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The Base-catalyzed Deuteration of Hydrogen in Methyl Group substituted at 3-Position of Pyrazolone Ring[1]

Tsuyoshi Goromaru[2a] and Atsuko Noda[2b]

Faculty of Pharmaceutical Sciences, Kyushu University[2]

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A simple method for labeling methyl group with deuterium at 3-position of pyrazolone ring was established. Trideuterium labeled aminopyrine obtained by the present method did not indicate the isotope effect. It is available for a tracer as well as an internal standard on gas chromatography–mass spectrometry.

Keywords——deuteration; base-catalyzed; C-methyl group of pyrazolones; d_3-aminopyrine; tracer

As a part of our studies on aminopyrine (AM) metabolism in man, two kinds of compounds labeled with deuterium and with carbon-13 were synthesized by modified procedures as shown in Chart 1. The compounds, trideuterium labeled AM (I) and carbon-13 labeled AM (II), were effectively employed as a tracer of metabolic study or an internal standard on gas chromatography–mass spectrometry (GC–MS).[3,4]

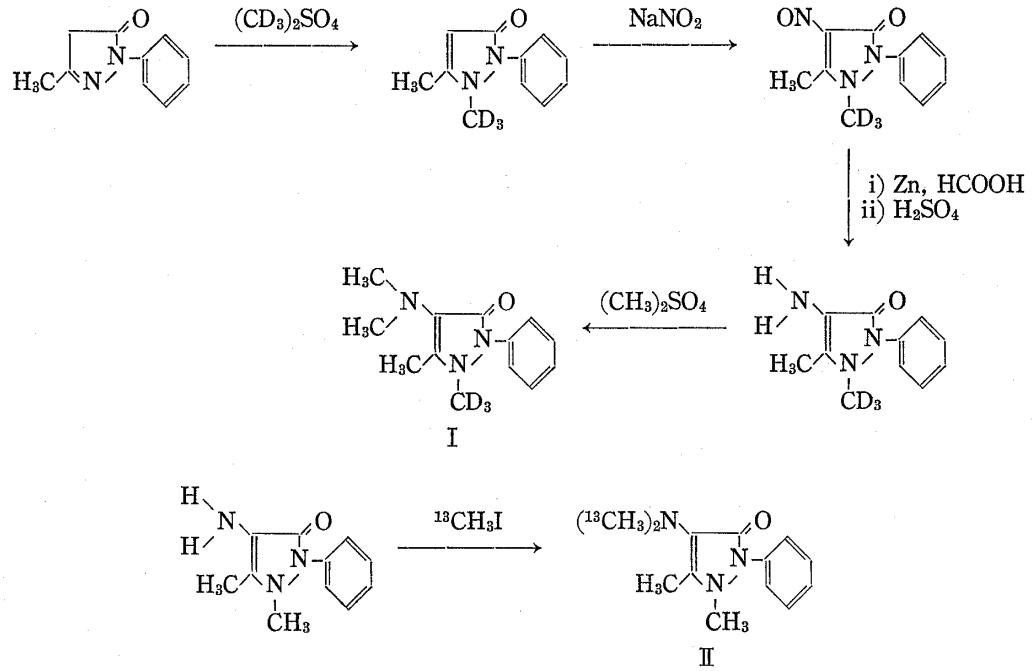


Chart 1

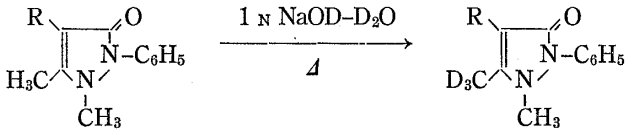
However, the synthetic procedures described in the previous paper were not only complex but also expensive in making a large amount of labeled compound. The present method is

1) This work was presented at 97th Meeting of Pharmaceutical Society of Japan, Tokyo in 1977.
2) Location: *Maidashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan*; *a*) Present address: *Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji-shi, Tokyo, 192-03, Japan*; *b*) To whom communications should be directed.
3) A. Noda, T. Goromaru, N. Tsubone, K. Matsuyama, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1502 (1976).
4) T. Goromaru, K. Matsuyama, A. Noda, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 33 (1978).

recommendable for labeling methyl group with deuterium at 3-position of pyrazolone ring, since it is very simple and inexpensive.

Results and Discussion

According to Kawazoe *et al.*,[5] hydrogen-deuterium exchange of α-hydrogens of a side chain alkyl group substituted in pyridine, quinoline, isoquinoline, their N-oxides and N-alkyl halides were carried out on heating them in D_2O solution containing sodium hydroxide or sodium carbonate. We examined the base-catalyzed hydrogen exchange in D_2O solution of AM and obtained trideuterium labeled AM (d_3-AM) in good yield as shown in Chart 2. As shown in Fig. 1, the deuteration took place in the methyl group at 3-position of pyrazole ring, whose signal disappeared from the nuclear magnetic resonance (NMR) spectrum after deuteration as shown in Table I.



R : H, NH_2, $NHCH_3$, $N(CH_3)_2$, $NHCOCHN(CH_3)_2$, $NHCOCH_3$, $CH(CH_3)_2$, OCH_3

Chart 2

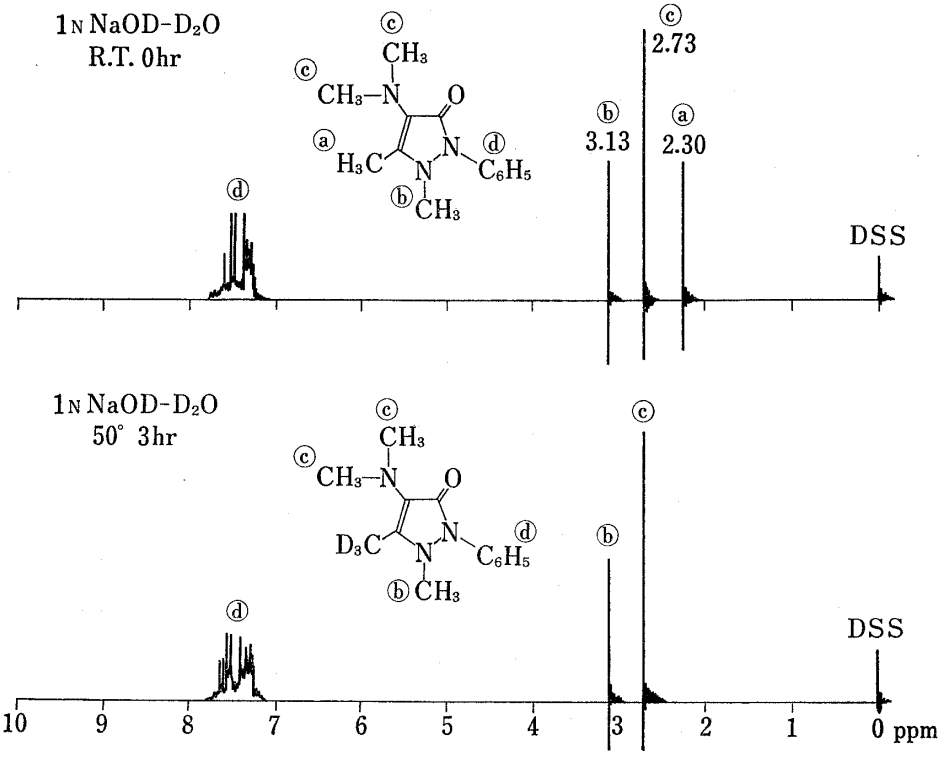


Fig. 1. NMR Spectra of d_0-AM and d_3-AM

5) Y. Kawazoe, M. Ohnishi, and Y. Yoshioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1225 (1976).

If the labeled compound has isotope effect, it can not be employed as a tracer. Isotope effect of d_3-AM (III) was examined prior to the metabolic studies. From the concentration profile of unlabeled AM (d_0-AM) and d_3-AM in plasma after the oral administration of their equimolar mixture (50 mg/kg) to rabbit, no isotope effect was observed as shown in Fig. 2. In this experiment, d_6-AM (IV) which was synthesized from AA and $(CD_3)_2SO_4$ was employed as an internal standard on GC–MS analysis. In order to calculate the concentration of d_0-AM and d_3-AM in plasma, mass fragmentography was performed by monitering three peaks at m/e 231 (d_0-AM), 234 (d_3-AM) and 237 (d_6-AM). Showing no isotope effect, d_3-AM can be used as a tracer.

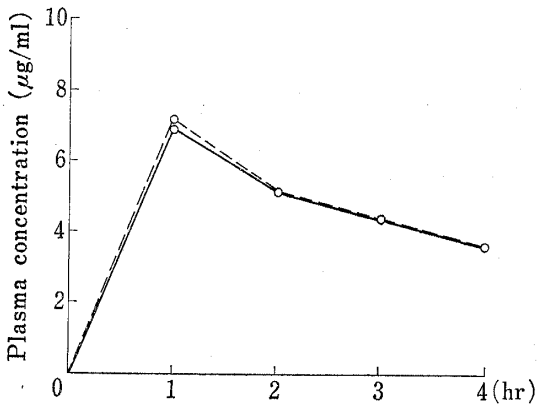
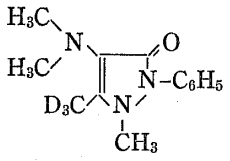
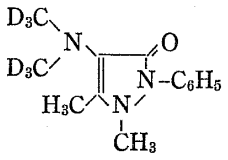


Fig. 2. Plasma Concentration of d_0-AM and d_3-AM in Rabbit after the Oral Administration of an Equimolar Mixture (50 mg/kg) of d_0-AM and d_3-AM

○——○: d_0-AM, ○——○: d_3-AM.



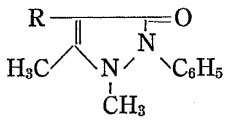
III



IV

On an extensive examination of pyrazolones, deuteration took place in the methyl group at 3-position of antipyrine (AN), sulpyrine, 4-isopropylaminoantipyrine (PA), 4-aminoantipyrine (AA), aminopropylone (AP), 4-monomethylaminoantipyrine (MAA) and 4-methoxyantipyrine under the similar condition as described in the case of AM. The ending of deuteration was judged from the disappearance of NMR signal due to a methyl group at 3-position of the ring. The deuterated amount was calculated from the peak heights of mass spectra, comparing the peak heights around the molecular ion peak of d_0-compound with those of labeled compound. The deuterated amounts of pyrazolones and the δ values of methyl substituent are summarized in Table I.

Table I.



Pyrazolones	R	δ (ppm) (1N NaOD–D_2O) 2-NCH_3	3-CCH_3	4-CH_3	Deuterated position	% of deuteration
Antipyrine	H	2.98(3H)[a]	2.17(3H)[a]	—[a]	3-CD_3	87
4-Isopropyl-antipyrine	$CH(CH_3)_2$	3.09(3H)	2.30(3H)	1.21, 1.28(6H)	3-CD_3	87
4-Amino-antipyrine	NH_2	3.00(3H)	2.24(3H)	—	3-CD_3	88
Aminopyrine	$N(CH_3)_2$	3.13(3H)	2.30(3H)	2.73(6H)	3-CD_3	93
4-Monomethyl-aminoantipyrine	$NHCH_3$	3.06(3H)	2.32(3H)	2.69(3H)	3-CD_3	94
4-Methoxy-antipyrine	OCH_3	3.09(3H)	2.90(3H)	3.80(3H)	3-CD_3	96
Amino-propylone	$NHCOCHN(CH_3)_2$ (CH3)	3.03(3H)[a]	2.27(3H)[a]	1.20, 1.32(3H)[a] 2.33(6H)	3-CD_3 4-$CDN(CH_3)_2$	96
4-Acetamino-antipyrine	$NHCOCH_3$	3.24(3H)	2.22(3H)	2.22(3H)	3-CD_3 4-$NHCOCD_3$	98

a) Measured in $CDCl_3$.

In the case of 4-acetylaminoantipyrine (AcAA), six hydrogens of methyl group at 3-position and acetyl group at 4-position were exchanged by deuterium. Four hydrogens were exchanged by deuterium in the case of AP. The expected deuteration did not take place in 4-formylantipyrine (FA), 4-formylaminoantipyrine (FAA) and 4-hydroxyantipyrine (HA). When sulpyrine was heated in 1 N NaOD–D_2O solution, was obtained d_3-MAA instead of d_3-sulpyrine. Therefore, d_3-sulpyrine was synthesized by another way, *i.e.* derivatization to methanesulfonate of d_3-MAA as shown in Chart 3.

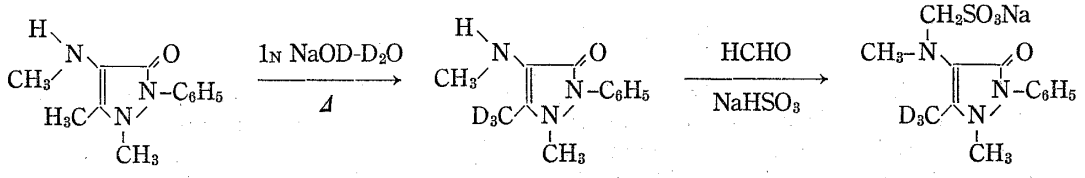


Chart 3

Experimental

Materials——J. P. VIII grade of AM, AN, sulpyrine and PA were used in this experiment. AA was purchased from Wako Pure Chemical Co. AP was given by the pharmacy of Kyushu University Hospital. MAA, FAA and AcAA were synthesized by the same way described in the previous paper.[3] FA and HA were synthesized by the known procedures.[5,6] 4-Methoxyantipyrine was synthesized as follows.

4-Methoxyantipyrine——HA was methylated quantitatively by diazomethane method in ether to obtain white needles from light petroleum, mp 75—77°. *Anal.* Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.05; H, 6.42; N, 12.84. Found: C, 66.17; H, 6.49; N, 12.83. Mass spectrum *m/e*: 218 (M^+). NMR (1N NaOD–D_2O) δ: 2.14 (3H, s, C–CH_3), 3.08 (3H, s, N–CH_3), 3.80 (3H, s, O–CH_3), 7.2—7.6 (5H, aromatic protons).

Method of Deuteration——Prior to the synthesis, the proper experimental condition of each compound was examined in a sealed NMR sample tube. In general, the reaction took place at 50°. However, the stronger condition was necessary to obtain a large amount of labeled compound in good yield. The synthetic procedure is as follows. Four gram of pyrazolone derivative was dissolved in 100 ml of 1 N NaOD–D_2O. The mixed solution was refluxed for an appropriate period and extracted with chloroform. The combined extracts were dehydrated with anhydrous sodium sulfate and evaporated to dryness. After the solvent was removed, the residue was recrystallized from a proper solvent.

Studies on the Isotope Effect of d_3-AM——In order to examine the isotope effect of d_3-AM, the blood samples were taken at 0, 1, 2, 3 and 4 hr after the oral administration of an equimolar mixture (50 mg/kg) of d_0-AM and d_3-AM to a rabbit. The quantitative data was obtained from mass fragmentogram by comparing the peak heights at *m/e* 231 (d_0-AM) and 234 (d_3-AM) with 237 (d_6-AM, internal standard).

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7) J. Hukki and J. Myry, *Acta Chem. Scand.*, **13**, 174 (1959).