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# Corticosteroid Derivatization : Unexpected Results Obtained Using N,N-Dimethylformamide Dimethyl Acetal on Dexamethasone.

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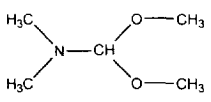
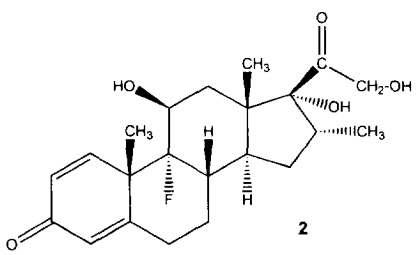
**Abstract :** N,N-dimethylformamide dimethyl acetal, a methylating reagent, has been used on artificial corticosteroid dexamethasone to improve its GC-MS responsiveness. The major reaction product gave unexpected low resolution mass spectra. Its structure has been unambiguously determined. This compound, a corticosteroid ester usually obtained after numerous reaction steps[1], could be of great interest in the fields of analytical chemistry and drug residues survey. Copyright © 1996 Elsevier Science Ltd

N,N-dimethylformamide dialkyl acetals have already been used to derivatize amino acids. They react with both the primary amino and the carboxylic acid groups[2]. Recent work on β-agonistic molecules has revealed that N,N-dimethylformamide dimethyl acetal (DMF-DMA, **1**) can also react with the primary alcohol group of the β-agonist salbutamol[3,4]. Taking into account this reaction, research has been carried out for its potential application as a derivatizing reagent for other alcohol-containing substances. Among these substances, artificial corticosteroids, a group of anti-inflammatory drugs which present some difficulties when assayed by Gas Chromatography-Mass Spectrometry (GC-MS), were chosen.

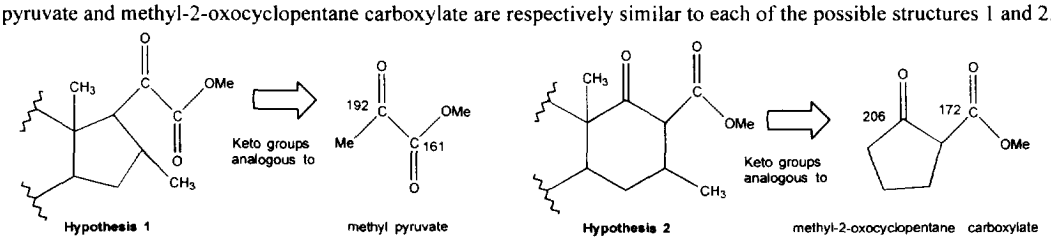


**1**

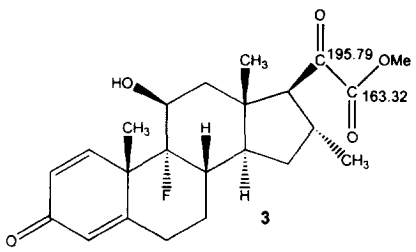


**2**

DMF-DMA reaction[7] has been carried out on dexamethasone (**2**). The major reaction product is $C_{23}H_{29}O_5F$ (HRMS[8]). $^1H$ and $^{13}C$ NMR[5,9] of dexamethasone were compared to NMR after DMF-DMA derivatization. Two hypothetical structures remained. In order to discard one of them, NMR spectra of related compounds have been taken. Indeed, the keto groups of methyl pyruvate and methyl-2-oxocyclopentane carboxylate are respectively similar to each of the possible structures 1 and 2.



Considering $^{13}C$ chemical shifts of the keto groups of the major reaction product (163.32 and 195.79), only hypothesis 1 could be retained. This enabled us to identify the major reaction product as 9α-fluoro-11β-hydroxy-21-methoxy-16α-methyl-pregna-1,4-diene-3,20,21-trione (**3**). These results were confirmed by a X-ray crystal structure determination which will be published later[10].




**3**

This reaction product is unexpected, as methylation of alcohol groups, or its transformation into a dimethyl amino group were thought to be more likely to happen. In order to enhance the GC-MS analysis ability of this molecule, a further derivatization step can be contemplated, such as methoxylamine-trimethylsilyl (MO-TMS) or simple trimethylsilyl derivative formation. The latter has been assayed using MSTFA/TMIS/DTE mixture[6,11] as silylating reagent. The spectrum of the bi-TMS derivative shows a very intense molecular ion peak (m/z=548). A characteristic fragment of artificial corticosteroids (m/z=206) can also be observed. The same reactions were applied to artificial corticosteroids prednisolone, 6α-methyl prednisolone and flumethasone, leading to the same type of derivatives with molecular ion m/z ratios of 516, 530 and 566 respectively. These molecules have been chosen because they are often used as human or veterinary drugs and are known to be illegally used as feed additives in livestock production.

## REFERENCES AND NOTES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7. The reaction was performed as follows : dexamethasone (1g ; 2.548 $10^{-3}$ mol) was dissolved in 100 ml of anhydrous pyridine. DMF-DMA (5 ml) was then added. The solution was warmed to 70°C for 3 h under magnetic stirring. The reaction mixture was then evaporated to dryness. The oily residue was taken up with 50 ml of dichloromethane and purified by chromatography (30 g silica gel in dichloromethane). The major reaction product was eluted using a dichloromethane / methanol (96 : 4 ; V/V) mixture. The resulting crystals were dissolved in diethyl ether and recrystallisation was completed by addition of hexane. The yield of this reaction is 49% (504 mg ; 1.247 $10^{-3}$ mol) and could probably be increased if the reaction conditions were optimised.
8. High Resolution Mass Spectrometry performed on the $[M-HF]^{+\bullet}$ fragment ion (base peak) gave the following results : m/z = 384.1916. Proposed formula : $C_{23}H_{28}O_5$ (error = -2.1 $10^{-3}$ u). Number of cycles or unsaturations : 10. Jeol SX 102® mass spectrometer, resolution ≈ 10,000.
9. Nuclear Magnetic Resonance experiments were carried out on a Bruker ARX 400® spectrometer operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$. Chemical shifts have been measured in ppm with tetramethylsilane as internal reference, and the compounds studied were dissolved in deutered dimethylsulfoxyde.
10. Radiocrystallographic study submitted to *Acta Crystallogr. C - Cryst. Str.* for publication.
11. N-methyl-N-trimethylsilyl-trifluoroacetamide/trimethyliodosilane/dithiothreitol (1000:5:5 ; v/v/w).


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