shift an the CMR signals of methyl carbons, no such effect occurs when two deuterium atoms are introduced into methyl groups ; a result opposed to previous reports of progressive increase in CMR deuterium isotope shifts along with the number of hydrogens substituted at the carbon atom of interest (29).

b) Substitution of N-H by $N-CH^2H_2$ affects position N_3 preferentially. However further M.S. studies are required for precise quantitation of this parasite substitution (to be published later).

Anyway one must bear in mind that, though more sterically hindered than position N_7 in xanthines ; position N_3 carries a more acidic proton and must thus be favoured for nucleophilic substitution (32). This, combined with the lesser electron-donor properties of ^1H relative to ^2H could possibly explain the marked susceptibility of N_3 to alkylation by CH^2H_2I, though we still lack evidence for it.

CONCLUSION

NMR analysis of the 7 caffeine analogues synthesized evidenced selective methyl deuteration at positions N-1, N-3 and N-7 but failed in quantitating isotopic purity, an obstacle M.S. studies will help overcome.

In four instances, Deuterium isotope effects on { ^1H } CMR signals of N-methyl carbons were observed, while the presence of CH^2H_2I impurity in alkylating reagent casually demonstrated the absence of associated CMR-deuterium isotope shift at N-3.

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Acknowledgments : We wish to thank Dr. Quang Tho Pham for reading the manuscript and Christiane Dufour for secretarial assistance in the preparation of the manuscript. We are also indebted to Jean-claude Fugier for careful spectra copying.