Metabolism of Leukotriene B₄ in Isolated Rat Hepatocytes

IN Volvement of 2,4-Dienoyl-Coenzyme A Reductase in Leukotriene B₄ Metabolism

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Radiolabeled leukotriene (LT) B₄ was incubated with isolated rat hepatocytes in order to assess the metabolism of this chemotactic leukotriene by the liver. At least eight radioactive metabolites were observed, three of which were previously identified as 20-hydroxy-, 20-carboxy-, and 18-carboxy-19,20-dinor-LTB₄. A less lipophilic major metabolite (designated H₄) was purified by two reverse phase high performance liquid chromatography separations and was found to exhibit maximal UV absorbance at 269 nm with shoulders at 260 and 280 indicating the presence of a conjugated triene chromophore. Negative ion electron capture gas chromatography/mass spectrometry analysis of the pentafluorobenzyl ester, trimethylsilyl ether derivative of H₄, and positive ion electron ionization mass spectra of the methyl ester trimethylsilyl derivative were consistent with a structure of this metabolite being 16-carboxy-14,15-dihydro-17,18, 19,20-tetranor-LTB₃. The appearance of this metabolite supports the concept of further β-oxidation of LTB₄ to the carbon 16 which requires the action of 2,4-dienoyl-CoA reductase to remove the 14,15-double bond located two carbon atoms removed from the CoA thioester moiety. One minor metabolite was analyzed by negative ion continuous flow fast atom bombardment mass spectrometry which revealed an ion at m/z 444 which by high resolution mass spectrometry was shown to contain both nitrogen and sulfur. Tandem mass spectrometry suggested the presence of SO₂ as well as other fragments corresponding to the amino acid taurine. Incubation of isolated rat hepatocytes with [³⁵S]taurine as well as [³H]LTB₄ revealed the incorporation of both radioactive isotopes into this metabolite. The data supported the identification of this metabolite as tauro-18-carboxy-19,20-dinor-LTB₄.

Amino acid conjugation of leukotrienes has not been previously reported and suggests that such intermediates might participate in enterohepatic circulation of LTB₄ metabolites in the intact animal and thus serve as an alternative metabolic route for LTB₄ elimination.

Leukotriene B₄, (5S,12R)-dihydroxy-6Z,8E,10E,14Z-eicosa-

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1 The abbreviations used are: LTB₄, (leukotriene B₄); 5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosa-5,12,20-trihydroxy-6,8,10,14-eicosatetraenoic acid; 20-OH-LTB₄, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; 20-OH-LTB₄, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; 20-COOH-LTB₄, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; 16-COOH-LTB₄, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; 16-COOH-LTB₄, 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; RP-HPLC, reverse-phase HPLC; FAB, fast atom bombardment; TMS, trimethylsilyl; PFB, pentafluorobenzyl; BSTFA, bis(trimethylsilyl)trifluoroacetamide.
Materials—LTB₄ was kindly provided by the Upjohn Co. (Kalamazoo, MI). [5,6,8,9,11,12,14,15-H]LTB₄ (196 Ci/mmol) was purchased from Du Pont-New England Nuclear. 20-Carboxy-LTB₄ was purchased from Cayman Chemical Co. (Ann Arbor, MI). [11C]Taurine (115 mCi/mmol) was purchased from Amersham Radiochemicals. Chromatographic supplies included octadeckylsilica (ODS) SepPak® cartridges from Waters Associates (Milford, MA). Reverse-phase HPLC was performed on an Apex II ODS column (150 × 4.6 mm, Jones Chromatography, Columbus, OH). HPLC solvents were obtained from Fisher Chemical Co. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Supelco. Diazald, used to prepare ethereal solutions of diazomethane for 5 min in methanol, was purchased from Aldrich (Milwaukee, WI).

Hepatocyte Isolation and Incubation—Hepatocytes were isolated by the collagenase perfusion method as described previously (18) with slight modifications. Male Sprague-Dawley rats (200–500 g) were anesthetized with pentobarbital (65 mg/kg). After opening the abdomen to expose the portal vein, the liver was perfused with Ca²⁺-free collagenase (Type II, 6.25 ml/ml min) and removed from the animal. The liver was then perfused with 4.5 units/ml collagenase (Type II, Cooper Biomedical, Freehold, NJ) and 1 mM CaCl₂-containing oxygenated buffer for 15 min. After a 5-min washout with collagenase/Ca²⁺-free buffer, hepatocytes were freed from the liver and purified by four steps of differential centrifugation using a separate wash buffer each time. The hepatocytes were resuspended in an incubation buffer for 5 × 10⁶ viable cells/ml. In all experiments, cell viability was >85% as determined by trypan blue exclusion. Incubations with various concentrations of LTB₄ (0.3–30 μM) were carried out at 37°C under an atmosphere of 95% O₂, 5% CO₂, between 5 and 60 min. The incubation buffer contained 129 mM NaCl, 5.2 mM KCl, 3.3 mM NaHPO₄, 0.9 mM MgSO₄, 1.1 mM CaCl₂, 4.4 mM glucose, 10 mM Tris, equilibrated with 95% O₂, 5% CO₂, pH 7.4. For the [14C]taurine experiments, cells were preincubated for 5 min with 25 μCi of [14C]taurine prior to the addition of LTB₄.

Metabolite Purification—Incubations were terminated by the addition of 1 volume of ethanol (−20°C). After approximately 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The pellet was resuspended, sonicated for 15 min in 80% methanol/water, and then centrifuged. The supernatant portions were pooled and analyzed separately by RP-HPLC. Residual radioactivity in the pellet was assayed following the addition of 4 volumes of ethanol (−20°C). After approximately 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The pellet was resuspended, sonicated for 15 min in 80% methanol/water, and then centrifuged. The supernatant portions were pooled and analyzed separately by RP-HPLC. Residual radioactivity in the pellet was assayed following the addition of 4 volumes of ethanol (−20°C). After approximately 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The pellet was resuspended, sonicated for 15 min in 80% methanol/water, and then centrifuged. The supernatant portions were pooled and analyzed separately by RP-HPLC. Residual radioactivity in the pellet was assayed following the addition of 4 volumes of ethanol (−20°C). After approximately 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The pellet was resuspended, sonicated for 15 min in 80% methanol/water, and then centrifuged. The supernatant portions were pooled and analyzed separately by RP-HPLC. Residual radioactivity in the pellet was assayed following the addition of 4 volumes of ethanol (−20°C). After approximately 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The pellet was resuspended, sonicated for 15 min in 80% methanol/water, and then centrifuged. The supernatant portions were pooled and analyzed separately by RP-HPLC.

The first stage of RP-HPLC utilized a two-step linear gradient (system I) from 10% solvent B (methanol/solvent A/acetic acid (90:10:0.1)) to 70% B in 50 min, followed by a gradient from 70%–100% B in 15 min. The mobile phase was then run at 1 ml/min. A 5% aliquot from the large incubations was analyzed using an on-line radioactivity detector (Flow One/Beta, Radiomatic, Tampa, FL). The remaining sample was then purified, and radioactive peaks were collected manually, based upon monitoring UV absorbance at 270 nm (HP-1040A photodiode array detector, Hewlett-Packard, Palo Alto, CA). Collected fractions were then evaporated to dryness and dissolved in a small volume (200–500 μl) of 1 mM ammonium formate, pH 4.5, and rechromatographed separately with a second stage of RP-HPLC. The second HPLC employed a linear gradient from 100% 1 mM ammonium formate, pH 4.5, to 50% acetonitrile in 50 min at 1 ml/min (system 2). Metabolites were detected by UV absorbance and colormetric assay. RP-HPLC was performed on purified metabolites using a HP8452A UV spectrophotometer (Hewlett-Packard, Palo Alto, CA).

Gas Chromatography-Mass Spectrometry—Samples analyzed by electron ionization (70 eV) were first methylated with ethereal diazomethane for 5 min in methanol/triethylamine, and then reacted with 100 μl of 50% BSTFA in acetonitrile for 20 min at 60°C. Electron ionization GC/MS was carried out on a Hewlett-Packard 5970 mass spectrometer interfaced with a DB-1 capillary column (10 m × 0.25 mm inside diameter, 0.2 μm film thickness) programmed from 100 to 320 °C at 15 °C/min using helium as carrier gas. Samples analyzed by negative ion electron capture mass spectrometry were first taken to dryness, then reacted with 20 μl of pentafluorobenzyl (Aldrich) and 10 μl of N,N-diisopropylethylamine (Aldrich), in 100 μl of 90% methanol/acetone for 30 min at room temperature. The benzyl esters were then reacted with BSTFA as described above. GC/MS analyses were performed on a Nermag 1010C (Delmar-Nermag, Paris, France) using methane as moderating gas at 0.5 Torr source pressure. Identical GC conditions were employed as described above for the methyl ester, trimethylsilyl, and other derivatives.

Fast Atom Bombardment-Mass Spectrometry—Fast atom bombardment (FAB)-mass spectrometry and continuous flow FAB mass spectrometry were performed on a Finnigan TSQ-70B (Finnigan Corp., San Jose, CA) using methanol/water/glycerol (40:10:1) as the mobile phase with a flow rate of 10 μl/min for continuous flow FAB. Samples were dissolved in this mobile phase and desorbed from the probe tip with a beam of xenon atoms from a sapphire field atom gun operated at 2 mA, 7-kV accelerating potential. Tandem mass spectrometry was performed also using the triple quadrupole TSQ-70B with argon at 0.5 mTorr in the second quadrupole collision cell and 29 eV collision energy. Daughter ions were recorded by scanning the third quadrupole mass spectrometer after selecting the specific LTB₄ metabolite ion with the first quadrupole mass spectrometer. High resolution FAB mass spectrometry of metabolite H₂ was kindly performed by Drs. C. Costello and S. Martin from the MIT Mass Spectrometry Facility and the Genetics Institute on a 4-sector XEOL NX110/HX110; peak matching was performed using a glycerol matrix ion at m/z 459.2588. The exact mass was the average of two determinations.

Results

Metabolite Isolation and Purification—Incubation of LTB₄ with rat hepatocytes led to the production of metabolites including several which have not been previously structurally characterized. The absolute amount of each metabolite depended upon the incubation conditions employed, concentration of LTB₄, and incubation time. Furthermore, each preparation of hepatocytes was somewhat variable in the relative amounts of each metabolite observed, likely due to the exact conditions for each hepatocyte preparation. In a large incubation of LTB₄ (119 nmol of LTB₄, 5 × 10⁶ cells/ml, 10 ml) carried out for 30 min, the majority of the radioactivity was recovered in the ethanolic supernatant of the hepatocyte suspension. As seen in Table I, 8% of the initial radioactivity from [3H]LTB₄ was recovered in the ethanolic supernatant of the hepatocyte suspension. Incubation supernatant 92% Volatile 33% Nonvolatile 59% Hepatocyte incubation pellet 8%

Table I

| Isolation step | [3H]LTB₄, % |
|----------------|------------|
| Hepatocyte incubation supernatant | 92         |
| Volatile | 33         |
| Nonvolatile | 59        |
| KP-SepPak (water wash) | 3         |
| RP-SepPak (methanol wash) | 56       |
| Hepatocyte incubation pellet | 8         |

*Expressed as the percent of starting [3H]LTB₄, added to hepatocyte incubation.
dioactivity showed the same reverse-phase HPLC metabolic profile as that of the supernatant (see below). Evaporation of the supernatant resulted in a loss of 33% of the initial radioactivity as volatile material, most likely water, while 59% was nonvolatile at reduced pressure (0.1 Torr for over 1 h). This residue was dissolved in acidic water and applied to a reverse-phase solid-phase extraction system with approximately 56% of the starting material (95% of the added radioactivity) being retained and then eluted with methanol. Reverse-phase HPLC separation (System 1) of this partially purified extract revealed four major and three minor radioactive components (Fig. 1). The reverse-phase conditions were altered from that previously reported (15) in order to retain the more polar metabolites longer on the HPLC column. The radioactivity which was not retained by the reverse-phase SepPak was designated Hx and corresponded to 3% of the initial LTB4 starting material in this experiment.

The radioactive peaks observed (Fig. 1) also were found to absorb ultraviolet light at 270 nm, consistent with the retention of the conjugated triene chromophore in each metabolite structure (Fig. 1). As seen in Fig. 1, five metabolites were designated H1 through H5, respectively, in order of decreasing lipophilicity, based upon their relative reverse-phase HPLC retention times. The relative amount of each metabolite obtained from several experiments is shown in Table II. In other experiments, specific incubation conditions were optimized for production of one or more metabolites in order to increase the quantity available for structural studies. A second HPLC purification step (System 2) was employed to further separate these metabolites from other biochemical impurities. This reverse-phase HPLC separation employed an acetonitrile/water gradient system, and separation of interfering substances was followed by UV absorbance. In addition, analysis of the metabolites following this second reverse-phase HPLC separation yielded a substantially reduced number of extraneous GC/MS components during subsequent mass spectrometric analyses.

When incubations were carried out for longer times or with a lower concentration of LTB4, a larger production of the component labeled Hx was observed. This component was not retained by the reverse-phase HPLC system and therefore it is not known if one or more components are present in this peak.

20-OH-LTB4 and 20-COOH-LTB4.—Although no 20-hydroxy-LTB4 remained in the supernatant following the 30-min incubation seen in Fig. 1, this metabolite was observed during shorter incubation periods. Based upon radioactive content, the UV absorption triene chromophore and co-elution with synthetic standards, the identity of 20-hydroxy-LTB4 was established. Correspondingly, the identity of 20-COOH-LTB4 (metabolite H11, Fig. 1) was established by the UV absorption spectrum and co-elution with synthetic standards. In addition, the electron ionization mass spectrum of the dimethyl ester, bis(trimethylsilyl) ether derivative (Me,TMS) and the negative ion electron capture mass spectrum of the bis(pentafluorobenzyl) ester, bis(trimethylsilyl) ether (PFB, TMS) derivative were identical for both the isolated hepatocyte metabolite (H11) and synthetic 20-carboxy-LTB4 (data not shown).

18-COOH-dimethyl LTB4—a The radioactive metabolite H11 had an UV absorbance spectrum similar to that observed for LTB4 and 20-carboxy-LTB4. Furthermore, the electron ionization mass spectrum of the Me, TMS derivative was identical with that which has been previously published (15) with a molecular ion at m/z 510 (M+) and fragment ions at m/z 479 (M-31, loss of OCH3) and m/z 420 (M-90, loss of Me3SiOH), as well as a very abundant ion above mass 200 at m/z 203 (Me3SiOCH2CH2CH2COOCH3). In addition, the PFB, TMS derivative of metabolite H11 had a negative ion electron capture mass spectrum revealed an abundant ion at m/z 661, corresponding to the carbonate anion (M-181, loss of PFB), and abundant ions at m/z 569 (M-181-92, loss of PFB-FSiMe3), m/z 499 (M-181-90-72, loss of PFB-HOTMS-TMS), m/z 481 (M-181-180, loss of both PFB es-

### Table II

| Metabolite | [%][H]LTB4 | % |
|------------|------------|---|
| 20-COOH-LTB4 | 9±2 |   |
| 18-COOH-LTB4 | 24±4 |   |
| H11 | 21±2 |   |
| H12 | 3±2 |   |
| H13 | 8±2 |   |
| H14 | 4±1 |   |
| H15 | 11±2 |   |
| Otherb | 18±5 |   |
| Starting material (LTB4) | 2±1 |   |

a Values are expressed as the average percent ± S.E. (6 experiments) for each metabolite, of the radioactivity eluting from the HPLC column after injection of a 5% aliquot of the radioactivity remaining after evaporation of the ethanolic incubation supernatant.

b Values less than 2% were classified as other.

**Fig. 2.** UV absorption spectra of LTB4 metabolites. Spectra were obtained using an on-line UV detector, from experiments using 12 μM LTB4, 5 x 10⁶ cells/ml, for 30 min. A, metabolite H11; B, metabolite H12; C, metabolite H13; and D, metabolite H14.

![Fig. 1. Reverse-phase HPLC chromatogram of [3H]LTB4 metabolites from a 30-min incubation with [3H]LTB4 (3 nmol, 0.1 μCi). System 1 was used for the separation as outlined in the text. Prostaglandin B2 (100 ng) was added to the ethanolic supernatant prior to evaporation as an internal standard.](http://www.jbc.org/Downloaded from)
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Fast atom bombardment analysis of the underivatized molecule (Fig. 3) yielded an abundant ion at m/z 337 corresponding to the carboxylate anion (M-H\textsuperscript{-}) consistent with a molecular weight of 338 for the underivatized molecule. These data were consistent with identification of this metabolite as 16-carboxy-19,20-dinor-LTB\textsubscript{4} (H\textsubscript{III}).

**Metabolite H\textsubscript{IV}**—The radioactive metabolite eluting with a HPLC retention time slightly less than that of the previously characterized 18-COOH-LTB\textsubscript{4} had a UV absorbance maximum at 269 nm with shoulders at 260 and 280 nm, consistent with the presence of a conjugated triene moiety (Fig. 2A). CC/MS analysis of the Me\textsubscript{3} TMS derivative using electron ionization (Fig. 4) revealed ions at m/z 484 (M\textsuperscript{+}), m/z 469 (M-15, loss of CH\textsubscript{3}), m/z 453 (M-31), and m/z 394 (M-90). The most abundant ion above mass 200 was observed at m/z 203 as discussed above for metabolite H\textsubscript{III}. The molecular ion for this metabolite corresponded to 26 mass units less than that of the mass data analyzed for 18-COOH-LTB\textsubscript{4}. When metabolite H\textsubscript{IV} was esterified with the pentafluorobenzyl group followed by derivatization of free hydroxyl groups to the trimethylsilyl ethers, GC/MS analysis of negative ions using electron capture conditions revealed major ions at m/z 635 (M-181), m/z 543 (M-181-92), m/z 473 (M-181-90-72), m/z 455 (M-181-180), and m/z 437 (M-181-198) as seen in Fig. 5. All abundant ions at high mass were 26 mass units less than the corresponding ions observed for the PFB, TMS derivative of 18-COOH-LTB\textsubscript{4}.

Underivatized metabolite H\textsubscript{IV} was analyzed by continuous flow FAB mass spectrometry in the negative ion mode, and an ion at m/z 311 (M-H\textsuperscript{-}) was observed. This ion also corresponded to a loss of 26 mass units from the ion obtained from 18-COOH-LTB\textsubscript{4} and suggested a molecular weight of 312 for underivatized H\textsubscript{IV}. Since \textbeta-oxidation typically results in the loss of two carbon units as methylene units, this would lead to a loss of 28 mass units from a homologous series of \textbeta-oxidation metabolites. However, as shown for the \textbeta-oxidation of LTB\textsubscript{4} (19), the chain-shortening process of \textbeta-oxidation results in loss of only 26 mass units when an isolated double bond is present two carbons from the corresponding CoA ester moiety. Based upon HPLC retention time and mass spectrometric analyses, the structure of metabolite H\textsubscript{IV} was consistent with a further \textbeta-oxidized metabolite of LTB\textsubscript{4} which has one double bond saturated during the \textbeta-oxidation process. Considering this metabolite retained a triene chromophore, the double bond reduced would correspond to the double bond in the 14,15-position. Assuming that the double bonds in the conjugated triene remain in their original conformation, we conclude that compound H\textsubscript{IV} is 16-carboxy-14,15-dihydro-17,18,19,20-tetranor-LTB\textsubscript{4} (16-COOH-LTB\textsubscript{4}).

**Metabolite H\textsubscript{V}**—It is noteworthy that metabolite H\textsubscript{V} was the most variable metabolite in terms of yield. In some experiments, this metabolite became a major metabolite, although, as shown in Fig. 1, in other experiments it was rather minor. H\textsubscript{V} retained the conjugated triene structure as evidenced by the UV absorption spectrum with maximal absorbance at 270 nm and shoulders at 261 and 281 nm (Fig. 2C). Attempts to analyze this molecule by gas chromatography/mass spectrometry as described for the previous metabolites, either through formation of the methyl ester, trimethylsilyl ether derivative, or pentafluorobenzyl ester, trimethylsilyl ether derivative, failed to reveal any gas chromatographic peaks which might be related to a LTB\textsubscript{4} metabolite. In contrast, negative ion
continuous flow FAB analysis of Hv revealed an abundant ion at m/z 444 (Fig. 6A). By high resolution mass spectrometry, the exact mass was determined to be m/z 444.1719, consistent with an elemental composition C_{30}H_{50}N_{10}S (2.7 millimass unit error, m/z 444.1692 calculated). When Hv was treated with diazomethane, this ion was shifted by 14 mass units to m/z 458 suggesting the presence of only one carboxylate moiety in this molecule (Fig. 6B). Since these ions were observed at even mass, this suggested an anion (M-H-) containing an odd number of nitrogen atoms.

One of the possibilities for metabolite Hv was that of a taurine conjugate. Therefore, hepatocytes were prelabeled with [14C]taurine prior to incubation with [3H]LTB$_4$ for 30 min. After isolation of LTB$_4$, metabolites, as described above, HPLC analysis revealed the co-elution of [14C], using an online scintillation detector, with metabolite Hv at the correct retention time as indicated by the tandem UV monitor (Fig. 7). As expected, there was a doublet at the retention time in the tritium channel resulting from separation of [3H]Hv from the [14C]Hv (label from taurine) detected at 4% efficiency in the tritium channel. This separation was due to a tritium isotope effect on HPLC retention times.

Collision-induced dissociation (0.5 mT argon, 29 eV laboratory collision energy) of the ion at m/z 458 (H$_v$-Me) resulted in major daughter fragment ions at m/z 219 (CH$_2$CONHCH$_2$CH$_2$SO$_3$), m/z 165 (CH$_2$CONHCH$_2$CH$_2$SO$_3$), and m/z 80 (SO$_3$)$^-$ as seen in Fig. 8. The formation of SO$_3$ by collision-induced dissociation of the taurocholate molecular anion has also been observed. Other collision-induced dissociation ions from Hv-Me correspond to positions which would be expected to be particularly labile either by being allylic to double bonds or α to a hydroxyl moiety. The

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2 M. A. Shirley and R. C. Murphy, unpublished observations.
Results, metabolite Hv was assigned the structure tauro-18-

m/z 210 ion lends evidence to support the conjugation of 

and a 29 eV collision energy offset.

FIG. 7. RP-HPLC chromatogram (32-40 min) of metabolites Hv (33 min), Hv (35 min), and Hv (39 min) derived from co-incubation of rat hepatocytes with 25 μCi of [14C]taurine and 2 μCi of [3H]LTB4 (120 nmol) for 40 min. The major [14C] peak in this 8-min chromatographic window co-eluted with metabolite Hv. Part of the double peak for [3H]Hv can be accounted for by a 4% overlap of the [14C] signal in the [3H] detecting window and part by a slightly shorter retention time for [3H]Hv.

FIG. 8. Daughter ion mass spectrum of the molecular anion (m/z 458) of the methyl ester of metabolite Hv (33 ag) obtained using a Finnigan TSQ-70B in the negative ion CF-FAB MS-MS mode. Continuous flow conditions were as outlined under "Experimental Procedures." Collision-induced dissociation was mediated using argon gas at 0.5 mTorr and a 29 eV collision energy offset.

carboxy-19,20-dinor-LTB4. The failure to observe this molecule during GC/MS analysis would be consistent with the inability to form a volatile derivative of taurine conjugates (20).

Metabolites Hv and Hv—In all experiments, metabolites Hv and Hv were obtained as minor metabolites (Fig. 1). Nevertheless, it was possible to obtain UV spectra (Fig. 2, B and D) of each metabolite which suggested retention of the characteristic triene chromophore of LTB4. Thus, both metabolites most likely retained the double bonds in LTB4 from carbon 6 to carbon 11. Insufficient material was available for further structure characterization.

DISCUSSION

Leukotriene B4 is metabolized in the isolated rat hepatocyte into several metabolites by ω- and β-oxidation (Fig. 9). In addition, taurine conjugation of one metabolite has been observed. The ω-oxidation of LTB4 has been studied in some detail in the human polymorphonuclear leukocyte where a unique cytochrome P-450LTB has been reported to catalyze both ω-oxidation of LTB4 as well as the formation of 20-CHO-LTB4 and 20-COOH-LTB4. ω-Hydroxylation of LTB4 has also been studied in the hepatic microsomal preparations which lead to the formation of 20-hydroxy- as well as 19-hydroxy-LTB4 (21). These studies suggest that a unique isozyme or P-450 may exist which is different from the cytochrome P-450s which catalyze similar ω-oxidations of prostaglandins and lactic acid. The conversion of 20-OH-LTB4 to 20-COOH-LTB4 in rat hepatocytes has been shown to be due to enzymatic activity in the cytosol which could be inhibited with 4-methylpyrazole, an inhibitor of alcohol dehydrogenase (16). These studies have implicated alcohol dehydrogenase and aldehyde dehydrogenase in the conversion of 20-OH-LTB4 to 20-COOH-LTB4. Cytosolic enzymes in hepatocytes may therefore play an important role in the metabolic formation of 20-COOH-LTB4.

Previous studies have shown that 18-COOH-LTB4 is also a major metabolite in this preparation suggesting that β-oxidation proceeds after ω-oxidation of the methyl terminus of LTB4 (15). However, several other metabolites were not characterized in these studies and structural studies reported here indicate that further β-oxidation indeed takes place in the isolated rat hepatocyte. In particular, β-oxidation of 18-COOH-LTB4 resulted in the formation of 16-COOH-LTB4, a metabolite shortened four carbons from native LTB4. Furthermore, this metabolite had the carbon 14-15 double bond saturated during the process of β-oxidation consistent with the previous observations of the β-oxidation of LTE and formation of 16-COOH-N-acetyl-LTE by isolated rat hepatocytes. The formation of this reduced metabolite of LTB4 strongly argues for the operation of 2,4-dienoyl-CoA reductase in the process of the CoA ester of 18-COOH-LTB4, following oxidation by either acyl-CoA dehydrogenase (mitochondrial process) (22) or acyl-CoA oxidase (peroxisomal process) (23). The resultant diene metabolite is identical with the 2,4-dienoyl-CoA esters observed in the metabolism of polyunsaturated fatty acids containing the nearest double bond, an even number of carbon atoms from the acyl-CoA ester (24). Following reduction of the 2,4-diene unit to the 2,3-mononeo and operation of cis-trans-3,2-enoic CoA isomerase, β-oxidation can proceed resulting in cleavage of the two carbon fragments and formation of the chain-shortened CoA ester of 16-COOH-LTB4.

Taurine (β-aminoethanesulfonic acid) is a ubiquitous molecule present in large amounts in various tissues. It is primarily recognized as being an important amino acid for con-
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Figure 9. Metabolism of LTB₄ by the isolated rat hepatocyte.

jugation of certain carboxylic acids, although it has also been implicated to have roles in the central nervous system (25), the heart (26), and other systems (27). Taurine typically forms conjugates with various bile acids (28), and taurine conjugates have been reported for various xenobiotics (20). Recently, the 15,15-dimethyl prostaglandin E₂ analog, trimoprostil, has been reported to be metabolized into four different taurine conjugates from this prostanoid. Considering the common structural features inherent in leukotrienes and prostaglandins, it is not surprising that leukotriene B₄ can be conjugated to taurine, although this is the first reported occurrence of a tauro-conjugate in the leukotriene class of arachidonic acid metabolites. Hepatocytes contain a large amount of taurine (mM) (30) and fatty acid intermediates which undergo coenzyme A thioester formation may serve as substrates for the acyl-CoA amino acid N-acyltransferase responsible for taurine conjugation. This soluble enzyme can also use glycine for the amino nitrogen atom acceptor which suggests that the glycine conjugate of 1-carboxy-LTB₄ may also be present. There were several minor metabolites observed in these isolated rat hepatocyte preparations, but they were not structurally characterized.

Previous studies of [³H]LTB₄ metabolism in vivo showed a relatively low excretion of nonvolatile radioactivity into the urine (17). In addition, a fair amount of radioactivity has been found in the bile of mice injected with LTB₄.² It is possible that a significant amount of LTB₄ remains in the body for quite some time due to enterohepatic circulation, perhaps involving various amino acid conjugates including taurine conjugates.

Several reduced metabolites of LTB₄ were observed when LTB₄ was incubated with porcine leukocytes (31). These cells lack ω-oxidation capacity for LTB₄. Reduced metabolites of ω-6-trans-LTB₄, and ω-9-epi-ω-trans-LTB₄, which are poorly ω-

² T. W. Harper and R. C. Murphy, unpublished observations.

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