

A Further Study of Identifying the Characteristic NMR Signals Detected at Lower Magnetic Fields*

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Abstract

A further attempt is made to reconfirm the previous identified proton NMR signals regarded as a cancer maker. The result of the attempt shows that the NMR signals clearly correspond to the four aromatic proton NMR signals of m-hydroxyphenyl.

INTRODUCTION

It has already been reported that the characteristic cancer urine proton NMR signals observed between 7 ppm and 8 ppm are used as a cancer marker and differentiate between cancer urine and normal and other diseased urine¹⁾. Furthermore the NMR signals which are relatively broader and sharper are more frequently observed in the cancer urine especially. It has also recently been reported that the signals²⁾ can be identified as m-hydroxyphenyl by comparing the computed spin coupling constants of the cancer urine signals with those of the signals of DL-m-tyrosine, DL-o-tyrosine and L-tyrosine shown in the Aldrich Library of NMR Spectra³⁾.

Since the above identified substance has not specifically indicated as one of the excretions of human urine⁴⁾, in this study a further attempt is made to reconfirm the previous identification using JNM-MY 60 MHz NMR spectrometer. The result of the attempt shows that the characteristic NMR signals still correspond to the ones of m-hydroxyphenyl.

EXPERIMENT

NMR Measurement

An NMR sample tube is filled with 0.5 milliliter of L-tyrosine or DL-m-tyrosine in powder form dissolved in D₂O and DCl to measure the proton NMR signals using JNM-MY 60 MHz NMR spectrometer. Tetramethylsilane is used as a reference standard for chemical shift measurements. The above two substances are, respectively, measured for the signals by 10-10 and 100-1 of spectrum amplitude with the same sweep time of 600 sec. All the data are taken up to three significant figures with round-off.

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Reagents for Detection

The following processes are taken to obtain the possible reagents for detecting the phenolic compounds (or the hydroxyphenyl compounds) : (a) 5 grams of one of the metals of Cu, Zn, Fe, Cd, and Ni are dissolved in 50 milliliters of nitric acid. (b) 50 grams of mercury are dissolved in 50 milliliters of nitric acid. (c) To obtain the reagent corresponding to each of the above first three metals the mixing ratio of the solution obtained by the process (a), the solution obtained by the process (b) and distilled water is 1 : 2 : 1. And the mixing ratio for Ni obtained by following the above same processes is 2 : 1 : 3.

RESULTS AND DISCUSSION

The following figure shows the proton NMR signal distribution of urine of stomach cancer with bilirubinuria : **

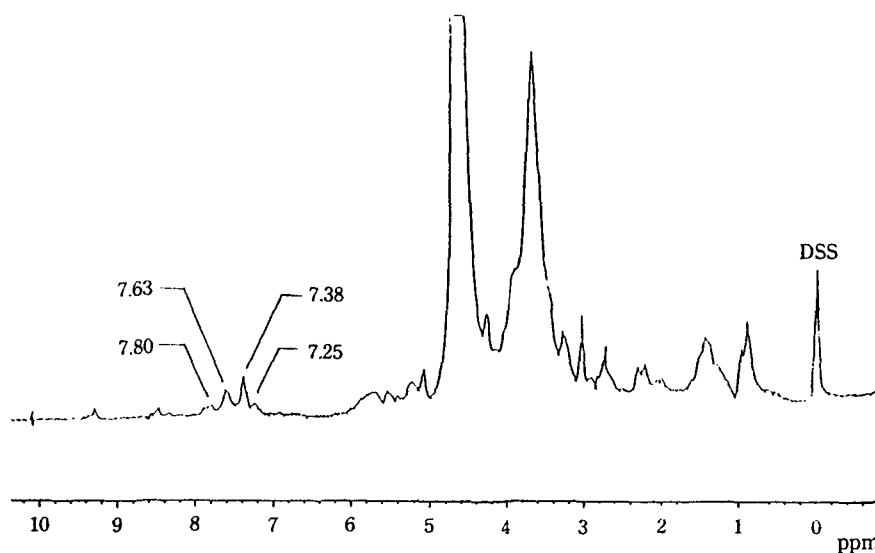


Fig.1 Proton NMR signal distribution of a patient's urine of stomach cancer with bilirubinuria.

It must here be noted that only the numerical values of the NMR signals of the cancer urine observed between 7 ppm and 8 ppm are concerned and indicated for the present work as mentioned in Section I.

It has been known that the excretions of tyrosine species in human urine are tyrosine and its metabolites such as p-hydroxyphenyl-pyruvate, -lactate, -acetate, -and other tyrosine derivatives^{3,4}. However it must be noted that there are many different kinds of tyrosine besides the tyrosine just mentioned.

Among these substances the reaction colors of DL-m-tyrosine and L-tyrosine caused by

**This NMR experimental result is finished by a joint research with Samil Pharmaceutical Co., Ltd. Seoul.

the reagents made by the processes in Section II are relatively close to the characteristic reaction color of the cancer urine which is red. It is noted that the red coloration is due to the phenolic compounds (or the hydroxyphenyl compounds) of the two tyrosine species reacted on the reagents. Because of this only the two substances can be restricted for the present work.

The proton NMR signal distributions of the above two substances are shown as follows :

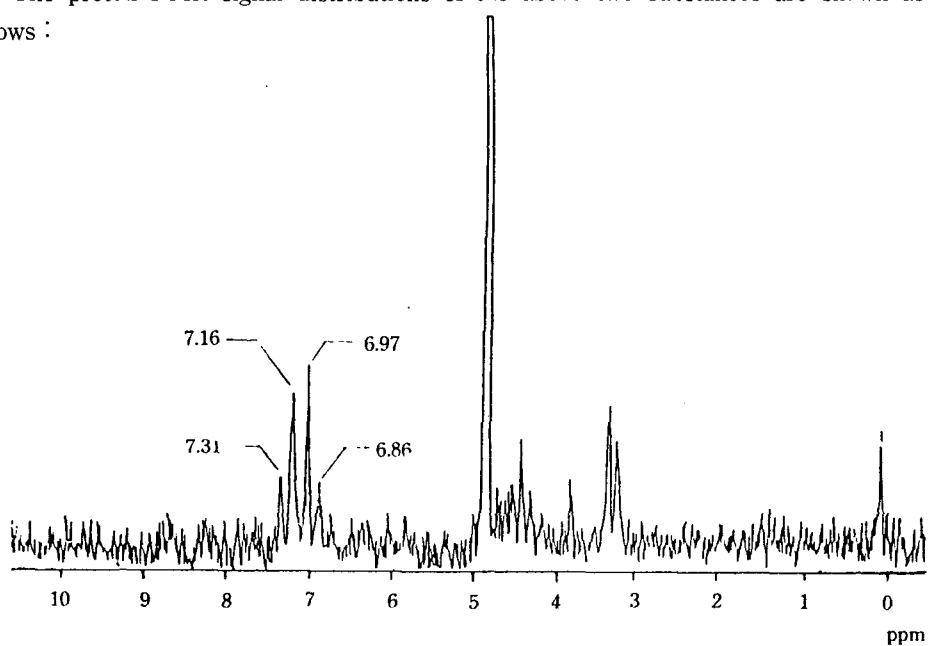


Fig. 2 Proton NMR signal distribution of DL-m-tyrosine.

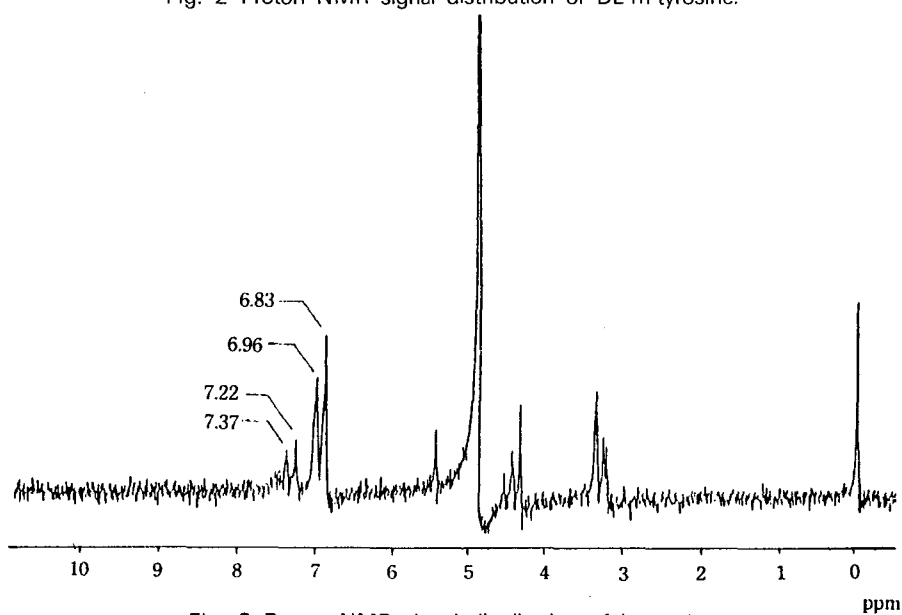


Fig. 3 Proton NMR signal distribution of L-tyrosine.

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The four numerical values specified on the resonance signals in the above two figures are the four aromatic proton NMR signals of the two hydroxyphenyl species.

The respective spin coupling constants⁵⁾ obtained from the signals are listed in the following table :

Table 1. The proton NMR signals and the spin coupling constants of the cancer urine and the two hydroxyphenyl species.

Cancer urine and hydroxyphen	Proton NMR signals(ppm)	Spin coupling constant(ppm)
Cancer urine	7.80 7.63 7.38 7.25	0.17 0.25 0.13
m-hydroxyphenyl	7.37 7.22 6.96 6.83	0.15 0.26 0.13
p-hydroxyphenyl	7.31 7.16 6.97 6.86	0.15 0.19 0.11

Here it must be noted that each spin coupling constant in the table is expressed in ppm rather than in frequency used customarily.

Since the constants are characteristic of given substances, they can be used for the substance identification. As shown in Table 1, the first two constants are very close to each other. Nonetheless, it is noticed that the cancer urine signals in Fig. 1 resonate at relatively lower magnetic fields in comparison with those of either m-hydroxyphenyl in Fig. 2 or p-hydroxyphenyl in Fig. 3. This can happen, for the resonance positions can be shifted considerably by the shielding effects⁵⁾ which are changed according to the environment made up by various excretions in human urine^{6)***}. It is concluded that the cancer urine signals regarded as a cancer maker correspond to the four aromatic proton NMR signals of m-hydroxyphenyl which agrees with the previous identified one²⁾.

As mentioned in this early section, it can be inferred from the consideration of the tyrosine metabolites that the ambiguously indicated tyrosine³⁾ belongs to L-tyrosine with p-hydroxyphenyl rather than DL-m-tyrosine with m-hydroxyphenyl. Therefore the substance drawn on the above conclusion must be a newly identified one which has not been reported as an excretion in cancer urine yet.

REFERENCE

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***Refer the proton NMR signals of the two stomach cancer patients' urine, one being the signal of 6.80 ppm shown on the extreme left-hand side of Fig. 2 and the other being the signal of 6.51 ppm shown on extreme left-hand side of Fig. 4 in reference 6.

Yong Jin Kim

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저자기장에서 검출된 암노의 특성 NMR 신호 규명에 관한 연구

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초 록

일차 규명한 암 지표가 되는 양성자 핵자기공명(NMR)신호를 재확인하기 위한 시도를 하였다. 이 시도의 결과에 의하면, 일차 규명한 물질인 m-hydroxyphenyl의 네 개의 방향족 양성자 NMR신호와 일치함을 재확인하게 되었다.