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Determination of Paeonol Metabolites in Man by the Use of Stable Isotopes[1)]

Kunio Mimura*,[2)] and Shigeo Baba

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo, 193-03, Japan

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The metabolism of paeonol (2-hydroxy-4-methoxyacetophenone: I) in man was studied by a stable isotope tracer technique. For the qualitative analysis of metabolites, an aliquot of 24 h urine from a man who had recived orally an equimolar mixture of I and I [methoxy-d_3, acetyl-$^{13}C_2$] was investigated by mass chromatography.

Paeonol, 2,5-dihydroxy-4-methoxyacetophenone (II) and resacetophenone (III) were identified as free, β-glucuronide, sulfate and enzyme-resistant conjugate form metabolites in the urine. In addition, by comparing the ion intensities of fragment ions of II and II-$^{13}C_2d_3$ and those of III and III-$^{13}C_2$, an isotope effect in the process of metabolism was found. This isotope effect was explained in terms of the metabolic switching phenomenon.

By dilution analysis using I [methoxy-d_3], II [methoxy-d_3] and III [acetyl-$^{13}C_2$] as internal standards, the amounts of metabolites in the urine were determined. The excretion of this drug was very rapid; the total amount of metabolites excreted in the urine within 24 h reached 78.8% of the dose (II 50.2%, III 21.0%, I 7.6%). Predominant metabolites were the β-glucuronide of II (28.8% of dose) and an enzyme-resistant conjugate form of II (23.9%).

Keywords——paeonol; metabolism; excretion; stable isotope; human urine; isotope effect

Radioisotope (RI) tracer techniques have been extensively used to investigate the metabolic fate of drugs in experimental animals. However, these techniques have inherent limitations. Administration of an RI-labeled drug to man is regulated by the International Commission on Radiological Protection. Stable isotope (SI) tracer techniques have advantages in safety, specificity and sensitivity.

The increased use of SI tracer techniques is due largely to the improvement or development of gas chromatography–mass spectrometry (GC–MS), high sensitivity detectors involving the accelerating voltage alternator[3)] and multiple ion detector,[4)] and application of GC–MS–computer systems such as mass chromatography.[5)]

The use of stable isotopes enables us to carry out qualitative and quantitative analysis with simple and rapid separation procedures. Thus, these techniques have been widely applied in the pharmaceutical and biomedical fields.[6)]

Paeonol (2-hydroxy-4-methoxyacetophenone; I) is a main component of *Botan-pi*,[7)] which has been shown to exhibit antipyretic, sedative, anti-inflammatory[8)] and antibacterial[9)] activities. 2,5-Dihydroxy-4-methoxyacetophenone (II), resacetophenone (III) and unchanged I were identified as metabolites in the rats.[10)]

In a previous paper we reported syntheses[11)] of I, II and III labeled with deuterium and carbon-13. Species differences[12)] in the metabolism of I were also studied in our laboratory by an RI tracer technique. This paper deals with studies on the metabolism and excretion of I in man.

Experimental

Labeled Compounds——2-Hydroxy-4-methoxy[d_3]acetophenone (I-d_3), 2,5-dihydroxy-4-methoxy[d_3]-acetophenone (II-d_3) and resacetophenone[acetyl-$^{13}C_2$] (III-$^{13}C_2$) were synthesized from methyliodide-d_3 (99.5 atom %, E. Merck), dimethylsulfate-d_6 (99.0 atom %, E. Merck), and acetic acid-1,2-$^{13}C_2$ (92.0 atom %, Prochem), respectively, by the methods described in a previous paper.[11)] 2-Hydroxy-4-methoxy[d_3]acetophenone[acetyl-$^{13}C_2$] (I-$^{13}C_2d_3$) was synthesized by the reaction of III-$^{13}C_2$ and methyliodide-d_3. The isotopic

compositions of I-d_3, II-d_3 and III-$^{13}C_2$ were 99.3% deuterium atoms (d_3: 98.5%, d_2: 1.35%, d_1: 0.11%), 98.9% deuterium atoms (d_3: 96.9%, d_2: 2.84%, d_1: 0.27%), and 92.0% carbon-13 atoms ($^{13}C_2$: 84.1%, $^{13}C_1$: 15.1%, $^{13}C_0$: 0.81%), respectively.

Nonlabeled Compounds——2,5-Dihydroxy-4-methoxyacetophenone (II) was prepared by the method described above. 2-Hydroxy-4-methoxyacetophenone (Aldrich Chemical; I) and resacetophenone (Wako Pure Chemical Industries, Tokyo; III) were used after recrystallization from EtOH, and from hot dilute (1: 11) hydrochloric acid, respectively.

Instrumentation and Conditions——GC–MS and GC–MS-selected ion monitoring (SIM) measurements were made with a Shimadzu LKB-9000 gas chromatograph–mass spectrometer equipped with a Shimadzu 9060S high speed multiple ion detector-peak matcher, and a Shimadzu GCMSPAC-300 data processing system coupled with an OKITAC 4300 computer. The GC column was a coiled glass column (2 m×3 mm i.d.) packed with 1.5% Silicone OV-1 on Shimalite WAW 60/80 mesh. The separation temperature, injection temperature, and ion source temperature were 280°C, 250°C, and 310°C, respectively. Helium was used as a carrier gas at a flow rate of 20 ml/min. Mass spectra were obtained at an accelerating voltage of 24 eV, and an ionizing current of 60 μA.

Quantitative analysis was carried out by SIM assay. The selected ion monitor was focused as described below and the peak height ratio was measured. The column temperature coniditions and m/z values of monitoring ions were: 210°C, m/z 166, 169 for I-OAc, I-d_3-OAc; 230°C, m/z 182, 185 for II-OAc_2, II-d_3-OAc_2; and 220°C, m/z 152, 154 for III-OAc_2, III-$^{13}C_2$-OAc_2, respectively.

In the case of mass chromatography, the column temperature was programmed from 160 to 280°C at the rate of 5°C min^{-1}. The mass spectra of gas chromatographic effluents were recorded every 5 s during a period of 2—20 min. Mass chromatograms were obtained by selecting the channels of m/z values expected to be derived from possible metabolites of I.

Detection of Urinary Metabolites——A male volunteer, 36 years of age and weighing 58 kg, orally received 100 mg of an equimolar mixture of I and I-$^{13}C_2d_3$ (I: I-$^{13}C_2d_3$), and for the following 24 h urine was collected. Free and conjugated metobolites were separated as described in a previous paper.[12] That is, a mixture of one-tenth of the urine and the same volume of 0.2 M acetate buffer (pH 5.0) was extracted 3 times with 300 ml of ether. The residual aqueous layer was incubated with β-glucuronidase (50000 Fishman units) for 24 h at 37°C, then extracted 3 times with 300 ml of ether. The residual aqueous layer was incubated with aryl-sulfatase (10 units) for 24 h at 37°C, and then extracted 3 times with 300 ml of ether. To the residual aqueous layer, conc. H_2SO_4 was added to make about 5 w/w %, then 100 mg of ferrous sulfate heptahydrate was added. After hydrolysis for 1 h at 80°C, the aqueous layer was cooled, and extracted 3 times with 300 ml of ether. Each ether fraction was evaporated to dryness at 40°C *in vacuo*, and the residue was acetylated[11] with acetic anhydride and anhydrous pyridine in the usual manner prior to mass chromatography.

Calibration Curves——Methanol solution containing a fixed amount of I from 1 μg to 1 ng was mixed with a solution containing 100 ng of I-d_3. After evaporation, each mixture was acetylated and SIM assay was carried out as described above. The peak height ratio of the m/z 166 ion to the m/z 169 ion was determined for each sample and the observed peak height ratio was plotted against the known molar ratio of I to I-d_3.

A calibration curve for II and II-d_3 was obtained in the same manner. In the case of III and III-$^{13}C_2$, however, the observed peak height ratio was corrected for the contribution of III to the m/z 154 channel and that of III-$^{13}C_2$ to the m/z 152 channel.

Sensitivity——SIM determination was carried out for known amounts of I-OAc so that the sample injected into the gas chromatograph–mass spectrometer covered the paeonol range of 10 pg to 10 ng. The signal-to-noise ratio was determined for each selected ion. The same experiment was performed for the acetylated derivatives of I-d_3, II, II-d_3, III and III-$^{13}C_2$.

In order to examine the possible interference of endogeneous substances with the quantification of urinary metabolites of I, investigations were carried out as follows; free and conjugated form fractions were separated from 100 ml of human control urine in the manner described above. One ml of MeOH solution containing a known amount of I, I-d_3, II, II-d_3, III or III-$^{13}C_2$ in the range of 100 μg to 10 ng was added to each fraction. Each compound added was purified by thin-layer chromatography (TLC) as described below, then an aliquot of acetylated sample was introduced into the GC–MS–SIM system.

Accuracy——A recovery test was carried out as follows; one-tenth aliquot of the 6—12 h urine described below was used for the separation of free and conjugated form fractions in the manner described above. To each fraction, known amounts of I, II and III were added. Furthermore, I-d_3, II-d_3 and III-$^{13}C_2$ were added as internal standards (I.S.) in the manner described below. Metabolites were purified by TLC and then aliquots of the acetylated samples were introduced into the GC–MS–SIM system.

Quantification of Urinary Metabolites——1) Excretion in Urine: Four male volunteers (41, 37, 35 and 24 years of age; weighing 56, 64, 58 and 62 kg, respectively) orally received 100 mg of I. Urine samples were then collected for the first 72 h at 6, 12, 24, 36, 48 and 72 h. Aliquots of urine samples (half by volume) were diluted with water and kept frozen until analysis. To a 100 ml of sample urine, 100 mg of ferrous sulfate heptahydrate and 2.7 ml of conc. H_2SO_4 was added, then 1.0 ml of MeOH solution containing I-d_3, II-d_3 and III-$^{13}C_2$ (0.05 mg, 0.20 mg and 0.10 mg, respectively) was added as I.S. The mixture was hydrolyzed for 1 h at 80°C, cooled, and extracted 3 times with 200 ml of ether. The ether layer was dried over

anhydrous sodium sulfate and evaporated to dryness at 40°C *in vacuo*. The residue was subjected to TLC. The spotted silica gel plates (Kieselgel $60F_{254}$, E. Merck, 0.5 mm thickness, 20×20 cm^2) were developed with benzene–$CHCl_3$–AcOH (10: 3: 2, by vol.). After the development, the spots corresponding to the *Rf* values of I, II and III (detected under UV irradiation) were scraped off and eluted with MeOH. The eluate was evaporated to dryness *in vacuo*, acetylated, and then an aliquot of the sample was introduced into the GC–MS–SIM system.

2) Quantification of Free and Conjugated Metabolites: Aliquots of urine samples (half by volume) excreted within 24 h were mixed and diluted with water to 1000 ml and kept frozen until analysis. A 100 ml of the sample urine was used for the separation of free and conjugated form fractions, and then I-d_3, II-d_3 and III-$^{13}C_2$ were added as I.S. to the free form, β-glucuronide and sulfate fractions in the manner described above. However, in the case of the enzyme-resistant conjugate form fraction, I.S. was added to the aqueous layer just before the acid hydrolysis step. Metabolites were purified by TLC, and acetylated, then an aliquot of the sample was introduced into the GC–MS–SIM system.

Results and Discussion

Identification of Urinary Metabolites

Prior to the identification of urinary metabolites of I in man, the mass spectra of I and I-$^{13}C_2d_3$ were studied. The mass spectra of I-OAc and I-$^{13}C_2d_3$–OAc (Fig. 1) showed that the mass differences between the nonlabeled compound and labeled one for the M^+ ions (*m/z* 208, 213), and $[M-CH_2CO]^+$ ions (*m/z* 166, 171) were 5 a.m.u. The mass difference of 4 a.m.u. for the $[M-CH_2CO-CH_3]^+$ ions (*m/z* 151, 155) was probably due to the elimination of one carbon-13 atom from the labeled compound. Although the isotope effect of I-$^{13}C_2d_3$ during mass fragmentation was investigated by the same method as for I-d_3,[11] no isotope effect was observed. Relative intensities of the ions from I-$^{13}C_2d_3$–OAc in the region of *m/z* 153 to 157 coincided well with those calculated[13] from the isotope content of the labeled materials used in the synthesis. Besides, the peaks at *m/z* 212, 170 and 154 (main peak minus 1 a.m.u.) were small compared to the corresponding main peaks. This means that the isotopic purity of I-$^{13}C_2d_3$ used in this study is sufficiently high.

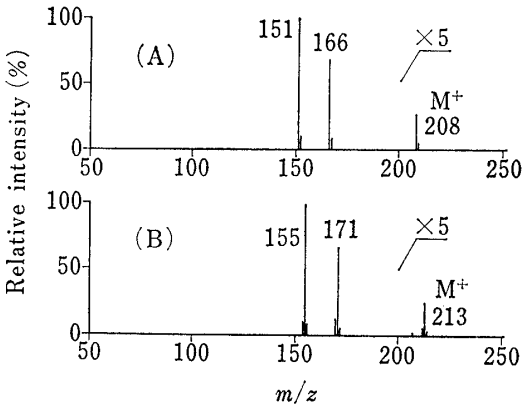

Fig. 1. Mass Spectra of 2-Hydroxy-4-methoxyacetophenone-OAc (A) and 2-Hydroxy-4-methoxy[d_3]acetophenone [acetyl-$^{13}C_2$]-OAc (B)

In a previous paper[12] 2,5-dihydroxy-4-methoxyacetophenone (II), resacetophenone (III) and unchanged I were identified as urinary metabolites of I in rabbits, guinea pigs, rats and mice, and remarkable species differences in the ratio of metabolites I, II and III were observed. Therefore, in this study we investigated I, II, III and analogous compounds as potential human metabolites of I.

As a typical example of a mass chromatogram (MC), an MC obtained from urinary β-glucuronide fraction of a man orally given I: I-$^{13}C_2d_3$ is presented in Fig. 2A. The following ions were selected to obtain this MC: fragment ions at *m/z* 166, 171, 151 and 155, originating from I-OAc and I-$^{13}C_2d_3$-OAc; fragment ions at *m/z* 152, 154, 137 and 138, originating from III-OAc_2 and III-$^{13}C_2$-OAc_2; and fragment ions at *m/z* 182, 187, 167 and 171, originating from II-OAc_2 and II-$^{13}C_2d_3$-OAc_2.

A peak originating from I: I-$^{13}C_2d_3$ was selected when the retention time of the total ion chromatogram (TIC) peak coincided with that of two or more ion chromatogram peaks and the mass spectrum of this peak showed ion clusters separated by 2—5 mass units. Although many peaks appeared in the TIC, peaks a, b and c were thus selceted as peaks originated from I: I-$^{13}C_2d_3$. For example, the retention time of TIC peak a (Fig. 2A) coincided with that

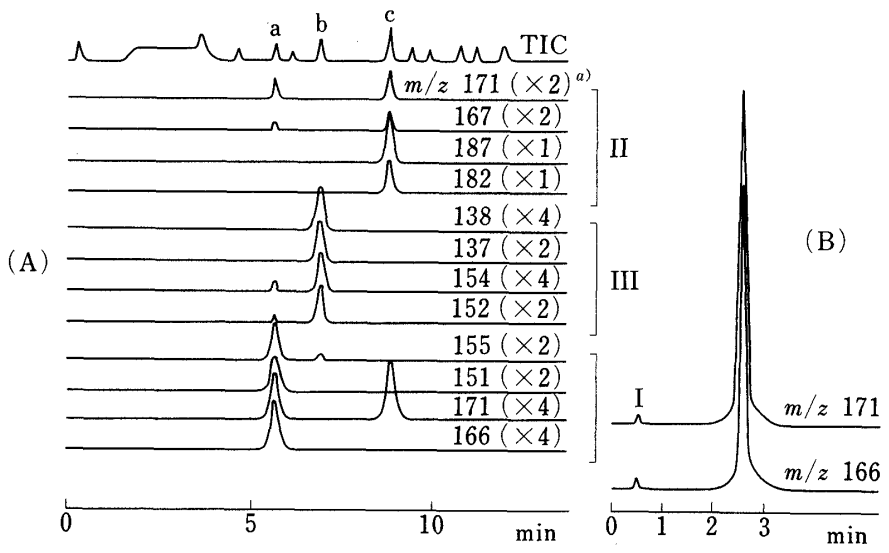

Fig. 2. Mass Chromatogram (A) of Acetylated Urinary Metabolites of an Equimolar Mixture of I and I-$^{13}C_2d_3$ in Man, and Selected Ion Monitoring (B) for the Measurement of I-OAc and I-$^{13}C_2d_3$-OAc (corresponding to TIC peak a)

a) Figures in parentheses indicate the channel gain of the monitored ion. Column temperature 160—280°C (5°C/min): A; 210°C: B.

of the *m/z* 166, 171, 151 and 155 ion chromatogram peaks. The mass spectrum of this peak (Fig. 3B) showed ion clusters at *m/z* 208: 213; 166: 171; and 151: 155 separated by 5 or 4 mass units each and was the same as the mass spectrum of I: I-$^{13}C_2d_3$-OAc (Fig. 3A). From these observations, it became clear that the TIC peak a had originated from I: I-$^{13}C_2d_3$-OAc.

By comparing the mass spectrum of TIC peak b (Fig. 3D) with that of an authentic equimolar mixture of III-OAc_2 and III-$^{13}C_2$-OAc_2 (Fig. 3C), ion clusters showing remarkably different ion intensity ratios (irregular IC) were observed at *m/z* 137: 138; 152: 154; 194: 196; and 236: 238. In each ion cluster, the ion intensity due to the nonlabled compound was higher than that from the labeled compound. Thus, it was suggested that the amount of III-OAc_2 in the sample was larger than that of III-$^{13}C_2$-OAc_2.

By comparing the mass spectrum of TIC peak c (Fig. 3G) with those of II-OAc_2 (Fig. 3E) and II-d_3-OAc_2 (Fig. 3F), possible irregular IC originating from II-OAc_2 and II-$^{13}C_2d_3$-OAc_2 was observed at *m/z* 167: 171; 182: 187; 224: 229; and 266: 271. However, in this case, it is of particular interest that the ion intensity due to the labeled compound was higher than that due to the nonlabeled compound.

The following interpretations are possible for the fact that ion clusters observed in the mass spectrum of the urinary metabolites of I in a man orally given I: I-$^{13}C_2d_3$ are remarkably irregular: (1) isotope effect affecting the gas chromatographic retention time; (2) isotope effect occurring during mass fragmentation; (3) isotope effect during the process of metabolism. It was confirmed in a previous investigation[11] that the second problem does not occur in the mass spectrometric analysis. Regarding the first possibility, the following experiments were carried out: an equimolar mixture of II and II-d_3 was acetylated and introduced into the gas chromatograph–mass spectrometer, then mass spectra were obtained by four scans at the same interval centering around the peak top of the TIC (Fig. 4A). Although irregular IC was observed in the mass spectra (Fig. 4B, 4E) obtained at the retention time corresponding to the peak edge, in the mass spectra (Fig. 4C, 4D) obtained at the rention time near the peak top, regular IC was observed.

Furthermore, SIM assay was carried out on the same sample used for mass chromatography. The peak height ratio of monitored ions from labeled and nonlabeled compounds

was almost 1:1 in the SIM chromatogram of the metabolites corresponding to I-OAc and I-$^{13}C_2d_3$-OAc (Fig. 2B). However, in SIM chromatograms corresponding to II-OAc_2, II-$^{13}C_2d_3$-OAc_2 or III-OAc_2, III-$^{13}C_2$-OAc_2, the peak height ratio was considerably different from 1:1. This is also shown in Fig. 2A. That is, the peak height ratio of the *m/z* 187 ion (II-$^{13}C_2d_3$-OAc_2) to the *m/z* 182 ion (II-OAc_2) was 1.49 at the retention time corresponding to TIC peak c. On the other hand, that of the *m/z* 154 ion (III-$^{13}C_2$-OAc_2) to the *m/z* 152 ion (III-OAc_2) was 0.53 at the retention time corresponding to TIC peak b. Therefore, it is suggested that the irregular IC observed in the mass spectra of II-OAc_2, II-$^{13}C_2d_3$-OAc_2; or III-OAc_2, III-$^{13}C_2$-OAc_2 was due to isotope effects in the metabolic process of I.

These results can be explained by the metabolic switching phenomenon observed by Horning *et al.*[14] in a metabolic study of caffeine. That is, the elimination rate of the $-CH_3$ group from the methoxy group of I is faster than that of the $-Cd_3$ group from the deuteromethoxy group of I-$^{13}C_2d_3$. Therefore, the amount of II-$^{13}C_2d_3$ produced in the metabolism of I-$^{13}C_2d_3$ is larger than that of II produced in the metabolism of I. On the contrary, the amount of III-$^{13}C_2$ produced from I-$^{13}C_2d_3$ is smaller than that of III from I.

In addition, mass chromatography was carried out by selecting ions formed from other possible metabolites of I such as 2,4,5-trihydroxyacetophenone, 2,3,4-trihydroxyacetophenone and 2,3,5-trihydroxy-4-methoxyacetophenone. However, IC-forming ion peaks originating from these compounds were not detected. In a similar manner, metabolites were investigated for the free form, sulfate and enzyme-resistant conjugate form fractions separated from the urine of a man orally given I: I-$^{13}C_2d_3$. Compounds I, II and III were identified in all cases, while other metabolites were not detected. The cumulative excretion rate of these metabolites in 0—72 h urine reached 84% (Fig. 7) of the administered dose, as described below. Therefore, it is suggested that no other major metabolite is produced from I in man.

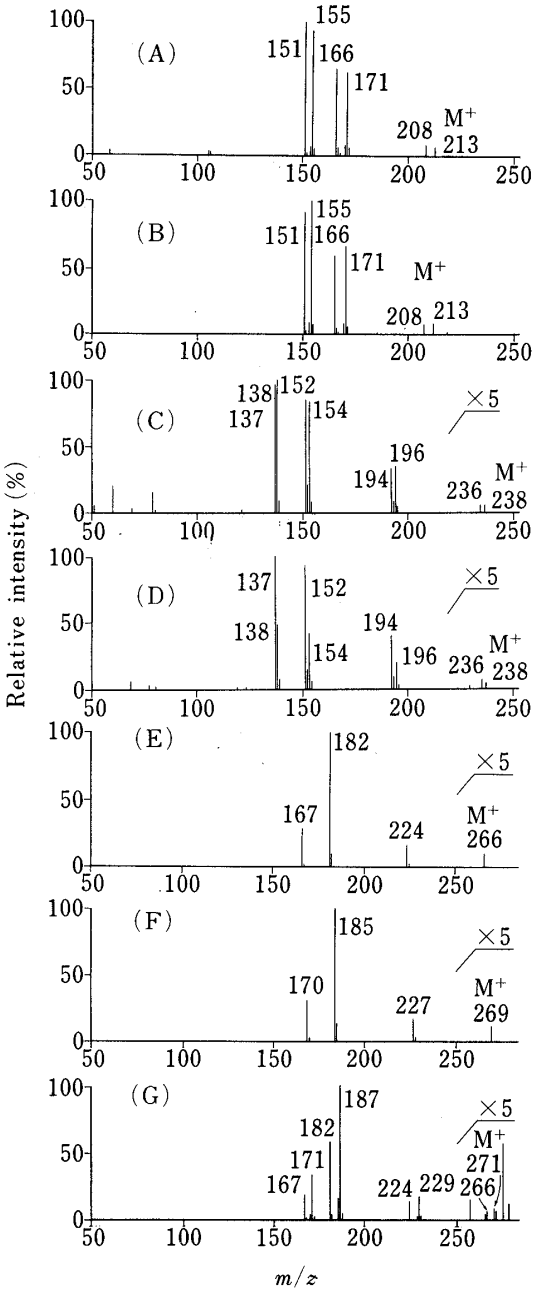

Fig. 3. Mass Spectra of Authentic I: I-$^{13}C_2d_3$-OAc (A), Metabolite Peak a (B), Authentic III: III-$^{13}C_2$-OAc_2 (C), Metabolite Peak b (D), Authentic II-OAc_2 (E), Authentic II-d_3-OAc_2 (F), and Metabolite Peak c (G)

Fundamental Studies on Quantification

1) **Calibration Curve**——As an example of a calibration curve, that of I, I-d_3 is shown in Fig. 5. Because of the high isotopic purities of I-d_3 and II-d_3, a good correlation was obtained between the mixed molar ratio and the observed peak height ratio without correcting for the contribution of d_3 form to d_0 form.

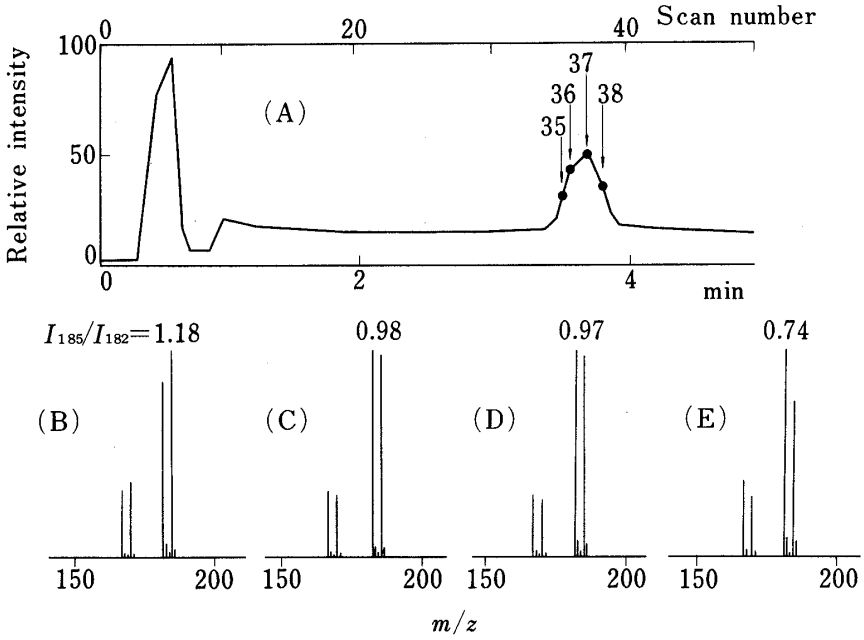

Fig. 4. Total Ion Chromatogram of an Equimolar Mixture of II-d_3-OAc_2 and II-OAc_2 (A), and Partial Mass Spectra of Scans 35 (B), 36 (C), 37 (D) and 38 (E)

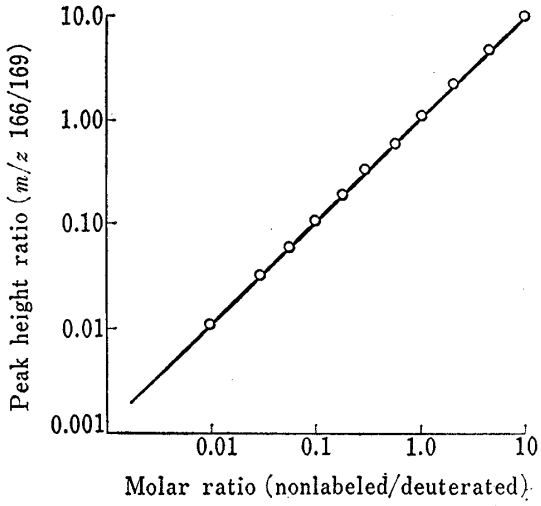

Fig. 5. Calibration Curve for Paenol [methoxy-d_3] and Paeonol

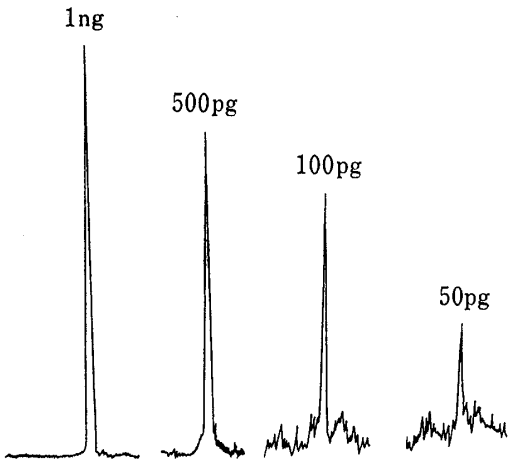

Fig. 6. Sensitivity of Selected Ion Monitoring of I-OAc (*m/z* 166)

2) **Sensitivity**——When the amount of I injected into the gas chromatograph–mass spectrometer was 100 pg or 50 pg, the signal-to-noise ratio (S/N) calculated from each SIM chromatogram (Fig. 6) was 6 or 3, respectively. Therefore, the lower limit ($S/N=2$) of detection in SIM assay of I was 30 pg. In a similar manner, the lower limits of detection for II and III were found to be 50 pg. In addition, interference by endogeneous substances in the urine during the quantification was studied as follows. One μg of I was added to 100 ml of human control urine. After purification and acetylation as described above, SIM assay was carried out on an aliquot of the sample corresponding to 1 ng of I. It was found that I could be quantified without any interference by endogeneous substances down to a lower limit of 10 ng/ml of urine. The same result was obtained for II and III in a similar manner. If 0.05% of I (corresponding to the amount of I excreted in 48—72 h urine) is excreted in 24 h urine (total volume, about 1600 ml), the concentration of I in the urine would be about 30 ng/ml.

Therefore, the quantitative method used here has adequate sensitivity.

3) **Accuracy**——An aliquot of the 6—12 h urine of a man orally given 100 mg of I was used for separation of the β-glucuronide fraction, and then known amounts of I, II and III were added to the fraction. The amounts of I, II and III in this sample were determined by dilution analysis as described above. The results presented in Table I show that the amount of each compound added was in good agreement with the amount measured, the relative error and variation coefficient being less than 4%.

Table I. Accuracy of Selected Ion Monitoring Assay of Metabolites of I in Human Urine

Metabolite	Added (μg/ml)	Expected (μg/ml)	Found (μg/ml) Individual values[a]			Mean±S.D.	CV%	Relative error (%)
I-G[b]	—	—	2.98	2.83	2.76	2.86±0.11	3.9	—
	1.31	4.17	4.40	4.13	4.38	4.30±0.15	3.5	+3.12
	2.62	5.48	5.62	5.56	5.42	5.53±0.10	1.8	+0.91
	3.93	6.79	6.82	7.00	6.82	6.88±0.10	1.5	+1.33
II-G	—	—	8.53	8.18	8.22	8.31±0.19	2.3	—
	4.27	12.58	12.44	12.02	12.40	12.29±0.23	1.9	−2.31
	8.54	16.85	16.82	16.51	16.40	16.58±0.22	1.3	−1.60
	12.81	21.12	21.29	20.91	20.71	20.97±0.29	1.4	−0.71
III-G	—	—	5.60	5.53	5.29	5.47±0.16	2.9	—
	2.87	8.25	7.98	8.20	7.87	8.02±0.17	2.1	−2.79
	5.56	11.03	10.82	10.96	10.60	10.79±0.18	1.7	−2.18
	8.34	13.81	13.58	13.78	13.53	13.63±0.13	1.0	−1.30

a) Each individual value represents the mean of triplicate measurements.
b) β-Glucuronide of I.

Excretion in Urine

The urinary excretions of metabolites for three days after oral administration of I (100 mg) to four volunteers are shown in Fig. 7. From these results, it is suggested that the excretion of I in man is rapid and that the urine is the main excretion route: the total amount of the three metabolites excreted in the urine after administration was 68.3% of the dose within 12 h, 78.8% within 24 h, and 83.4% in three days. Therefore, the authors used 24 h urine for the quantification of free and conjugated metabolites. By comparing the results on the excretion of I in man with those in experimental animals,[12] it was concluded that the excretion pattern in man was similar to that in rats.

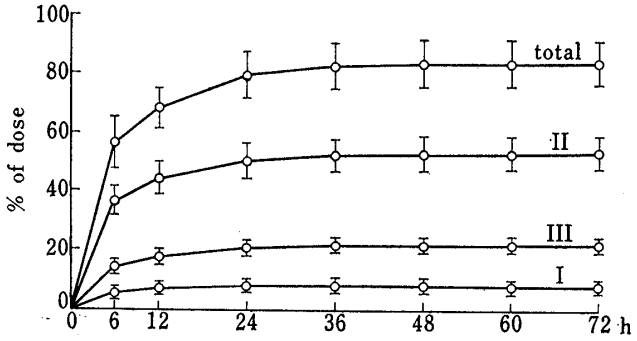

Fig. 7. Cumulative Urinary Excretions of 2-Hydroxy-4-methoxyacetophenone and Its Metabolites in Man

Values are expressed as means ±S.D. (n=4).

Quantification of Free and Conjugated Metabolites

Urinary metabolites of I in man were analyzed by mass chromatography in the manner described above. It was clarified that β-glucuronide and enzyme-resistant conjugate were major metabolites, and that the amounts of free form and sulfate were only small. Therefore, free and conjugated metabolites were quantified by SIM assay. The results, presented in Table II, show that the predominant metabolite is the β-glucuronide of II (28.8% dose), followed by the enzyme-resistant conjugate of II (23.9%). Among the other metabolites,

Table II. Metabolism of 2-Hydroxy-4- methoxyacetophenone in Man

	% of dose[a]			
	I	II	III	total
Free form	0.0±0.0	0.3±0.1	0.2±0.0	0.6±0.1
β-Glucuronide	7.9±1.4	28.8±3.7	11.9±1.0	48.6±5.0
Sulfate	0.3±0.1	0.0±0.0	0.2±0.0	0.5±0.1
Enzyme-resistant conjugate	0.5±0.1	23.9±3.5	5.7±0.2	30.1±3.4
Total	8.6±1.3	53.1±6.1	18.0±1.1	79.7±6.4

a) Mean±S.D. (n=4).

the β-glucuronide of I (7.9%) and enzyme-resistant conjugate of III (5.7%) were significant, but other metabolites amounted to less than 1%.

On the other hand, the predominant metabolites classified by the type of conjugation were β-glucuronide (48.6%) and enzyme-resistant conjugate (30.1%). The amounts of free form and sulfate were small. The total amounts of II and its conjugated form reached 66.6% of all the metabolites, while the total amounts of III and I were 22.6% and 10.8%, respectively. By comparing the results obtained here with those[12] in rabbits, guinea pigs and mice, it was found that the ratio of free metabolites to conjugated ones in these animals was higher than that in man. In addition, the composition of metabolites in animals was remarkably different from that in man, except in the case of the rat, which is similar to man in the ratio of free metabolites to conjugated ones, the composition of metabolites, and the excretion rate.

References and Notes

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