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METABOLISM OF DIBUCaine: ISOLATION AND IDENTIFICATION OF URINARY BASIC METABOLITES IN THE RAT, RABBIT AND MAN

KAZUO IGARASHI, FUMIYO KASUYA, AND MIYOSHI FUKUI

Faculty of Pharmaceutical Sciences, Kobegakuin University, 518, Arise, Ikawadani, Nishi, Kobe, 673, Japan

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The metabolism of dibucaine was studied in the rat, rabbit and man. A total of ten basic metabolites other than dibucaine were detected in the urine samples of three species by thin-layer chromatography (TLC) and gas chromatography (GC), and structures of these metabolites were identified by comparison of the properties given by TLC, GC and gas chromatography-mass spectrometry (GC-MS) with those of authentic compounds. Four of these metabolites were new metabolites which were found in the rabbit or human urine; two were identified as the 2’, 3’-dihydroxybutoxy product (M-6, diol) and its N-deethyl product (M-2), and others were identified as the 2’-hydroxyethoxy product (M-8, alcohol) and its N-deethyl product (M-3). One of the two hydroxyl groups on M-5 was at 6-position on the quinoline ring, while another was assumed to be at 3’-position on the O-alkyl side chain. There were apparent species differences with regard to the major metabolites found in each species; i.e., M-10 and M-5 in rat, M-6 and M-4 in rabbit, and M-8 and M-4 in man. Small amounts of the conjugated basic metabolites were observed in the urine of all three species. The new metabolic pathways to the diols (M-2 and M-6) or the alcohols (M-3 and M-8) were also discussed.

Keywords — dibucaine; dibucaine metabolism; urinary metabolite; human; animal; GC-MS analysis; isolation; identification; species difference

INTRODUCTION
Dibucaine, 2-butoxy-N-(2-diethylaminooethyl)cinchoninamide has been used as a local anesthetic since 1930, but the drug metabolism has not been studied in detail. This is probably because of the lack of sensitive and selective methods of detection and determination of the compound in biological fluids.

Recently, Kuhara et al. 1) and Shinohara et al. 2) reported the several metabolites of dibucaine in the urine of animals using gas chromatography-mass spectrometry (GC-MS). Shinohara et al. 3) determined these metabolites including the unchanged drug excreted in urine by high performance liquid chromatography (HPLC) and found that they amounted to less than a few percent of the dose. The fate of the major portion of dibucaine administered has not been explained yet.

The present paper describes the isolation and identification of the urinary metabolites in rat, rabbit and man. Four novel metabolites found in rabbit or man are described in detail.

MATERIALS AND METHODS
Materials — Dibucaine hydrochloride was purchased from Teikoku Chemical Industry Co., Ltd. (Osaka, Japan) and purified by silica gel column chromatography to remove the impurity such as 2-butoxy-N-(2-ethylaminooethyl)cinchoninamide. Authentic reference compounds of the various metabolites were synthesized in our laboratory. The reference samples of 2,6- and 2,8-dihydroxycinchoronic acids were kindly supplied by Sankyo Co., Ltd. (Tokyo, Japan). Amberlite XAD-2 resin was obtained from
Rohm and Haas Co., Ltd. (USA). β-Glucuronidase (type H-2) was obtained from Sigma Chemical Co. (USA). N,O-Bis(trimethylsilyl)acetamide (BSA) was purchased from Tokyo Kasei Chemical Co. (Tokyo, Japan). All other chemicals and solvents used in this study were of reagent grade.

Synthesis of Reference Compounds — 2-Hydroxy-cinchonic acid (I), 2-chloro-cinchonic acid (II) and 2-chloro-N-(2-diethylaminoethyl)cinchinonamide (III) were prepared according to the methods described by Aeschlimann and Miescher.

2-3′-Hydroxybutoxy-N-(2-diethylaminoethyl)cinchinonamide (M-10): A solution of III (305 mg) in abs. tetrahydrofuran (THF) was added to a solution of sodium (0.05 g) in 1,3-butanediol (0.9 g). The mixture was refluxed at 80°C for 2h and the solvent was removed in vacuo. The residue was diluted with water and extracted with CHCl₃. The extract was chromatographed on silica gel. Elution with ethyl acetate-heptane (4:3) gave M-10 (250 mg), mp 80–85°C.

2-2′-Hydroxyethoxy-N-(2-diethylaminoethyl)cinchinonamide (M-8): To a solution of sodium (0.2 g) and ethylene glycol (2 g) in abs. THF was added a solution of III (1 g) in abs. THF. The mixture was refluxed at 80°C for 10h and then added a small amount of water. After extraction with CHCl₃, the solvent was removed in vacuo to yield M-8 (0.89 g) as a white crystal, mp 120–122°C.

2-2′,3′-Dihydroxybutoxy-N-(2-diethylaminoethyl)cinchinonamide (M-6): 2′-Butoxy-N-(2-diethylaminoethyl)cinchinonamide (IV) was prepared from III (0.5 g) and sodium 2-butenoxide (0.45 g) by a similar method for the preparation of M-10. A solution of IV (0.3 g) in abs. ether was added to a solution of osmium tetroxide (0.25 g) in abs. ether and allowed to stand at room temperature for 2h. The resulting solid was collected and added in 10 ml of 10% Na₂SO₃ solution. The mixture was heated at 80°C for 3h. After cooling, the reaction mixture was washed with heptane and the product was extracted with ethyl acetate. The extract was purified by preparative TLC using ethyl acetate-methanol-conc. NH₄OH (70:10:1) to give M-6 (0.12 g), mp 75–76°C.

2-Hydroxy-N-(2-diethylaminoethyl)cinchinonamide (M-4): A mixture of I (1 g) and SOCl₂ (6.3 g) was heated at 80°C for 2h. The residue obtained after removal of SOCl₂ in vacuo was dissolved in abs. benzene and the solution was added dropwise to a stirred solution of N,N-diethylaminoethylamine (3.1 g) in 10% NaOH solution at 4°C. The mixture was stirred at 4°C for 1h. The benzene layer was separated and washed with water. The product was extracted into 1 N HCl and the acid extract was made basic with 1 N NaOH to yield M-4 (0.93 g), mp about 220°C (dec.).

2-Butoxy-N-(2-ethylaminoethyl)cinchinonamide (M-9): 2-Butoxy-cinchonic acid (V) was prepared from II (1.7 g) and sodium butoxide (1.8 g) by a similar method for the preparation of M-10. Then V (1 g) was treated with SOCl₂ and N-ethylaminoethylamine as described for the preparation of M-4. The resulting product was purified by preparative TLC using ethyl acetate–methanol-conc. NH₄OH (90:5:4) to give 0.85 g of M-9 as a yellow oil.

2-3′-Hydroxybutoxy-N-(2-ethylaminoethyl)cinchinonamide (M-7) and 2-2′-hydroxyethoxy-N-(2-ethylaminoethyl)cinchinonamide (M-3): These compounds were prepared from II, the corresponding alcohohates and N-ethylaminoethylamine by a similar method for the preparation of M-9.

All reference compounds were analyzed by GC and GC-MS. The chemical ionization (CI) — and electron impact (EI) —GC-MS characteristics of these compounds were consistent with the expected structures.

Treatment of Animals and Human Subjects — Male Wistar rats (200–250 g) and male white rabbits (2.5–3.0 kg) were intraperitoneally given dibucaine hydrochloride in 0.9% saline solution at a dose of 10 mg/kg after fasting for 24h. Rabbits were also intraperitoneally given the synthesized M-10 in 0.9% saline solution at a
dose of 10 mg/kg. They were housed in individual metabolic cages permitting the separate collection of urine and feces. Food and drinking water were supplied ad libitum.

In the case of human (22–32 years old), three healthy men (weight 55–60 kg) participated in this study as volunteers. A dose of 20 mg of dibucaine hydrochloride or the synthesized M-10 in 5% sucrose solution was orally administered to each person.

Urinary samples were collected for 0–24h after administration of the drug and stored at −20°C until analysis. Blanck urine for analysis was also collected as control.

Extraction of Metabolites — Urine collected for 24h after administration of the drug was passed through an Amberlite XAD-2 resin column (3.5 × 40 cm). The column was washed with deionized water, and the metabolites were eluted with methanol. The methanol fraction was evaporated almost to dryness in vacuo, and the residue was dissolved in deionized water. The solution was adjusted to about pH 9 with 1N NaOH and extracted twice with an equal volume of methylene chloride. The organic layer was evaporated, and the residue was redissolved in methanol and subjected to preparative TLC with solvent system (A), ethanol–water (90:10). For analysis of the conjugated metabolites, the remaining aqueous layer was adjusted to pH 5 with 2N sodium acetate buffer and incubated with an excess of β-glucuronidase for 24h at 37°C. Enzymatically hydrolyzed compounds were extracted into methylene chloride under alkaline condition and applied to TLC as described above.

The metabolites visualized under ultraviolet (UV) light after development in solvent system (A) were scraped off from the plate and extracted with methanol. After removal of the solvent in vacuo, the residue was subjected to rechromatography using solvent system (B), ethyl acetate–methanol–conc. NH₄OH (85:10:5), and separated into the several fractions. These fractions were subjected to various analyses for identification of the metabolites.

Thin-Layer Chromatography (TLC) — Preparative TLC was performed on glass plate with silica gel (Wacogel-B-5FM, 0.5 mm). Analytical TLC was also performed on the pre-coated glass plate (silica gel 60 F254, 0.25 mm, E. Merck). The solvent systems used were those as described above. The resulting chromatograms were examined by UV light and Dragendorff's reagent. Dibucaine and its metabolites gave orange color by spraying with the reagent.

Gas Chromatography (GC) — GC was performed with a Shimadzu model GC-6A equipped with a hydrogen flame ionization detector.

![Graph showing densitometric profiles of thin-layer chromatograms of urinary metabolites in rat, rabbit, and human](image)

**FIG. 1.** Densitometric Profiles of Thin-Layer Chromatograms of Urinary Metabolites in Rat, Rabbit, and Human

Metabolism of Dibucaine

Following clean-up on Amberlite XAD-2, the methylene chloride extracts of urine were examined by TLC with solvent systems (A) and (B). The several basic metabolites were visualized under UV light or Dragendorff’s reagent on the chromatogram by solvent system (B) as compared with those of control urine. For the remaining aqueous layer after methylene chloride extraction of urine, it was hydrolyzed with β-glucuronidase. The hydrolysate was extracted with methylene chloride under alkaline condition followed by examining by TLC with solvent systems (A) and (B). The chromatogram by solvent system (B) indicated the faint presence of some basic metabolites, but no further experiment was performed.

The metabolite patterns in three species were assessed by TLC with solvent system (B) (Fig. 1). Unchanged drug and six metabolites were found in all urine samples of rat, rabbit and human. These metabolites were isolated by preparative TLC, and subjected further to analytical TLC and GC. The results indicated that total ten basic metabolites other than unchanged drug were found in three species. These metabolites were named as M-1, M-2, — and M-10 in the order of the Rf value on TLC using solvent

### RESULTS

1. Isolation of the Metabolites in Urine

#### TABLE I. Chromatographic Properties of Dibucaine and Its Metabolites

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A</th>
<th>B</th>
<th>GC (relative t&lt;sub&gt;R&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>Underiv.</td>
</tr>
<tr>
<td>Dibucaine</td>
<td>0.30</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>M-1</td>
<td>0.10</td>
<td>0.21</td>
<td>1.00</td>
</tr>
<tr>
<td>M-2</td>
<td>0.19</td>
<td>0.31</td>
<td>—</td>
</tr>
<tr>
<td>M-3</td>
<td>0.20</td>
<td>0.35</td>
<td>—</td>
</tr>
<tr>
<td>M-4 (dibucyl)</td>
<td>0.23</td>
<td>0.44</td>
<td>—</td>
</tr>
<tr>
<td>M-5</td>
<td>0.27</td>
<td>0.46</td>
<td>—</td>
</tr>
<tr>
<td>M-6</td>
<td>0.28</td>
<td>0.48</td>
<td>—</td>
</tr>
<tr>
<td>M-7</td>
<td>0.22</td>
<td>0.54</td>
<td>1.53</td>
</tr>
<tr>
<td>M-8</td>
<td>0.26</td>
<td>0.58</td>
<td>1.17</td>
</tr>
<tr>
<td>M-9 (N-deethyl)</td>
<td>0.24</td>
<td>0.61</td>
<td>0.90</td>
</tr>
<tr>
<td>M-10 (α-1 OH)</td>
<td>0.28</td>
<td>0.79</td>
<td>1.62</td>
</tr>
</tbody>
</table>

<sup>a)</sup> Plate: Silica gel pre-coated (20× 20 cm).
<sup>b)</sup> The t<sub>R</sub> of dibucaine was 3.45 min. on 2% OV-101 column.
system (B). TLC and GC chromatographic properties of dibucaine and its metabolites were shown in Table I.

The predominant metabolites differed in each species: M-10 and M-5 in rat, M-6 and M-4 in rabbit, and M-8 and M-4 in man. M-1, M-4, M-9 and M-10 were commonly found in all three species. M-5 and M-7 were found only in rat, M-2 and M-6 only in rabbit. M-3 and M-8 were also found only in man.

2. Identification of the Metabolites in Urine

Metabolites M-1, M-4, M-7, M-9 and M-10
—These metabolites except for M-7 were commonly present in the urine samples of three species. The chromatographic and GC-MS characteristics of M-4, M-7, M-9 and M-10 coincided well with those of authentic compounds. The characteristic fragment ions in EI mass spectra of these compounds were shown in Table II. The mass spectrum of dibucaine showed characteristic fragment ions at m/z 86[base peak, corresponded to the ion \(+\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2\), 341 (M\(^+\)-2), 326 (M\(^+\)-17), 271 (M\(^+\)-72, corresponded to the loss of N(\text{C}_2\text{H}_5)_2) and 228 (M\(^+\)-115, corresponded to the loss of NHCH\(_2\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2\), but the molecular ion at m/z 343 (M\(^+\)) was less intensive. The mass spectra of M-4 and M-10 revealed characteristic fragment ions which showed that the compound had an intact amide chain. The mass spectra of M-7 and M-9 indicated that the base peak was m/z 58 corresponding to the ion \(+\text{CH}_2\text{NHCH}_2\text{H}_5\), and some other characteristic ions, (M\(^+\)-57), (M\(^+\)-70), (M\(^+\)-86) and (M\(^+\)-114) revealed the compounds were deethylated at the amide side chain.

The relative t\(_R\) value on GC and the mass spectrum of M-1 were identical with those of dibucaine (Table I, II). However, the Rf value of TLC was not identical. From these findings it was deduced that M-1 was N'-oxide product, which was a thermally labile compound and

### TABLE II. Mass Spectral Data for Dibucaine and Its Metabolites

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M(^+)</th>
<th>M(^+)-2</th>
<th>M(^+)-15</th>
<th>M(^+)-17</th>
<th>M(^+)-72</th>
<th>M(^+)-115</th>
<th>Base peak</th>
<th>Other fragment ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibucaine</td>
<td></td>
<td>341</td>
<td>326</td>
<td>271</td>
<td>228</td>
<td>86</td>
<td>172, 144, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-1</td>
<td>341</td>
<td>326</td>
<td>271</td>
<td>228</td>
<td>86</td>
<td>172, 144, 113, 99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-10 (ω-1 OH)</td>
<td>357</td>
<td>344</td>
<td>342</td>
<td>287</td>
<td>244</td>
<td>86</td>
<td>215, 172, 144, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-10-TMS</td>
<td>429</td>
<td>416</td>
<td>414</td>
<td>359</td>
<td>316</td>
<td>86</td>
<td>344, 144, 129, 117, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-4-TMS (debutyl)</td>
<td>357</td>
<td>344</td>
<td>342</td>
<td>287</td>
<td>244</td>
<td>86</td>
<td>201, 144, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-5-TMS</td>
<td>517</td>
<td>504</td>
<td>502</td>
<td>447</td>
<td>404</td>
<td>86</td>
<td>432, 375, 303, 144, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-6-TMS</td>
<td>517</td>
<td>504</td>
<td>502</td>
<td></td>
<td></td>
<td>86</td>
<td>402, 344, 287, 147, 117, 99</td>
<td></td>
</tr>
<tr>
<td>M-8</td>
<td>329</td>
<td></td>
<td>314</td>
<td>259</td>
<td>216</td>
<td>86</td>
<td>189, 172, 144, 113, 99</td>
<td></td>
</tr>
<tr>
<td>M-8-TMS</td>
<td>401</td>
<td>388</td>
<td>386</td>
<td>331</td>
<td>288</td>
<td>86</td>
<td>246, 215, 172, 144, 113, 99</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M(^+)</th>
<th>M(^+)-15</th>
<th>M(^+)-57</th>
<th>M(^+)-70</th>
<th>M(^+)-86</th>
<th>M(^+)-114</th>
<th>Base peak</th>
<th>Other fragment ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-9 (N-deethyl)</td>
<td>300</td>
<td>258</td>
<td>245</td>
<td>229</td>
<td>201</td>
<td>58</td>
<td>145, 71</td>
<td></td>
</tr>
<tr>
<td>M-7</td>
<td>316</td>
<td>274</td>
<td>261</td>
<td>245</td>
<td>217</td>
<td>58</td>
<td>145, 71</td>
<td></td>
</tr>
<tr>
<td>M-7-TMS</td>
<td>388</td>
<td>346</td>
<td>333</td>
<td>317</td>
<td>289</td>
<td>58</td>
<td>145, 71</td>
<td></td>
</tr>
<tr>
<td>M-2-TMS</td>
<td>476</td>
<td>434</td>
<td>421</td>
<td>405</td>
<td>377</td>
<td>58</td>
<td>374, 316, 259, 143, 117, 71</td>
<td></td>
</tr>
<tr>
<td>M-3-TMS</td>
<td>360</td>
<td>318</td>
<td>305</td>
<td>289</td>
<td>261</td>
<td>58</td>
<td>172, 145, 71</td>
<td></td>
</tr>
</tbody>
</table>

\(a) \quad M^+ \) is not detected or very less intensive.
Metabolism of Dibucaine

easily was converted into a parent compound under GC conditions.\textsuperscript{6} To confirm this fact M-1 was treated with titanium trichloride according to the method of Beckett \textit{et al.}\textsuperscript{7} and analyzed by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{UV Spectra of Dibucaine and Its Metabolites (about 20\(\mu\)g/ml) in Ethanol \(a\) — dibucaine, — — M-10, — — — M-6, \(b\) — dibucaine, — — M-4, — — — M-5.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Mass Spectra of Urinary Metabolites M-2 and M-6, and Authentic Compound M-6 as the TMS Derivatives}
\end{figure}
TLC and GC. The results revealed that treated M-1 had the same chromatographic behavior as dibucaine. Consequently, metabolites M-1, M-4 and

**FIG. 4. Mass Spectra of Urinary Metabolites M-3 and M-8, and Authentic Compounds M-3 and M-8**

**TABLE III. High-resolution Mass Spectral Data of Metabolite M-8**

<table>
<thead>
<tr>
<th>Nominal</th>
<th>Observed</th>
<th>Calculated</th>
<th>Error</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>329</td>
<td>329.17304</td>
<td>329.17380</td>
<td>-0.7</td>
<td>C_{18}H_{23}N_{3}O_{3}</td>
</tr>
<tr>
<td>314</td>
<td>314.15420</td>
<td>314.15034</td>
<td>+3.8</td>
<td>C_{17}H_{20}N_{3}O_{3}</td>
</tr>
<tr>
<td>259</td>
<td>259.10834</td>
<td>259.10817</td>
<td>+0.1</td>
<td>C_{15}H_{18}N_{2}O_{3}</td>
</tr>
<tr>
<td>216</td>
<td>216.06775</td>
<td>216.06600</td>
<td>+1.1</td>
<td>C_{12}H_{10}N_{1}O_{3}</td>
</tr>
<tr>
<td>189</td>
<td>189.07562</td>
<td>189.07891</td>
<td>-0.8</td>
<td>C_{11}H_{11}N_{1}O_{2}</td>
</tr>
<tr>
<td>172</td>
<td>172.03791</td>
<td>172.03981</td>
<td>-1.9</td>
<td>C_{10}H_{9}N_{1}O_{2}</td>
</tr>
<tr>
<td>144</td>
<td>144.04057</td>
<td>144.04490</td>
<td>-4.3</td>
<td>C_{9}H_{9}N_{1}O_{1}</td>
</tr>
<tr>
<td>113</td>
<td>113.10884</td>
<td>113.10780</td>
<td>+1.0</td>
<td>C_{8}H_{13}N_{2}</td>
</tr>
<tr>
<td>99</td>
<td>99.10100</td>
<td>99.10473</td>
<td>-3.7</td>
<td>C_{8}H_{13}N_{1}</td>
</tr>
<tr>
<td>86</td>
<td>86.09250</td>
<td>86.09691</td>
<td>-4.4</td>
<td>C_{8}H_{12}N_{1}</td>
</tr>
</tbody>
</table>
Metabolites M-2 and M-6 — These metabolites were found only in rabbit urine, M-6 being prominent. The UV spectrum of M-6 was similar to that of dibucaine (Fig. 2). The CI mass spectrum of the TMS derivative of M-6 showed the prominent ion at m/z 520 (M+ +1). The EI mass spectrum did not show the molecular ion (M+), but showed characteristic fragment ions at m/z 86 (base peak), 504 (M+−15), 502 (M+−17), 402 (M+−117, corresponded to the loss of CH(OTMS)CH₃ in the O-alkyl side chain) and 287 (M+−232, corresponded to the loss of CH₃CH(OTMS)CH(OTMS)CH₃ minus one hydrogen atom) (Fig. 3). The base peak at m/z 86 represents that the diethylaminoethyl side chain of the parent drug is intact. In addition, a molecular weight of 519 by the CI mass spectrum indicated that M-6 had two hydroxyl groups on the parent drug, and the fragment ions at m/z 402 and m/z 287 in the EI mass spectrum suggested the presence of two hydrox-

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**FIG. 5. Mass Spectra of Non-hydrolyzed Urinary Metabolite M-5, Hydrolyzed Metabolite M-5 and Reference Compounds (A, B and C) as the TMS Derivatives**

Reference compounds: (A) 2-hydroxycinchonic acid, (B) 2,6-dihydroxycinchonic acid, (C) 2,8-dihydroxycinchonic acid.
FIG. 6. Gas Chromatograms of the Hydrolyzed Urinary Metabolite M-5 and Reference Compounds (A, B and C) as the TMS Derivatives

Hydrolysis; at 130°C, for 2 h in 6N HCl. GC condition; 2% OV-101 (3 mm×1 m), Col. temp., 210°C.

yl groups in the butoxy side chain. The $t_R$ on GC and the mass spectrum of authentic M-6 (as TMS derivative) were identical with those of metabolite. From these evidences, it was concluded that M-6 was 2',3'-dihydroxybutoxy product of dibucaine (dihol).

The mass spectrum of the TMS derivative of M-2 showed characteristic fragment ions for the compound having a monoethyloaminoethyl side chain; $m/z$ 58 (base peak), 434 ($M^+\!-\!57$), 422 ($M^+\!-\!70$), 405 ($M^+\!-\!86$) and 377 ($M^+\!-\!114$) (Fig. 3). The CI mass spectrum showed the prominent ion at $m/z$ 492 ($M^+\!+\!1$). The fragment ions at $m/z$ 374 ($M^+\!-\!117$) and $m/z$ 259 ($M^+\!-\!232$) suggested the presence of two hydroxyl groups in the butoxy side chain. Thus, without comparison with authentic compound, it was concluded that M-2 was the N-deethyl product of M-6.

Metabolites M-3 and M-8 — These metabolites were found only in human urine, and particularly M-8 was prominent. The UV spectra of M-3 and M-8 were similar to that of dibucaine. The CI mass spectrum of M-8 showed the prominent ion at $m/z$ 332 ($M^+\!+\!1$) which was 12 mass units lower than that of dibucaine. The EI mass spectrum did not show the molecular ion ($M^+$), but showed the fragment ions at $m/z$ 329, 314, 259 and 216 to be 12 mass units lower than the corresponding ions in dibucaine (Fig. 4 and Table II). The base peak at $m/z$ 86 indicated that the diethylaminoethyl side chain of the parent drug was not modified. The mass spec-

FIG. 7. Thin-Layer Chromatograms of Metabolites Obtained from Rabbit and Human Urine after Administration of the Synthesized M-10

Plate; Silica gel 60 F254, precoated (0.25 mm thick). Solvent system; ethyl acetate–methanol–conc. NH₄OH (85:10:5).
trum of the TMS derivative of M-8 showed characteristic fragment ions at m/z 401, 388, 386, 331 and 288. These fragment ions were 28 mass units lower than the corresponding ions in the TMS derivative of M-10 (Table II). Therefore, it was suggested that the molecule of M-8 was 28 \( (C_2H_4) \) smaller than M-10 in side chain at 2-position of quinoline ring. The high-resolution mass spectral data of M-8 are shown in Table III. The characteristic fragment ions at m/z 329 \( (M^+ - 2) \), 314 \( (M^+ - 17) \), 259 \( (M^+ - 72) \) and 216 \( (M^+ - 115) \), shown to be \( C_{18}H_{23}N_3O_3 \), \( C_{17}H_{20}N_3O_3 \), \( C_{14}H_{15}N_2O_3 \) and \( C_{12}H_{10}NO_3 \), respectively, indicated that there was the loss of \( C_2H_6 \) and addition of \( OH \) in the dibucaine molecule. The fragment ion at m/z 144 \( (C_9H_6NO) \) suggested that the quinoline ring of the parent drug was intact. From these facts, it was considered that M-8 must be the 2'-hydroxyethoxy product of dibucaine. Finally, the \( t_R \) on GC and the mass spectra of authentic M-8 and its TMS derivative were identical with those of urinary metabolite (Fig. 4, Table I and II).

The mass spectrum of the TMS derivative of M-3 showed a base peak at m/z 58 and the fragment ions at m/z 360 \( (M^+ - 15) \), 318 \( (M^+ - 57) \), 305 \( (M^+ - 70) \), 289 \( (M^+ - 86) \) and 261 \( (M^+ - 114) \). These fragment ions are characteristic of the compound containing the monoethylaminoethyl side chain and are also 28 mass units smaller than those of the TMS derivative of M-7. In addition, the CI mass spectrum of the TMS derivative of M-3 showed a prominent ion

![Diagram of Metabolic Pathway of Dibucaine](image)

**FIG. 8. Postulated Metabolic Pathway of Dibucaine**
at m/z 376 (M⁺ + 1) which was 28 mass units smaller than those of the TMS derivative of M-7. A comparison with the mass spectrum of authentic M-3 indicated approximate agreement with the mass peaks of the metabolite (Fig. 4). Thus, it was concluded that M-3 was the N-deethyl product of M-8.

**Metabolite M-5** — This metabolite was found only in rat urine as the major metabolite. The CI mass spectrum of the TMS derivative of M-5 showed a prominent ion peak at m/z 520 (M⁺ + 1). This corresponds to addition of two O-TMS groups to the parent drug, suggesting that two additional oxygen atoms are present in M-5. The EI mass spectrum showed a base peak at m/z 86 and characteristic fragment ions at m/z 517 (M⁺ - 2), 504 (M⁺ - 15), 502 (M⁺ - 17), 447 (M⁺ - 72) and 404 (M⁺ - 115) (Fig. 5, Table III). These fragment ions revealed that the diethylaminoethyl side chain was intact in the parent drug. The UV spectrum of M-5 was similar to that of unchanged drug, M-6 and M-10 (Fig. 2); the maximum absorption was observed at 350 nm which shifted to longer wavelength than that of dibucaine (320 nm). This suggested the presence of a hydroxy group on the quinoline ring.

To elucidate further the position of the hydroxy group on M-5, M-5 was hydrolyzed in 6N HCl for 2h at 130°C. The TMS derivative of the hydrolysate was chromatographed on GC (Fig. 6). The TMS derivatives of reference compounds (mono and dihydroxycinchoninic acids) were also chromatographed on GC. The result demonstrated that the \( t_R \) of the hydrolysate was identical with 2,6-dihydroxycinchoninic acid. Based on these results, M-5 was assumed to be the 6-hydroxy-3'-hydroxybutoxy product of dibucaine.

3. **Metabolism of M-10 (ω-1 OH) by Rabbit and Man**

In order to examine the metabolic pathways from dibucaine to diols (M-2 and M-6) and alcohols (M-3 and M-8), the synthesized M-10 was administered to rabbit and man. The urinary metabolites were isolated in the same procedures as described previously and analyzed by TLC, GC and GC-MS.

As shown in Fig. 7, three metabolites other than unchanged M-10 were detected chromatographically from rabbit urine and human, respectively. These metabolites were identified as diols (M-2 and M-6), alcohols (M-3 and M-8) and O-debetyl (M-4) products of dibucaine by GC-MS analysis. Of these metabolites, M-6 or M-8 was also found respectively in the urine of rabbit or human as the major basic metabolite. It is therefore considered that the diols and the alcohols are produced via M-10.

**DISCUSSION**

The urinary metabolites of dibucaine in the rat, rabbit and man have been examined in the present study. The metabolites found in the urine of three species are summarized in Fig. 8. Four of these metabolites, M-6 and M-2 in rabbit, M-8 and M-3 in man, were new and were not reported by the other investigators. Among these new metabolites, M-6, M-8 and M-3 were identified by comparison of TLC, GC and GC-MS properties with those of the authentic compounds. Metabolites M-6 and M-2 were identified as the 2',3'-dihydroxybutoxy and its N-deethyl product (diol). M-8 and M-3 were identified as the 2'-hydroxyethoxy and its N-deethyl product (alcohol). The chemical structure of M-8 was elucidated by high-resolution mass spectral data, the decrease of 12 mass units in the dibucaine molecule indicated the loss of \( C_2H_5 \) and addition of OH in the parent compound. In regard to M-5, Kuhara _et al._ reported that M-5 was di-hydroxylated dibucaine, and the hydroxylation occurred both at aromatic ring and at alkyl side chain. However, the position of the hydroxy group on the aromatic ring has been unconfirmed. In the present study, the position of the aromatic hydroxyl group on metabolite M-5 was identified as the 6-hydroxy product of dibucaine by comparison of the GC and GC-MS data on the TMS derivative of hydrolyzed M-5 with those of the reference compounds (mono and dihydroxycinchoninic acids).
Also, it was assumed that the hydroxyl group on the alkyl side chain of both M-5 and M-10 was on the same position, because M-5 must be produced via M-10. Thus, we deduced that M-5 was the 6-hydroxy-3'-hydroxybutoxy product of dibucaine.

It was very interesting that M-2 and M-6 (diols) were found in rabbit urine, M-3 and M-8 (alcohols) in human urine, as the new basic metabolites of dibucaine. These diols and alcohols were also isolated from the urine of rabbit and man given the synthesized M-10 (ω-1 OH), respectively. These findings suggest that the diols and the alcohols are produced via ω-1 hydroxylation. These metabolic pathways to the diols or the alcohols via ω-1 hydroxylation of the side chain have not been observed in other drug metabolism. In general, ω-1 and ω hydroxylations are well known metabolic pathways in many drugs having alkyl side chains. Yoshi- mura et al. reported that p-nitrophenyl ethers having various alkyl group such as propyl, butyl and isoamyl were metabolized mainly through ω-1 or ω hydroxylation of the O-alkyl groups in rabbit. In addition, Tsuji et al. investigated the metabolism in vitro of butyl p-nitrophenyl ether with rabbit liver preparation and obtained the results that butyl p-nitrophenyl ether was metabolized in rabbit through the three different pathways, α, ω-1 and ω hydroxylations, and the ω-1 hydroxylation derivative was further metabolized to p-nitrophenol through the ω-1 oxo derivative. Considering from these evidences, it seems to be usual that the compounds possessing the O-butyl side chain are metabolized mostly by ω-1 hydroxylation followed by dealkylation. But, in the present study, we found the other unusual metabolic pathways in the O-butyl side chain of dibucaine.

The metabolic reactions to the diols or the alcohols via ω-1 hydroxylation found in the present study are novel in the metabolism of foreign compounds. The formation of the diol compound at alkyl side chain is generally considered to result from the oxidation of double bond in allyl group, with an epoxide as an intermediate product. Practically, the diol is formed via an epoxidation of allyl group in the drug such as secobarbital, alphenal and alprenolol. However, the formation of the diol compound via ω-1 hydroxylation at alkyl side chain but not allyl side chain has not been shown in other drug metabolism except for cyclohexane.

About the formation of the alcohols, we assumed that there were some intermediate compounds in the metabolism of ω-1 OH (M-10) to alcohol (M-8). As one of the probable intermediates, we considered the presence of the diol compound (M-6). Attempts were made to obtain the cleavage via diol (M-6) to alcohol (M-8) by administering the synthesized M-6 to man orally, but we could not find the metabolite M-8 in urine although M-4 and minor metabolites were detected. It is probably considered that this negative result is due to the hydrophilic property of the diol compound and easily to undergo the O-dealkylation. Further study on this metabolic pathway is now in progress.

In the present study, we found ten basic metabolites in the urine of three species, but total amounts of these metabolites including unchanged dibucaine accounted for 10% the dose after 24h. Fukui reported that 1-2% of the dose was excreted within 12h in the urine of patients infused dibucaine in the spinal cord. Shinohara et al. demonstrated that the urinary excretion of the metabolites detected in rat accounted for less than several percent of the dose. More than 90% of the dose is therefore not explained, and we consider that most of dibucaine may be metabolized to unknown polar compounds.

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REFERENCES


