STEREOSELECTIVE METABOLISM OF BENOXAPROFEN IN RATS

Biliary Excretion of Benoxaprofen Taurine Conjugate and Glucuronide

KIMINORI MOHRI, KENJI OKADA, AND LESLIE Z. BENET

Clinical Pharmaceutics Laboratory, Department of Pharmaceutics, Meiji College of Pharmacy (K.M., K.O.), and Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco (L.Z.B.)

(Received August 8, 1997; accepted November 24, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:

Benoxaprofen (BOP) was administered iv to bile duct-cannulated rats at a dose of 10 mg/kg. BOP and its metabolites in plasma, urine, and bile were quantified using HPLC. A previously unidentified BOP metabolite was found in HPLC chromatograms of rat bile, and the metabolite was isolated chromatographically. Positive-ion fast-atom bombardment (FAB) MS analysis of the compound showed [M+H]+ at m/z 409, i.e. 108 mass units greater than the molecular weight of BOP (301 mass units). In the 1H NMR spectrum of the compound, two signals assigned to two methylene groups appeared at 2.53 ppm and 3.30 ppm, in addition to BOP signals. Analysis of FAB mass spectra and 1H-1H and 1H-13C correlated NMR spectra of the isolated metabolite suggested that the new metabolite was a BOP taurine conjugate (BOP-T). A BOP-T standard was chemically synthesized, and physicochemical data were compared with those for the isolated metabolite. Identical results, i.e. Rf values from TLC, Rt values from HPLC, and FAB MS and 1H-13C correlated NMR findings, were obtained, establishing that the new metabolite found in rat bile was BOP-T. In five rats, mean values for per cent excretion of the dose in bile over 12 hr for BOP glucuronide (BOP-G), BOP-T, and unchanged BOP were 13.2 ± 2.3, 2.54 ± 0.80, and 0.33 ± 0.09%, respectively. Furthermore, the optical isomers of BOP and its metabolites in plasma and bile were analyzed using a chiral HPLC column. (R)-BOP showed rapid plasma elimination, whereas the plasma elimination of (S)-BOP was very slow. The amounts of BOP, BOP-G, and BOP-T enantiomers excreted into the bile were as follows: (S)-BOP and (R)-BOP-G, 12.5 ± 1.8 and 2.1 ± 0.6% of the dose; (R)-BOP-T and (S)-BOP-T, 2.0 ± 0.6 and 0.3 ± 0.05% of the dose; (R)-BOP and (S)-BOP, 0.02 ± 0.03 and 0.2 ± 0.1% of the dose, respectively. (S)-BOP was metabolized mainly to BOP-G, and BOP-T excreted into the bile was produced mainly from (R)-BOP.

BOP[1] [2-(4-chlorophenyl)-a-methyl-5-benzoxazolacetic acid] is a 2-arylpropionic acid derivative that exhibits anti-inflammatory effects (Cashin et al., 1977; Tsurumi et al., 1980, 1982) and was withdrawn from the market because of unexplained fatal liver toxicity (Duthie et al., 1982; Goudie et al., 1982). The metabolic fate of BOP has been studied in mice, rats, rabbits, dogs, rhesus monkeys, and humans (Chatfield and Green, 1978; Ohtsuki et al., 1981a,b; Furlanet al., 1985). Results obtained in rats with the 14C-labeled compound showed that BOP was readily absorbed from the gastrointestinal tract and 11–24% and 79–85% of the dose was excreted in the urine and feces, respectively, within 192 hr (Ohtsuki et al., 1981b). Only BOP-G has been identified as a major metabolite of BOP in mammals (Chatfield and Green, 1978; Ohtsuki et al., 1981b; Spahn et al., 1989). Chatfield and Green (1978) and Ohtsuki et al. (1981b) examined the biliary excretion of BOP using radioactive [14C]BOP, and they reported the presence of BOP and BOP-G in bile.

In this study, we describe the isolation from rat bile of a new BOP metabolite, BOP-T, the physicochemical characteristics of which were compared with those of the synthesized authentic standard. Furthermore, the biliary excretion of BOP after iv administration of a 10 mg/kg dose to bile duct-cannulated rats was investigated. BOP and its metabolites in plasma and bile were analyzed using reverse-phase analytical and chiral HPLC columns.

Materials and Methods

Measurements. Elemental analyses were performed using a Perkin-Elmer model 240B elemental analyzer (Perkin-Elmer Corp., Norwalk, CT). High-resolution mass spectra were obtained with a JEOL JMS-DX302 instrument (JEOL, Tokyo, Japan), using a direct-inlet system. Operating conditions were as follows. The accelerating voltage and ionization current were 3 kV and 300 μA, respectively. Ionization was by electron impact with 70-eV electrons, with a source temperature of 150°C. Perfluorokerosene was used as a standard compound. Positive-ion FAB mass spectra were obtained with an Auto Spec mass spectrometer (Micromass UK Ltd., Manchester, England) equipped with a FAB source using cesium atoms. The instrument was operated with an accelerating voltage of 8 kV, and glyceral was used as the FAB matrix for the acquisition of positive-ion spectra. 1H, 13C and 1H-13C COSY NMR spectra were obtained using a JEOL JNM-LA500 spectrometer (JEOL), with tetramethylsilane as the IS. Chemical shifts are expressed in ppm downfield from tetramethylsilane, and coupling constants are given in hertz. Column chromatography was carried out with silica gel (230–400 mesh; Kanto Chemical Co.,
Tokyo, Japan). TLC was undertaken using Kieselgel 60 F 254 plates (Merck, Darmstadt, Germany). Spots on the TLC plates were detected under UV light (253.6 nm). The HPLC system consisted of a model BIP-I pump (JASCO Co., Tokyo, Japan) equipped with a model 820-FP fluorometer (JASCO), a model SIL-9A autoinjector (Shimadzu Co., Kyoto, Japan), and a model C-R4A Chromatopac integrator (Shimadzu). The excitation and emission wavelengths (xenon lamp) were set at 315 and 365 nm, respectively.

Chemicals. TBA hydrogen sulfate was purchased from Aldrich Chemical Co. (Milwaukee, WI). Naproxen was purchased from Sigma Chemical Co. (St. Louis, MO). BOP was kindly donated by Eli Lilly Co. (Indianapolis, IN) before withdrawal of the drug from the market. MeOH, MeCN, and tetrahydrofuran were HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other chemicals were reagent grade. The water used had been double-distilled in a glass still. NAP-Me (IS) and reference BOP-T were synthesized as reported previously (Spahn et al., 1989). Standard (R)- and (S)-BOP enantiomers were obtained by the method described previously (Spahn et al., 1989). Small amounts of standard (R)- and (S)-BOP-T were synthesized from the enantiomers by the Schotten-Baumann method (Idle et al., 1978). Small amounts of (R)- or (S)-BOP enantiomers were administered to rats, and standard (R)- or (S)-BOP-G, respectively, was obtained from rat bile.

Animals and Drug Administration. Male Sprague-Dawley rats (Sankyo Labo Service Co., Tokyo, Japan), weighing 300–350 g, were used throughout the study. The rats were housed in stainless steel cages in groups of 10, in a temperature-controlled (20–28°C) room with a 12-h light/dark cycle. The rats were allowed free access to standard rat chow (Sankyo) and water for 1 week before the experiments. Each animal was anesthetized with 20% (w/v) urethane (1 g/kg body weight, ip). The femoral vein was cannulated with PE-10 tubing (Clay Adams, Parsippany, NJ) for instillation of saline solution. An abdominal incision was made and the common bile duct was cannulated with PE-10 tubing (Clay Adams), for collection of bile samples, and closed with surgical clips. Bile samples were collected in 100-ml plastic tubes and stored at −80°C. During experimental procedures, body temperatures were maintained at 38 ± 0.5°C with a heating lamp, to prevent hypothermic alterations of bile flow. The solution of BOP for injection was prepared by dissolving 500 mg of BOP in a 20-mixture of 1 M NaOH, ethanol, and saline (8:8:1, v/v). Fifty rats received 20–50 mg/kg doses of BOP. Saline was injected into the rats through the cannula to supplement body fluids.

For the pharmacokinetic study, five male Sprague-Dawley rats, weighing 250–300 g, were used. BOP was administered at a dose of 10 mg/kg body weight through the femoral vein. The femoral artery was cannulated with PE-50 tubing (Clay Adams) and a heparin-lock was established, using 100 units/ml heparin in saline. Blood samples (each approximately 0.2 ml) were collected from the femoral artery at 0, 5, 10, 15, 20, 30, and 45 min and 1 hour, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 12 hr after iv administration. The collected blood was immediately centrifuged at 15,000 g for 15 min at 4°C, and plasma was separated. Bile was collected into 2-ml plastic containers containing 100 μl of 10% metaphosphoric acid, in successive 30-min periods. Urine samples were collected through a PE-50 tubing bladder cannula into 5-ml glass tubes containing 100 μl of 10% metaphosphoric acid, continuously for 12 hr. Plasma samples were stored in 2-ml plastic tubes, 180 μl of MeCN, 30 μl of IS (100 μM/ml in DMSO), and 30 μl of distilled water were added, with vigorous mixing. After centrifugation of the sample for 10 min at 15,000g at 4°C, 10 μl of the supernatant was injected directly into the HPLC system.

HPLC Conditions. The analysis was performed at room temperature (23°C) with a reverse-phase analytical Capcell Pak C18 column (4.6 mm i.d. × 25 cm; particle size, 5 μm; Shiseido Co., Tokyo, Japan) equipped with a guard column packed with Capcell Pak C18 material (4.6 mm i.d. × 1 cm; particle size, 5 μm; Shiseido). Mobile phase, consisting of 10 mM TBA buffer/MeCN/MeOH (30:5:97, v/v), was pumped through the column at a rate of 1.0 ml/min. The excitation and emission wavelengths were set at 315 and 365 nm, respectively.

The migration of the different compounds was determined using a Chromatopac integrator (Shimadzu). The excitation and emission wavelengths (xenon lamp) were set at 315 and 365 nm, respectively.

Steroselective Metabolism of Benoxaprofen in Rats

Results

HPLC. Under the HPLC conditions described above, BOP, BOP-G, BOP-T, IS, and their enantiomers exhibited symmetrical

Table 1. Relative peak areas and retention times for BOP and its metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak Area</th>
<th>RT (min)</th>
<th>RF</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP-G</td>
<td>1.39</td>
<td>5.9</td>
<td>0.19</td>
<td>409</td>
</tr>
<tr>
<td>BOP-T</td>
<td>7.70</td>
<td>7.38</td>
<td>0.19</td>
<td>409</td>
</tr>
<tr>
<td>IS</td>
<td>7.67</td>
<td>7.44</td>
<td>0.19</td>
<td>409</td>
</tr>
<tr>
<td>BOP</td>
<td>8.19</td>
<td>8.44</td>
<td>0.19</td>
<td>409</td>
</tr>
</tbody>
</table>

Note: RT = retention time, RF = resolution factor.
signal/noise ratio of 10). Analytical reproducibility (per cent coeffi-
cient of variation) for (S)-BOP was 10.3, 11.8, and 4.4 min, respectively. The retention times
injection of authentic compounds into the HPLC system. The retention times
illustrates the analysis of a bile sample obtained 30 min after BOP
administration. The retention times, with the ODS column, for
BOP-T, and BOP-G were 16.7, 16.1, 4.3, 22.5, and 26.0, respectively. The mobile phases
used for the separation of enantiomers are described in
HPLC Conditions.

Quantification and Analytical Variables. The BOP concentration
in urine was below the detection limit of this HPLC assay method. For
the ODS column, the correction factors to calculate BOP-T and
BOP-G concentrations in bile were 0.24 ± 0.002 and 0.21 ± 0.004, respectively. The calibration curves for BOP (1–100
µg/ml), prepared as described above, were linear and apparently
passed through the origin, as follows: y = 0.066x −0.001 (r = 1.000)
for BOP in bile and y = 0.065x −0.002 (r = 1.000) for BOP in
plasma. The lower detection limit for BOP was 10 pg/ml (with
a signal/noise ratio of 10). Analytical reproducibility (per cent coeffi-
cient of variation) values for intra- and interday assay variations were
0.34 and 1.80%, respectively, for 50 µg/ml BOP in bile.

Characterization of a New BOP Metabolite. The compound was recrystallized from hot MeOH as a white powder (TLC: Rf 0.19
(CHCl3/MeOH, 4:1, v/v); HPLC: Rf 5.9 min (fig. 1C, first peak);
FAB MS: m/z 409 ([M+H]+); 1H NMR (DMSO-d6): 8 1.39 (d, 3H,
J = 7, CH-CH3), 2.53 (m, 2H, CH2-NO2), 3.30 (m, 2H, NH-CH2-),
3.70 (q, 1H, J = 7, CH-CH3), 7.38 (dd, 1H, J = 1.8 and 8.5, Ar-H),
7.67 (d, 2H, J = 8.5, Ar-H2), 7.69 (d, 1H, J = 1.8, Ar-H), 7.70 (d,
1H, J = 1.8, Ar-H2), 7.88 (dd, 1H, J = 5.2 and 5.5, NH), and 8.19 (d,
2H, J = 8.5, Ar-H2)).

Characterization of NAP-Me. The compound was recrystallized
from MeOH [TLC: Rf 0.27 (n-hexane/ethyl acetate, 20:1, v/v);
HPLC: Rf 13.2 min (fig. 1C, third peak); elemental analysis for
NAP-Me: C, 73.75; H, 6.63; calculated for C13H25O3 (molecular
weight, 244.1106) 73.70, 6.60; high-resolution MS: m/z 244.1106
(M+, 45.6% relative intensity), 229 (M−15), 213 (M−31), and
185 (M−59); 1H NMR (CDCl3): δ 1.56 (d, 3H, J = 7.0, CH-CH3),
3.65 (s, 3H, COOCH3), 3.84 (q, 1H, J = 7, CH-CH3), 3.89 (s, 3H,
Ar-OCH3), 7.09 (d, 1H, J = 2.4, Ar-H), 7.12 (dd, 1H, J = 2.4 and 8.5,
Ar-H), 7.38 (dd, 1H, J = 1.5 and 8.5, Ar-H), 7.64 (d, 1H, J = 1.5,
Ar-H), and 7.68 (d, 2H, J = 8.5, Ar-H2)].

Biliary Excretion of BOP and Its Metabolites. Characterization of the biliary metabolites of BOP was performed by subjecting bile
samples to HPLC analysis, as described in Materials and Methods.

Pharmacokinetic Data. Twelve-hour cumulative amounts of BOP,
BOP-G, and BOP-T (as percentages of the dose) excreted into bile
were 0.33 ± 0.09, 13.22 ± 2.28, and 2.54 ± 0.80%, respectively.
It is apparent from fig. 3 that BOP exhibits a long-half in rat plasma. If
the estimated 31-hr terminal half-life (determined from 12-hr sam-
pling only) is correct, >78.3 ± 13.0% of the BOP dose would remain
to be excreted from the body after 12 hr, as estimated from a
preliminary fit of the data representing the solid line drawn through
the plasma concentration measurements. The same plasma and bile
samples were analyzed using a chiral column (figs. 4 and 5). (R)-BOP
showed rapid plasma elimination, whereas the plasma elimination of
(S)-BOP was very slow. The amounts of BOP, BOP-G, and BOP-T
enantiomers excreted into bile were as follows: (R)-BOP-G and (S)-
BOP-G, 2.1 ± 0.6 and 12.5 ± 1.8% of the dose; (R)-BOP-T and
(S)-BOP-T, 2.0 ± 0.6 and 0.3 ± 0.05% of the dose; (R)-BOP and
(S)-BOP, 0.02 ± 0.03 and 0.2 ± 0.1% of the dose, respectively.

Discussion
After observing an unidentified peak in the HPLC chromatograms
(fig. 1C, first peak) for bile samples from rats administered BOP, we
isolated (from bile) the compound corresponding to the peak and we
studied its chemical structure. When the bile samples (1 ml) were
incubated with 1 M NaOH (1 ml) at 37°C for 1 hr, no change was
observed in the HPLC chromatograms of the samples. When the bile
samples (1 ml) were incubated with 1 M HCl (1 ml) at 37°C for 1 hr, a change was observed in the HPLC chromatograms of the samples. When the bile samples (1 ml) were incubated with 1 M NaOH (1 ml), the second peak (fig. 1C) in the HPLC chromatograms disappeared and the BOP peak (fig. 1C, fourth peak) increased. The retention time of the second peak in the HPLC chromatograms was identical to that of standard
BOP-G. Thus, the second peak was identified as BOP-G. The first
peak in the chromatogram (fig. 1C) was stable under not only acidic
but also alkaline conditions.

Positive-ion FAB MS analysis of the compound showed a [M+H]+ ion at m/z 409, i.e. 108 mass units greater than the molecular weight of BOP (301 mass units), suggesting that the unknown compound might be a taurine conjugate of BOP [from the molecular weight of taurine (125 mass units), with an assumed loss of water]. In the
1H-13C COSY NMR spectrum of the compound in DMSO-d6, signals
at 2.80 and 3.13 ppm, each two protons. The doublet of doublets signal at 7.88 ppm (corresponding to one proton) was
coupled with the methylene group at 3.30 ppm and was assigned
to the proton of the amide moiety (-CONH-CH2CH2-). Because
the compound eluting as the first peak in the chromatogram shown in fig.
1C was suspected to be BOP-T, standard BOP-T was chemically

Fig. 1. HPLC chromatograms of BOP, BOP metabolites (BOP-T and BOP-G), and NAP-Me (IS).
synthesized by the Schotten-Baumann method (see Synthesis of Standard BOP-T) (Idle et al., 1978). The TLC Rf and HPLC Rt values for the unknown compound were identical to those for synthesized BOP-T. Furthermore, positive-ion FAB MS and 1H-13C COSY NMR findings for the metabolite were also identical to those for synthesized BOP-T.

To investigate the biliary excretion of BOP and its metabolites, especially BOP-T, the parent drug was administered iv to male Sprague-Dawley rats at a 10 mg/kg dose. The plasma elimination profile (fig. 3) and the half-life (approximately 31 hr) for BOP were almost identical to previously reported results (Ohtsuki et al., 1981b). Ohtsuki et al. (1981b) reported that a large amount of BOP-G and a small amount of BOP were excreted in rat bile. We showed here that BOP-T was also excreted in rat bile. Because no measurable levels of BOP or its metabolites were excreted in urine, it is clear that the disposition of BOP in rats is the result of mainly biliary clearance.

Initially, in this preliminary animal study, we analyzed BOP and its metabolites as racemates, by HPLC with a reverse-phase ODS column (fig. 3). Interestingly, BOP-T excretion in rat bile did not appear to continue beyond 4 hr after BOP administration. In contrast, the glucuronic acid conjugation of BOP proceeded continuously after 4 hr. To test whether this might be the result of depletion of substrates for BOP-T conjugation, taurine (100, 250, 500, and 1000 mg/kg body weight, \(N = 3\) for each dose) was injected into rats through the femoral cannula 4 hr after BOP administration. However, the amount of BOP-T excreted into the bile was not altered (data not shown).

Bopp et al. (1979) and Simmonds et al. (1980) reported that BOP exhibits stereoselective plasma disposition and \((R)\)-BOP is converted unidirectionally to \((S)\)-BOP in the body. Thus, to investigate the changes with time for each BOP enantiomer, as well as their metabolites, in plasma and bile, we reanalyzed the plasma and bile samples...
using a chiral HPLC column (figs. 4 and 5). (R)-BOP showed rapid plasma elimination, whereas the plasma elimination of (S)-BOP was very slow. These results are similar to those observed for many 2-arylpropionic acid derivatives (Jamali, 1988).

Analysis of BOP conjugates in bile yielded interesting results with respect to the configuration of the propionic acid moiety of the conjugates. Of the glucuronide excreted into the bile, 85.6% had the S-configuration at the propionic acid moiety (figs. 4 and 5). This finding is quite similar to the results reported for humans by Spahn et al. (1989). Those workers reported that the cumulative excretion of the (S)-glucuronide in urine over 96 hr after administration of 300 mg of racemic BOP was about twice that of the (R)-glucuronide. Iwakawa et al. (1991) reported stereoselective disposition of caprofen, flunoxaprofen, and naproksen in rats, whereas the cumulative excretion of the (S)-glucuronide in bile over 8 hr was greater than that of the (R)-glucuronide after administration of racemic flunoxaprofen (a BOP derivative) and naproksen. Excretion of flunoxaprofen (S)-glucuronide was about 2.5-fold greater than that of the (R)-glucuronide, whereas the excretion of naproksen (S)-glucuronide was 3.4-fold greater than that of the (R)-glucuronide. Cumulative excretion of (R)-BOP-T into bile over 12 hr was about 6.7-fold greater than that of (S)-BOP-T (figs. 4 and 5).

In recent years, stereoselective amino acid conjugation of 2-arylpropionic acid derivatives (profens) has been considered together with the metabolic chiral inversion mechanism, because both reactions involve drug-CoA thioester formation. Hutt and Caldwell (1983) have reviewed the metabolic chiral inversion of 2-arylpropionic acids. The chiral inversion of BOP occurs unidirectionally, from the R-enantiomer to the S-enantiomer, in the body, as described previously (Bopp et al., 1979; Simmonds et al., 1980). This is hypothesized to occur as a result of the R-enantiomer of BOP serving as a substrate for the enzyme “acyl-CoA ligase” during acyl-CoA thioester formation, whereas the S-enantiomer is not a substrate for the enzyme (Hutt and Caldwell, 1983; Nakamura et al., 1984).

In the process of conjugation of BOP with taurine, BOP-CoA thioester formation might be required as the first step, with BOP-T being produced from BOP-CoA thioester by taurine N-acetyltransferase as a second process. This could explain why taurine conjugation of BOP stopped approximately 4 hr after BOP administration. This might be the result of substrate depletion. However, the substrate is not taurine but (R)-BOP, which is the precursor for BOP-CoA thioester formation in the first step. It is clear from fig. 4 that about 95% of (R)-BOP was converted to (S)-BOP by metabolic chiral inversion within approximately 4 hr after BOP administration.

A small amount of (S)-BOP-T was identified in rat bile. This may be the result of conversion of (R)-BOP-CoA thioester to (S)-BOP-CoA thioester by epimerase, followed by metabolism to (S)-BOP-T by taurine N-acetyltransferase.

Although a variety of 2-arylpropionic acid derivatives are widely used in clinical practice, only a few reports of taurine conjugation of these drugs have been published. Taurine conjugates of suprofen (Sakai et al., 1984; Mori et al., 1985), trans-hydroxyloxoprofen (Tanaka et al., 1983), trans-hydroxy-CS-670 (Asami et al., 1995), and M-5011 (Kitamura et al., 1996) were found in dog urine, and a taurine conjugate of ibuprofen was identified in human urine (Shirley et al., 1994). The only taurine conjugate reported in rat and mouse urine was that of pirprofen pyrrole (Egger et al., 1982).

Similarly, only a few reports of stereoselective amino acid conjugation of 2-arylpropionic acid derivatives have appeared in the literature. Shirley et al. (1994) reported that the taurine conjugate of ibuprofen excreted in human urine exhibits the S-configuration at the propionic acid moiety (approximately 87% of the dose after oral administration of the racemate). Asami et al. (1995) reported that trans-hydroxy-CS-670 excreted in dog urine mainly has the S-configuration. The S/R ratios of taurine conjugates after administration of (2R)-CS-670 and (2S)-CS-670 were 75.9:1 and 249:1, respectively. Recently, Kitamura et al. (1996) noted that the taurine conjugate of M-5011 excreted in dog urine and feces exhibited only the S-configuration (15.4 and 13.5% of the dose was excreted in dog urine and feces, respectively). Tanaka et al. (1992) reported that a 2-arylpropionic acid glycine conjugate excreted in dog urine has the S-configuration. All of the aforementioned authors suggested that amino acid N-acetyltransferase, rather than acyl-CoA ligase, was responsible for the stereoselective taurine and glycine conjugation of 2-arylpropionic acid derivatives. BOP-T excreted into rat bile, as reported here, mainly had the R-configuration. This is the first report of stereoselective taurine conjugation of 2-arylpropionic acid derivatives in rats, although, as noted above, such results have been described for dog and human urine. Although BOP was withdrawn from the market, we believe it to be a useful compound for the study of stereoselective amino acid conjugation mechanisms.

In conclusion, we identified BOP-T in rat bile and showed that approximately 2.5% of the BOP dose was excreted in bile, over a 4-hr period, as this newly confirmed metabolite. The configuration at the propionic acid moiety of BOP-T was mainly the R-configuration.

Acknowledgments. The authors are grateful to Lo Lin Ip at the School of Pharmacy, University of California, San Francisco, and Kazumi Udagawa and Yukiko Ido at Meiji College of Pharmacy for skilful technical assistance.

References


