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SYNTHESIS AND E.I.M.S. FRAGMENTATION ANALYSIS OF [1,3- $^{15}N_2$] XANTHINE AND [1,3- $^{15}N_2$] CAFFEINE

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Summary

HPLC and mass spectrometry can be used to isolate and identify all metabolites of caffeine in plasma of patients. The synthesis of [1,3- $^{15}N_2$] xanthine and [1,3- $^{15}N_2$] caffeine are of interest in the elucidation of mass spectrometry fragmentation pathways and the unambiguous determination of metabolites, especially uric acid which exists as a natural constituent of human plasma.

Key Words: caffeine, [^{15}N] labelling, mass spectrometry.

INTRODUCTION

Caffeine (1,3,7-trimethyl-xanthine) is commonly consumed in beverages such as coffee, tea, cocoa and certain soft drinks. Its use in therapy as replacement of theophyllin (1,3-dimethyl-xanthine) has been successfully developed especially in the treatment of apnea in premature infants[1]. Thus the absorption, distribution and excretion of this drug in humans , as well as in various experimental animals, have been the subject of various investigations[2-7]. It has been found that considerable variations appear to exist in the rate of elimination of caffeine from human plasma, which can be responsible for severe intoxications[8-10].This discrepancy in the natural disposition may be due to individual differences in the metabolism and/or environmental factors[11].

Thus it appears necessary to develop efficient methods to gain rapid and precise information on the pharmacokinetics and metabolism of the drug in order to define the sensibility of each patient in a long term treatment.

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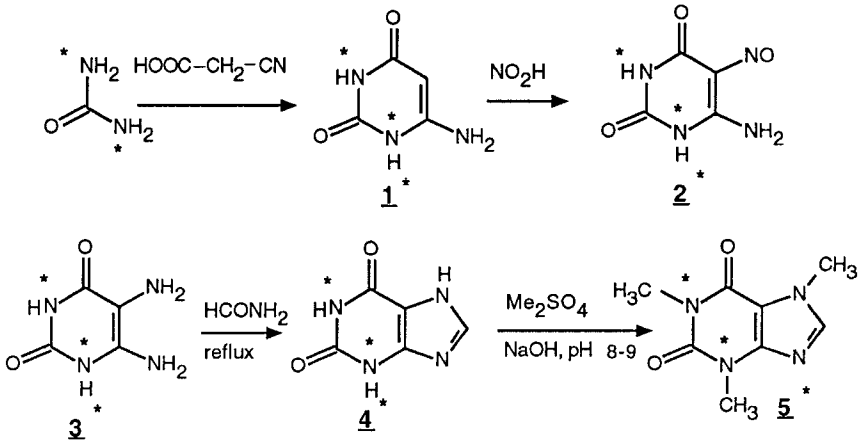
Gas chromatography and liquid chromatography /mass spectrometry methods are convenient methods currently used for the quantification of biodisponibility of drugs and their metabolites. These methods require the synthesis of labelled molecules either as internal standard for mass spectrometry or as unambiguous markers for the identification of metabolites which may arise either from the drug or be naturally present such as uric acid in the case of caffeine. For this purpose some deuterated isotopomers of caffeine have already been described[12-13].

We propose here a more appropriate synthesis of [1,3- $^{15}N_2$] caffeine which was useful in the interpretation of fragmentation patterns in electron impact mass spectrometry (E. I. M. S.).

The synthesis of [1,3- $^{15}N_2$] caffeine 5* followed scheme 1:



SCHEME 1: Synthesis of [1,3- $^{15}N_2$] caffeine

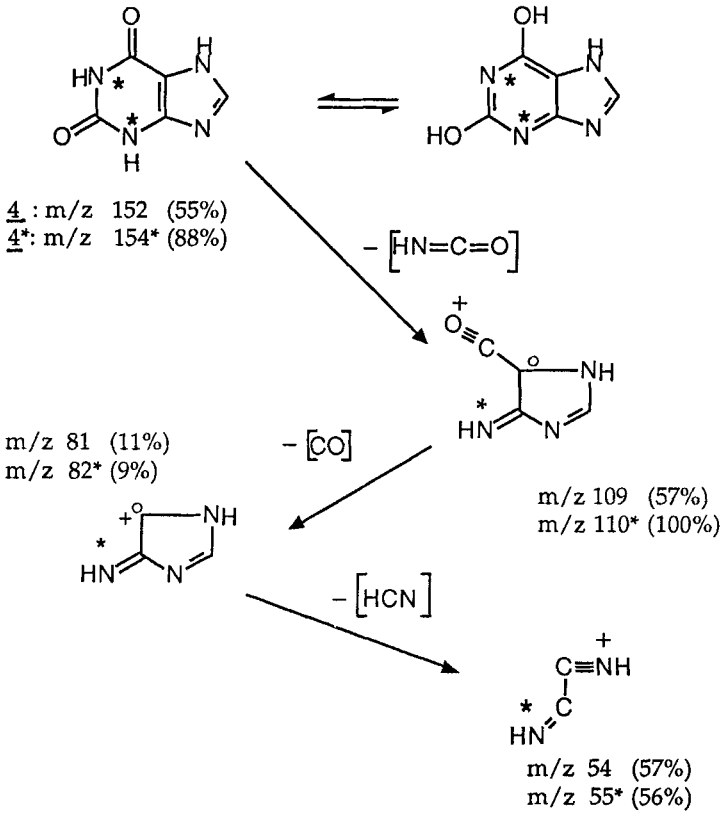
It started as we previously described in [14] from [$^{15}N_2$] urea via 6-amino-uracil 1*, through 2*, 3*[15], which was cyclized by reflux in formamide[16] to 4*, the N-permethylation[16] of which with Me_2SO_4 gave 5*.

RESULTS AND DISCUSSION

Fragmentations of xanthine 4 and [1,3-$^{15}N_2$] xanthine 4* such as fragmentation of caffeines 5 and 5* patterns were compared in order to verify unambiguously some decompositions previously proposed[17-21]. Molecular ions (M^+ and M^++2) were detected with a high relative intensity. A loss of radical (.CHO) from the N_1 position after initial cleavage of the N_1-C_6 bound, was observed for 5 and 5* (scheme 3) and not for 4 and 4* (scheme 2). The correctness of this postulated mecanism was proved by Votiky et al [21] using caffeine [N_1-CD_3]. They observed a loss of 30 mass units (instead of 29 mass units with unlabelled caffeine). It can be interpreted as a proof that the radical °CDO was split off as a result of deuterium transfer from the methyl group at nitrogen N_1 to the adjacent C_6 atom. The absence of the peak at m/z (M-29) for compound 4 (here) and theobromine[21] can be well rationalized, this fragmentation having been accepted.

A retro Diels-Alder decomposition with loss of a cyanate fragment (R-N_1=C=O) (where R= H or CH_3) was observed for all compounds, in agreement with our previous works on electron-impact fragmentation of 6-amino-uracils[14] and in Votiky's work [21] with caffeine [N_1-CD_3].

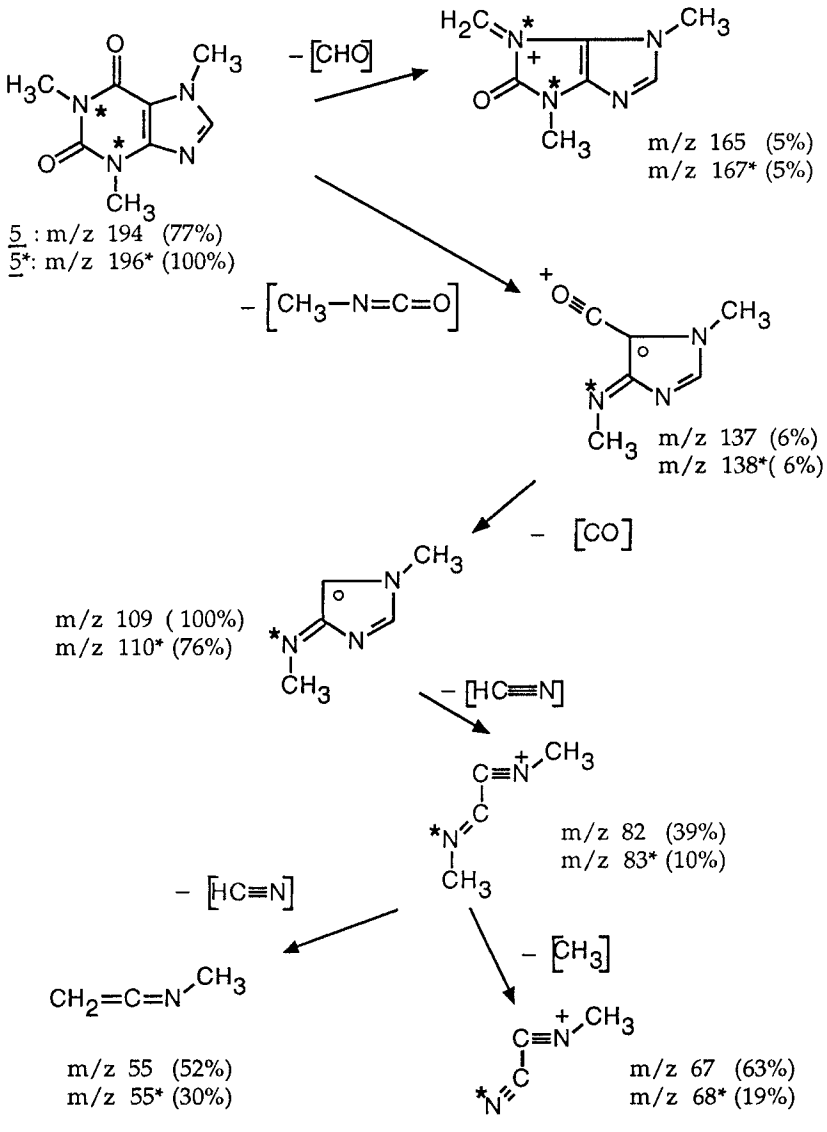
Then, successive losses of neutral (CO) and (HCN) gave ions at m/z = 54, 55 (for 4 and 4*) and 82, 83 (5 and 5*). Ultime fragmentations occur for 5 and 5* ions at m/z = 82 and 83, to give the ions at m/z = 67 and 68 by a demethylation process, and the ions at m/z = 55 after expelling of (HCN) as a result of an internal rearrangement.



SCHEME 2: General fragmentation pattern of xanthine (m/z values and relative intensity %)

CONCLUSION

[1,3-$^{15}N_2$] xanthine and [1,3-$^{15}N_2$] caffeine help to identifying the fragmentation pattern of both compounds under the electron impact. Moreover [1,3-$^{15}N_2$] caffeine can be used to determine its metabolic profiles in human and thanks to an accurate method of detection of all known metabolites of caffeine by HPLC may permit to elucidate some metabolic pathways, especially the formation of uric acid which can arise from other natural sources. Such an investigation is in progress but need recruitment of a lot of patients. The results of this clinical program will be published later on.



SCHEME 3: General fragmentation pattern of caffeine
(m/z values and relative intensity %)

EXPERIMENTAL

[1,3-$^{15}N_2$] Caffeine : synthesis and purification.

[1,3-$^{15}N_2$] caffeine was prepared from [$^{15}N_2$] urea (98%) obtained from CEA (Saclay, France). The intermediate [1,3-$^{15}N_2$]-6-aminouracil **1*** was obtained as we

described previously[14]. From **1***, xanthine and caffeine were obtained in excellent yields. For this purpose **1*** was treated with nitrous acid to give 5-nitroso-6-aminouracil **2*** and subsequently reduced by sodium hydrosulfite into 5,6-diaminouracil **3***[15]. The cyclization to xanthine **4*** was performed by simple reflux in formamide[16]. N-trimethylation into caffeine **5*** was obtained by the simultaneous addition of (i) dimethyl sulfate and (ii) 60% sodium hydroxide, to an aqueous solution of xanthine **4*** at 35°C. The pH was maintained at 8-9[16]. The crude product was obtained by extraction with methylene chloride and crystallization of the residue from absolute ethanol with an overall yield of 85%.
All products exhibited consistent spectral data and elemental analyses within ± 0.4%.

2*: 90 % yield; m.p > 300°C; IR: 1290 cm^{-1}(ν NO); MS (EI): 158 (M^++ 2)
3*: 87 %; m.p > 300°C; IR: 3420, 3100 cm^{-1}(ν NH) 1700, 1640 cm^{-1}(ν C=O); MS (EI): 144 (M^++2)
4*: 75 %; m.p > 300°C; IR: 2900-3000 cm^{-1}(ν CH), 1720,1660 cm^{-1}(ν C=O); MS (EI): 1254 (M^++2)
5*: 85 %; m.p : 232°C (238°C litt.); IR: 3100 cm^{-1}(ν NH), 2900 cm^{-1}(ν CH), 1700, 1650 cm^{-1} (νC=O) ; MS (EI): 196 (M^++2); 1H NMR (δ ppm,$DMSO_{d6}$): 3.1 (s, 3 H, N_1-CH_3), 3.3 (s, 3H, N_3-CH_3), 3.75 (s, 3H, N_7-CH3), 7.8 (s, 1H, C_8-H).

Chromatography.

The purity of the various products was assessed by liquid chromatography. A gradient elution HPLC system was used, consisting of Hewlett-Packard 1090 chromatograph (Hewlett-Packard, Orsay, France) connected with HP 85 B integrator and an autosampler injector.
Chromatography was performed using a 70 mm x 4.5 mm ID stainless steel column containing 5 µm ODS (Beckman, France) protected by a precolumn packed with the same phase. The eluted peaks were detected at 274 nm using diode array fitted with a 100 µl flow cell.
Labelled caffeine* was dissolved into mobile phase: water containing 0.05% acetic acid/methanol (92: 8, WV). Methanol concentration increased from 8% to 30% in 25 min. Under these conditions only one peak was obtained.

Mass Spectrometry

Electron impact mass spectra were recorded with a Ribermag R10-10 quadrupole mass spectrometer combined with a Riber 400 data system, using a direct insertion inlet and operating at 70 eV and source temperature of 160 + 20°C.

REFERENCES

(1) Aranda J.V.; Gorman W.; Bergsteinson H.; Gunn. T.- J. Pediatr., 90, 467, (1977).
(2) Cornish H.H.; Christman A.A.- J. Biol. Chem., 228, 315, (1957).
(3) Burg A.W.- Drug Metab. Rev., 4, 199, (1975).
(4) Aldridge A.; Aranda J.V.; Neims A.H.- Clin. Pharmacol. Ther., 25, 447, (1979).
(5) Monks T.J ; Caldwell C.H.; Smith R.L.- Clin. Pharmacol. Ther., 26, 513, (1979).
(6) Latini R.; Bonati M.; Castelli D.; Garattini S.- Toxicol. Lett., 2, 267, (1978).
(7) Aldridge A.; Parsons W.D.; Naims A.H.- Life Sci., 21, 967, (1978).
(8) Aranda J.V.; Collinge J.M.; Zinman A.; Watters G.- Arch. Dis. Child., 54, 946,(1979).
(9) Le Guennec; J.C.; Billon B.; Pare C.- Pediatrics, 76, 834, (1985).
(10) Lhermitte M.; Houdret N.; Broly F.; Roussel P.; Carpentier C.; Zaoui C.; Lequien P. - J. Pediatr., 110, 666, (1977).
(11) Grant D.H.; Tang B.K.; Kalow W.- Clin. Pharmacol. Ther., 33, 591, (1983).
(12) Falconnet J.B.; Brazier J.L.; Desage M.- J. Label. Compounds Radiopharm., 23, 267,(1986).
(13) Cherrah Y.; Falconnet J.B.; Desage M.; Brazier J.L; Zini R.; Tillement J.P.- Biomed. Mass Spectrom., 14, 653, (1978).
(14) Bernier J.L.; Hénichart J.P- Biomed. Mass Spectrom., 10, 626, (1983).
(15) Blicke F.F. and Godt Jr. H.C.- J. Am. Chem. Soc., 76, 2798, (1954).
(16) Bredereck H.; V. Schuh H.; Martini A.L.- Chem. Ber, 83, 201, (1950).
(17) Budzikiewicz H.; Djerassi C.; Williams D.H. "Structure Elucidation of Natural Products by Mass Spectrometry", (1964), Vol. 1, pp. 214-217, Holden-Day, San-Francisco.
(18) Rao G.S.; Khanna K.L.; Cornish H.H.- J. Pharm. Sci., 61, 1822, (1972).
(19) Midka K.K.; Sved S.; Mc Gilveray L. J.- Biomed. Mass Spectrom., 4, 172, (1977).
(20) Lifshitz C.; Bergmann E.D.; Sheinok V.- Isr. J. Chem., 6, 827, (1968).
(21) Voticky Z.; Kovacik V.; Rybar A.; Antos K.- Collect. Czech Chem. Commun., 34, 1657, (1969).