[1H]Thromboxane B2 was biosynthesized and infused into an unanesthetized monkey. Several urinary metabolites were isolated and their structures elucidated using gas chromatography-mass spectrometry. In addition to the major urinary metabolite, dinor-thromboxane B2, a series of metabolites resulting from dehydrogenation of the alcohol group at C-11 were identified: 11-dehydro-thromboxane B2, 11-dehydro-15-keto 13, 14-dihydro-2,3-dinor-thromboxane B2, and 11-dehydro-15-keto-13, 14-dihydro-19-carboxyl-2,3,4,5-tetranor-thromboxane B2, 6-(1,3-dihydroxypropyl)-7-hydroxy-10-oxo-3-pentadecanoic acid was also identified. Three mono-O-ethylated metabolites were formed from thromboxane B2, which in this study was infused in an ethanolic solution. A small quantity of thromboxane B2 was excreted unchanged into the urine.

The thromboxanes, thromboxane A2 and TxB2, are a recently described class of compounds shown to be derived from PGH2 (1). The major pathway of PGH2 metabolism in human platelets, guinea pig lung, and rat and guinea pig brain is to the thromboxanes (2-4). In the metabolic sequence, PGH2 is converted to TXA2, which subsequently degrades to TxB2 by the incorporation of a molecule of water with a t1/2 of 32 s at 37°C in aqueous media (1). TXA2 possesses significant biological activity, inducing irreversible platelet aggregation (1) and causing potent contraction of vascular smooth muscle (5). Except for possibly acting as a chemotactic agent (6), TxB2, however, has not been shown to possess any significant biological activity. Because of the biological significance of TXA2, quantification of its synthesis under various conditions has potential significant biological application. Accurate quantification of TXA2 directly is difficult because of its lability. TxB2, however, is a stable compound which can be isolated, and quantification of TxB2 should directly reflect the quantity of TXA2 that has been formed (1, 2).

Because of very rapid metabolism and formation from the formed elements in blood, direct analysis of the primary prostaglandins, PGE2 and PGF2α, has proved to be an unreliable indicator of their biosynthesis, a more reliable index being the measurement of the circulating and urinary prostaglandins metabolites (7-8). These same problems would be anticipated in attempting to assess the quantity of thromboxane synthesis by direct measurement of TxB2 in plasma. It, therefore, seemed of importance to identify the urinary metabolites of TxB2, the structures of which could provide a basis for future quantitative studies of thromboxane synthesis in vivo. This paper describes the identification of several urinary metabolites of TxB2 in the monkey. The nonhuman primate was used in this initial study because species differences in prostaglandin metabolism have been demonstrated and, at least regarding metabolism of PGFi2, the monkey has been found to be the animal species which most closely approximates human prostaglandin metabolism (9). A preliminary report of part of this work describing the identification of the major urinary metabolite as dinor-TxB2 has been published earlier (10).

**EXPERIMENTAL PROCEDURES**

**Materials**

Sheep seminal vesicle glands were the generous gift of Dr. John Pike, The Upjohn Co., Kalamazoo, Mich. We are indebted to Dr. N. A. Nelson of The Upjohn Co. for a generous gift of 11-dehydro-thromboxane B2 methyl ester. Arachidonic acid was obtained from Nu-Chek Prep., Inc., Elysian, Minn. and [1H8]Thromboxane B2, [3H]Arachidonic acid (833 μCi/μmol) was prepared and the prostaglandin endoperoxides (PGH2, PGH2) were biosynthesized using sheep seminal vesicle glands as previously described (11). TxB2 was biosynthesized from the prostaglandin endoperoxides using guinea pig lung microsomes by a procedure recently described in detail (10), yielding a total of 1.25 mg of [1H]TxB2 (6.90 μCi/μmol).

**Methods**

**Infusion of Thromboxane B2**—[1H]TxB2 (1.25 mg, 6.90 μCi/μmol) was infused into a cynomolgus male monkey as previously described (10). Amblerite XAD-2 and Silicic Acid Chromatography—Extraction of urine was performed on Amberlite XAD-2 and the crude extract subjected to silicic acid chromatography as previously described (10). Reversed Phase Partition Chromatography—Reversed phase partition chromatography was performed as previously described (12). Solvent systems used are listed in Table I. Stationary phases were supported on 4.5 g of hydrophobic Hydro-Super-Cel (Fisher Scientific Co., Fair Lawn, N.J.). Fractions (5 ml) were collected.

**High Performance Liquid Chromatography**—High performance liquid chromatography was performed using a model U6K injector, model 660 solvent programmer, and two model 6000A solvent pumps (Water Associates, Milford, Mass.). A flow rate of 1 ml/min was used, and 1-ml fractions were collected. Chromatography performed on a μ-Porsil column (30 cm × 6 mm outer diameter) (Water Associates) was accomplished by linear programming of 100% chloroform to 100% chloroform/methanol/acetic acid (500:50:11) (v/v/v) in 2 h as previously described (13). Chromatography performed on μ-Bondapak

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column (Water Associates) was accomplished under isocratic conditions with the solvent system water/acetoneitrile/benzene/acetic acid (400:100:15:5) (v/v/v).

Gas-Liquid Chromatography-Mass Spectrometry—Mass spectrometry was performed using an LKB 9006-PDP 12 gas chromatography-mass spectrometer computer system (10). C values were obtained using mixtures of methyl esters of normal saturated fatty acids as standards (14). Radioactivity detection and C value determination of gas chromatography peaks was accomplished by analyzing a portion of the samples on a Nuclear-Chicago 4740 gas chromatograph coupled with a series 3000 radioactivity monitor (10).

Preparation of Derivatives for Gas-Liquid Chromatography and Mass Spectrometry—Methyl ester, ethyl ester, O-methoxylamine, and trimethylsilyl ether derivatives of every metabolite identified were analyzed by mass spectrometry to determine the total number of carbonyl, methine, and trimethylsilyl groups present and to aid in the interpretation of structures of various fragment ions in the mass spectra.

Assay of Radioactivity—Radioactivity was determined with a Searle Mark III 6880 liquid scintillation counter. The scintillation fluid consisted of 3a20 (Research Products International Corp., Elk Grove Village, Ill.) for nonaqueous samples and 3a40 for aqueous samples.

Reaction with Ethanolic HCl—An ethanolic HCl solution was prepared by dissolving 50 μl of redistilled acetic chloride in 1 ml of ethanol. Approximately 20 μg of the ethyl ester of the compound was dissolved in 0.5 ml of ethanol and 0.5 ml of the ethanolic HCl solution added. After 30 min at room temperature, the reaction mixture was evaporated to dryness under a stream of nitrogen, and the residue treated with excess ethereal diazomethane.

Reaction with Sodium Methoxide—A 0.5 N solution of sodium methoxide was prepared by dissolving sodium in methanol. Freshly cut sodium was washed extensively with toluene and then hexane before addition to the methanol. The sodium methoxide solution was then passed through a 0.45 Millipore filter. The solution (100 μl) was added to 1 to 10 μg of the methyl ester of the compound. After 20 min at room temperature, 1.95 ml of 0.01 M sodium phosphate (pH 5.0) was added and the mixture acidified to pH 5.0 with 0.01 N HCl. The compound was then extracted into ethyl acetate and the solution evaporated under reduced pressure.

Nomenclature and Carbon-numbering System—Chemical nomenclature and carbon numbering of the TxB2 molecule in the past has usually referred to the acyclic aldehyde alcohol form of TxB2 instead of the hemiacetal. Several of the TxB2 metabolites identified in this work, however, although retaining a 6-member ring (tetracydropryan), are not hemiacetals. Although these metabolites can be described by established chemical nomenclature, this nomenclature and the carbon numbering is necessarily totally different than that previously used for TxB2. This complicates the structural comparison of these metabolites with TxB2 and hinders lucid description of the metabolic pathways. These problems could be eliminated if a basic system of nomenclature was adopted for the thromboxanes similar to the prostanoic acid system used for the prostaglandins. We, therefore, are proposing that the basic 20-carbon-substituted tetrahydropryan with the carbon-numbering system as illustrated in Fig. 1 be called thrombanoic acid. Using this system, for example, the chemical nomenclature for TxB2 would be 9α,11,15(S)-trihydroxy-thromb-5,13-tienoic acid. Such a system provides both a useful common nomenclature for describing the thromboxanes and their metabolites, as well as a nomenclature and carbon-numbering system similar to that used for the prostanoic acids and is used in this paper unless otherwise indicated or obvious.

RESULTS

At no time during the infusion of TxB2 was there any significant change in blood pressure or pulse rate of the monkey. No clinically apparent adverse effects were observed. Eighty per cent of infused radioactivity was recovered in the urine in 8 h, the majority of which was excreted within 2 h.

A flow diagram outlining the urine purification procedures that are subsequently described and the isolation and letter-number designation of identified TxB2 metabolites is illustrated in Fig. 2.

The urine collected was extracted by Amberlite XAD-2 chromatography. Ninety per cent of the applied radioactivity was eluted with 400 ml of ethanol. The residue obtained after evaporation of the ethanol was dissolved in ethyl acetate and purified by silicic acid chromatography. Radioactivity was eluted quantitatively with 300 ml of ethyl acetate.

Further sample purification and initial compound isolation was achieved by reversed phase partition chromatography using Solvent System D (Table I). The sample was dissolved in 0.5 ml of mobile phase with 1 drop of acetic acid added immediately prior to application to the column. Two polar peaks emerged, designated A and B, followed by elution of a substantial amount of less polar material, designated Area C, that was not resolved by this solvent system (Fig. 3). Additional radioactivity was eluted from the stationary phase with 50 ml of methanol. Total radioactivity recovered from that applied to the column was 65% with 10% in Peak A, 18% in Peak B, 38% in Area C, and 34% was eluted from the stationary phase with methanol. The total radioactivity recovered at this stage from the amount originally infused was 47%.

Identification of Compounds Eluted with Methanol from Stationary Phase—Material eluted with methanol was subjected to reverse phase partition chromatography using Solvent System C-45 (Table I). Four radioactive peaks appeared (Fig. 4) designated M1 (40 to 70 ml of eluate, 9% of recovered radioactivity), M2 (75 to 115 ml, 25%), M3 (120 to 180 ml, 30%), and M4 (185 to 295 ml, 36%). Material in Peak M1 was subsequently subjected to high performance liquid chromatography on μ-Porasil, but sufficient quantity of material was not present in any radioactive peak to permit identification.

Structures of Compounds in Peak M3—Material in Peak M3 was further purified by high performance liquid chromatography on μ-Porasil. Two prominent peaks emerged, one of which was designated Compound M3 (40 to 45 ml of eluate, 59% of total recovered radioactivity) and the other (31 to 34 ml, 13%) was subsequently determined to be a minor amount of Compound C3 (see below). Compound M3 represented approximately 6% of the total radioactivity present in the urine. Using an identical solvent program, TxB2 elutes from μ-Porasil at 48 to 53 ml. It was, therefore, determined that Compound M3 was less polar than parent TxB2 in this chromatographic system. Gas-liquid chromatographic analysis of the Me3Si ether derivative of the methyl ester of Compound M3 showed a major peak designated M3a and a minor peak...
designated M3b, with C values of 26.5 and 24.0, respectively. Although significantly less polar than parent TxB2 on µ-Porasil chromatography, M3a has a significantly higher C value than the Me:Si ether derivative of the methyl ester of parent TxB2 (C 23.6) (10).

The mass spectrum obtained for the Me:Si ether derivative of the methyl ester of M3b is shown at the top of Fig. 6. Ions were present at m/e 630 (M); 615 (M-15), loss of -CH3; 599 (M-31), loss of -OCH3; 586 (M-31), loss of -OCOCH3; 559 (M-71), loss of -O(CH2)4CH=CH2; 540 (M-90), loss of MeSiOH; 509 [M-(90 + 31)]; 469 [M-(90 + 71)]; 450 [M-(2 x 90)]; 438 [M-(90 + 71 + 31)]; 399 [M-(141 + 90)], loss of [CH2-O-CH-CH-CH(OCH3)]+; 379 [M-(2 x 90 + 71)]; 367 [M-(173 + 90)], loss of [MeSiO-CH-(CH=CH2)CH3OCH2 + 90]; 355 [M-(175 + 90), loss of [MeSiO-CH-(CH=CH2)CH3OCH2 + 90]; 301 [MeSiO]=CH-CH=CH(OH)(CH2)4CH=CH2]. A dicarboxylic acid structure was suggested by an increase in C value of 0.9 on gas-liquid chromatographic analysis of the Me:Si ether derivative of the ethyl ester, since a difference of 0.45 to 0.5 C value has been observed for methyl and ethyl esters of monocarboxylic acid TxB2 derivatives (10). This was confirmed by analysis of the ethyl ester by mass spectrometry where the molecular ion of the methyl ester was found to shift from m/e 630 to m/e 658. The location of the second carboxyl group at the original C-11 position was suggested by the prominent ion at m/e 175 in the mass spectrum of the methyl ester and its shift to m/e 189 in the corresponding mass spectrum of the ethyl ester derivative. The δ-lactone, 11-dehydro-TxB2, should be in equilibrium with a dicarboxylic acid structure in the presence of water, and the mass spectral data on M3b indicated that this was the dicarboxylic acid form of M3a or 3-hydroxy-4-(1,4-dihydroxy-2-nonenyl)-6-undecenoic acid.

The mass spectrum recorded for the Me:Si ether derivative of the methyl ester of M3b is shown at the top of Fig. 6. Ions were present at m/e 630 (M); 615 (M-15), loss of -CH3; 599 (M-31), loss of -OCH3; 586 (M-31), loss of -OCOCH3; 559 (M-71), loss of -O(CH2)4CH=CH2; 540 (M-90), loss of MeSiOH; 509 [M-(90 + 31)]; 469 [M-(90 + 71)]; 450 [M-(2 x 90)]; 438 [M-(90 + 71 + 31)]; 399 [M-(141 + 90)], loss of [CH2-O-CH-CH-CH(OCH3)]+; 379 [M-(2 x 90 + 71)]; 367 [M-(173 + 90)], loss of [MeSiO-CH-(CH=CH2)CH3OCH2 + 90]; 355 [M-(175 + 90), loss of [MeSiO-CH-(CH=CH2)CH3OCH2 + 90]; 301 [MeSiO]=CH-CH=CH(OH)(CH2)4CH=CH2]. A dicarboxylic acid structure was suggested by an increase in C value of 0.9 on gas-liquid chromatographic analysis of the Me:Si ether derivative of the ethyl ester, since a difference of 0.45 to 0.5 C value has been observed for methyl and ethyl esters of monocarboxylic acid TxB2 derivatives (10). This was confirmed by analysis of the ethyl ester by mass spectrometry where the molecular ion of the methyl ester was found to shift from m/e 630 to m/e 658. The location of the second carboxyl group at the original C-11 position was suggested by the prominent ion at m/e 175 in the mass spectrum of the methyl ester and its shift to m/e 189 in the corresponding mass spectrum of the ethyl ester derivative. The δ-lactone, 11-dehydro-TxB2, should be in equilibrium with a dicarboxylic acid structure in the presence of water, and the mass spectral data on M3b indicated that this was the dicarboxylic acid form of M3a or 3-hydroxy-4-(1,4-dihydroxy-2-nonenyl)-6-undecenoic acid.
When the methyl ester of M3 was treated with methoxyamine HCl for approximately 18 h at room temperature and then converted to a MeSi ether derivative and analyzed by gas chromatography, a peak with a C value of 24.5 was present in addition to the two peaks previously noted for M3a and M3b at C values of 26.5 and 24.0, respectively. The M3a peak had also been apparently reduced in relative size proportional to the size of the peak at C 24.5, approximately 30 to 50%. This suggested that M3a had been partially converted to a less polar derivative by treatment with methoxyamine HCl. A known reaction of a lactone with hydroxyamine is the formation of a hydroxamic acid (16). Methoxyamine would be expected to react in a similar fashion. It, therefore, seemed reasonable to suspect that the lactone 11-dehydro-TxB2 had been converted to the acyclic amide derivative illustrated in Fig. 7. The expected molecular ion of this compound as a trimethylsilyl ether derivative is m/e 645. However, when this derivative at C 24.5 was analyzed by mass spectrometry, the highest ion seen was m/e 614, and no deuterium-containing ions were detected in the mass spectrum of the corresponding derivative formed after treatment with trideuteromethoxyamine. These data suggested that the ion at m/e 614 was not the molecular ion. If we assume a molecular ion of m/e 645, the interpretations of the prominent ions seen in the mass spectrum of this derivative are as follows: m/e 614 (M-31), loss of OCH3; 613 (M-32), loss of CH3OH; 598 (M-(32 + 15)), loss of [32 + CH3]; 542 (M-(71 + 32)), loss of [CH3CH3 + 32]; 523 (M-(90 + 32)), loss of [MeSiOH + 32]; 382 (M-(141 + 90 + 32)), loss of [-CH2-CH=CH-(CH2)3COOCH3 + 90 + 32]; 365 (M-(190 + 90)); 350 (M-(173 + 90 + 32)); 301 (base) [MeSiOCH=CH-CH=CH-CH(OSiMe3)(CH2)CH3]; 211 (301-90), and 173 [MeSiO-CONH2]. This mass spectrum is consistent with the acyclic amide derivative, and we hypothesize that OCH3 or CH3OH is very readily eliminated from the amide upon electron impact. This would explain the absence of a visible molecular ion, the presence of many ions formed as a result of the combined loss of methanol plus another moiety, and the absence of deuterium-retaining ions in the derivative formed by treatment with trideuteromethoxyamine. It is interesting that amide derivatives have not been described after methoxyamine treatment of the α-lactone of δα,7α-dihydroxy-11-keto-tetranorprosta-1,16-dioic acid (17).

To obtain further evidence for the structure of M3a, chemically synthesized 11-dehydro-TxB2 methyl ester was converted to a MeSi ether derivative and analyzed by gas chromatography-mass spectrometry. This gave a peak with a C value of 26.5 (identical with M3a) and the mass spectrum recorded is seen at the bottom of Fig. 5. It is apparent that this mass spectrum is identical with that of the same derivative of metabolite M3a seen at the top of Fig. 5. When chemically synthesized 11-dehydro-TxB2 methyl ester treated with methoxyamine, converted to a MeSi ether derivative, and analyzed by gas chromatography-mass spectrometry, an additional peak with a C value of 24.5 was present, and the
mass spectrum obtained was identical with that obtained for the derivative of M3 at C 24.5 formed after treatment with methoxyamine.

To obtain additional evidence for the lactone ring structure and the apparent dicarboxylic acid structure of M3b, the chemically synthesized 11-dehydro-TxB2 methyl ester was treated with sodium methoxide in methanol. This would be expected to convert the lactone to the dicarboxylic acid methyl ester structure as illustrated in Fig. 8. The product of this reaction when converted to a Me3Si ether derivative and analyzed by gas chromatography-mass spectrometry gave a peak with a C value of 24.0 and the mass spectrum recorded is seen at the bottom of Fig. 6. When compared to the mass spectrum of metabolite M3b at the top of Fig. 6, it is apparent that the two spectra are essentially the same except for minor variation in low mass ions, presumably due to co-eluting impurity.

The additional confirmatory evidence obtained from the analysis of the chemically synthesized compound lead us to conclude that Compound M3a was the δ-lactone, 11-dehydro-TxB2, and that its corresponding dicarboxylic acid was Compound M3b. Using thrombanoic acid nomenclature, 11-dehydro-TxB2 is 9α,15(S)-dihydroxy-11-oxothromba-5,13-dienoic acid.

It is unlikely that the δ-lactone and the dicarboxylic acid would co-chromatograph throughout all of the chromatographic systems employed during urine processing and only be separated by gas-liquid chromatography. As a free acid or methyl ester the δ-lactone 11-dehydro-TxB2 would be predicted to be less polar than parent TxB2 in both the straight phase and reversed phase partition chromatographic systems employed. The methyl esters of TxB2 and chemically synthesized 11-dehydro-TxB2 were subjected to thin layer chromatography with the use of the solvent system benzene/dioxane.

Fig. 6. Top, mass spectrum of the methyl ester, Me3Si ether derivative of Metabolite M3b. Bottom, mass spectrum of the methyl ester, Me3Si ether derivative of the product formed after treatment of the methyl ester of 11-dehydro-thromboxane B2 with sodium methoxide.

Fig. 7. Amide derivative formed by treatment of the methyl ester of 11-dehydro-thromboxane B2 (Metabolite M3a) with methoxyamine hydrochloride for 18 h at room temperature.

The additional confirmatory evidence obtained from the analysis of the chemically synthesized compound lead us to conclude that Compound M3a was the δ-lactone, 11-dehydro-TxB2, and that its corresponding dicarboxylic acid was Compound M3b. Using thrombanoic acid nomenclature, 11-dehydro-TxB2 is 9α,15(S)-dihydroxy-11-oxothromba-5,13-dienoic acid.

It is unlikely that the δ-lactone and the dicarboxylic acid would co-chromatograph throughout all of the chromatographic systems employed during urine processing and only be separated by gas-liquid chromatography. As a free acid or methyl ester the δ-lactone 11-dehydro-TxB2 would be predicted to be less polar than parent TxB2 in both the straight phase and reversed phase partition chromatographic systems employed. The methyl esters of TxB2 and chemically synthesized 11-dehydro-TxB2 were subjected to thin layer chromatography with the use of the solvent system benzene/dioxane.
Identification of Compounds Eluting in Peak M4—Material in Peak M4 was further purified by μ-Porasil high performance liquid chromatography, and two discrete peaks emerged. The less polar peak (25 to 28 ml of effluent, 15% of recovered radioactivity) was designated Compound M4a, and the more polar peak (34 to 36 ml, 44%) was designated Compound M4b. M4a represented approximately 1.8% and M4b approximately 5.4% of the total radioactivity in the urine.

The methyl ester of M4a was converted to an O-methyloxime, Me₃Si ether derivative and analyzed by gas chromatography-mass spectrometry. Essentially a single peak was found with a C value of 22.8. The mass spectrum of this derivative of M4a (Fig. 9) showed prominent ions at m/e 603 (M); 588 (M-15), loss of CH₂; 572 (M-31), loss of OH; 513 (M-90), loss of Me₃SiOH; 482 [M-(90 + 57) + 1], loss of [90 + (CH₂)₂CH₂=CH; 462 [M-(90 + 31 + 57) + 1]; 400 [M-(113 + 90)], loss of CH₂=CH=CH₂; 349 [M-(2 x 90 + 31)]; 371 [M-(142 + 90)], loss of [CH₂=CH=CH₂; 328 (M-113); 297 (M-142)]; 371 [M-(142 + 90)], loss of [CH₂=CH=CH₂; 328 (M-113); 297 (M-142)]; 276 [M-113]; 258 [M-(90 + 113)]; 258 [M-113]; 258 [M-(90 + 113)]; 238 (M-142]; 219 [M-113]; 219 [M-113]; 219 [M-113]. Clues to the structure of M4a were found in the base ion m/e 258 and the prominent ion at m/e 219. Confirmation that the ion at m/e 219 contained two Me₃Si groups and the ion at m/e 258 contained one Me₃Si and one O-methyloxime group was obtained by analysis of [²H₄]Me₃Si and trideuteromethoxime derivatives. A base ion at m/e 258 is also found in the mass spectrum of the O-methyloxime, Me₃Si ether derivative of the methyl ester of 15-keto-13,14-dihydro-TxB₂ (18), and, therefore, suggested that M4a was an acyclic compound in which dehydrogenation of the alcohol group at the original C 15 position and reduction of the original Δ₁₃ double bond had occurred. The conspicuous absence of a prominent ion at m/e 174 suggested that an acyclic aldehyde-alcohol structure was not present as is typically seen after formation of O-methyloxime, Me₃Si ether derivative of TxB₂ and TxB₂ metabolite with a hemiacetal ring (cf. Compound C1) (10, 18). However, a prominent ion at m/e 219 is also found in the mass spectrum of the methyl ester, Me₃Si ether derivative of the product formed by treatment of TxB₂ with sodium borohydride and indicates an acyclic structure with Me₃Si groups attached at the original C-11 and C-12 positions. The ion at m/e 400 formed in part by the loss of 113 mass units from the carboxyl side chain also suggested that M4a had undergone one step of β oxidation to a C18 derivative.

From these data it was, therefore, concluded that Compound M4a was 6-(1,3-dihydroxypropyl)-7-hydroxy-10-oxo-3-pentadecanoic acid.

The methyl ester of M4b was then converted to a Me₃Si ether derivative and analyzed by gas chromatography-mass spectrometry. A single radioactive peak was present with a C value of 22.2. The mass spectrum of this derivative of M4b
FIG. 10. Mass spectrum of the methyl ester, Me3Si ether derivative of Compound M4b.

(Fig. 10) showed prominent ions at m/e 528 (M); 513 (M-15), loss of CH2=CH-COOC2H5; 482 (M-46), loss of CH3CH2OH; 457 (M-71), loss of CH2=CH-COOC2H5; 432 (M-90), loss of a MeSi group, eliminated from the ring; 321 [M-(90 + 71 + 46)]; 301, probably [Me3SiO'-CH=CH-CH=CH(OSiMe3)-CH=CH2-COOCH3]; and 228, probably derived from two sources: (a) [Me3SiO'=CH-(CH2)CH2(OH)2], and (b) Me3SiO'=CH-CH=CH-CH(OSiMe3)-H. The base ion at m/e 228 suggested that this compound had in part been formed by one step of β oxidation resulting in the loss of 2 carbons from the upper side chain (see structure of Compound C1). Two Me3Si groups were indicated by analysis of the [1H]Me3Si ether derivative and were assigned locations at C-7 by a shift of m/e 228 to m/e 237 and at C-13 by a shift of m/e 217 to 218. The absence of a trimethylsilyl group at C-9 was suggested by the absence of the ion m/e 217 which is prominent in the mass spectrum of the Me3Si ether derivative of the methyl ester of dinor-TxB2. Dehydration of the hydroxyl group at C-9 would account for the loss of the C-9 hydroxyl group and would render the molecule less polar than dinor-TxB2. However, the expected molecular ion for the Me3Si ether derivative of the methyl ester of this compound would be m/e 482. The molecular ion seen in the mass spectrum of M4 was m/e 528. These data suggested, therefore, that instead there was a relatively nonpolar group comprising 43 mass units attached to C-9. A reasonable structure that would satisfy these requirements would be 9-O-ethyl-dinor-TxB2. Using thrombanoic acid nomenclature, M4b is Sa,15(S)-dihydroxy-11-ethoxy-2,3-dinorthromba-5,13-dienoic acid.

Fig. 11. Conversion of the ethyl ester of thromboxane B2, to the two epimers of mono-O-ethyl-thromboxane B2 ethyl ester by reaction with acetyl chloride in ethanol.
been generated artificially in vitro from dinor-TxB₂ in the presence of ethanol under acidic conditions during the urine purification procedures. To investigate this possibility, 200 μg of [³H]TxB₂ (6.9 μCi/μmol) were dissolved in a 20% solution of ethanol in 0.9% NaCl solution (saline), kept at room temperature for 3 h, added to 100 ml of human urine, and then subjected to identical urine purification procedures as employed in the processing of the monkey urine. This resulted only in the recovery of unchanged TxB₂. We were, therefore, not able to reproduce the formation of a mono-O-ethylated derivative from TxB₂ by our urine purification procedures.

The other predominant ring structure found in several metabolites, δ-lactone, would not be expected to form a mono-O-ethylated derivative at the original C-11 position in the presence of ethanol and acid. To confirm this, however, 11-dehydro-TxB₂ methyl ester was reacted with methanolic HCl analogous to the ethanolic HCl treatment of TxB₂. Methanolic HCl was used because of the presence of the methyl ester in our chemically synthesized compound. We have shown that methanolic HCl is as equally effective in converting TxB₂ to mono-O-methyl-TxB₂ as ethanolic HCl is in forming the mono-O-ethyl derivative. When the products of this reaction were converted to Me₃Si ether derivatives and analyzed by gas chromatography-mass spectrometry, 11-dehydro-TxB₂ was found to have been converted to two major compounds. The identification of these compounds was not rigorously pursued, but neither had a retention time or mass spectrum compatible with 11-O-methyl-TxB₂.

It appears that M₄b was formed prior to the first reversed phase partition chromatographic step (Solvent System D) since mono-O-ethyl-dinor TxB₂ (M₄b) was separated from dinor-TxB₂ (Cl) by this chromatography. If mono-O-ethyl-dinor-TxB₂ was formed in vitro prior to this chromatography, the only possible procedure where compounds were possibly exposed to ethanol in the presence of acid is XAD-2 chromatography. To evaluate this possibility, TxB₂ as well as 11-dehydro-TxB₂ methyl ester were added to water, acidified to pH 3.2, and subjected to XAD-2 chromatography. Two prominent peaks emerged and were designated Compound M₂a (40 to 43 ml of eluate, 18% of recovered radioactivity) and Compound M₂b (48 to 53 ml, 41%). M₂a represented about 1.5% and M₂b about 3.5% of the total radioactivity in the urine.

M₂b had an identical elution volume on μ-Porasil chromatography as parent TxB₂. The methyl ester of M₂b was converted to a O-methyloxime, Me₃Si ether derivative, and analyzed by gas chromatography-mass spectrometry. A single radioactive peak was present with an assigned C value of 23.7, identical with that of TxB₂. The mass spectrum recorded for this derivative of M₂b showed ions at m/e 629 (M), 614, 598, 558, 539, 526, 508, 468, 449, 436, 418, 366, 310 (base), 211, 174, and 173. This mass spectrum was identical with that of the O-methyloxime, Me₃Si ether derivative of the methyl ester of authentic TxB₂. In addition, when the Me₃Si ether derivative of the methyl ester of M₂b was analyzed by gas chromatography-mass spectrometry, a mass spectrum was recorded that was identical with that obtained for the same derivative of TxB₂ (10, 18). It was, therefore, concluded that M₂b was unchanged TxB₂.

The methyl ester of M₂a was converted to a Me₃Si ether derivative and analyzed by gas chromatography-mass spectrometry. A prominent peak was found with a C value of 20.2 and the mass spectrum recorded for this derivative of M₂a (Fig. 12) showed prominent ions at m/e 502 (M), 487 (M-15), loss of CH₃OH; 456 (M-46), loss of CH₂OH; 431 (M-71), loss of (CH₂)₂CH, 412 (M-90), loss of Me₃SiOH; 341 [(M-90 + 71)], 312 (M-190), loss of [O(CH₂)₃CH₂CH₂CH=O(SiMe₃)], eliminated from the ring; 301, probably [(Me₃SiO)=CH-CH=CH-(CH₂)₂COOCH₃], formed by rearrangement; 295 [(M-90 + 71 + 46)]; 292 [(Me₃SiO)=CH-CH=CH-CH₂CH₂OH; 287, probably derived from two sources: (a) Me₃SiO=CH-(CH₂)₂CH₂OH, and (b) [(Me₃SIO)=CH-CH=CH-(CH₂)₂COOCH₃]; and 173, probably derived from two sources: (a) Me₃SiO=CH-(CH₂)₂CH₂OH, and (b) [(Me₃SIO)=CH-CH=CH-(CH₂)₂COOCH₃].

In contrast, as described above, in the presence of ethanol and acid, the two mono-O-ethyl epimers are formed in approximately equal abundance and are separated by high performance liquid chromatography and gas-liquid chromatography. Two prominent peaks emerged and were designated Compound M₂a (40 to 43 ml of eluate, 18% of recovered radioactivity) and Compound M₂b (48 to 53 ml, 41%). M₂a represented about 1.5% and M₂b about 3.5% of the total radioactivity in the urine.

![Fig. 12. Mass spectrum of the methyl ester, Me₃Si ether derivative of Compound M₂a.](https://example.com/f12.png)
the loss of 46 mass units, and the ion m/e 312, formed by the loss of 190 mass units. This pattern of fragmentation is analogous to that resulting in the formation of ions m/e 482, 321, and 338, respectively, in the mass spectrum of the Me3Si ether derivative of the methyl ester of mono-O-ethyl-dinor TxBz (see structure of M4). These data and other reasoning analogous to that outlined for the determination of the structure of M4 suggested that this metabolite had an ethoxy group attached at C-7.

It was, therefore, concluded that Compound M2a was 11-O-ethyl-2,3,4,5-tetranor TxBz, or 2 (3 hydroxyl 1 octenyl) 3 (2-carboxyethyl)-4-hydroxy-6-ethoxy-tetrahydropyran. Using thrombanoic acid nomenclature, M2a is 9α,15(S)-dihydroxy-11-ethoxy-2,3,4,5-tetranorthromb-13-enoic acid.

Identification of Compounds Eluting in Area C—Material that eluted in Area C (Fig. 3) was subjected to reverse phase partition chromatography using Solvent System C-45 (Table I). Three radioactive peaks were present (Fig. 13), C1 (5 to 85 ml of effluent, 67% of recovered radioactivity), C2 (90 to 135 ml, 18%), and C3 (140 to 225 ml, 15%).

Structure of Compound C1—Material in Peak C1 was further purified by υ-Porasil high performance liquid chromatography. A single prominent peak emerged (50 to 56 ml of effluent, 45% of recovered radioactivity), designated Compound C1, along with several less polar unresolved peaks. Compound C1 isolated from this chromatography represented approximately 12% of the total radioactivity present in the urine. An additional amount of Compound C1, 2.5% of the total radioactivity in the urine, was also isolated from Peak B (see “Structure of Compounds in Peak B2”). The total amount of C1, therefore, represented approximately 14.5% of the total radioactivity present in the urine. The identification of Compound C1 was recently reported as dinor-TxBz, the hemiacetal derivative of 6-(1-hydroxy-3-oxopropyl)-7,10(S)-dihydroxy-3,8-pentadecadienoic acid (10). Using thrombanoic acid nomenclature, dinor-TxBz is 9α,11,15(S)-trihydroxy-2,3-dinorthromb-5,13-dienoic acid. Identification was based on mass spectral analysis of C1 and its reduced product and the formation of an identical compound from TxBz in vitro by incubation with rat liver mitochondria.

Material in Peak C2 could not be sufficiently purified by subsequent chromatographic procedures to permit identification.

Structure of Compound C3—Material in Peak C3 was further purified by υ-Porasil high performance liquid chromatography. A single prominent peak was present (31 to 35 ml of effluent, 65% of recovered radioactivity) which was designated Compound C3. C3 represented approximately 4% of the total radioactivity present in the urine.

The methyl ester of C3 was converted to an O-methyloxime, Me3Si ether derivative and analyzed by gas chromatography-mass spectrometry. Two peaks were present with C values of 25.0 and 25.2. The mass spectra recorded for these two peaks were essentially the same except for minor variation in ion intensities and most likely represented syn-anti isomerism of the O-methyloxime groups. The mass spectrum of the less polar isomer is shown in Fig. 14. Ions of high intensity were found at m/e 455 (M); 440 (M-15), loss of -CH2; 424 (M-31), loss of -OCH3; 412 (M-43), loss of -(CH2)2CH:; 399 (M-56), loss of [-(CH2)2CH-CH2-H]; 368 [M-(31 + 56)]; 342 (M-113), loss of -(CH2)2CH=CH-CH2OH; 313 (M-142), loss of -C=CH2-O=NOCH2CH2CH2CH3; 294, formed either by M-(90 + 71), loss of [Me3SiOH + (CH2)2CH-], or M-(160 + 1), loss of [-(OCOCH2CH=CH=O) + H], eliminated from the ring; 238 [M-(160 + 56 + 1)], loss of [-(OCOCH2CH=O) + Me3Si]+; 223 (M-(90 + 142)); 156 *(CH2)2-C-NO-
CH3)(CH2)3CH2OH], 143 [{CH3-CH=CH(COOCH3)CH2OCH2CH2-CH2OCH2CH2-CH2OH} - 11, and 128 [{CH3-CH=CH(COOCH3)CH2OCH2CH2-CH2OH} - 26]. From the behavior of C3 on \( \mu \)-Porasil and gas-liquid chromatography systems C3 was judged significantly less polar than TxBz on the former and more polar than TxBz on the latter. This apparent disparity regarding polarities in these systems suggested that C3 may be a lactone (cf. Compound M3a). The presence of a methoxime group and its assignment to the various fragmentation ions was confirmed by mass spectral analysis of the triester-four-omethoxime derivative. The ion at m/e 342, formed by the loss of 113 mass units from the carboxyl side chain, indicated that the formation of C3 had probably involved one step of \( \beta \) oxidation, resulting in the loss of 2 carbons from the upper side chain. These data, therefore, suggested that C3 had been formed from TxBz by dehydrogenation of the alcohol group at C 11, reduction of the \( \Delta^\alpha \) double bond, dehydrogenation of the alcohol group at C 15, and \( \beta \) oxidation to a C 18 derivative.

To obtain additional evidence for the proposed structure of C3, the methyl ester was treated with sodium methoxide in methanol, converted to an O-methyloxime, Me3Si ether derivative, and analyzed by gas chromatography-mass spectrometry. This treatment would be expected to convert the \( \delta \)-lactone structure to a dicarboxylic acid methyl ester form (cf. Fig. 8). When the product of this reaction was analyzed by gas chromatography, a peak with a C value of 23.0 was present, and the mass spectrum showed ions of high intensity at m/e 559 (M); 544 (M-19), loss of -CH3; 528 (M-31), loss of -OCH3; 469 (M-90), loss of Me3SiOH; 438 [M-(M-90 + 31)]; 258 (base ion) [Me3SiO'CH(CH2)3C-CHCH3OCH2CH2CH2OH], and 175 [Me3SiO'CHCH2COOCH2CH2CH2OH]. The difference in C values of C3 and the product formed after treatment with sodium methoxide (\( \Delta^\alpha \)) is compatible with C3 being converted from a \( \delta \)-lactone to a dicarboxylic acid methyl ester form (cf. Compounds M3a and M3b). The base ion at m/e 258 was predicted since the same major fragment ion is seen in the mass spectrum of the O-methyloxime, Me3Si ether derivative of the methyl ester of 15-keto-13,14-dihydro-TxB2, which has a \( \delta \)-lactone structure. The ion at m/e 175 was also expected and is indicative of a methyl ester group at C 9 (cf. Compound M3b).

From these data it was, therefore, concluded that Compound C3 was the \( \delta \)-lactone of 3-hydroxy-4-(1-hydroxy-4-oxononyl)-5-nonenedioic acid or 11-dehydro-15-keto-13,14-dihydro-TxBz, which has a \( \delta \)-lactone structure. The base ion at m/e 175 was also expected and is indicative of a methyl ester group at C 9 (cf. Compound M3b).

Identification of Compounds Eluting in Peak B—Fifty per cent of the material eluting in Peak B was converted to a methyl ester and 50% to an ethyl ester. The methyl and ethyl esters were separately subjected to reverse phase partition chromatography using Solvent System F-50 (Table I). Analogous chromatographic results were obtained for both esters. Two prominent peaks emerged in the chromatography of the methyl esters, designated B1 (20 to 35 ml of effluent, 20% of recovered radioactivity) and B2 (40 to 70 ml, 30%) (Fig. 15). Thirty-three per cent of recovered radioactivity remained on the stationary phase. Material in Peak Bl was subsequently subjected to p-Porasil high performance liquid chromatography, a peak with a C value of 23.9 was present, and the mass spectrum showed ions of high intensity at m/e 559 (M), 544 (M-19), loss of -CH3; 528 (M-31), loss of -OCH3; 469 (M-90), loss of Me3SiOH; 438 [M-(M-90 + 31)]; 258 (base ion) [Me3SiO'CH(CH2)3C-CHCH3OCH2CH2CH2OH], and 175 [Me3SiO'CHCH2COOCH2CH2CH2OH]. The difference in C values of C3 and the product formed after treatment with sodium methoxide (\( \Delta^\alpha \)) is compatible with C3 being converted from a \( \delta \)-lactone to a dicarboxylic acid methyl ester form (cf. Compounds M3a and M3b). The base ion at m/e 258 was predicted since the same major fragment ion is seen in the mass spectrum of the O-methyloxime, Me3Si ether derivative of the methyl ester of 15-keto-13,14-dihydro-TxB2, which has a \( \delta \)-lactone structure. The ion at m/e 175 was also expected and is indicative of a methyl ester group at C 9 (cf. Compound M3b).

From these data it was, therefore, concluded that Compound C3 was the \( \delta \)-lactone of 3-hydroxy-4-(1-hydroxy-4-oxononyl)-5-nonenedioic acid or 11-dehydro-15-keto-13,14-dihydro-TxBz, which has a \( \delta \)-lactone structure. The base ion at m/e 175 was also expected and is indicative of a methyl ester group at C 9 (cf. Compound M3b).

Identification of Compounds Eluting in Peak B—Fifty per cent of the material eluting in Peak B was converted to a methyl ester and 50% to an ethyl ester. The methyl and ethyl esters were separately subjected to reverse phase partition chromatography using Solvent System F-50 (Table I). Analogous chromatographic results were obtained for both esters. Two prominent peaks emerged in the chromatography of the methyl esters, designated B1 (20 to 35 ml of effluent, 20% of recovered radioactivity) and B2 (40 to 70 ml, 30%) (Fig. 15). Thirty-three per cent of recovered radioactivity remained on the stationary phase. Material in Peak Bl was subsequently subjected to \( \mu \)-Porasil high performance liquid chromatography, and only multiple small unresolved peaks were present, which were not studied further.

Structures of Compounds in Peak B2—Material in Peak B2 was further purified by \( \mu \)-Porasil high performance liquid chromatography, and two prominent peaks emerged. The more polar peak (42 to 45 ml of effluent, 24% of recovered radioactivity) was designated Compound B2 and represented approximately 1% of the total radioactivity present in the urine. The less polar peak (37 to 41 ml of effluent, 41% of recovered radioactivity) was additionally purified by \( \mu \)-Porasil high performance liquid chromatography under isocratic conditions using the solvent ether/methanol/acetic acid (90:1:2) (v/v/v). Two radioactive peaks emerged at 14 to 18 ml of effluent (60% of recovered radioactivity) and 22 to 25 ml of effluent (30%). Material in the less polar peak was subsequently analyzed by gas chromatography-mass spectrometry and found to have an identical retention time and mass spectrum as Compound C1 and, therefore, was identified as dinor-TxBz. The elution of a relatively small quantity of dinor-TxBz in Peak B was undoubtedly due to incomplete resolution of compounds in Peak B and Area C (see Fig. 9).
Thromboxane B₂ Metabolism

FIG. 16. Mass spectrum of the methyl ester, Me₃Si ether derivative of Compound B₂.

1 and ω-2 hydroxylated metabolites (20, 21).

We, therefore, concluded that compound B₂ is 11-O-ethyl-19-hydroxy-2,3-dinor-TxB₂ or 2-(3,7-dihydroxy-1-octenyl)-3-(4-carboxy-2-buteryl)-4-hydroxy-6-ethoxy-tetrahydropyran. Using thrombanoic acid nomenclature, Compound B₂ is 9α, 15(S),19-trihydroxy-11-ethoxy-2,3-dinorthromboxa-5,13-dienoic acid.

Identification of Compounds Eluting in Peak A—Sixty percent of the material in Peak A was converted to a methyl ester and 40% to an ethyl ester before subsequent purification. The methyl and ethyl esters were purified separately by identical procedures with analogous chromatographic results. The methyl ester fraction was initially subjected to high performance liquid chromatography using a μ-Bondapak column (see under "Experimental Procedures"). A single prominent peak emerged, designated A₁ (29 to 33 ml of effluent, 20% of recovered radioactivity), along with more polar unresolved material (Fig. 17). Material in Peak A₁ was further purified by μ-Porsil high performance liquid chromatography. A single prominent peak was present which was designated Compound A₁ (25 to 26 ml of effluent, 70% of recovered radioactivity). Compound A₁ represented approximately 1.5% of the total radioactivity present in the urine.

Structure of Compound A₁—The methyl ester of A₁ was converted to an O-methyloxime, Me₃Si ether derivative, and analyzed by gas chromatography-mass spectrometry. A radioactive peak was present with a C value of C 26.2. As noted previously, the apparent disparity in polarity characteristics of A₁ as judged by its behavior on μ-Porsil and gas-liquid chromatography again suggested the possibility of a lactone ring structure. The mass spectrum recorded for this derivative (Fig. 18) showed ions of high intensity at m/e 473 (M); 458 (M-15), loss of CH₃; 442 (M-31), loss of OCH₃; 386 (M-87), loss of (CH₂)₃COOCH₃; from either upper or lower side chain; 373 (M-100), loss of [(CH₂)₃COOCH₃ - H]; 352 [M-(90 + 31)], loss of [Me₃SiOH + 31]; 312 [M-(160 + 1)], loss of [(OCOCH₃CH=O(SiMe₃) + H], eliminated from ring; 296 [M-(90 + 87)]; 22 [M-(160 + 101)], loss of [160 + (CH₂)₃COOCH₃]; 187 [(CH₂)=NO(CH₂)₃COOCH₃ + H] and 115 [(CH₂)₃COOCH₃]. The presence of one methoxime group was confirmed by analysis of the trideuteromethoxime derivative. A dicarboxylic acid structure was suggested by an increase in C value of 1.0 on gas-liquid chromatography of the ethyl ester compared to the methyl ester and was confirmed by mass spectrometry where the molecular ion of the methyl ester, m/e 473, was found to shift 28 mass units to m/e 501 in the mass spectrum of the ethyl ester. The formation of ions at m/e 312 and m/e 212 by elimination in part of 160 mass units is characteristic of the δ-lactone ring structure (see identification of structures of M₃a and C₃).

This study has demonstrated that TxB₂ in the monkey is converted to a variety of metabolites that are rapidly excreted into the urine. We had previously identified the major urinary metabolite as dinor-TxB₂ (10) as has also been found by Kindahl (22). The present paper has described the isolation and structural identification of seven additional urinary metabolites as well as the excretion of a small amount of unchanged TxB₂.

DISCUSSION

This study has demonstrated that TxB₂ in the monkey is converted to a variety of metabolites that are rapidly excreted into the urine. We had previously identified the major urinary metabolite as dinor-TxB₂ (10) as has also been found by Kindahl (22). The present paper has described the isolation and structural identification of seven additional urinary metabolites as well as the excretion of a small amount of unchanged TxB₂.

One major pathway of metabolism of TxB₂ is via one step of β oxidation resulting in the formation of dinor-TxB₂, the most abundant metabolite. The second major pathway of metabolism results from dehydrogenation of the alcohol group at C-11, reduction of the Δ¹³ double bond, dehydrogenation of the alcohol group at C-15, ω oxidation, and β oxidation to a C₁₆ derivative. A₁ is, therefore, the δ-lactone of 3,5-dihydroxy-4-(2-carboxyethyl)-8-oxo-tridecanedioic acid or 11-dehydro-15-keto-13,14-dihydo-19-carboxyl-2,3,4,5-tetranor-TxB₂. Using thrombanoic acid nomenclature, A₁ is 9α-hydroxy-11,15-dioxo-2,3,4,5-tetranorthromboxa-1,20-dienoic acid.

Double bond reduction. A minor metabolite reflects that metabolic transformation of TxB2 also occurs by a process of reduction, resulting in an acyclic compound with hydroxyl groups attached at the original C-11 and C-12 positions. This metabolite has also undergone one step of \( \delta \) oxidation, dehydrogenation of the alcohol group at the original C-15 position, and reduction of the original \( \Delta^{15} \) double bond. Three additional metabolites were also identified that were mono-\( \Delta \)-ethylated derivatives at the original C-11 position of TxB2. Table II lists the percentage of total recovered radioactivity represented by each compound identified.

The origin and significance of the three mono-\( \Delta \)-ethylated derivatives is not clear at this time. It is not entirely certain whether the ethoxy group was adducted in vivo or in vitro. If the derivatives had been formed in vitro, one might expect to find the same ratio of parent compound to mono-\( \Delta \)-ethylated compound for all three compounds as was found for dinor TxB2 and mono-\( \Delta \)-ethyl-dinor-TxB2 (approximately 2.5:1). However, no parent compounds with a hydroxyl group at the original C-11 position were found corresponding to \( \delta \)-ethyl-tetranor-TxB2 or \( \delta \)-ethyl-19-hydroxy-dinor-TxB2. In addition, as previously outlined, because we were not able to reproduce the formation of a mono-\( \Delta \)-ethylated derivative by our urine purification procedures, there is evidence they were present before the initial reversed phase partition chromatography, and the fact that only one epimer of each compound was isolated all strongly suggest that these derivatives were not formed in vitro. The other remaining possibility is that an ethoxy group was adducted in vivo, either from the small amount of ethanol (1 ml) used to make a 20% solution of ethanol in saline as a vehicle for infusion of TxB2 or from some endogenous source. Future experiments are being planned to investigate these various possibilities.

It is assumed that the double bond in the dinor metabolites is in the \( \Delta^1 \) position. Although the amount of these metabolites did not permit chemical degradation to confirm this assumption, it seems unlikely that double bond isomerization from the \( \Delta^1 \) to the \( \Delta^2 \) position by action of the enzyme \( \Delta^1 \)-cis=\( \Delta^2 \)-trans-eneoyl-CoA isomerase (23) has occurred, as such isomerization has not been described for dinor prostaglandin metabolites previously identified (24, 25).

The proposed pathways of TxB2 metabolism in the monkey are illustrated in Fig. 19. A small quantity of unchanged TxB2 was recovered from the urine. The three mono-\( \Delta \)-ethylated metabolites have been excluded from the figure because of their somewhat obscure mechanism of formation. The major pathway of metabolism is through \( \delta \) oxidation to dinor-TxB2 (C1). Although we have demonstrated that in vitro TxB2 can in part be additionally \( \delta \)-oxidized to a \( \Delta \)-derivative and have identified two minor \( \Delta \)-oxidized urinary metabolites (A1, M2a), in contrast to PGE2 and PGF2\(_\alpha\), the formation of \( \Delta \)-metabolites apparently is not a major pathway of thromboxane metabolism in vivo in the monkey.

The other major pathway of thromboxane metabolism involves dehydrogenation of the alcohol group at the C-11 position. The second most abundant metabolite isolated was the \( \delta \)-lactone 11-dehydro-TxB2 (M3). The other two metabolites identified with the \( \delta \)-lactone ring structure had been degraded by additional processes of \( \delta \) oxidation, dehydrogenation of the alcohol group at the original C-15 position, and reduction of the \( \Delta^{15} \) double bond. The \( \Delta \)-compound had also undergone \( \omega \) oxidation. Since \( \delta \)-oxidized prostaglandins have been shown to be poor substrates for the prostaglandin dehydrogenase enzyme (26), it seems reasonable to propose that 11-dehydro-TxB2 initially undergoes dehydrogenation of the alcohol group at C-15 and \( \Delta^{15} \) double bond reduction as an intermediate step prior to subsequent \( \delta \) oxidation to a \( \Delta \)-derivative (C3). C3 is probably further degraded by an additional step of \( \delta \) oxidation to a \( \Delta \)-derivative as well as \( \omega \) oxidation, resulting in the formation of metabolite A1. As indicated in Fig. 19, it is suggested that this second step of \( \delta \) oxidation occurs prior to \( \omega \) oxidation, although the reverse sequence of events cannot be excluded.

![Figure 18](http://www.jbc.org/)

**Fig. 18.** Mass spectrum of the methyl ester, methoxime, Me3Si ether derivative of Metabolite A1.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Percentages of total recovered urinary radioactivity represented by identified compounds</th>
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<tbody>
<tr>
<td>Compound</td>
<td>Percentage</td>
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<tr>
<td>A1</td>
<td>1.5</td>
</tr>
<tr>
<td>B2</td>
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<td>M4a</td>
<td>1.8</td>
</tr>
<tr>
<td>M4b</td>
<td>5.4</td>
</tr>
</tbody>
</table>

A minor pathway of metabolism was indicated by metabolite M4a. This compound had been formed by a process of reduction resulting in an acyclic structure with hydroxyl groups attached at the original C-11 and C-12 positions. This is envisioned to occur by reduction of the aldehyde group in the acyclic aldehyde-alcohol form of TxB2. In addition, dehydration of the original C-15 alcohol group and reduction of the original Δ15 double bond had occurred. As indicated in Fig. 19, we have proposed that reduction of the aldehyde group occurs prior to dehydration of the C-15 alcohol group and Δ13 double bond reduction. This seems reasonable since there were no 15-keto-13,14-dihydro metabolites identified with an intact hemiacetal ring structure, suggesting that structural modification of the ring improves the ability of the compound to act as a substrate for dehydration of the C-15 alcohol group. The 15-keto-13,14-dihydro derivative is also proposed as being formed prior to β oxidation to the C18 derivative, since β-oxidized prostaglandins have been shown to be poor substrates for the prostaglandin dehydrogenase enzyme than parent TxB2. Further in vitro studies will be necessary, however, to confirm these assumptions. In addition, although 15-keto-13,14-dihydro-TxB2 has been shown to be formed by the immune-challenged guinea pig lung (19), formation of this metabolite in vivo by the nonimmunologically challenged monkey apparently is not a significant pathway of metabolism.

β oxidation, ω oxidation, dehydrogenation of the alcohol group at C-15, and reduction of the Δ15 double bond have all been described as common processes in the metabolism of prostaglandins (24, 25). Although TxB2 is not a prostaglandin because of the hemiacetal ring, dehydration of the alcohol group at C-11 has not been previously described in the metabolism of prostaglandins. Therefore, the enzyme that may be responsible for this transformation of TxB2 is at present unknown. In addition the enzyme responsible for reduction of the aldehyde group in the acyclic aldehyde-alcohol form of TxB2 is at present unknown. In addition to conversion to 8-(1,3-dihydroxypropyl)-9-hydroxy-12-oxo-5-heptadecaenoic acid, we have also been able to demonstrate conversion of TxB2 to 11-dehydro-TxB2 by incubation of TxB2 with the 100,000 g supernatant of guinea pig liver. A boiled control resulted in no conversion. These initial in vitro experiments, therefore, suggest that 11-dehydrogenation and aldehyde reduction are at least enzymatic processes involving soluble enzymes.

The structural identification of the TxB2 metabolites described in the present study has provided the initial background chemical information necessary for future detailed studies of the sequences and mechanisms of these biochemical transformations and has provided a basis for the eventual quantification of the excretion of TxB2 metabolites as an index of TxB2 synthesis in vivo.

Acknowledgments—We gratefully acknowledge the valuable advice and suggestions of Dr. Douglas S. Taber. We are indebted to Dr. N. A. Nelson of the Upjohn Co. for the generous gift of 11-dehydro-TxB2 methyl ester. The skillful technical assistance of J. L. Morgan and N. A. Payne was greatly appreciated.

Addendum—Since submission of this manuscript additional data has been obtained that has helped to clarify the somewhat uncertain origin of the three mono-O-ethylated derivatives described in the present work. We have now performed a similar study of TxB2 metabolism in man. In this study, however, TxB2 was infused as a sodium salt instead of dissolved in ethanol/saline and wherever ethanol had been used in the isolation and purification of metabolites from the monkey, methanol was substituted. Compounds identified...
in the study in man have included mono-O-methyl-TxBz, mono-O-methyl-dinor-TxBz, and mono-O-butyl-dinor-TxBz. No mono-O-ethylated derivatives have been found. These data imply that the formation of these compounds in the study in man occurred in vitro from the exposure of the hemiacetal ring to methanol or butanol (Solvent System D) under acidic conditions and strongly suggest that the mono-O-ethylated compounds isolated from monkey urine were not formed in vivo but were formed in an analogous fashion in vitro from exposure of dinor-TxBz, tetranor-TxBz, and 19-hydroxy-dinor-TxBz to ethanol under acidic conditions during urine processing.

REFERENCES

Metabolism of thromboxane B2 in the monkey.
L J Roberts, 2nd, B J Sweetman and J A Oates


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