PREPARATION OF DEUTERIUM-LABELED RUTIN BY HYDROGEN EXCHANGE REACTION

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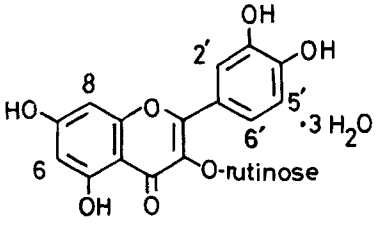
SUMMARY

Preparation of deuterium-labeled rutin by hydrogen exchange reaction under alkaline condition is described. Hydrogens at positions 2', 5' and 6' of rutin were replaced with deuteriums only on heating, while hydrogens at positions 6 and 8 were readily replaced at room temperature. On the basis of these findings rutin-2',5',6',6,8-d_5 was first prepared and then treated with alkaline water at room temperature to obtain rutin labeled with deuteriums at positions 2',5' and 6'.

Key Words : Rutin-2',5',6'-d_3, Deuterium, NMR spectrum, Basic hydrogen exchange reaction

INTRODUCTION

Rutin, a flavonol glycoside, has long been used in the treatment of disease states characterized by capillary bleeding associated with increased capillary fragility. The structure is as follows:



A tracer method provides a useful means for the metabolic studies of drugs. There have been several reports concerning the synthesis of radio isotope-labeled rutin. The Wilzbach method has been applied to prepare tritiated rutin.[1,2]

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Blanquet et al.[3] obtained biosynthetically ^{14}C-labeled rutin from Viola tricolor using $^{14}CO_2$. However, radioactive rutins thus obtained appeared not to be suitable for the metabolic study because of their low specific activity and of the random labeling. In order to elucidate the metabolic fate of this drug in man, rutin labeled with stable isotopes would be useful. At present there are no reports available concerning the synthesis of stable isotope-labeled rutin.

We report here a convenient and inexpensive method for the preparation of three forms (rutin-6,8-d_2, rutin-2',5',6',6,8-d_5, rutin-2',5',6'-d_3) of selectively labeled rutins with deuterium by hydrogen-deuterium exchange reaction under mild alkaline condition.

EXPERIMENTAL

All melting points are uncorrected. NMR spectra were determined with a JEOL JM-MH-100 spectrometer for solutions in methanol-d_4 with tetramethylsilane as an internal standard. Column chromatogragpy was performed on a Sephadex LH-20 with methanol as eluent. Thin layer chromatography was performed on Merck DC-Fertigplatten Kieselgel $60F_{254}$. Rf values refer to the following solvent systems ; n-BuOH : AcOH : H_2O = 4 : 1 : 2, AcOEt : HCO_2H : H_2O = 10 : 2 : 3.

Rutin-6,8-d_2

To a solution of sodium hydroxide (0.04 g, 1.0 mmole) and deuterium oxide (3.0 g, 150 mmole) was added rutin (0.30 g, 0.49 mmole). The mixture was stirred for 2 hr at 25° and then acidified with 10 % acetic acid (10 mL). The resultant yellowish solid was collected, washed well with water, and dried. The crude solid was chromatographed on a Sephadex LH-20 column with methanol as eluent. The methanol solution was concentrated and to this was added water (50 mL). The product was crystallized from the solution to give yellowish needles (0.26 g, 87 %) : mp 193° ; NMR δ 1.15 (3H, d, J=6.0 Hz, rhamnosyl-CH_3), 6.83 (1H, d, J=9.0 Hz, 5'-H), 7.58 (1H, d of d, J=9.0 Hz, 2.0 Hz, 6'-H), and 7.63 (1H, broad s, 2'-H) ; Rf 0.45 (n-BuOH : AcOH : H_2O = 4 : 1 : 2), Rf 0.35 (AcOEt : HCO_2H : H_2O = 10 : 2 : 3).

Rutin-2',5',6',6,8-d_5

A solution of sodium hydroxide (0.58 g, 14.5 mmole) and rutin (6.0 g, 9.8 mmole) in deuterium oxide (60.0 g, 3.0 mole) was heated at 95° for 8 hr in a nitrogen-filled sealed tube. The mixture was completely freeze-dried and was added deuterium oxide (60.0 g, 3.0 mole). The solution was heated for 8 hr in a similar manner, and acidified with 10 % acetic acid (200 mL). The reaction mixture was then worked up in the same manner as the procedure of rutin-6,8-d_2. The purified product was obtained as yellowish needles (4.80 g, 80 %) : mp 193° ; NMR δ 1.15 (3H, d, J=6.0 Hz, rhamnosyl-CH_3); UV λ_{max}^{EtOH} : 258, 267 (sh.), 297 (sh.), 362 nm ; Rf 0.45 (n-BuOH : AcOH : H_2O = 4 : 1 : 2), Rf 0.35 (AcOEt : HCO_2H : H_2O = 10 : 2 : 3).

Rutin-2',5',6'-d_3

To a solution of sodium hydroxide (0.64 g, 16.0 mmole) and water (50.0 g, 2.8 mole) was added rutin-2',5',6',6,8-d_5 (4.80 g, 7.9 mmole). The mixture was stirred for 1 hr at 25° and then acidified with 10 % acetic acid (150 mL). After repetition of this procedure the crude solid was chromatographed on a Sephadex LH-20 column with methanol as eluent. The product was crystallized from the solution to give yellowish needles (3.60 g, 75 %) : mp 193° ; NMR δ 1.15 (3H, d, J=6.0 Hz, rhamnosyl-CH_3), 6.16 (1H, d, J=2.5 Hz, 6-H), 6.38 (1H, d, J=2.5 Hz, 8-H) ; UV λ_{max}^{EtOH} : 258, 267 (sh.), 297 (sh.), 362 nm ; Rf 0.45 (n-BuOH : AcOH : H_2O = 4 : 1 : 2), Rf 0.35 (AcOEt: HCO_2H : H_2O = 10 : 2 : 3).

DISCUSSION

To a suspended solution of rutin in deuterium oxide (1 : 10, w/w) a minimum amount of sodium hydroxide (2.2 molar) to dissolve rutin was added and the reaction mixture was stirred at 25°. The reaction was terminated at 0.5, 1 and 2 hr by the addition of 10 % acetic acid and the resulting yellowish crystalline needles were collected by filtration.

The use of n.m.r. to follow a time cource of the exchange reaction revealed that hydrogens at positions 6 and 8 were exchanged with deuteriums. That is ; signals at 6.16 and 6.38 ppm corresponding to the 6- and 8-hydrogens, respectively, decreased with time and eventually disappeared in about 2 hr. There was not an

extensive exchange with deuterium at the other positions under these experimental conditions.

The yellowish crystals obtained above were washed well with water, dried and chromatographed on a Sephadex LH-20 column with methanol as eluent. This purification procedure gave rise to deuterated rutin (rutin-6,8-d_2) in 90 % yield, the purity of which being checked by thin layer chromatography (Rf 0.45, n-BuOH : AcOH : H_2O = 4 : 1 : 2). The extent of deuteration at positions 6 and 8 was about 90 % when the reaction was allowed for 2 hr. These results were consistent with those described by Hand et al.[4] who treated resorcinols with deuterium oxide under mild alkaline conditions to observe rapid exchange of aryl hydrogens with deuteriums. These authors also found that the exchange reaction did not occur in catechols.

Ingold et al.[5] and Kirby et al.[6] reported that phenols, when heated about 100° under reflux in alkaline deuterium oxide, were labeled with deuterium at positions ortho and para to phenolic hydroxyl group. We employed the reaction temperature of 95° to obtain deuterium-labeled rutin by the reaction in the catechol ring in the presence of varying amounts of sodium hydroxide (1.0, 1.6, 2.2 and 3.4 molar). The effect of the amount of sodium hydroxide and of the reaction time on the exchange reaction is shown in Table I. As expected, almost complete exchange with deuteriums of hydrogens at positions 6 and 8 occurred under any of these reaction conditions. The changes in the n.m.r. spectra indicated that hydrogens at positions 2', 5' and 6' were also exchanged. An effective change was observed when the amount

NaOH (molar)[a)]	Time (h)	Deuterium Content (%) C-6	C-8	C-5'	C-2' + 6'	Chemical Yield (%)
1.0	1	80	75	5	10	91
1.0	4	85	85	45	35	84
1.0	8	85	80	50	40	90
1.6	1	85	80	5	15	90
1.6	4	85	85	50	50	92
1.6	8	85	85	55	50	89
2.2	1	90	90	0	15	87
2.2	4	85	85	10	35	83
2.2	8	85	80	15	45	90
3.4	1	85	80	0	25	62
3.4	4	90	90	10	30	46
3.4	8	90	90	10	40	43

Table I Deuteration of Rutin at 95° in 10-fold D_2O (w/w)

a) Molar ratio of NaOH against rutin

of sodium hydroxide was 1.6 molar and the extent of deuteration increased with increasing reaction time. The use of 3.4 molar sodium hydroxide resulted in the unfavorable degradation products increased with the prolonged reaction time. To obtain deuterated rutin (rutin-2',5',6',6,8-d_5) with a higher deuterium content, rutin was first heated with 1.6 molar sodium hydroxide and 10.0-fold deuterium oxide for 8 hr. The reaction mixture was completely freeze-dried and to this was added 10.0-fold deuterium oxide. The solution was then heated for 8 hr. The identification of the product was performed by m.p. measurement, UV absorption and thin layer chromatography. The deuterium contents are given in Table II.

Deuterated Rutin	Deuterium Content (%)[a]			
	C-6	C-8	C-5'	C-2'+ 6'[b]
Rutin-6,8-d_2	90	90	2	5
Rutin-2',5',6',6,8-d_5	95	95	95	70
Rutin-2',5',6'-d_3	2	2	90	70

Table II Deuterium content at each position of prepared deuterated rutins
a) The extent of deuteration was calculated from the integrated intensity in the n.m.r. spectra.
b) Average deuterium content (%) at positions 2' and 6' (the proton signals at positions 2' and 6' were overlapped in the n.m.r. spectra)

While hydrogens in the resorcinol ring were readily replaced with deuterium at room temperature, heating was needed to replace hydrogens in the catechol ring. The mechanism involved in the exchange reaction is probably via ketone-enolate intermediate which must be influenced by the attribute of aromaticity.

Two forms of labeled rutins (rutin-6,8-d_2, rutin-2',5',6',6,8-d_5) were thus obtained. However, these rutins do not appear to be suitable for the metabolic study, because the stability of deuterium label at positions 6 and 8 is questionable during the metabolic pathway.

Based on the experiments described above, the preparation of deuterium-labeled rutin (rutin-2',5',6'-d_3) was then made. When rutin-2',5',6',6,8-d_5 was treated with 2.2 molar sodium hydroxide in water at 25° for 1 hr, the deuterium contents at positions 6 and 8 decreased to about 15 % and 20 %, respectively. On repetition of this procedure, deuterium atoms at these positions were almost completely replaced with hydrogens. This was clearly observed by the reappearance of the

signals corresponding to hydrogens at positions 6 and 8.

The chemical purity of rutin-2',5',6'-d_3 obtained in this experiment was confirmed by n.m.r. spectroscopy and thin layer chromatography. The n.m.r. spectra of rutin-2',5',6'-d_3 and unlabeled reference are given in Fig. I. Fig. I shows that the signals corresponding to hydrogens at positions 2' and 5' disappeared. The signal corresponding to hydrogen at position 6' appeared to be affected only to a small extent. The deuterium contents at each position of rutin-2',5',6'-d_3 are shown in Table II.

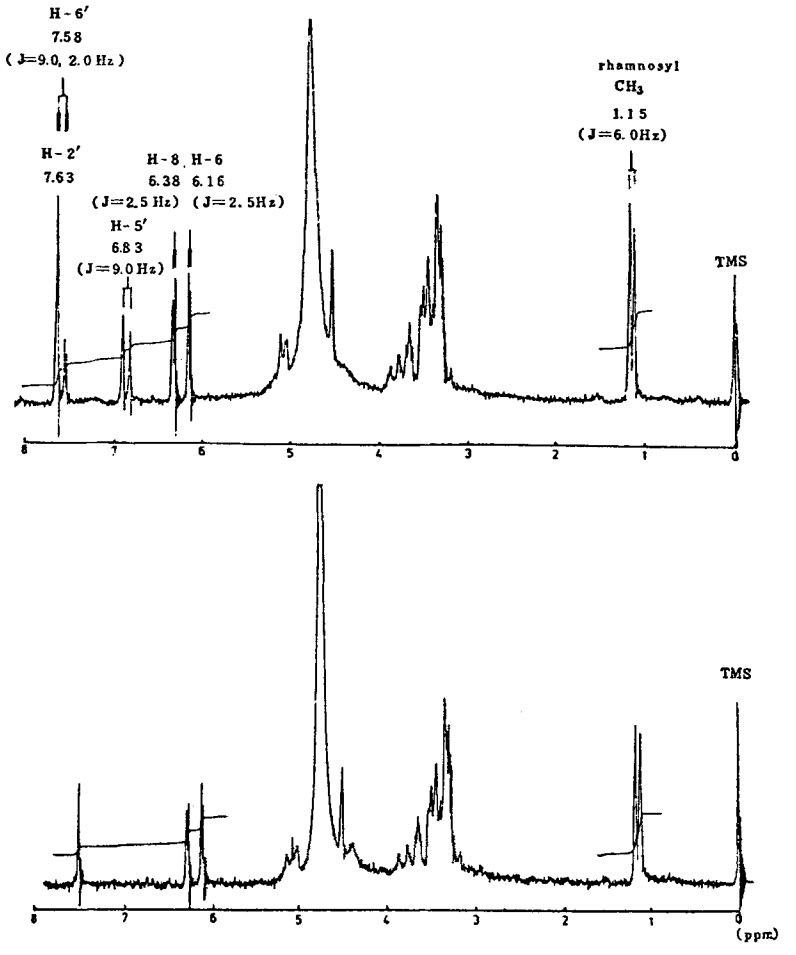

Fig. I NMR Spectra of Unlabeled Rutin (Upper) and Rutin-2',5',6'-d_3 (Lower)

Rutin-2',5',6'-d_3 was heated at about 60° for 2 hr in methanol or water. Loss of deuterium at any position was not observed.

The present procedure provides a simple and inexpensive method for the preparation of deuterium-labeled rutin in a good yield. Deuterated rutin thus obtained, especially rutin-2',5',6'-d_3, should be useful for the metabolic study of rutin in man.

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