[Chem. Pharm. Bull. 23(4) 920—922 (1975)]

UDC 547.94.09 : 615.22.033.076.9

Studies on Drug Metabolism by Use of Isotopes. XV.[1] Stability of Deuterium-Label in *p*-Hydroxylation of *l*-Ephedrine

Kunio Kawai and Shigeo Baba

Tokyo College of Pharmacy[2]

(Received July 30, 1974)

Stability of the deuterium-label in the *p*-hydroxylation of *l*-ephedrine[arom.-$^2H_5$] was investigated as one of problems on the applicability of this labeled compound to metabolic studies in man. *p*-Hydroxyephedrine liberated by enzymic hydrolysis from its glucuronide in the 24-hr urine of rats injected deuterated *l*-ephedrine (97.9 atom% ^2H) was purified and oxidized to *p*-hydroxybenzoic acid. The deuterium content on the aromatic ring of the latter compound was found to be 98.1 atom% ^2H by determining accurately by mass spectrometry. This fact indicates that the deuterium-label at meta and ortho positions of the substrate was retained during the *p*-hydroxylation and that the deuterium-label of the resulting *p*-hydroxyephedrine was stable during the glucuronide formation and the subsequent enzymic hydrolysis of the glucuronide to its free form.

A series of fundamental problems on the applicability of a drug labeled with deuterium on the benzene ring to the metabolic studies in man have been investigated.[1, 3–6] The previous data[3] obtained from a double tracer experiment by use of *l*-ephedrine[α-^{14}C, arom.U-^3H] and *l*-ephedrine[α-^{14}C, *p*-^3H] in the rat indicated a notable retention of tritium in the *p*-hydroxylation due to an NIH-shift.[7] In addition, it was suggested that appreciable loss of tritium (5—20%) occurred during the formation of glucuronide of *p*-hydroxyephedrine and its enzymic hydrolysis. If this occurs in the case of deuterium-label, the loss of the label would lead to a trouble in the mass spectral quantification of *p*-hydroxylated metabolites formed from a drug deuterated on the benzene ring. From the present study, the deuterium-label of *p*-hydroxyephedrine, a metabolite of *l*-ephedrine[arom.-$^2H_5$] in rats, was demonstrated to be stable during the glucuronide formation and the subsequent enzymic hydrolysis.

Experimental

Materials——*l*-Ephedrine[arom.-$^2H_5$] hydrochloride (I-d_5·HCl) was a sample prepared previously from $C_6{}^2H_6$ (99.5 atom% ^2H).[4] *l*-Ephedrine hydrochloride (JP grade) and *p*-hydroxybenzoic acid (GR grade) were purchased from Sanko Seiyaku Kogyo Co., Tokyo, and Tokyo Kasei Kogyo Co., Tokyo, respectively. *p*-Hydroxyephedrine hydrochloride was supplied from Hoechst Japan Ltd., Tokyo.

Isolation of Urinary Deuterated Ephedrine and *p*-Hydroxyephedrine——I-d_5·HCl (40 mg/kg) was subcutaneously injected to 10 male Wistar albino rats (230—270 g). A 24-hr urine was collected and combined. Unlabeled ephedrine hydrochloride and *p*-hydroxyephedrine hydrochloride were added as carriers (100 mg) to the combined urine. After deproteinization as described previously,[1] ephedrine was extracted with $(C_2H_5)_2O$ at pH 12 and then *p*-hydroxyephedrine with AcOEt at pH 9. The aqueous phase was evaporated at 40° under a reduced pressure and the residue was dissolved in 50 ml of 0.2M acetate buffer. After incubation with β-glucuronidase (120,000 Fishmann units, Tokyo Zoki Co., Tokyo) at 37° for 24 hr, unlabeled *p*-hydroxyephedrine hydrochloride was dissolved as a carrier (100 mg) in the medium. After deproteinization, *p*-hydroxyephedrine was extracted with AcOEt at pH 9. Ephedrine and *p*-hydroxyephedrine recovered were recrystallized as hydrochlorides from EtOH-$(C_2H_5)_2O$ and MeOH–$(CH_3)_2CO$, respectively.

1) Part XIV: K. Kawai and S. Baba, *Chem. Pharm. Bull.* (Tokyo), **23**, 289 (1975).
2) Location: *20-1, Kitashinjuku 3-Chome, Shinjuku-ku, Tokyo.*
3) S. Baba and M. Horie, *Yakugaku Zasshi*, **94**, 779 (1974).
4) S. Baba and K. Kawai, *Yakugaku Zasshi*, **94**, 783 (1974).
5) S. Baba, K. Kawai, and Y. Shida, *Yakugaku Zasshi*, **94**, 826 (1974).
6) K. Kawai and S. Baba, *Chem. Pharm. Bull.* (Tokyo), **22**, 2372 (1974).
7) G. Guroff, J.W. Daly, D. Jerina, J. Renson, S. Udenfriend, and B. Witkop, *Science*, **157**, 1524 (1967).

Oxidation of Ephedrine to Benzoic Acid and of *p*-Hydroxyephedrine to *p*-Hydroxybenzoic Acid——*p*-Hydroxyephedrine hydrochloride (20 mg) recovered from the urine and purified was converted into *p*-hydroxybenzaldehyde with $NaIO_4$. The method used was virtually identical with that described for phenylephrine by Chafetz.[8] $(C_2H_5)_2O$ extract containing *p*-hydroxybenzaldehyde was evaporated to dryness and the product was oxidized to *p*-hydroxybenzoic acid with Ag_2O.[9] $(C_2H_5)_2O$ extract from the reaction mixture was applied on a plate (5 cm × 20 cm) having a 0.25-mm layer of silica gel containing a fluorescent indicator (Wakogel B-5F, Wako Pure Chemical Ind. Ltd., Osaka) in order to remove coexisting *p*-hydroxybenzaldehyde. The plate was developed with $CHCl_3$–$(CH_3)_2CO$ (5: 1, v/v), and *p*-hydroxybenzoic acid was detected on the chromatoplate by its absorption of short-wave ultraviolet ray. The band of *Rf* 0.0—0.2 was scraped off the plate and the compound was eluted with $(C_2H_5)_2O$. The eluate was evaporated and the residue was recrystallized from water.

Ephedrine hydrochloride (20 mg) was oxidized to benzoic acid *via* benzaldehyde by the above method. Benzoic acid obtained was recrystallized from water without purification by thin-layer chromatography. Oxidation of ephedrine to benzoic acid was also performed with $KMnO_4$ as described for 1-phenyl-1,2-propanediol in the previous paper.[1]

Mass Spectral Analysis——All mass spectra were obtained with an ionization voltage of 30 eV on a Hitachi RMU-7L Mass Spectrometer. Benzoic acid and *p*-hydroxybenzaldehyde were introduced into an ion source from the indirect inlet system, and *p*-hydroxyephedrine hydrochloride and *p*-hydroxybenzoic acid from the direct inlet system. While a total ion current was being maintained constant, partial mass spectra were scanned repetitively at a slow speed over a mass range of *m/e* 120 to 130 and *m/e* 136 to 146, respectively, for the determination of deuterium content in deuterated benzoic acid and *p*-hydroxybenzoic acid.

Results and Discussion

The prominent molecular ion (M^+) of a deuterated compound would be most suitable for the determination of deuterium content because a fragment ion may be formed by a fragmentation process accompanied with an intramolecular exchange between deuterium and hydrogen as described previously.[5] It was impossible to determine the deuterium content in I-d_5 from its mass spectrum[4] as the M^+ ion does not appear. I-d_5 was, therefore, oxidized to deuterated benzoic acid and the deuterium content in the former was determined in the latter chemical form from the M^+ ion. As shown in Table I, the deuterium content[10] (97.9 atom% ^2H) in deuterated benzoic acid formed from I-d_5 by oxidation with $KMnO_4$ aggreed with that by oxidation with $NaIO_4$ followed with Ag_2O. From this fact and a finding[3] that tritium labeled on the benzene ring of ephedrine was stable to $KMnO_4$ oxidation, it was indicated

Table I. Deuterium Content in *l*-Ephedrine[a] and *p*-Hydroxyephedrine[b]

Source	Peak intensity (%)					Atom % ^2H[c]
	M−2(*A*)	M−1(*B*)	M	M+1	M+2	
Ephedrine as substrate[d]	0.9	8.9	82.0 (*m/e* 127)	7.4	0.8	97.9
Ephedrine as substrate[e]	0.9	8.8	82.0 (*m/e* 127)	7.5	0.8	97.9
Ephedrine as metabolite[d]	0.7	8.7	82.3 (*m/e* 127)	7.5	0.8	98.0
p-Hydroxyephedrine from glucuronide[e]	0.3	7.1	83.8 (*m/e* 142)	7.8	1.0	98.1

Each value indicates an average of 3 measurements.
a) determined as benzoic acid
b) determined as *p*-hydroxybenzoic acid
c) calculated approximately according to the following equation; $100-(A\times2+B)/N$, where N is the number of hydrogen isotope atoms attached to the carbon atoms constituting the aromatic ring
d) subjected to oxidation with $KMnO_4$
e) subjected to oxidation with $NaIO_4$ followed with Ag_2O

8) L. Chafetz, *J. Pharm. Sci.*, **52**, 1193 (1963).
9) I.A. Pearl, *J. Org. Chem.*, **12**, 85 (1947).
10) The deuterium content is expressed as 100 atom% ^2H when all the hydrogen atoms attached to the carbon atoms constituting the aromatic ring of a compound were replaced by deuterium atoms.

that no deuterium-label in I-d_5 was lost during both oxidation processes. In addition, it was found that deuterium on the labeled benzene (99.5 atom% ^{2}H) was only slightly lost during the reaction sequence for synthesis of I-d_5.[4]

The mass spectra of unlabeled *p*-hydroxyephedrine, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzoic acid are shown in Fig. 1. *p*-Hydroxyephedrine, which did not reveal the M^+ ion (*m/e* 181), was converted into *p*-hydroxybenzaldehyde with $NaIO_4$ because of decomposition of the phenol moiety by $KMnO_4$ oxidation. The intense M^+ ion (*m/e* 122) of unlabeled *p*-hydroxybenzaldehyde was as abundant as the fragment ion (*m/e* 121) resulting from loss of the aldehydic hydrogen atom. The calculation of deuterium content in its labeled compound would be more complicated because of the presence of the intensive M-1 species. *p*-Hydroxybenzaldehyde was, therefore, oxidized to *p*-hydroxybenzoic acid with Ag_2O and the deuterium content was determined in this chemical form.

p-Hydroxyephedrine before and after hydrolysis with β-glucuronidase were separately recovered from the urine with the aid of a carrier. The M^+ ion (*m/e* 142) of deuterated *p*-hydroxybenzoic acid derived from *p*-hydroxyephedrine, which was liberated from its glucuronide, had a mass increased by 4 atomic mass units compared to that of its unlabeled compound. The ratio of the carrier to deuterated *p*-hydroxybenzoic acid derived from the glucuronide was found to be approximately 10: 1, while the ratio in the case of free *p*-hydroxyephedrine was too large to determine the deuterium content. The mass spectrum of the former was, therefore, recorded at two scales (×1 and ×10). As the peak intensity at *m/e* 140 is due to both the M−2 species of the labeled metabolite and the M+2 species of the unlabeled carrier, the net peak intensity of the former was obtained by substracting the relative intensity of the M+2 species to the M species in the unlabeled compound from the observed peak intensity. The deuterium content in *p*-hydroxyephedrine regenerated from its glucuronide and that in unchanged ephedrine closely agreed with that in I-d_5 used as the substrate (*cf.*, Table I). These data indicated that all of the deuterium labeled on the benzene ring were retained during the formation of glucuronide of *p*-hydroxyephedrine and its enzymic hydrolysis except for *p*-hydroxylation, in which one deuterium atom at *para* position was lost. From the present study, one explanation for the lower $^3H/^{14}C$ ratios in *p*-hydroxyephedrine and its glucuronide observed in the previous paper[3] will be a quasi-counting of radioactivity in these compounds by any cause, *e.g.*, chemiluminescence, rather than the loss of the tritium.

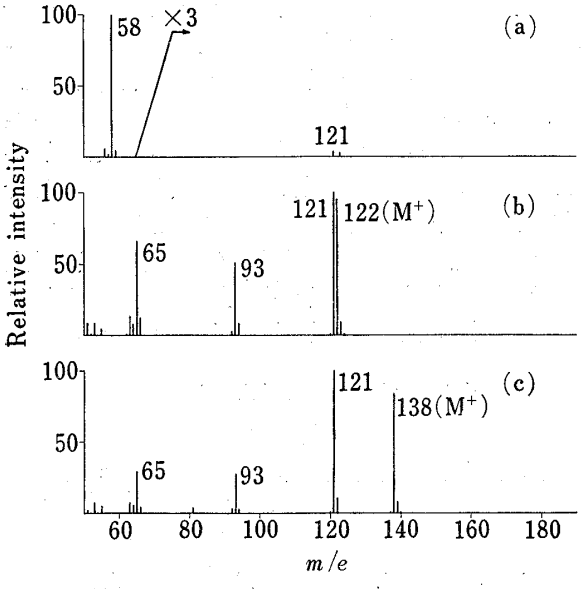

Fig. 1. Mass Spectra of *p*-Hydroxyephedrine·HCl (a), *p*-Hydroxybenzaldehyde (b), and *p*-Hydroxybenzoic Acid (c)