STUDIES ON THE METABOLISM OF DILTIAZEM IN MAN

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(Received June 10, 1983)

The human urinary metabolites of diltiazem were analyzed by thin-layer chromatography (TLC) and gas chromatography-mass spectrometry.

Diltiazem was metabolized by deacetylation, N-demethylation, O-demethylation and conjugation. Metabolite $M_A$, $N$-monodemethyl-diltiazem, was identified as a new major metabolite in human urine, and four metabolites were identified as deacetyl-diltiazem ($M_1$), deacetyl-$N$-monodemethyl-diltiazem ($M_2$), deacetyl-$O$-demethyl-diltiazem ($M_4$), deacetyl-$N,O$-demethyl-diltiazem ($M_6$) which were known as rat urinary metabolites. Metabolite $M_2$, $M_4$ and $M_6$ were converted in part to glucuronides and/or sulfates.

Unchanged diltiazem and metabolite $M_A$ were determined in human plasma and urine by TLC-densitometry. Diltiazem and metabolite $M_A$ excreted in 24-h urine were 44.4 and 48.5% of the total unconjugated form, respectively. The mean plasma level of metabolite $M_A$ was approximately one-third of diltiazem level. On the basis of these findings, a probable metabolic pathway of diltiazem in man is presented.

Keywords — diltiazem; calcium antagonist; human urinary metabolite; urinary excretion; plasma concentration; GC-MS; TLC-densitometry

Diltiazem hydrochloride, cis-(+)-3-(acetyl-oxy)-5-[2-(dimethylamino) ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5$H$)-one hydrochloride, is a new potent coronary vasodilator known as a calcium channel blocker or calcium antagonist which was developed in our company.$^{1,2}$

The metabolic fate and pharmacokinetics of diltiazem have been studied in mice,$^9$ rats,$^4$ dogs$^6$ and men.$^6$ In a previous paper, we reported that diltiazem was extensively metabolized in rat and the major metabolites were deacetyl-$O$-demethyl-diltiazem, deacetyl-$N$, $O$-demethyl-diltiazem and deacetyl-$N$, $O$-demethyl-methoxyl-diltiazem.$^4$ Morselli et al.$^6$ reported that diltiazem was deacetylated and subsequently $O$-demethylated and/or $N$-demethylated in men, similarly in rats.$^4$

In the present paper, we report a new metabolite of diltiazem, $N$-monodemethylated diltiazem ($M_A$), which was first found in human urine, and the results of detailed study on the metabolism of diltiazem in man.

EXPERIMENTAL

Reference Compounds of Diltiazem — The reference compounds, shown in Fig. 1, were synthesized in our Organic Chemistry Research Laboratory and were used for identification of human urinary metabolites. Their $R_f$ values on thin-layer chromatography (TLC) and retention times ($t_R$) on gas chromatography (GC) are summarized in Table I.

Human Subjects — For isolation of metabolites, three normal healthy male subjects, aged 25—40 years and weighing 60—68 kg, received two 30 mg-tablets of diltiazem hydrochloride after submission of a written informed consent and the urine sample was collected for 24 h.

For quantitative analysis of diltiazem and its major metabolites in urine, five normal healthy
Metabolism of Diltiazem in Man

![Chemical Structures of Diltiazem (dil.) and Synthetic Compounds](image)

Diltiazem: \( R^1 = R^3 = R^4 = CH_3, R^2 = COCH_3 \)

**synthetic compounds**

*N*-monodemethyl-dil. (M₄)
: \( R^1 = R^3 = CH_3, R^2 = COCH_3, R^4 = H \)

Deacetyl-dil. (M₁)
: \( R^1 = R^3 = CH_3, R^2 = COCH_3, R^4 = H \)

Deacetyl-*N*-monodemethyl-dil. (M₂)
: \( R^1 = R^3 = CH_3, R^2 = CH_3, R^4 = H \)

Deacetyl-*O*-demethyl-dil. (M₃)
: \( R^1 = R^2 = CH_3, R^3 = R^4 = CH_3 \)

Deacetyl-*N*, *O*-demethyl-dil. (M₅)
: \( R^1 = R^2 = CH_3, R^3 = R^4 = CH_3 \)

Deacetyl-*N*-didemethyl-dil. (Mₓ)
: \( R^1 = CH_3, R^2 = CH_3, R^3 = R^4 = H \)

**FIG. 1. Chemical Structures of Diltiazem (dil.) and Synthetic Compounds**

Male subjects, aged 29–36 years and weighing 58–69 kg, received a 30 mg-tablet of diltiazem hydrochloride as above mentioned. The urine samples were collected in the following periods: 0–2, 2–4, 4–6, 6–8, 8–10 and 10–24 h after administration. For determination of plasma concentration of diltiazem and its metabolites, plasma samples were obtained from patients suffering from angina pectoris under treatment with diltiazem in a dose of two 30 mg-tablets three times daily.

*Rats* — Five male Sprague-Dawley rats, weighing about 200 g and aged six weeks, were administered orally \(^{14}\text{C}-\text{diltiazem hydrochloride} 100 \text{ mg/kg}. \) Rats were housed in cages constructed to permit separate collection of urine and feces, and the urine sample was collected for 24 h.

**Isolation of Urinary Metabolites** — The procedure is outlined in Chart 1. The urine sample was applied on Amberlite XAD-2 column. The column was washed with water and the metabo-

**TABLE I. Rf Values and Retention Times (tᵣ) of Diltiazem and Its Synthetic Reference Compounds on TLC and GC**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rf (^{a}))</th>
<th>tᵣ (^{b}) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem (dil.)</td>
<td>0.44</td>
<td>32.8</td>
</tr>
<tr>
<td><em>N</em>-monodemethyl-dil. (M₄)</td>
<td>0.48</td>
<td>35.0</td>
</tr>
<tr>
<td>M₄-TFA</td>
<td></td>
<td>35.6</td>
</tr>
<tr>
<td>Deacetyl-dil. (M₁)</td>
<td>0.29</td>
<td>26.0</td>
</tr>
<tr>
<td>M₁-TMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deacetyl-<em>N</em>-monodemethyl-dil. (M₂)</td>
<td>0.31</td>
<td>26.0</td>
</tr>
<tr>
<td>M₂-TMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deacetyl-<em>O</em>-demethyl-dil. (M₃)</td>
<td>0.20</td>
<td>27.6</td>
</tr>
<tr>
<td>M₃-TMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deacetyl-<em>N</em>, <em>O</em>-demethyl-dil. (M₅)</td>
<td>0.24</td>
<td>25.4</td>
</tr>
<tr>
<td>M₅-TMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deacetyl-<em>N</em>-didemethyl-dil. (Mₓ)</td>
<td>0.88</td>
<td>26.0</td>
</tr>
<tr>
<td>Mₓ-TMS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a)}\) Chromatography was performed on Silica gel 60 GF₂₅₄ with solvent system: chloroform–n-butanol–ethanol–methanol–acetic acid (8:3:3:1:1, v/v). The plate was developed twice with the same solvent.

\(^{b)}\) Retention time (min) on 3% OV-17 column.
lites were eluted with methanol. The methanol eluate was concentrated to dryness under vacuum and the residue was dissolved in a small volume of water. The aqueous solution was adjusted to pH 8.0 with NaHCO₃ and extracted three times with three volumes of ethyl acetate. The ethyl acetate layer contained lipophilic metabolites (fraction A). The residual aqueous solution was adjusted to pH 5.0 with 0.2 M acetate buffer and incubated with β-glucuronidase (5000 units, type IV; Sigma Chemical Co.) for 24 h. The hydrolyzed metabolites were extracted with ethyl acetate in a similar manner as fraction A. The ethyl acetate layer contained aglycones (fraction B). The residual aqueous solution after addition of saccharo-1,4-lactone (5 mg) was incubated similarly with arylsulfatase (50 units, type IV; Sigma Chemical Co.) and the hydrolyzed metabolites were extracted similarly as described above (fraction C). Each ethyl acetate extract (fraction A, B and C) was concentrated to dryness under vacuum. The resulting residues were dissolved in a small volume of methanol and spotted on pre-coated silica gel plates (Merck, Silica gel 60 GF₂₅₄, 0.25 mm thick). The plates were developed once with the solvent system, chloroform-n-butanol-ethanol-methanol-acetic acid (8:3:3:1:1, v/v) and redeveloped in same solvent after air-dried. The zones corresponding to diltiazem and its metabolites were visualized under ultraviolet (UV) light (254 nm), and the silica gel of each zone was scraped from the plates and the metabolites were eluted with chloroform-methanol (1:1, v/v). The eluates were concentrated to dryness under vacuum. The residues were derivatized and examined by gas chromatography-mass spectrometry (GC-MS).

**Derivatization for GC-MS** — The residues obtained above were dissolved in a mixture (30 µl) of ethyl acetate-trimethylsilylimidazole (1:1, v/v) or in a mixture (30 µl) of ethyl acetate-trifluoroacetyltrimazole (1:1, v/v). The reaction mixture was left at room temperature for 30 min and injected into the gas chromatograph-mass spectrometer.

**CHART 1. Isolation Procedure of Metabolites in Human Urine**

**Gas Chromatography-Mass Spectrometry** —
A Hitachi RMU-6MG gas chromatograph-mass
spectrometer equipped with a Hitachi data processing system 002B was used. A coiled glass column (1 m × 3 mm i.d.) packed with 3% OV-17 on Gas-chrom Q (100–120 mesh) was used. The flow rate of carrier gas (He) was 40 ml/min. The column oven temperature was programmed at 3°C/min from 200 to 280°C. The temperatures of injection port, separator oven and ion source were 250, 280 and 180°C, respectively. The mass spectra were taken in EI mode. Ionizing voltage, trap current and accelerating voltage were 30 eV, 70 µA and 3 kV, respectively.

Determination of Diltiazem and Its Metabolites in Plasma and Urine — Quantitative analysis of diltiazem and its metabolites in plasma and urine was carried out by TLC-densitometry with slight modification of the procedure reported previously. One or 2 ml of plasma was shaken twice with ether (10 ml) for 5 min. Urine sample, after 10 fold-dilution with 0.5 M phosphate buffer (pH 8), was treated in a similar manner as plasma sample. After centrifugation, the combined ether layer was evaporated to dryness in a tapered microtube. The residue was dissolved in 50 µl of CHCl₃ and the tube was rinsed successively with two portions of 50 µl CHCl₃. The combined CHCl₃ solution was applied to TLC plate. The plate was developed with the solvent system, chloroform-n-butanol-ethanol-methanol-acetic acid (8:3:3:1:1, v/v). The spots corresponding to unchanged diltiazem and its metabolites were detected and analyzed by densitometry at 237 nm using a Shimadzu CS-910 Chromatoscanner. The reference wave length was set at 340 nm for this assay. The lower limits of determination for diltiazem on this assay method were about 1 ng/ml in plasma and 50 ng/ml in urine.

RESULTS

Identification of Metabolites in Human Urine

1) Lipophilic Metabolites (Fraction A) — We investigated the fragmentation on mass spectrometry of diltiazem and the reference compounds which were synthesized as authentics of rat urinary metabolites (M₁–M₇) and possible metabolites of diltiazem in man (M₈ and M₉). The structures of the major fragment ions were assigned as follows:

\[
m/z 44 \left[ \text{CH}_2\text{N}^{+}\text{H}_3 \right]^+, 57 \left[ \text{CH}_2=\text{CHN}^{+}\text{H}_3 \right]^+, 58 \left[ \text{CH}_2\text{N}^{+}\text{H}_3 \right]^+, \text{or} \left[ \text{CH}_2\text{CH}_2\text{N}^{+}\text{H}_3 \right]^+, 71 \left[ \text{CH}_2=\text{CHN}^{+}\text{H}_3 \right]^+, 72 \left[ \text{CH}_2\text{CH}_2\text{N}^{+}\text{H}_3 \right]^+, 150 \left[ \text{HOCH}=\text{CH}^{+}\text{OCH}_3 \right]^+, 192 \left[ \text{CH}_3\text{COOCH}=\text{CH}^{+}\text{OCH}_3 \right]^+, 222 \left[ \text{TMSO-CH}=\text{CH}^{+}\text{OCH}_3 \right]^+ \text{and} 280 \left[ \text{TMSO-CH}=\text{CH}^{+}\text{OCH}_3 \right]^+.
\]

The urinary metabolites were searched by means of mass chromatography in which those characteristic ions described above were selected. The unchanged diltiazem, metabolite M₈, M₉, M₁₀, M₁₁ and M₁₂ were identified by comparing their mass spectra, Rf value on TLC and tR on GC with those of corresponding synthetic compounds.

Unchanged Diltiazem: The compound having the identical Rf value on TLC with that of authentic diltiazem had a peak at tR 32.8 min on GC which was identical with that of authentic compound. The mass spectrum (Fig. 2) of this compound, showing a molecular ion at m/z 414 and the prominent ions at m/z 192, 150, 121 [ (O-OCH₃), 71 and 58, was the same as that of authentic diltiazem.

Metabolite M₈: The Rf value of metabolite M₈ was identical with that of synthetic N-monodemethyl-diltiazem (M₈). In addition, tR of metabolite M₈ and its trifluoroacetic acid (TFA) derivative were also identical with those of the synthetic compound. The mass spectrum (Fig. 2) of metabolite M₈ showed a molecular ion at m/z 400 and characteristic fragment ions at m/z 343 (M⁺–57), 310 (343–COCH₃), 284, 283, 161 [O=O=O-CH=CH=O(O-OCH₃), 150, 136, 121 [(O-OCH₃), 57 and 44. The mass spectrum of TFA-metabolite M₈ showed the base peak at m/z 150 and other important ions at m/z 436 (M⁺–CH₃COOH), 408 (436–CO), 296, 283, 240, 154 [CH₂CH₂N(CH₃)₂]^+ +, 153 TFA⁺.
Metabolism of Diltiazem in Man

\[ \text{[CH}_2\text{=CHN}<\text{CH}_3]^{+}, \text{150, 136, 121 and 43} \]
\[ \text{[COCH}_3]^{+} \]. These mass spectra of metabolite \( M_A \) and its TFA derivative were identical with those of the synthetic \text{N-monomethyl-diltiazem} (\( M_A \)), respectively. Therefore, it could be concluded that metabolite \( M_A \) was \text{N-monomethyl-diltiazem} (\( M_A \)).

Metabolite \( M_1 \): The \( R_f \) value of metabolite \( M_1 \) and \( t_R \) of trimethylsilyl (TMS)-metabolite \( M_1 \) were identical with those of synthetic deacetyl-diltiazem (\( M_1 \)) and its TMS derivative, respectively. The mass spectrum of TMS-metabolite \( M_1 \) (Fig. 2), showing a molecular ion at \( m/z \) 502 and the prominent ions at \( m/z \) 500 (\( M^+ - 2\text{H} \)), 487 (\( M^+ - 15 \)), 432 (\( M^+ - 71 + \text{H} \)), 342 (\( 432 - \text{TMSOH} \)), 301, 179 (\( \text{OTMS} \)), 72, 71 and 58, was identical with that of the synthetic deacetyl-diltiazem (\( M_1 \')).

Metabolite \( M_2 \): The \( R_f \) value of metabolite \( M_2 \) and \( t_R \) of TMS-metabolite \( M_2 \) were identical with those of synthetic deacetyl-N-monomethyl-diltiazem (\( M_2 \)) and its TMS derivative, respectively. The mass spectrum (Fig. 2) of TMS-metabolite \( M_2 \) was identical with that of the synthetic deacetyl-N-monomethyl-diltiazem (\( M_2 \)). A molecular ion was observed at \( m/z \) 430 and characteristic fragment ions were observed at \( m/z \) 415 (\( M^+ - 15 \)), 374 (\( M^+ - 57 + \text{H} \)), 284 (\( 374 - \text{TMSOH} \)), 222, 136 (\( S\text{NH} - \text{CH}^{+} \)), 121, 73, 58 and 44.

Metabolite \( M_4 \): The \( R_f \) value of metabolite \( M_4 \) and \( t_R \) of TMS-metabolite \( M_4 \) were identical with those of synthetic deacetyl-O-demethyldiltiazem (\( M_4 \)) and its TMS derivative, respectively. The mass spectrum of TMS-metabolite \( M_4 \) (Fig. 2), showing a molecular ion at \( m/z \) 502 and the prominent ions at \( m/z \) 500 (\( M^+ - 2\text{H} \)), 487 (\( M^+ - 15 \)), 432 (\( M^+ - 71 + \text{H} \)), 342 (\( 432 - \text{TMSOH} \)), 301, 179 (\( \text{OTMS} \)), 72, 71 and 58, was identical with that of the synthetic deacetyl-O-demethyldiltiazem (\( M_4 \)).

Metabolite \( M_6 \): Metabolite \( M_6 \) had the same \( R_f \) value and \( t_R \) as the synthetic deacetyl-N-O-demethyldiltiazem (\( M_6 \)). The mass spectrum of TMS-metabolite \( M_6 \), shown in Fig. 2, was also identical with that of synthetic compound 2.

2) Conjugated Metabolite — The hydrolyzed metabolites in fraction B and C were treated and analyzed in a similar manner as lipophilic metabolites in fraction A. The aglycones formed by hydrolysis with \( \beta \)-glucuronidase (fraction B) were identified as the synthetic compound \( M_2 \), \( M_4 \) and \( M_6 \). On the other hand, the metabolites in the hydrolysate after treatment by aryl sulfatase (fraction C) were identified as the synthetic compounds \( M_4 \) and \( M_6 \).

Therefore, it could be concluded that metabolite \( M_2 \) was excreted as free and glucuronide.

### TABLE II. Urinary Excretion of Diltiazem and Its Unconjugated Metabolites in 5 Volunteers after Oral Administration of Diltiazem (30 mg-tablet × 2)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Diltiazem</th>
<th>( M_A ) (mg)</th>
<th>( M_2 ) (mg)</th>
<th>Total (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—2</td>
<td>0.371 ±0.297</td>
<td>0.248 ±0.144</td>
<td>0.041 ±0.021</td>
<td>0.660 ±0.445</td>
</tr>
<tr>
<td>2—4</td>
<td>0.712 ±0.383</td>
<td>0.678 ±0.253</td>
<td>0.090 ±0.035</td>
<td>1.48 ±0.65</td>
</tr>
<tr>
<td>4—6</td>
<td>0.535 ±0.250</td>
<td>0.504 ±0.080</td>
<td>0.076 ±0.036</td>
<td>1.12 ±0.32</td>
</tr>
<tr>
<td>6—8</td>
<td>0.379 ±0.157</td>
<td>0.510 ±0.099</td>
<td>0.062 ±0.037</td>
<td>0.951 ±0.238</td>
</tr>
<tr>
<td>8—10</td>
<td>0.272 ±0.096</td>
<td>0.387 ±0.101</td>
<td>0.047 ±0.023</td>
<td>0.706 ±0.194</td>
</tr>
<tr>
<td>10—24</td>
<td>0.549 ±0.273</td>
<td>0.749 ±0.487</td>
<td>0.132 ±0.057</td>
<td>1.43 ±0.78</td>
</tr>
<tr>
<td>0—24</td>
<td>2.82 ±1.02</td>
<td>3.08 ±1.22</td>
<td>0.45 ±0.20</td>
<td>6.35 ±1.31</td>
</tr>
</tbody>
</table>

*The values indicate the mean ± S.D. of 5 volunteers.*
forms, and metabolite $M_4$ and $M_6$ were excreted as free, glucuronide and sulfate forms into urine. Determination of Diltiazem and Its Metabolites in Man

1) Urinary Excretion of Diltiazem, Metabolite $M_A$ and Metabolite $M_6$ — Diltiazem, metabolite $M_A$ and metabolite $M_6$, in free forms, were determined in 24-h urine after oral administration of two 30 mg-tablets of diltiazem to five volunteers. Other minor unconjugated metabolites (metabolite $M_1$, $M_4$ and $M_9$) were detected but by our assay methods the levels were below the lower limits of determination. The urinary excretion of each compound is given in Table II. Among these three compounds, the major components in human urine were diltiazem and metabolite $M_A$; the contents of diltiazem and metabolite $M_A$ were 2.82 mg (44.4 % of the total unconjugated form), 3.08 mg (48.5 % of the total unconjugated form), respectively. The content of the minor component (metabolite $M_6$) was 0.45 mg (7.1 % of the total unconjugated form). Metabolite $M_A$ and $M_2$ were excreted already in the 0—2 h urine, and thereafter the ratios of these metabolites and unchanged diltiazem appeared to be nearly constant.

2) Concentration of Diltiazem and Metabolite $M_A$ in Plasma — Diltiazem and metabolite $M_A$ in plasma of patients under treatment with diltiazem was determined. Three cases of the plasma levels of diltiazem and metabolite $M_A$ in patients are shown in Fig. 3. Metabolite $M_A$ was not detected in the case of patient C. But in the case of patient A and B diltiazem appeared in blood early, while metabolite $M_A$ appeared after a time lag of $1 - 2$ h; the level of metabolite $M_A$ was about one-third of that of diltiazem, when metabolite $M_A$ appeared over a period of 6 h.

Essentially similar observation was obtained in the case of healthy subjects. Metabolite in Rat Urine

Metabolite $M_A$, which was found in human urine, was not detected in rat urine when examined by TLC and by mass chromatography using the characteristic ions of synthetic $N$-monodeethyl-diltiazem ($M_A$).

The structure of metabolite $M_6$, which was found in rat urine, had been presumed by UV, nuclear magnetic resonance (NMR) and MS in our previous study. It was identified in the present study as deacetyl-$N$, $O$-demethyl-diltiazem by comparing the mass spectrum with that of
synthetic reference compound.

DISCUSSION

In the present study, the major metabolite (metabolite $M_A$) of diltiazem in humans was identified as $N$-monodemethyl-diltiazem, but this metabolite was not detected in rats. Recently, Morselli et al. reported that a new metabolite, $M_X$ (deacetyl-$N$-didemethyl-diltiazem), was isolated from human urine as a major metabolite of diltiazem, but this metabolite $M_X$ was not detected in rat urine. Therefore, we first thought that metabolite $M_A$ might be identical with metabolite $M_X$. However, by comparison of the mass spectra of synthetic compound $M_A$ and $M_X$ we concluded that they are different from each other. In addition, we could not detect metabolite $M_X$ in our human urine samples in spite of the detailed examination.

In addition, metabolite $M_1$, $M_2$, $M_4$ and $M_4$ were detected in human urine, as well as in rat urine. In the previous paper, we also found two other urinary metabolites, $M_3$ and $M_5$, in rat. Their structures were presumed as follows; metabolite $M_3$ and metabolite $M_5$. In this investigation, we re-examined the structures of these metabolites by mass spectrometry. The mass spectra of TMS

FIG. 4. Possible Metabolic Pathways of Diltiazem in Man
derivatives of metabolite M₃ and M₅ are not presented in this paper, though, they showed molecular ions and characteristic fragment ions and indicated that fragmentation patterns were similar to those of TMS derivatives of metabolite M₄ and M₆ (Fig. 2), respectively. TMS-metabolite M₃: m/z 532 (M⁺), 517 (Mᵖ⁻⁻⁻⁻⁻⁻相聚), 461 (Mᵖ⁻⁻⁻⁻⁻⁻相聚 +H), 371 (461-TMSOH), 310 (TMSOCH=CH(C=OCH₃)⁺, 209 (C=OCH₃)⁺, OTMS, 72, 71 and 58. TMS-metabolite M₅: m/z 518 (M⁺), 503 (Mᵖ⁻⁻⁻⁻⁻相聚), 462 (Mᵖ⁻⁻⁻⁻⁻相聚 +H), 372 (462-TMSOH), 310, 209, 58 and 44. We searched for metabolites M₃ and M₅ in human urine on mass chromatography by these characteristic ions described above; however, neither metabolites M₃ nor M₅ could be detected. Therefore, it seems that the hydroxylation of the 2-phenyl ring scarcely occurs in humans.

From these results it is concluded that diltiazem was metabolized by deacetylation or N-demethylation, followed by O-demethylation or deacetylation; in part these metabolites were converted to glucuronides and/or sulfates in man. A probable metabolic pathway of diltiazem in man is summarized in Fig. 4.

Biological activity of the new metabolite M₆ has been studied by Nagao et al. after administration of the synthetic compound (M₆). It was found that metabolite M₆ showed about one-fifth of the activity of diltiazem as a coronary vasodilator with the same duration of activity as that of diltiazem.

Acknowledgement The authors thank Dr. A. Kiyomoto, Director of Products Planning and Development Division, Dr. T. Ida, Director of Products Formulation Research Laboratory and Dr. H. Nakajima, Director of Pharmacological Research Laboratory for their interest and encouragement, and Mrs. M. Choei for typewriting of the manuscripts.

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