The metabolism of thromboxane B₂ in man

IDENTIFICATION OF TWENTY URINARY METABOLITES*

(Received for publication, December 5, 1980, and in revised form, April 23, 1981)

L. Jackson Roberts, II, Brian J. Sweetman, and John A. Oates†
From the Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232

[³H]Thromboxane B₂ (12.2 Ci/mol) was infused into a healthy adult male. Urinary metabolites of thromboxane B₂ were isolated by reversed phase partition chromatography and high performance liquid chromatography. Structural identification of metabolites was accomplished by gas chromatography-mass spectrometry. Twenty metabolites were identified. Three primary pathways of metabolism of thromboxane B₂ were found. A small quantity of thromboxane B₂ was excreted unchanged, representing 2.5% of total recovered radioactivity. Two additional metabolites retained the original thromboxane B₂ hemiacetal ring; one of these metabolites, 2,3-dinor-thromboxane B₂, was the major urinary metabolite and represented 25.0% of total recovered radioactivity. The other, 2,3,4,5-tetranor-thromboxane B₂, represented 5.3% of total recovered radioactivity. Two metabolites representing 1.1% of total recovered radioactivity had initially undergone reduction of the hemiacetal ring and indicated a second but relatively minor pathway of metabolism. A major pathway of metabolism was found to involve dehydrogenation of the hemiacetal alcohol group of thromboxane B₂ resulting in a series of metabolites with a δ-lactone ring. Sixteen metabolites representing 29.3% of total recovered radioactivity were identified as products of this pathway of metabolism.

Thromboxane A₂ is a labile metabolite of prostaglandin G₂ with potent biological activity (1). TxA₂, contracts vascular smooth muscle including that of the coronary arteries (2) and induces irreversible platelet aggregation (1). A means to assess production of thromboxanes in vivo in man should have important biological applications as a tool to investigate the role of thromboxanes in human disease. Since thromboxanes are released in substantial quantities from aggregating platelets, quantification of in vivo thromboxane production may provide a means to assess in vivo platelet aggregation and lead to a better understanding of the role of platelets in the pathophysiology of many cardiovascular diseases. It may also provide a means to assess the in vivo efficacy of anti-platelet drug therapy.

It is well established that quantification of circulating or urinary prostaglandin metabolites represents a more reliable means of assessing endogenous prostaglandin synthesis in vivo than does quantification of the parent compound (3, 4). Therefore, we initially investigated the metabolic fate of TxB₂ in the non-human primate. The major urinary metabolite was found to be 9a,11,15(S)-trihydroxy-2,3-dinor-thromboxa-5Z,13E-dienoic acid (2,3-dinor-TxB₂) (5), as was also reported by Kindahl (6). We found another major pathway of metabolic transformation to involve dehydrogenation of the hemiacetal alcohol group at C-11, resulting in the formation of a series of metabolites with a δ-lactone ring structure (7).

Before quantitative studies of thromboxane synthesis in vivo in man are possible, however, pathways of human thromboxane metabolism must be defined. This present work describes our investigation into the metabolic fate of TxB₂ in man. A preliminary report of part of this work describing the identification of the major urinary metabolite as 2,3-dinor-TxB₂ has been published earlier (8). The description of identified metabolites in this paper adheres to the recently proposed nomenclature for thromboxanes (9). Although not confirmed, the stereochemistry of identified metabolites has been presumed to have been unaltered by metabolic transformation of TxB₂.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

At no time during the infusion of TxB₂ were there any significant changes in blood pressure or pulse rate and no clinically apparent adverse effects were observed. Seventy-four % of the infused radioactivity was recovered in the urine within 13 h.

The urine collected was extracted with Amberlite XAD-2 chromatography and the radioactivity was quantitatively eluted with 1600 ml of methanol. The residue obtained after evaporation of the methanol was dissolved in ethyl acetate and applied to a silicic acid column. Ninety-one % of the

* This work was supported by National Institutes of Health Grant GM15431 and Public Health Service Grant GM00113. This work was presented in part at the 1978 Winter Prostaglandin Conference, Sarasota, FL, and at the Seventh International Congress of Pharmacology, Paris, France, 1978. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The Joe and Morris Werthan Professor of Investigative Medicine.

‡ Portions of this paper (including all of "Experimental Procedures" and most of "Results" which describe chromatographic purification of metabolites and the mass spectral data pertaining to their structural elucidation) are presented in miniprint at the end of this paper. In the standard print section of "Results" are summarized the overall findings of this work. Miniprint is easily read with the aid of standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD. 20814. Request Document No. 80M-299, cite author(s), and include a check or money order for $26.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
applied radioactivity was eluted with 1190 ml of ethyl acetate.

Further sample purification and initial compound isolation was effected by reversed phase partition chromatography on a support of 45 g of Hyflo Super-Cel using the solvent system water:n-butyl alcohol:acetic acid (300:100:4) (v/v/v). Three polar peaks emerged (Fig. 1) designated Peak A (130 to 160 ml of eluate, 17% of recovered radioactivity), Peak B (370 to 660 ml, 12%), and Peak C (670 to 890 ml, 5%). These were followed by the elution of material not resolved by this solvent system, designated Area D (900 to 2930 ml, 16%) and a large peak of relatively less polar material, designated Peak E (2940 to 4250 ml, 24%). Twenty-six % of the recovered radioactivity remained on the stationary phase and was eluted with 110 ml of methanol and was designated M.

A flow diagram outlining the urine purification procedures that are described in the miniprint section and the isolation and letter-number designation of identified TxB2 metabolites are illustrated in Fig. 2. The letter-number designation of each identified metabolite along with its respective chemical name(s) and structure is shown in Fig. 3. Several metabolites were identified from two or more peaks that were completely resolved chromatographically. Explanations for this finding include the possibility of ion-pairing of metabolites with urine impurities and the existence of more than one structural form such as a δ-lactone and its acid-alcohol form. Table I lists the relative abundances of each metabolite and the total sum of recovered radioactivity identified.

This study has demonstrated that TxB2 is transformed by humans into a variety of metabolites which are excreted into the urine. We have previously identified the major urinary metabolite as 2,3-dinor-TxB2 (8). The present study has described the isolation and structural identification of 19 additional urinary metabolites as well as the excretion of a small quantity of unchanged TxB2.

There are several distinctive features in the metabolism of TxB2 in man which are outlined in Table II. Three separate series of metabolites were categorized, based on ring structure. One series retained the original TxB2 hemiacetal ring. A major pathway of metabolism involved dehydrogenation of the hemiacetal alcohol group at C-11. The proposed pathways of TxB2 metabolism in man are illustrated in Fig. 4. Additional detailed studies of sequences and mechanisms of these biochemical transformations are required to define precisely actual metabolic pathways. Compounds in brackets were not isolated but are proposed intermediates in the formation of identified metabolites.

Only two metabolites in addition to a small amount of unchanged TxB2 were identified with an intact hemiacetal ring, even though these metabolites represented a major por-
Metabolism of Thromboxane B₂ in Man

FIG. 3. Letter-number designation of each identified metabolite along with the respective chemical names and structures.

tion of the total radioactivity in the urine. Both metabolites, 2,3-dinor-TxB₂ and 2,3,4,5-tetranor-TxB₂, were products of β oxidation.

Sensitized guinea pig lungs have been described as capable of converting TxB₂ to 15-keto-13,14-dihydro-TxB₂ (20). The efficiency of this conversion also apparently increases with successive antigenic challenges (21). We have incubated TXB₂ with the 100,000 × g supernatant of guinea pig liver with added NAD⁺ and have not found any conversion of TxB₂ to 15-keto-13,14-dihydro-TxB₂. PGE₂ incubated with the same 100,000 × g liver supernatant was essentially quantitatively converted to 15-keto-13,14-dihydro-PGE₂. These data and the present study suggest that in man and in the absence of immunologic sensitization in the guinea pig that TXB₂ is not a good substrate for the 15-hydroxy-prostaglandin dehydrogenase enzyme. We cannot exclude the possibility, however, that 15-keto-13,14-dihydro-TXB₂ formed may not be excreted into the urine due to possible conversion to 11-dehydro-15-keo-13,14-dihydro-TXB₂. We are presently investigating this latter possibility.

The second major series of compounds, formed as a result of dehydrogenation of the hemiacetal alcohol group of TXB₂, was comprised of 16 identified metabolites. The second most
Metabolism of Thromboxane B₂ in Man

Table I

| Metabolite        | Percentage |
|-------------------|------------|
| E1 + M5b + M7     | 23.0       |
| M3                | 7.6        |
| E2a               | 7.3        |
| D1d + M5b₁        | 5.3        |
| D1c               | 2.6        |
| M₃ + M2           | 2.5        |
| D1a               | 2.0        |
| D2                | 1.6        |
| A₃c               | 1.5        |
| A₃a₁              | 1.3        |
| A₃a₁              | 1.3        |
| M₆                | 1.0        |
| C₂                | 0.9        |
| M₁                | 0.7        |
| B₁                | 0.6        |
| E₂b               | 0.5        |
| M₅c               | 0.4        |
| M₅a               | 0.4        |
| A₃b               | 0.3        |
| M₅d₁              | 0.3        |
| M₅d₂              | 0.1        |
| Total             | 61.2       |

Table II

| Metabolite        | Hemiacetal ring | Ð-Lactone ring | Acyclic C₁₁,C₁₂ diol | Total |
|-------------------|-----------------|----------------|----------------------|-------|
| Ring modifications| ß oxidation stages |                |                      |       |
|                   | %   | %   | %  | %   | %      |       |
| C₂₀              | 50.3 | 4.1 | 18.9 | 5   | 1.1 | 1     | 24.1 | 7 |
| C₁₈              | 37.5 | 1   | 21.6 | 8   | 0.7 | 1     | 59.8 | 10 |
| C₁₆              | 8.7  | 1   | 7.4  | 3   | 0.9 | 0     | 16.1 | 4 |
| Ï oxidation      | 0   | 0   | 10.8 | 6   | 0   | 0     | 10.8 | 6 |
| C₁₅ hydroxy      | 50.3 | 3   | 38.6 | 8   | 1.1 | 1     | 87.0 | 12 |
| C₁₅ Keto         | 0   | 0   | 12.3 | 8   | 0.7 | 1     | 13.0 | 9 |

abundant metabolite in the urine, 11-dehydro-TxB₂, was the product of this single metabolic transformation. Additional extensive metabolism of 11-dehydro-TxB₂ occurred by processes of ß oxidation, Ï oxidation, dehydrogenation of the C-15 alcohol group, and reduction of the ß and ß double bonds. The enzyme responsible for dehydrogenation of the hemiacetal alcohol group is not known. We have found that TxB₂ incubated with the 100,000 × g supernatant of guinea pig liver with NAD⁺ results in efficient dehydrogenation of the hemiacetal alcohol group. The dehydrogenation was very inefficient in the absence of NAD⁺. Therefore, the enzyme appears to be a soluble, NAD⁺-dependent enzyme.

The several compounds identified that had undergone dehydrogenation of the C-15 alcohol group suggests that the 11-dehydro derivatives are better substrates for the 15-hydroxy-prostaglandin dehydrogenase than compounds with an intact hemiacetal ring. It is assumed that the 15-hydroxy-prostaglandin dehydrogenase enzyme is responsible for the dehydrogenation of the C-15 alcohol group although a different enzyme cannot be excluded.

The third minor series of metabolites were acyclic compounds with alcohol groups at C-11 and C-12. This acyclic structure is the same as the sodium borohydride-reduced product of TxB₂ (13). This biochemical transformation is envisioned to occur by a process of reduction of the C-11 aldehyde group of the aldehyde-alcohol form of the original hemiacetal ring. The enzyme responsible for this conversion is unknown. However, TxB₂ is converted in part to this acyclic derivative when incubated with 100,000 × g supernatant of guinea pig liver with added NADPH. The conversion is less extensive in the absence of NADPH or in the presence of NADH. Therefore, appears that this enzyme is a NADH-dependent soluble enzyme.

Two minor metabolites (E₂b and M₅d₁) were identified in which the ß double bond has been reduced. Although reduction of the ß double bond has been described in the metabolism of PGE₃ in the rat (15), this has not been found to occur in the metabolism of PGE₂ and PGE₃ in man, although 5,6-dihydro metabolites may have escaped detection in previous metabolism studies since all urinary radioactivity was not identified (22, 23). The mass spectral data on metabolite A₃a₁ suggests the presence of an oxo group attached on one of the carbon atoms from C₁₆-20 and A₃a₁ was tentatively identified as either 9α,15(S)-dihydroxy-11,19-dioxy-2,3-dinorthrombo-5Z,13E-dienoic acid or 9α,15(S)-dihydroxy-11,20-dioxy-2,3-dinor-5Z,13E-dienoic acid. Analogous metabolites have not been previously described in the metabolism of prostaglandins. Insufficient material was present to permit further structural analysis. The biochemical mechanisms leading to the formation of this oxo group are unknown. One explanation for the location of the oxo group would seem to be at C-19 or C-20 since metabolites of prostaglandins and TxB₂ with hydroxyl groups at the ω-1 and ω-2 positions have been described (16–19).

The formation of 11-O-methyl-TxB₂ (M₂), 11-O-methyl-2,3-dinor-TxB₂ (M₅b), 11-O-buty1-2,3-dinor-TxB₂ (M₇), and 2,3,4,5-tetranor-TxB₂ butyl ester (M₅d₆) are all considered to have formed artifactually from TxB₂, 2,3-dinor-TxB₂, and 2,3,4,5-tetranor-TxB₂ during chromatography. We had previously found that the hemiacetal alcohol group of the thromboxane ring is highly reactive with an alcohol in the presence of acid and identified several 11-O-ethyl derivatives in our study of the metabolism of TxB₂ in the monkey (7). In that study, the original XAD-2 column was eluted with ethanol and ethanol was frequently used during evaporation procedures to form an azetrop with water. In the present study, ethanol was purposely avoided throughout urine processing and the XAD-2 column was eluted with methanol. This explains the presence in the present study of 11-O-methyl derivatives and the absence of NADPH. The two most likely sites for the location of the oxo group would be at C-19 or C-20 since metabolites of prostaglandins and TxB₂ with hydroxyl groups at the ω-1 and ω-2 positions have been described (16–19).
collection after chromatographic losses to obtain a complete
mass spectrum would suggest that several micrograms of the
endogenous compound are excreted during a 24-h period.

This study has provided the background biochemical in-
formation necessary to begin quantification of urinary metabo-
lites of TxB2 as a means to assess in vivo production of TxB2
in man. In this regard, we have now developed a stable isotope
dilution assay for 2,3-dinor-TxB2 using combined gas chro-
matography-mass spectrometry. Six normal adult males have
been found to excrete a few hundred picograms/mg of creat-
ine of 2,3-dinor-TxB2. These initial studies document the
excretion of endogenous 2,3-dinor-TxB2 in human urine and
indicate the possibility that an assay for urinary 2,3-dinor-
TxB2 may prove to be a useful index of in vivo TxB2 produc-
tion in man.

Acknowledgments—We are grateful to Dr. N. A. Nelson of the
Upjohn Co. for the generous gift of chemically synthesized TxB2. The
skilful technical assistance of N. A. Payne was greatly appreciated.

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TABLE 1

| Vehicle Ondine or E45 in Dose (mg/kg) | Metabolite | Time (hr) | Control | 0.1 mg/kg | 0.3 mg/kg | 1.0 mg/kg |
|------------------------------------|------------|----------|--------|----------|----------|----------|
|                                   |            | 0        | 3.6    | 4.5      | 5.2      | 6.4      |
|                                   |            | 6        | 3.5    | 4.2      | 5.3      | 6.3      |
|                                   |            | 24       | 3.6    | 4.5      | 5.2      | 6.4      |

**Fig. 1.** Lipid biosynthesis in human skin as measured by the decrease in the content of the 24-nor-15,16,17-trihydroxy-18,20,24-trienoic acid (24:3T) of the fatty acids. Each point is the mean ± SEM of five experiments. (A): Control; (B): 0.1 mg/kg; (C): 0.3 mg/kg; (D): 1.0 mg/kg.
Metabolism of Thromboxane B2 in Man

Processes of Metabolism of TXB2: The metabolic fate of TXB2 was examined as follows: thromboxane, thrombin; other stimuli; and activated or gas-inactivated platelets (Fig. 1). The results show that the metabolic rate of thromboxane (Fig. 2) is lower than that of thrombin (Fig. 3) in the in vitro system. However, the results of both studies suggest that thromboxane is the major metabolite of thrombin in the in vitro system.

Production of TXB2: The method of TXB2 was also tested on human platelets and stimulated by ADP, thrombin, and other stimuli (Fig. 4). The results showed that the production of TXB2 is significantly higher in the stimulated platelets than in the resting platelets. The production of TXB2 is dependent on the concentration of ADP and the activation of platelets.

Metabolism of TXB2: The metabolism of TXB2 was examined in vivo in normal and thromboxane A2 (TXA2) depleted rats (Fig. 5). The results showed that the metabolism of TXB2 is significantly higher in the thromboxane A2 depleted rats than in the normal rats. The metabolism of TXB2 is dependent on the concentration of TXA2.

Stimulation of TXB2: The method of TXB2 was also tested on human platelets and stimulated by ADP, thrombin, and other stimuli (Fig. 6). The results showed that the production of TXB2 is significantly higher in the stimulated platelets than in the resting platelets. The production of TXB2 is dependent on the concentration of ADP and the activation of platelets.

Conclusion: The results of this study suggest that the metabolism of TXB2 is dependent on the concentration of thromboxane A2 and the activation of platelets. The production of TXB2 is also dependent on the concentration of ADP. Therefore, the metabolism of TXB2 is a complex process that is influenced by multiple factors.
Metabolism of Thromboxane B₂ in Man

Further purification and mass spectrometric analysis of metabolites of thromboxane B₂ has revealed the formation of a derivative designated as thromboxane B₂-11-sulfate. This derivative is present in plasma and urine and is formed by a sulfotransferase that is present in the liver. The formation of this derivative is catalyzed by the enzyme thromboxane B₂-sulfotransferase. The enzyme is present in high activity in the liver and is also present in the kidney. The enzyme activity in the liver is higher than in the kidney. The enzyme is also present in the intestine and in the lung. The enzyme activity in the intestine is higher than in the lung. The enzyme activity in the lung is lower than in the intestine and the liver.

Further evidence for the metabolism of thromboxane B₂ has been obtained by the use of a new radioactive tracer, thromboxane B₂-14C. The tracer is administered by intravenous injection and the plasma levels of the tracer are measured by a sensitive radiochemical assay. The results indicate that thromboxane B₂ is rapidly metabolized in man. The major metabolite is thromboxane B₂-11-sulfate. The metabolite is present in plasma and urine and is formed by a sulfotransferase that is present in the liver. The formation of this metabolite is catalyzed by the enzyme thromboxane B₂-sulfotransferase. The enzyme is present in high activity in the liver and is also present in the kidney. The enzyme activity in the liver is higher than in the kidney. The enzyme is also present in the intestine and in the lung. The enzyme activity in the intestine is higher than in the lung. The enzyme activity in the lung is lower than in the intestine and the liver.

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Metabolism of Thromboxane B₂ in Man

The main function of TxA₂ is to be converted to a thromboxane, intracellularly to the thromboxane D₂. The thromboxane D₂ is a stable form of the enzyme, which is then further metabolized to a thromboxane A₂. The thromboxane A₂ is a stable form of the enzyme, which is then further metabolized to a thromboxane B₂. The thromboxane B₂ is then further metabolized to a thromboxane C₂. The thromboxane C₂ is then further metabolized to a thromboxane D₂.

Structure of TxA₂ - The main function of TxA₂ is to be converted to a thromboxane, intracellularly to the thromboxane D₂. The thromboxane D₂ is a stable form of the enzyme, which is then further metabolized to a thromboxane A₂. The thromboxane A₂ is a stable form of the enzyme, which is then further metabolized to a thromboxane B₂. The thromboxane B₂ is then further metabolized to a thromboxane C₂. The thromboxane C₂ is then further metabolized to a thromboxane D₂.

Figure 13 - TLC chromatography on Lichroarb 100-100 of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Further purification and characterization of TxA₂ using HPLC (Fig. 15)

Figure 15 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Figure 16 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Figure 17 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Further purification and characterization of TxA₂ using HPLC (Fig. 18)

Figure 18 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Further purification and characterization of TxA₂ using HPLC (Fig. 19)

Figure 19 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |

Further purification and characterization of TxA₂ using HPLC (Fig. 20)

Figure 20 - HPLC chromatography on D,A,Z column of the mobile phase of the solvent system, Ethanol, n-hexane, Water (7:7:6). Flow rate: 70 μL/min, detection with UV light (λ = 254 nm).

ELUTION VOLUME (ML)

| Volume (ml) | Retention Volume (ml) |
|-------------|-----------------------|
| 0.0          | 0.0                   |
| 20.0         | 20.0                  |
| 40.0         | 40.0                  |
| 60.0         | 60.0                  |
| 80.0         | 80.0                  |
| 100.0        | 100.0                 |
| 120.0        | 120.0                 |
Metabolism of Thromboxane B2 in Man

Further Purification and Structure of Metabolite in 4C1 - Material in 4C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system A. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 5C1 - Material in 5C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system B. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 6C1 - Material in 6C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system C. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 7C1 - Material in 7C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system D. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 8C1 - Material in 8C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system E. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 9C1 - Material in 9C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system F. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 10C1 - Material in 10C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system G. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.

Further Purification and Structure of Metabolite in 11C1 - Material in 11C1 was subjected to high performance liquid chromatography on a reversed phase column using solvent system H. A single peak was observed at 33.6 min of elution (C-18 column, methanol-water-acetonitrile). The peak was further purified by a preparative ultraviolet spectroscopy. The purified peak was identified as the metabolite by mass spectrometry and NMR. The mass spectrum obtained at 33.6 min was identical to that recorded for thromboxane B2 (m/z 512). Therefore, the metabolite was identified as the metabolite of thromboxane B2.