Studies on Drug Metabolism by Use of Isotopes XX: Ion Cluster Technique for Detection of Urinary Metabolites of 1-Butyryl-4-cinnamylpiperazine by Mass Chromatography

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Abstract □ An ion cluster technique and mass chromatography were used for the structural elucidation of several unidentified urinary metabolites of 1-butyryl-4-cinnamylpiperazine (I) in mice and guinea pigs. The urinary metabolites in guinea pigs and mice receiving a single 100-mg/kg dose of an equimolar mixture of 1-butyryl-4-[aromatic-d_5]-cinnamylpiperazine (I-d_5) hydrochloride and I hydrochloride (I:I-d_5) were purified and subjected to trimethylsilyl derivatization with bis(trimethylsilyl)acetamide, followed by GLC–mass spectrometry–computer analysis. Appropriate fragment and molecular ions of unidentified metabolites were selected based on the major mass fragment ions appearing in the mass spectra of the previously identified metabolites of I to provide the mass chromatograms. The presence of ion clusters as doublet peaks in the mass chromatograms indicated that the corresponding total ion peaks originated from the administered I:I-d_5. The mass spectra of selected scans from the total ion chromatogram were plotted, and the structures of unidentified metabolites were readily determined by the presence of ion clusters separated by 3–5 mass units and by the shift of the fragment ions. By using this technique, six previously unidentified metabolites were identified in the urine of guinea pigs and two were identified in the urine of mice.

Keyphrases □ 1-Butyryl-4-cinnamylpiperazine—ion cluster–mass chromatographic analyses of urinary metabolites in mice and guinea pigs □ Ion cluster–mass chromatography—analyses, urinary metabolites of 1-butyryl-4-cinnamylpiperazine in mice and guinea pigs □ Mass chromatography–ion cluster technique—analyses, urinary metabolites of 1-butyryl-4-cinnamylpiperazine in mice and guinea pigs □ Metabolites, urinary—of 1-butyryl-4-cinnamylpiperazine, ion cluster–mass chromatographic analyses in mice and guinea pigs □ Analgesics—1-butyryl-4-cinnamylpiperazine, ion cluster–mass chromatographic analyses of urinary metabolites in mice and guinea pigs

Metabolism of 1-butyryl-4-cinnamylpiperazine (I) hydrochloride, developed as an analgesic (1), was studied in the rat by GLC (2) and a radioactive isotope tracer technique (3). With the latter method, the following metabolites of I were identified and quantified in rat urine: 1-butyryl-4-(4-hydroxycinnamyl)piperazine (II), 1-cinnamylpiperazine (III), 1-(4-hydroxycinnamyl)piperazine (IV), benzoic acid, hippuric acid, 4-hydroxybenzoic acid, and 4-hydroxyhippuric acid. Furthermore, studies on the species differences in I metabolism among experimental animals such as mice, guinea pigs, and rabbits by inverse radioactive isotope dilution analysis suggested that there also were several unidentified metabolites in the urine of mice and guinea pigs (4).

Structures of drug metabolites commonly are elucidated by mass and GLC–mass spectrometry and NMR and IR spectroscopy. It is, however, difficult to decide whether the spectra obtained are from the drug itself or other biological impurities. In mass spectrometry, an ion cluster technique (5–9), using a drug labeled with a stable isotope, facilitates the differentiation of the drug metabolites from other impurities by the presence of doublet peaks and offers information about the structures of metabolites by the shift of ion cluster-forming fragment ions. The metabolites isolated by TLC from the urine of animals receiving an equimolar mixture of I and 1-butyryl-4-[aromatic-d_5]-cinnamylpiperazine (I:I-d_5) were analyzed previously (10) with a mass spectrometer equipped with a direct inlet system. In this experiment, ^{14}C-labeled I was used to locate the metabolites on TLC. However, as is often the case, isolation by TLC alone was not sufficient to obtain a clear mass spectrum.

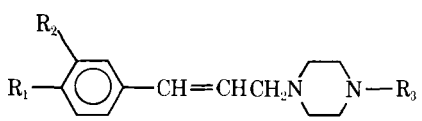
In the present experiment, metabolites of I:I-d_5 were separated by GLC and the metabolites were readily recognizable from the presence of the doublet peaks on the mass chromatograms (11, 12) obtained by a GLC–mass spectrometer–computer system. The present paper describes a mass chromatographic procedure coupled with an ion cluster technique for the identification of the unidentified metabolites of I.

EXPERIMENTAL

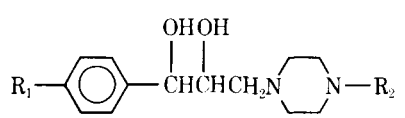
Labeled Compound—1-Butyryl-4-[aromatic-d_5]-cinnamylpiperazine (I-d_5) was synthesized according to the method described previously (10).

Authentic Compounds—Compound II hydrochloride (2), III dihydrochloride (13), IV dihydrochloride monohydrate (2), and 1-butyryl-4-(3-methoxy-4-hydroxycinnamyl)piperazine (V) (10) were synthesized in this laboratory.

1-Butyryl-4-(3-phenyl-2,3-dihydroxypropyl)piperazine (VI) was synthesized as follows. An absolute ether (10 ml) solution of I (2.20 g) was added, dropwise, to osmium tetroxide (2.00 g) in 25 ml of absolute ether at room temperature. The brown cyclic osmate ester formed immediately. To complete the reaction, the reaction mixture was allowed to stand for



I: $R_1 = R_2 = H$, $R_3 = COC_3H_7$
II: $R_1 = OH$, $R_2 = H$, $R_3 = COC_3H_7$
II′: $R_1 = H$, $R_2 = (OH)$, $R_3 = COC_3H_7$
III: $R_1 = R_2 = R_3 = H$
IV: $R_1 = OH$, $R_2 = R_3 = H$
V: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = COC_3H_7$
VII: $R_1 = R_2 = H$, $R_3 = COCH(OH)C_2H_5$
VIII: $R_1 = R_2 = H$, $R_3 = COCH_2CH(OH)CH_3$
IX: $R_1 = OH$, $R_2 = H$, $R_3 = COCH(OH)C_2H_5$
X: $R_1 = OH$, $R_2 = H$, $R_3 = COCH_2CH(OH)CH_3$
XII: $R_1 = R_2 = OH$, $R_3 = COC_3H_7$



VI: $R_1 = H$, $R_2 = COC_3H_7$
XI: $R_1 = OH$, $R_2 = COC_3H_7$

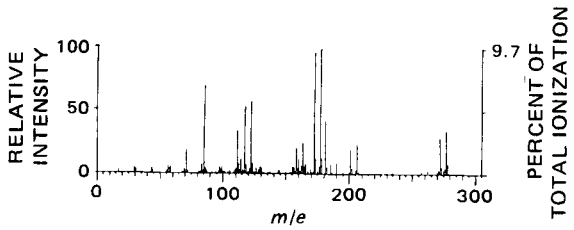


Figure 1—*Mass spectrum of an equimolar mixture of I and I-*d_5.

12 hr at room temperature. The product was separated, washed with absolute ether, and hydrolyzed with a solution of sodium sulfite (5 g) in ethanol–water (1:2 v/v, 200 ml) under reflux for 3 hr. After cooling, a dark-brown precipitate was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in water and the solution was extracted with ether.

After evaporation of the ether, the oily residue was subjected to column chromatography [silica gel C-200[1], 100–200 mesh, 3.2 cm i.d. and 30 cm in height, eluting solvent of chloroform–methanol (95:5 v/v)]. Colorless oily liquids, VI (750 mg) and I (85 mg), were collected in the 228–288-ml and 144–216-ml fractions, respectively. TLC[2] (silica gel 60 F-254) of VI thus separated showed a single spot with two different solvent systems, butanol–acetic acid–water (4:1:2 v/v, R_f 0.27) and chloroform–methanol–28% ammonium hydroxide (90:9:1 v/v, R_f 0.59). The mass spectrum of VI exhibited ions at *m/e* 306 ($M^{+}\cdot$), 288 ($M^{+}\cdot - H_2O$), 169, and 99. The hydrochloride of VI recrystallized from ethanol–ether (1:3 v/v) gave a colorless hygroscopic powder.

Anal.—Calc. for $C_{17}H_{27}ClN_2O_3$: C, 59.55; H, 7.94; N, 8.17. Found: C, 59.28; H, 8.06; N, 7.66.

Preparation of Biological Samples—Three DDY strain[3] male mice, ~20 g, and Hartley male guinea pigs, ~400 g, were injected with a single 100-mg/kg sc dose of I:I-d_5 hydrochloride dissolved in water. Urine samples were then collected for 24 (mice) or 12 (guinea pig) hr and kept frozen until analysis. Aliquots of urine samples (half in volume) were adjusted to pH 8 with a saturated sodium carbonate solution and subjected to column chromatography on an ion-exchange resin[4] (glass column of 8 mm i.d., 5 cm in height for mouse urine and 30 cm in height for guinea pig urine).

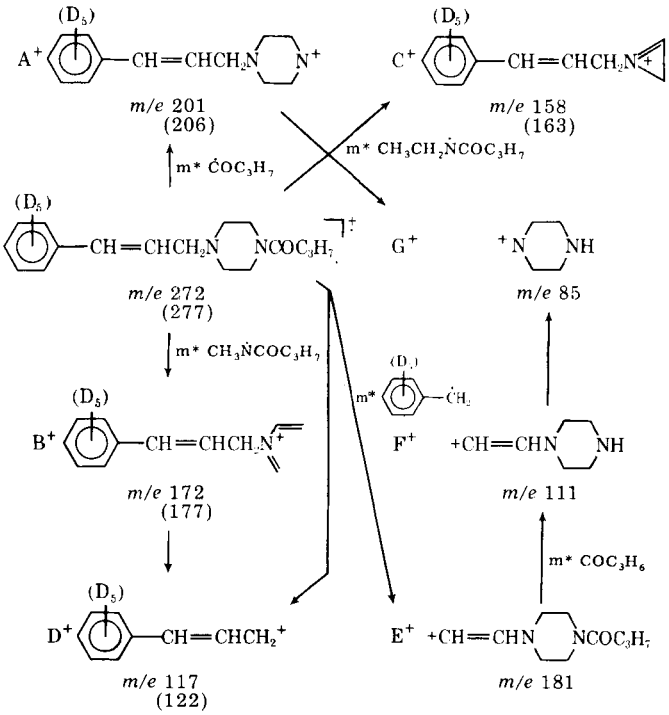
The column was washed with water (100 ml for mice urine and 600 ml for guinea pig urine), and I and its metabolites were eluted with methanol. The methanol eluate was concentrated to dryness under reduced pressure. The residue was dissolved in dry pyridine (0.2 ml for mice and 0.5 ml for guinea pigs), and 60-μl portions of the urine samples were trimethylsilylated with 20 μl of bis(trimethylsilyl)acetamide at 60° for 10 min. These samples were concentrated to 40 μl under a stream of dry nitrogen, and aliquots (1–2 μl) were injected into the gas chromatograph.

Analysis—A GLC–mass spectrometer[5] and a data processing system[6] connected to a minicomputer[7] were used. The GLC conditions were: column, 1-m × 3-mm i.d. glass column packed with 1.5% SE-30 on 60–80-mesh Chromosorb W; column temperature, programmed at 5°/min from 100 to 270°; flash heater temperature, 250°; and helium flow rate, 20 ml/min. The mass spectrometer conditions were: separator temperature, 280°; ionization source temperature, 310°; and ionization energy, 20 ev. For peak e and the trimethylsilyl derivative of VI, mass spectra were scanned under the following conditions: separator temperature, 240°; ionization source temperature, 270°; and ionization energy, 17 ev.

Eight minutes following injection of the sample, automatic magnet scanning was initiated, covering an *m/e* range of 50–550 every 8 sec. All scanning data were stored in the data processing system. Mass chromatograms were obtained on a digital plotter for selected mass numbers. Background-subtracted mass spectra also were obtained on the digital plotter.

RESULTS AND DISCUSSION

When an equimolar mixture of labeled and unlabeled compounds is used, fragment ions give their respective peaks in doublet form at the same retention time on the mass chromatogram. However, if there is a marked isotope effect on the retention time, this type of doublet peak is not formed. Therefore, to detect the drug metabolites by an ion cluster technique coupled with mass chromatography, the isotope effect of the labeled compound on the GLC retention time must be negligible.



Scheme I—Fragmentation pathways of I and I-d_5. *Figures in parentheses indicate the* m/e *value of deuterium-labeled compound.*

The retention time of a deuterium-labeled compound is shorter than that of the unlabeled compound (14); this isotope effect is influenced by the number and position of deuterium atoms in the labeled molecule. For instance, compounds with deuterium-labeled benzene rings, such as *l*-[aromatic-d_5]-ephedrine (oxazolidone derivative), L-[aromatic-d_5]-phenylalanine (enamine methyl ester derivative), and I-d_5 showed 1–2-sec shorter retention times than the unlabeled compounds under the GLC conditions employed. However, in mass chromatography, the scanning interval is generally much longer than this isotope effect. In the present study on I metabolism by an ion cluster technique and mass chromatography, the doublet peaks observed on the mass chromatograms were derived from ion cluster-forming ions of I:I-d_5 and its metabolites.

The mass spectrum of I:I-d_5 and its fragmentation pathways are shown in Fig. 1 and Scheme I, respectively. Ions produced from I:I-d_5 were divided into two groups, an ion cluster-forming group retaining the benzene ring and a nonion cluster-forming group lacking the benzene ring. Rearrangement of deuterium attached to the benzene ring was not observed in the fragmentation of I:I-d_5 (Fig. 1). The fragment ions of the former and the latter groups were named as indicated in Scheme I.

Typical examples of mass chromatograms obtained from the urine of guinea pigs and mice administered I:I-d_5 are presented in Fig. 2. The following ions were selected to obtain these mass chromatograms: a fragment ion at *m/e* 85 (ion G), which appeared in all mass spectra of I and its metabolites identified by a radioactive isotope tracer technique (2); fragment ions at *m/e* 117:122 (ion C), corresponding to the cinnamyl moiety, and at *m/e* 205:209, corresponding to the 4-hydroxylated cinnamyl moiety; the molecular ions of monohydroxylated I:I-d_5 at *m/e* 360:364, 365 and of dihydroxylated I:I-d_5 at *m/e* 448:452; and other ions of possible metabolites of I:I-d_5.

Not all peaks appearing on the total ion chromatogram originated from I:I-d_5. A peak originating from I:I-d_5 was selected when the retention time of the total ion chromatogram peak coincided with the retention time of two or more ion chromatogram peaks and the mass spectrum of this peak showed ion clusters separated by 3–5 mass units. Introduction of hydroxyl groups into the benzene ring is indicated by reduction in the spacing of the ion cluster.

For example, the retention time of the total ion chromatogram peak c in Fig. 2A (scan number 79) coincided with that of *m/e* 85, 117, and 122 ion chromatogram peaks. The mass spectrum of this peak c showed ion

[1] Wako Pure Chemical Industries, Tokyo, Japan.
[2] Merck.
[3] National Institute of Health, Shinagawa-ku, Tokyo, Japan.
[4] Amberlite XAD-2.
[5] LKB-9000, Shimadzu Seisakusho Ltd., Kyoto, Japan.
[6] GCMSPAC-300, Shimadzu Seisakusho Ltd., Kyoto, Japan.
[7] OKITAC-4300, Oki Electric Industry Co., Tokyo, Japan.

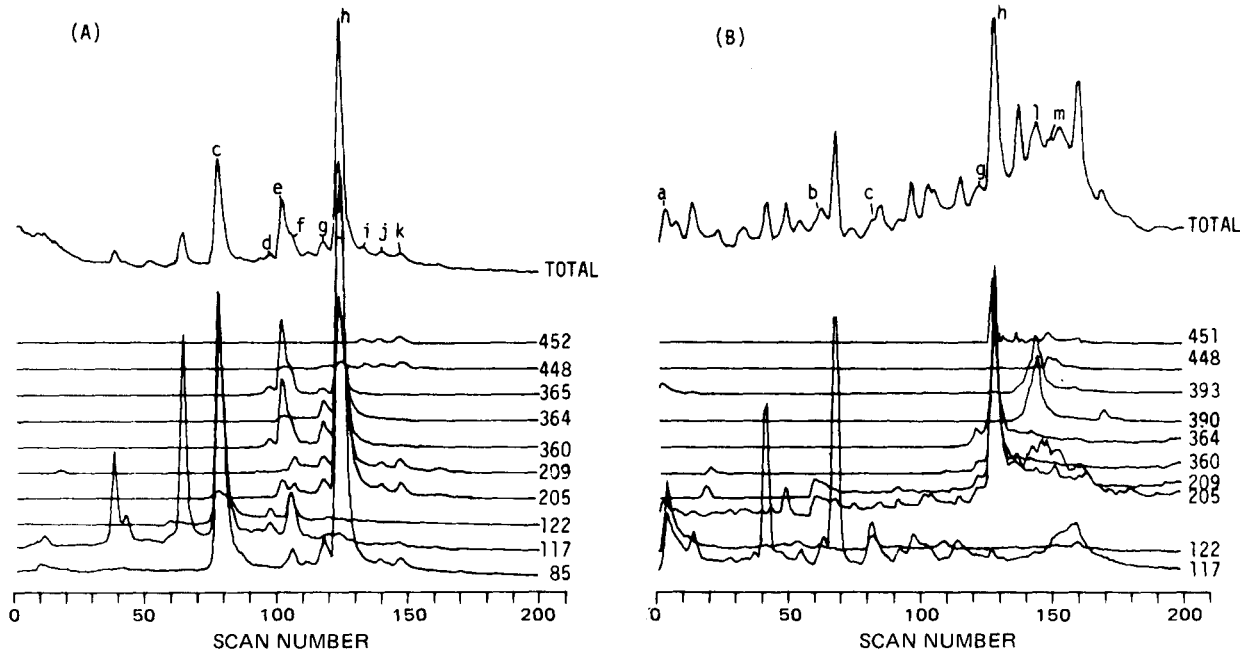


Figure 2—*Mass chromatograms of urinary metabolites of an equimolar mixture of I and I-*d_5 *in guinea pigs (A) and in mice (B).*

clusters separated by 5 mass units each and was the same as the mass spectrum of I:I-d_5 (Fig. 1). From these observations, it became apparent that the total ion chromatogram peak c originated from I:I-d_5. In a similar manner, eight other total ion chromatogram peaks (peaks d–k) shown in Fig. 2A and seven total ion chromatogram peaks (peaks a–c, g, h, l, and m) in Fig. 2B originated from I:I-d_5 or its metabolites. Total ion chromatogram peaks a, b, and h corresponded to Metabolites III, IV, and II, respectively, which had been identified by a radioactive isotope tracer technique. Their identity was confirmed by investigating the retention time on GLC and the mass spectrum of trimethylsilylated authentic compounds.

Mass spectra of total ion chromatogram peaks d, f, e, i, j, k, and l are shown in Figs. 3A, 3B, 4A, 4B, 5A, 5B, and 6, respectively. Whether or not singlet ions appearing in these mass spectra originated from the metabolites in question was determined as follows. Mass chromatograms were recorded by assigning the *m/e* values of the singlet ions (*e.g.*, ions at *m/e* 131, 180, and 269 of the mass spectrum of the total ion chromatogram peak j, Fig. 5A) and those of the ion cluster-forming ions (*e.g.*, *m/e* 205:209, 260:264, and 448:452, Fig. 7). When the scan number of the singlet ion peak coincided with that of the ion cluster-forming ion peak, the singlet ions in question were confirmed to have originated from the drug itself. Figure 7 shows that ions at *m/e* 131 and 269 belong to the metabolite of peak j, while an ion at *m/e* 180 does not belong to the peak. In Figs. 3B, 4B, and 5B, the star (*) indicates an ion peak not associated with the metabolites in question.

The structural elucidation of unidentified metabolites of I:I-d_5 (total ion chromatogram peaks d–g and i–m) was carried out as follows.

Total Ion Chromatogram Peaks d and f—In these mass spectra (Fig. 3), ion clusters separated by 5 mass units each were observed. The *m/e* values of the molecular ion (*m/e* 360:365) and the fragment ion (*m/e* 269) were 88 mass units higher than those of the molecular ion (*m/e* 272:277) and the fragment ion E (*m/e* 181) of I:I-d_5. These ions possessed a butyryl group. These facts indicated the introduction of a hydroxyl group into the butyryl group of I:I-d_5.

In the mass spectrum of peak d, a very intense peak at *m/e* 131 [$(CH_3)_3SiO^+{=}C_3H_6$], which would be formed from α-cleavage of the butyryl moiety, indicated that the hydroxylation occurred at the α-position of the butyryl group. On the other hand, in the mass spectrum of peak f, a fragment ion at *m/e* 117 was of higher intensity than a fragment ion at *m/e* 122, although these two ions (*m/e* 117:122) formed an ion cluster of equal intensity in the mass spectrum of peak d. This result is explained by the contribution of the fragment ion of *m/e* 117 [$(CH_3)_3SiO^+{=}C_2H_4$] resulting from the α-cleavage of the butyryl moiety. From these facts, the metabolites of peaks d and f were assigned as 1-(1-hydroxybutyryl)-4-cinnamylpiperazine (VII) and 1-(2-hydroxybutyryl)-4-cinnamylpiperazine (VIII), respectively.

Total Ion Chromatogram Peak e—The mass spectrum of peak e (Fig. 4A) gave dominant singlet peaks at *m/e* 169 and 271. Ion clusters at *m/e* 360:365 (I plus trimethylsilyloxy group minus one proton), 435:440 (I plus two trimethylsilyloxy groups minus 15), and 450:455 (I plus two trimethylsilyloxy groups) were of low intensity but were observed clearly. The presence of the molecular ion at *m/e* 450:455 and of the intensive singlet ion at *m/e* 169 and 271 suggested the possibility of the introduction of two hydroxyl groups into the double bond of the cinnamyl group to give rise to VI. This reaction was confirmed by investigating the retention time on GLC and the mass spectrum of trimethylsilylated authentic VI.

Total Ion Chromatogram Peak g—The mass spectrum of peak g showed the same molecular and fragment ions as that of peak h, although

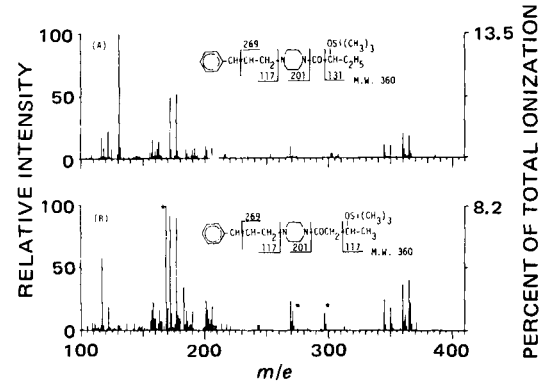


Figure 3—*Mass spectra of peak d (A) and peak f (B).*

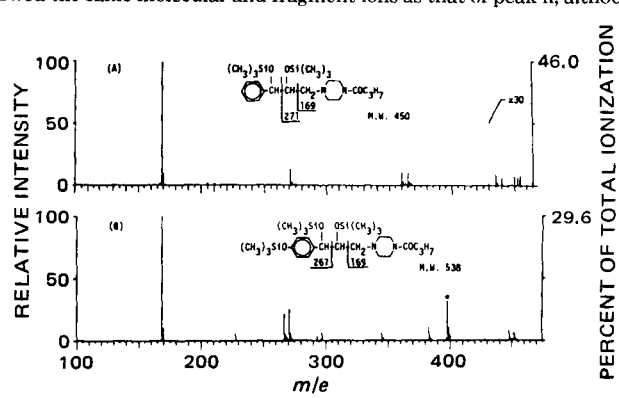


Figure 4—*Mass spectra of peak e (A) and peak i (B).*

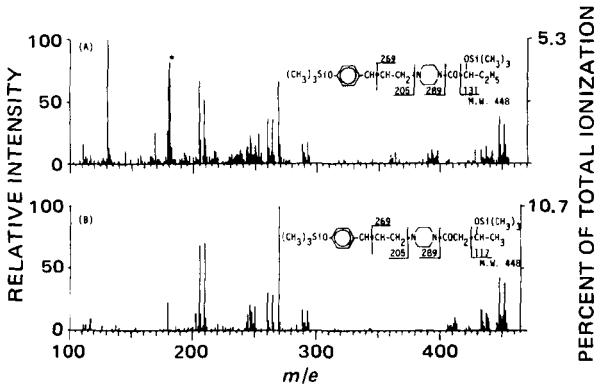


Figure 5—*Mass spectra of peak j (A) and peak k (B).*

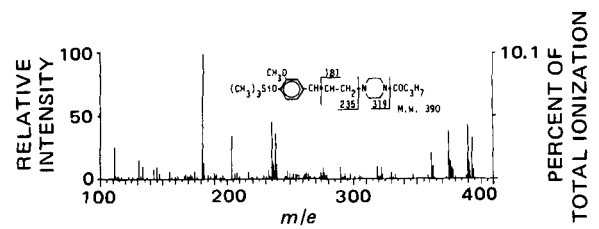


Figure 6—*Mass spectrum of peak l.*

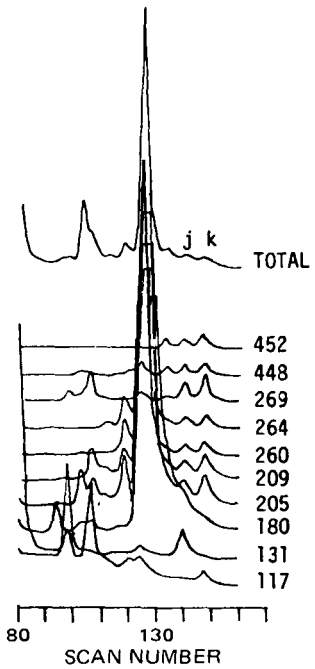


Figure 7—*Mass chromatogram of urinary metabolites of an equimolar mixture of I and I-d_5 in guinea pigs.*

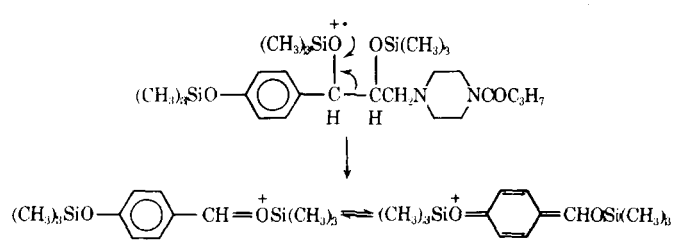
the relative intensities were different. Each ion cluster was 4 mass units apart in both mass spectra. These observations indicated that the metabolite of peak g was an *ortho*- or a *meta*-isomer of II.

Total Ion Chromatogram Peak i—The mass spectrum of peak i (Fig. 4B) gave an intense singlet peak at *m/e* 169 as observed in the mass spectrum of peak e. In this mass spectrum, the ion cluster at *m/e* 360:365 of peak e shifted to *m/e* 448:452, suggesting the introduction of three hydroxyl groups into the molecule, one in the benzene ring and two in the double bond of the cinnamyl group. The occurrence of an ion cluster at *m/e* 267:271 (in the mass spectrum of peak i), which was not observed in the mass spectrum of peak e, can be reasonably explained by resonance stabilization of the fragment ion produced by an α-cleavage (Scheme II). The metabolite of peak i was then assigned as 1-butyryl-4-[3-(4-hydroxyphenyl)-2,3-dihydroxypropyl]piperazine (XI).

Total Ion Chromatogram Peaks j and k—The mass spectra of peaks j and k (Fig. 5) showed the same ion peaks, except for the ion at *m/e* 131 that was only in the former and the ion at *m/e* 117 that was only in the latter (Fig. 7). In these mass spectra, the molecular ion (*m/e* 448:452) was 176 mass units higher (two trimethylsilyloxy groups) than that of I:I-d_5, and the fragment ions possessing either a benzene ring or a butyryl group (ions A–E) were 88 mass units (trimethylsilyloxy group) higher than the corresponding fragment ions of I:I-d_5. In addition, ion clusters in the mass spectra of peaks j and k were 4 mass units apart, whereas ion clusters in the mass spectrum of I:I-d_5 were 5 mass units apart.

These facts suggested the introduction of two hydroxyl groups into the molecule, one in the butyryl group and the other in the benzene ring. Moreover, the presence of the fragment ion at *m/e* 131 in the mass spectrum of peak j and the fragment ion at *m/e* 117 in the mass spectrum of peak k indicated that peaks j and k were derived from 1-(1-hydroxybutyryl)-4-(4-hydroxycinnamyl)piperazine (IX) and 1-(2-hydroxybutyryl)-4-(4-hydroxycinnamyl)piperazine (X), respectively.

Total Ion Chromatogram Peak l—The mass spectrum of peak l (Fig. 6) showed ion clusters 3 mass units apart (*m/e* 235:238, 319:322, and 390:393). In this mass spectrum, ion clusters at *m/e* 272:277 ($M^{+\cdot}$), *m/e* 201:206 (ion A), and 117:122 (ion C) of I:I-d_5 shifted by 118 mass units



Scheme II

(trimethylsilyloxy plus methoxy group), giving rise to ion clusters at *m/e* 390:393, 319:322, and 235:238, respectively. These facts suggested that peak l originated from V. The GLC retention time and the mass spectrum of trimethylsilylated authentic V confirmed the structure of the metabolite of peak l as V.

Total Ion Chromatogram Peak m—High background resulting from biological materials obscured part of the mass spectrum of peak m. However, an ion cluster at *m/e* 448:451, separated by 3 mass units, was observed clearly, suggesting that the metabolite of peak m might be 1-butyryl-4-(3,4-dihydroxycinnamyl)piperazine (XII).

The I metabolites identified by the present method from guinea pigs and mice are summarized in Table I. Oxidation of the cinnamyl moiety to give VI and XI and hydroxylation of the butyryl group to give VII–X occurred in guinea pigs; in mice, 3,4-dihydroxylation of the benzene ring occurred and a hydroxyl group at the 3-position readily methylated, resulting in V. In a previous study (4), it was demonstrated that the metabolism of I in rabbits proceeded predominantly by 4-hydroxylation of the benzene ring and *N*-deacylation of the butyryl group.

There are, therefore, marked species differences in I metabolism among the experimental animals studied. Furthermore, in studies on the species differences in I metabolism (4), significant amounts of benzoic acid and hippuric acid originating from this compound were detected in the urine of guinea pigs, while these metabolites were found in negligible amounts in mice. These facts suggest that benzoic acid is formed through dihydroxylation of the olefinic moiety of I.

The present study applied mass chromatography combined with an ion cluster technique for the separation and differentiation of the unidentified metabolites in a complex mixture of biological materials. The

Table I—Sources of the Metabolites of I Elucidated by Mass Chromatography Coupled with an Ion Cluster Technique

Peak	Metabolite	Guinea Pigs	Mice
a	III		+
b	IV		+
c	I	+	+
d	VII[a]	+	
e	VI[a]	+	
f	VIII[a]	+	
g	II'[a]	+	+
h	II	+	+
i	XI[a]	+	
j	IX[a]	+	
k	X[a]	+	
l	V[a]		+
m	XII[a]		+

[a] New metabolites.

ion cluster technique was effective not only for the differentiation of the metabolites from other biological impurities but also for the structural elucidation. The metabolic transformation of the drug molecule was easily recognized by the shift of the fragment ions. Labeling the benzene ring of I with deuterium atoms readily clarified the metabolic transformations occurring in the benzene ring, since the elimination of each deuterium atom due to the biotransformation reduced the spacing of the ion clusters by 1 mass unit.

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Effect of Salicylamide and Acetaminophen on Dextromethorphan Hydrobromide Metabolism: Possible Pharmacological Implications

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Abstract □ The effect of salicylamide and acetaminophen on the metabolic fate of dextrorphan, the primary metabolite of dextromethorphan, was studied *in vivo* in the rat. Plasma dextrorphan levels were measured at 5-min intervals up to 20 min and at longer intervals up to 2 hr after dextromethorphan hydrobromide was administered orally either alone or in combination with salicylamide and acetaminophen. The combination gave rise to higher plasma dextrorphan levels than did dextromethorphan hydrobromide alone at most sampling times. Conjugation of dextrorphan was inhibited almost quantitatively by salicylamide and acetaminophen at the 5-min sampling time. Salicylamide alone increased the plasma dextrorphan levels when it was coadministered with dextromethorphan, but the differences were not statistically significant. The antitussive activity of dextromethorphan hydrobromide in the unanesthetized dog was faster in onset, greater in intensity, and longer in duration when it was coadministered with salicylamide and acetaminophen. It is suggested that salicylamide and acetaminophen may inhibit the metabolic inactivation of dextrorphan, thereby improving the cough-inhibiting potential of dextromethorphan hydrobromide.

Keyphrases □ Dextromethorphan hydrobromide—metabolism, effect of salicylamide and acetaminophen, rats □ Metabolism—dextromethorphan hydrobromide, effect of salicylamide and acetaminophen, rats □ Salicylamide—effect on metabolism of dextromethorphan hydrobromide in rats □ Acetaminophen—effect on metabolism of dextromethorphan hydrobromide in rats □ Antitussives—dextromethorphan hydrobromide, metabolism, effect of salicylamide and acetaminophen, rats □ Analgesics—acetaminophen and salicylamide, effect on metabolism of dextromethorphan hydrobromide in rats

Many drugs are eliminated from the body by biotransformation to glucuronide and sulfate conjugates that are pharmacologically inactive. The enzyme systems responsible for the formation of such metabolites are saturable at relatively low drug concentrations (1, 2). Concomitant administration of drugs that undergo conjugation *in vivo* was reported to result in a competitive inhibition of the respective enzymatic processes (3–5).

The main routes of metabolism of dextromethorphan, a widely used nonnarcotic antitussive agent, are *O*- and *N*-demethylation followed by subsequent conjugation of the desmethyl metabolites to glucuronides and sulfates, with *O*-demethylation being the predominant pathway (6–10). The *O*-demethylation product, dextrorphan (*d*-3-hydroxy-*N*-methylmorphinan), was reported to possess antitussive activity in the dog (11). Salicylamide, a weak analgesic and antipyretic agent, is metabolized primarily to the ether glucuronide and ester sulfate (2). The main metabolites of the analgesic drug acetaminophen are the glucuronide and sulfate conjugates (12, 13).

Self-administration of analgesic preparations along with antitussives is common. This investigation determined, in experimental animals, if the biotransformation of dextromethorphan is affected by concurrent administration of salicylamide or a combination of salicylamide and acetaminophen and if the antitussive activity of dextromethorphan is altered by such multidrug therapy.

EXPERIMENTAL

Materials—The drugs and chemicals used were: dextromethorphan