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SYNTHESIS OF 1-C^2H_3 THEOPHYLLINE AND 3-C^2H_3 THEOPHYLLINE

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SUMMARY

This article describes a three step-selective deuteration method of 1-methyl and 3-methylxanthine, after protection of the N-7 position by chloromethylpivalate and alkylation by trideuteromethyl iodide ; yielding 1-C^2H_3 and 3-C^2H_3 theophylline.

Key Words : Theophylline, deuterium, labelling.

INTRODUCTION

Theophylline (2,6-dihydro-1,3-dimethyl purine ; 1-3 dimethyl xanthine) is an alkaloïd long known for its diuretic, cardiotonic and coronary-dilating properties.

At present, theophylline is mainly used in therapeutics as a bronchodilator in all kinds of asthma (17-23) and against idiopathic apnea of the newborn (12).

Despite its long use for alimentary and therapeutic purposes, its intimate mechanism of action still remains unclear (13-14). In the same way, since the discovery of an original methylation pathway into caffeine, its metabolism in the fetus must be precised (1-6-11).

On account of its harmlessness, stable isotope-labelling of theophylline constitutes a precious tool, allowing numerous pharmacolo-

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gical applications for in vivo studies as much for qualitative (8), as quantitative (14-18-24) and mechanistic studies (3).

Deuterium-labelling may induce isotopic effects that can be exploited for explanatory purpose in metabolism, distribution studies (2-14) and drug-design (10).

The object of this work was to synthetize two deuterated analogs of theophylline : 1-C^2H_3 and 3-C^2H_3 theophylline (figure 1) meant for isotopic effect-studies.

1-CD3 Theophylline 3-CD3 Theophylline

Figure 1 : Structure of the trideuteromethyl-theophylline molecules (for clearness of presentation ^2H was noted D in figures).

Several methods for theophylline synthesis have been described (4-7-19-25), but none of these permitted selective labelling on positions N-1 or N-3.

This article suggests a three step-synthesis of deuterated theophyllines from monomethylxanthines : 1-methylxanthine and 3-methylxanthine to give 3-C^2H_3 and 1-C^2H_3 theophylline respectively.

As a first step, chloromethyl pivalate protection of N-7 position in monomethylxanthines was conducted through alkaline medium-nucleophilic substitution, that was shown to be stable under alkylating conditions (16-22).

The second step, was a trideuteromethyliodide-nucleophilic substitution on positions N-1 or N-3, depending on the concerned substituted monomethyl xanthine, following N-hydrogene mobilization in alkaline medium (15).

At last, in the third step, alkaline hydrolysis of the N-C bond on position N-7 was carried out to release labelled-theophylline and the protecting group.

EXPERIMENTAL

APPARATUS

. A Nermag R 10-10 C high-resolution Mass Spectrometer (MS) (70 e.v. - electron impact ionisation) was used.
All spectra were obtained through direct introduction (source temperature : 210°C ; programming : direct introduction probe from 40 to 300°C).

. Bruker WP-80-FT high-resolution Nuclear Magnetic Resonance Spectrometer (NMR) operating at 80 MHz. Solvent used was C^2HCl_3, TMS internal lock, spectrum-recording at ambiant temperature ([0,15 ppm]), 1 to 64 scans, pulse angle 30°.

. Melting Point determination apparatus Electrothermal I.A. 6304

REAGENTS

. 1-methyl xanthine, 3-methyl xanthine and chloromethyl pivalate were provided from Fluka, (Buchs, Suisse).

. Trideuromethyl iodide (isotopic purity 99,25 %) was provided from C.E.A. (Saclay, France).

SYNTHESIS

. First Step : Protection of position N-7 of the monomethyl xanthines (1a, 1b) yielding 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (2a) and 1-methyl, 7[(pivaloyloxy)methyl] xanthine (2b) (figure 2).

1g monomethyl xanthine (6.02 mmol) and 638 mg sodium carbonate (6.02 mmol) were stirred in dry N,N-dimethylformamide (DMF, 20 ml). To the mixture was added, under nitrogen, at room temperature, lightscreen, a solution of 955 ul chloromethylpivalate (6.6 mmol) in DMF (3 ml). After 1 and 2 h. additional aliquots of chloromethyl pivalate were added in the same conditions.

After 16 h., the milky solution obtained was filtered. The residue was washed with 1N hydrochloric acid and distilled water to give unreacted monomethylxanthine. The DMF-filtrate, enclosing reaction products, was evaporated under nitrogen (B.M., 70°C). Part of the

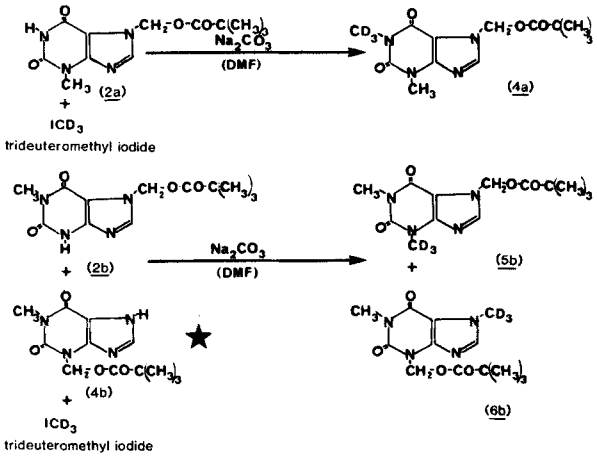

Figure 2 : Reactional scheme for protection of the position N-7 in monomethylxanthines.
★ : non isolated molecule.

unreacted monomethylxanthine being dissolved in DMF, its separation was performed by redissolving the evaporation residue in 100 ml acetone and filtration.

The collected monomethylxanthine-residues may be recristallised in 25 % ammonia-methanol-ethyl acetate (10-10-30) mixture.

The acetone filtrate was evaporated under vacuum (B.M., 40°C) and the residue was dissolved in a minimum of methanol to be chromatographied on silica HF.254 preparative plates [chloroform-ethanol (90-10)].

The products obtained were :

* Starting from 3-methylxanthine (1a) : 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (2a) with 29 % yield (489 mg), Rf = 0.47, M.P. = 245-246°C, NMR shifts (ppm) : 1.18 (S,9H), 3.56 (S,3H), 6.18(S,2H), 7.86 (S,1H), 8.25 (wide resonance signal, 1H) ; and traces of 3-methyl, 1,7 bis-[(pivaloyloxy)methyl] xanthine (3a), Rf = 0.65.

* Starting from 1-methyl xanthine (1b) : 1-methyl,7-[(pivaloyloxy)methyl] xanthine (2b) with 22 % yield (371 mg), Rf = 0.49, M.P. = 195-196°C, NMR shifts (ppm) : 1.19(S,9H), 3.43 (S,3H), 6.23 (S,2H), 7.25 (S,1H), 10.98 (wide resonance signal, 1H) ; and

1-methyl, 3,7 bis-[(pivaloyloxy)methyl] xanthine (3b) with 15 % yield (356 mg), Rf = 0.80, M.P. = 89-90°C, NMR shifts (ppm) : 1.19 (S,18H), 3.43 (S,3H), 6.12 (S,2H), 6.22 (S,2H), 7.85 (S,1H).

Second Step : Deuteromethylation of the protected monomethyl xanthines yielding 1-C^2H_3, 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (4a) on one side and 1-methyl, 3-C^2H_3, 7-[(pivaloyloxy)methyl] xanthine (5b), plus 1-methyl, 3-[(pivaloyloxy)-methyl], 7 C^2H_3 xanthine (6b) on the other (figure 3).

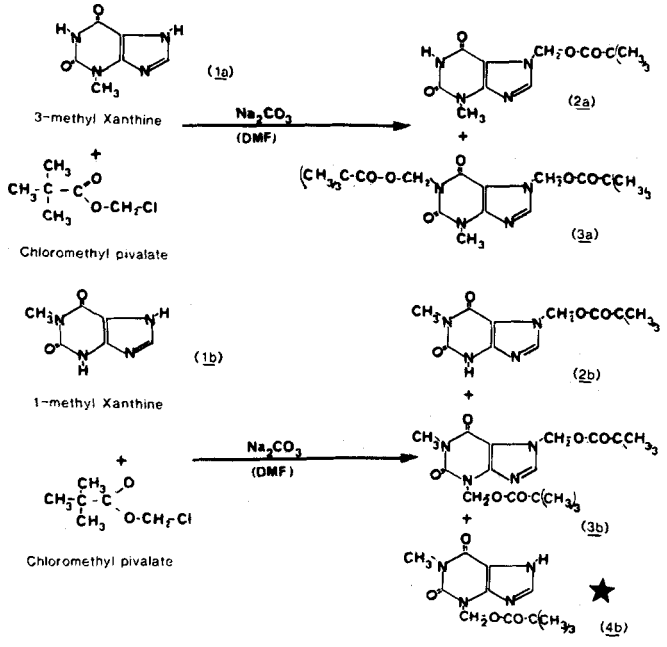

Figure 3 : Reactional scheme for deuteromethylation of the protected monomethyl xanthines.
★ : non-isolated molecule.

A mixture of 489 mg 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (2a) (1.75 mmol) and 371 mg sodium carbonate (3.5 mmol) was stirred until dissolution in dry DMF (10 ml). To the solution were added 115 µl trideuteromethyl iodide (1.8 mmol) and after 1 and 2 h. additional aliquots of 115 µl trideuteromethyl iodide.
The reactional mixture was kept 48 h. at room temperature, light-screen, under magnetic stirring. DMF was then evaporated under nitrogen (B.M., 70°C). To the dry residue it was added 50 ml distilled water and the deuterated product was extracted by chloroform (6 x 50 ml). The chloroformic phases were evaporated under vacuum (B.M. 50°C). The resulting yellow oil was dried under nitrogen. After residue redissolving in a minimum of methanol, preparative

TLC chromatographic purification was conducted in the same conditions as precedently.

It was obtained 1-C^2H_3, 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (4a) with 78 % yield (403 mg), Rf = 0.68, M.P. = 107-108°C, NMR shifts (ppm) : 1.18 (S,9H), 3.57 (S,3H), 6.20 (S,2H), 7.82 (S,1H).

Deuteromethylation of 1-methyl, 7-[(pivaloyloxy)methyl] xanthine (2b) was performed in similar conditions to those described for labelling of product (2a). A mixture of 1-methyl, 3-C^2H_3, 7-[(pivaloyloxy)methyl] xanthine (5b) and 1-methyl, 3-[(pivaloyloxy)methyl], 7-C^2H_3 xanthine (6b) was obtained with a 78 % overall yield (307 mg), respective Rf 0.64 and 0.60, NMR shifts (ppm) : 1.18 (S,18H), 3.43 (S,3H), 6.12 (S,2H), 6.23 (S,2H), 7.85 (S,1H). These two isomers are separated in the next stage.

. Third Step : Alkaline hydrolysis of N-[(pivaloyloxy)methyl] bonds in labelled xanthines yielding 1-C^2H_3 theophylline (5a) on one side and 3-C^2H_3 theophylline (7b), plus 7-C^2H_3 paraxanthine (8b) on the other (figure 4).

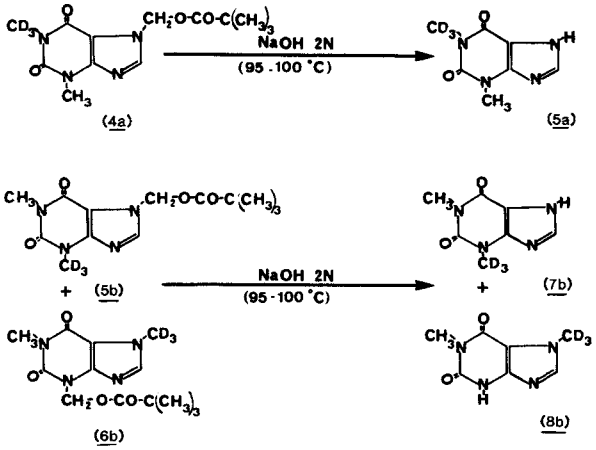

Figure 4 : Reactional scheme for alkaline hydrolysis of protected trideuteromethyl xanthines.

403 mg 1-C^2H_3, 3-methyl, 7-[(pivaloyloxy)methyl] xanthine (4a) (1,36 mmol) were reflux-heated for 2h. in NaOH 2N (15 ml). After cooling, the solution was acidified to pH 2-3 using small

drops 10 % HCl : the formed pivalic acid was extracted by means of chloroform (6 x 5 ml) :

- the chloroformic phase was then washed (HCl 0,5 N, 5 ml),
- the aqueous phases were gathered, pH was brought to 10 using NaOH 2N with subsequent vacuum solvent evaporation (B.M. 95-100°C).

The residue was then recristallized with distilled water, 1-C^2H_3 theophylline (5a) was recovered with 34 % yield (84 mg), Rf : 0.30, M.P. = 266-267°C.

The scheme adopted for alkaline hydrolysis of the mixture of the two isomers (5b) and (6b) was identical to the one described precedently, but in the present case isolation of the two products asked for a two-stage procedure : pH 10 recristallization-purification of the two isomer-mixture in distilled water, followed by preparative TLC separation with lost solvent front in chloroform-ethanol (9-1) and elution using the same phase.

3-C^2H_3 theophylline (7b) with 27 % yield (51 mg), Rf = 0.28, M.P. = 247-250°C and 7-C^2H_3 paraxanthine (8b) with 2 % yield (4 mg), Rf = 0,19 were obtained.

RESULTS AND DISCUSSION

MASS SPECTROMETRY

Mass spectra of standard theophylline and synthesis products (5a) and (7b) are presented in figure 5 :

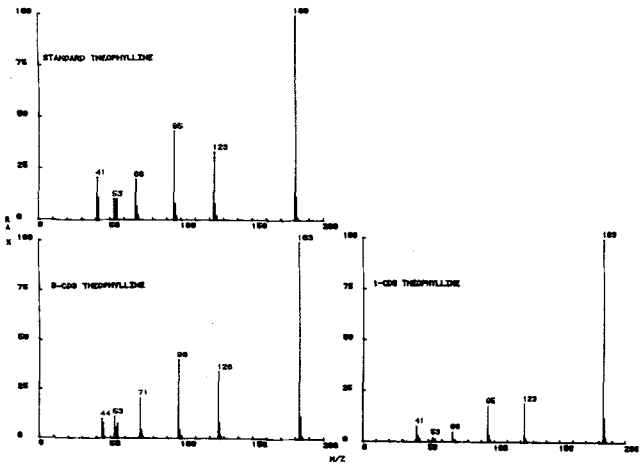

Figure 5 : Mass spectra of standard and trideuteromethyl theophyllines.

Table I displays the structures of the major fragment-ions for standard, 1-C^2H_3 and 3-C^2H_3 theophyllines with corresponding masses.

Observed m/z values			Structures of corresponding fragment-ions		
Standard Theophylline	1-C^2H_3 Theophylline	3-C^2H_3 Theophylline	Standard Theophylline	1-C^2H_3 Theophylline	3-C^2H_3 Theophylline
180	183	183	$C_7H_8N_4O_2^{+\cdot}$	$C_7H_5{}^2H_3N_4O_2^{+\cdot}$	$C_7H_5{}^2H_3N_4O_2^{+\cdot}$
123	123	126	$C_5H_5N_3O^{+\cdot}$	$C_5H_5N_3O^{+\cdot}$	$C_5H_2{}^2H_3N_3O^{+\cdot}$
95	95	98	$C_4H_5N_3^{+\cdot}$	$C_4H_5N_3^{+\cdot}$	$C_4H_2{}^2H_3N_3^{+\cdot}$
68	68	71	$C_3H_4N_2^{+\cdot}$	$C_3H_4N_2^{+\cdot}$	$C_3H^2H_3N_2^{+\cdot}$
53	53	53	$C_2HN_2^{+\cdot}$	$C_2HN_2^{+\cdot}$	$C_2HN_2^{+\cdot}$
41	41	44	$C_2H_3N^{+\cdot}$	$C_2H_3N^{+\cdot}$	$C_2{}^2H_3N^{+\cdot}$

Table I : m/z values and corresponding structures of fragment-ions of theophylline and deuteromethyl analogs.

The major fragment-ions observed for standard theophylline are in agreement with litterature data (20-21). The observed fragment-ions in mass spectra of products (5a) and (7b) are those expected for fragmentation of two theophyllines trideuteromethylated on positions N-1 and N-3.

PROTON-NUCLEAR MAGNETIC RESONANCE (PMR)

PMR spectra of standard and deuterated theophyllines are shown in figures 6-8

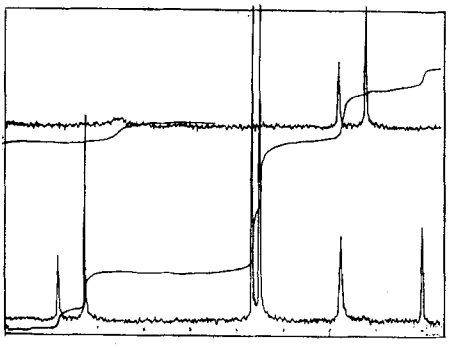

Figure 6 : PMR Spectrum of standard theophylline.

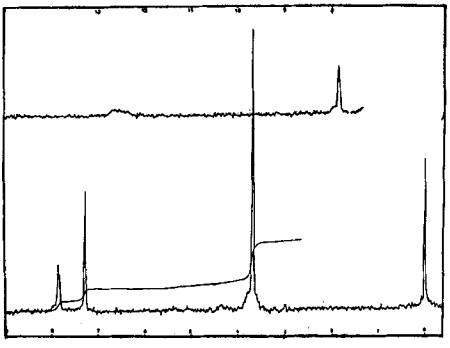

Figure 7 : PMR Spectrum of 1-C^2H_3 theophylline.

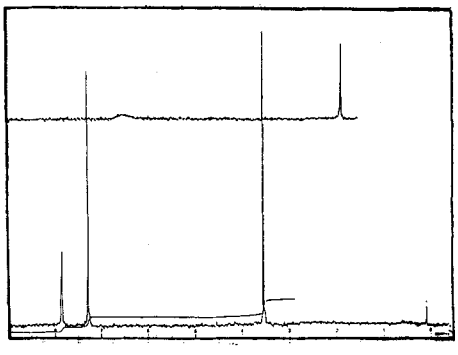

Figure 8 : PMR Spectrum of 3-C^2H_3 theophylline.

Resonance data pertaining to the above spectra are indicated in tables II - IV.

δ (ppm)	Intensity	Multiplicity	Attribution
3.49	3 H	S	CH_3 on N-1
3.66	3 H	S	CH_3 on N-3
7.83	1 H	S	H on C-8
12.66	1 H	wide resonance signal	H on N-7

Table II : Resonance data for standard theophylline.

δ (ppm)	Intensity	Multiplicity	Attribution
3.66	3 H	S	CH_3 on N-3
7.84	1 H	S	H on C-8
12.67	1 H	wide resonance signal	H on N-7

Table III : Resonance data for 1-C^2H_3 theophylline.

δ (ppm)	Intensity	Multiplicity	Attribution
3.49	3 H	S	CH_3 on N-1
7.83	1 H	S	H on C-8
12.66	1 H	wide resonance signal	H on N-7

Table IV : Resonance data for 3-C^2H_3 theophylline.

PMR data presented above show that the intensity of resonance signals of N-1 and N-3-methyl protons is three times higher than that of hydrogen on C-8 in the spectrum of standard theophylline as well as deuterated analogs. Moreover individual labelling of each of these positions brings about disparition of corresponding PMR signal permitting unequivocal attribution of methyl-proton signals naimely 3.50 and 3.70 ppm for positions N-1 and N-3 respectively

DISCUSSION

Obtention of the two theophylline isotopomers : 1-C^2H_3 and 3-C^2H_3 theophylline asked for a method permitting selective labelling of these two positions with a restricted number of stages in order to limit losses of labelled products.

The combined use of 1- and 3-monomethyl xanthines as starting products and chloromethylpivalate as protecting agent of position N-7 in a three-stage process meets these two objectives, labelling taking place in the penultimate step.

Protection of position N-7 is rendered necessary by the differential reactivities of N-H positions in methylxanthines : previous spectroscopic studies (9) suggest reactivities ranking N-3 N-7 N-1. Nevertheless, our and other experiments (16) favor reactivity of position N-7 as being equal, if not superior to that of position N-3.

The choice of chloromethylpivalate as protecting agent was governed by both the stability of the resulting protected mono-methyl xanthine formed in the present alkylation conditions, and the fact that its liberation through high-temperature alkaline hydrolysis does not affect labelling.

In the case of 3-C^2H_3 theophylline synthesis from 1-methyl xanthine, free N-3 and N-7 positions in the starting product induce formation of two protected derivatives, position N-7 being affected preferentially to N-3 leading to joint synthesis of 3-C^2H_3 theophylline and 7-C^2H_3 paraxanthine. Theses two products on account of their very similar physico-chemical properties could not be separated before the last stage.

Kinetic studies conducted in our laboratory concerning the alkylating stage of protected monomethyl xanthines through IC^2H_3-nucleophilic substitution in alkaline medium have shown that complete reaction required at least 24 h. Despite the restricted number of steps, synthesis of these two products is a rather long process.

The formation of side products demanded numerous purification stages. Recristallization trials performed leading to unsatisfactory purification, we opted for preparative TLC, a longer but more discriminative method.

The need for these purifications induces rather important product losses but allows obtention of chemically pure substances as shown by NMR and MS analysis.

CONCLUSION

The synthesis method proposed permits obtention of 3-C^2H_3 and 1-C^2H_3 theophylline from 1-methyl and 3-methyl xanthine respectively. The three-step process described include chloromethylpivalate protection of position N-7, followed by IC^2H_3 alkylation and liberation of position N-7 through high-temperature alkaline hydrolysis, yielding two chemically pure theophylline isotopomers.

These two labelled theophyllines as well as 7-C^2H_3 paraxanthine, a side-product in the synthesis of 3-C^2H_3 theophylline, are both molecules of great interest as tracers in pharmacological studies.

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