Synthesis of Thromboxane B_3 and Its Direct Separation from Thromboxane B_2 by Reversed-Phase High Performance Liquid Chromatography

Nobuyuki NAKAMURA, Hiroko NAGAI, Makiko YONEDA, Hisashi SUGA, Masanori KAWAMURA, and Sadahiko IGUCHI*

Minase Research Institute, Ono Pharmaceutical Co., Ltd., Shimamoto, Mishima, Osaka 618, Japan.
Received March 12, 1993

The first chemical synthesis of thromboxane B_3 starting with the Corey lactone is described. A reversed-phase high-performance liquid chromatographic method was developed for the direct determination without derivatization, of thromboxane B_3 in the presence of thromboxane B_2.

Keywords thromboxane B_3; thromboxane B_2; eicosapentaenoic acid; reversed-phase HPLC; chemical synthesis; separation

Recently, much effort has been devoted to elucidation of the role of all *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and its metabolite, thromboxane A_3 (TXA_3), in the prostanoid pathways.[1] EPA is a polyunsaturated fatty acid of the ω-3 series found in marine food.[2] Nutritional effects of EPA (as free acid, triglycerides or phospholipids from fish oil) are associated with lowering of cardiovascular risk and incidence of arterial thrombosis.[3] More recently, studies have suggested that EPA may also have a favorable effect on the other human diseases such as arthritis,[4] renal disorders,[5] asthma,[6] eczema,[7] and possibly also cancer.[8]

EPA is metabolized *via* prostaglandin H_3 (PGH_3) to TXA_3, and immediately to the final product TXB_3, while arachidonic acid containing the four *cis* double bonds in a molecule is metabolized *via* PGH_2 in a similar way to TXA_3, and then to TXB_2.[3a,9] TXA_3 is reported to possess different biological activities from those of TXA_2: *e.g.*, TXA_3 showed the less potent platelet aggregating activity than TXB_2.[10] The biological roles of EPA and its metabolite TXA_3 are not yet fully understood.

The both arachidonic acid (the precursor of TXB_2) and EPA (the precursor of TXB_3) exist in the body of mammalians, and the extracts obtained in *in vivo* biological experiments contain both TXB_2 and TXB_3. It would be very useful to develop an analytical method for TXB_3 in the presence of TXB_2. In the course of our studies on eicosaenoic acid-related compounds, aiming to elucidate their important physiological roles in the body of man, we needed to detect not only TXB_2 and various other metabolites but also TXB_3 by HPLC analysis. Therefore, we have conducted the first chemical synthesis of TXB_3, and developed an HPLC separation of TXB_3 and TXB_2.

Synthesis TXB_2 was synthesized according to the reported synthetic method,[11] and TXB_3 was synthesized by means of a modification of TXB_2 preparation, starting with the Corey lactone aldehyde **1**,[12] as shown in Chart 2. In the literature so far, the chemical synthesis of TXB_3 is not previously reported. The optically active aldehyde **1** was converted to the alcohol **2** by Wittig coupling with the β-oxido ylide[13] derived from (2*S*-hydroxy-5-heptenyl)-triphenylphosphonium iodide and methyllithium in tetrahydrofuran (THF) at -78 to $-30\,^{\circ}C$ in 40% yield. In general, the linkage from C_{13} through C_{18} including the hydroxy function at C_{15} is labile to acid to form the triene. In the final step of this synthesis, ring closure between the aldehyde and the hydroxy functions in a molecule with phosphoric acid in water was required. Therefore, at this stage, we examined the stability of the alcohol **2** to these conditions: **2** was exposed to 85% H_3PO_4–H_2O–THF (1 : 10 : 12) at 50 °C for 2 h or a mixture of 65% aqueous acetic acid–THF (10 : 1) at 45 °C for 2 h. In either condition, the hydroxy group at C_{15} was stable, and only the THP (tetrahydropyranyl) group at C_{11} was cleaved to produce the hydroxy function. After the hydroxy group at C_{15} was protected as the (1-methoxy-1-methyl)ethyl ether which can be selectively cleaved in the presence of the THP group by

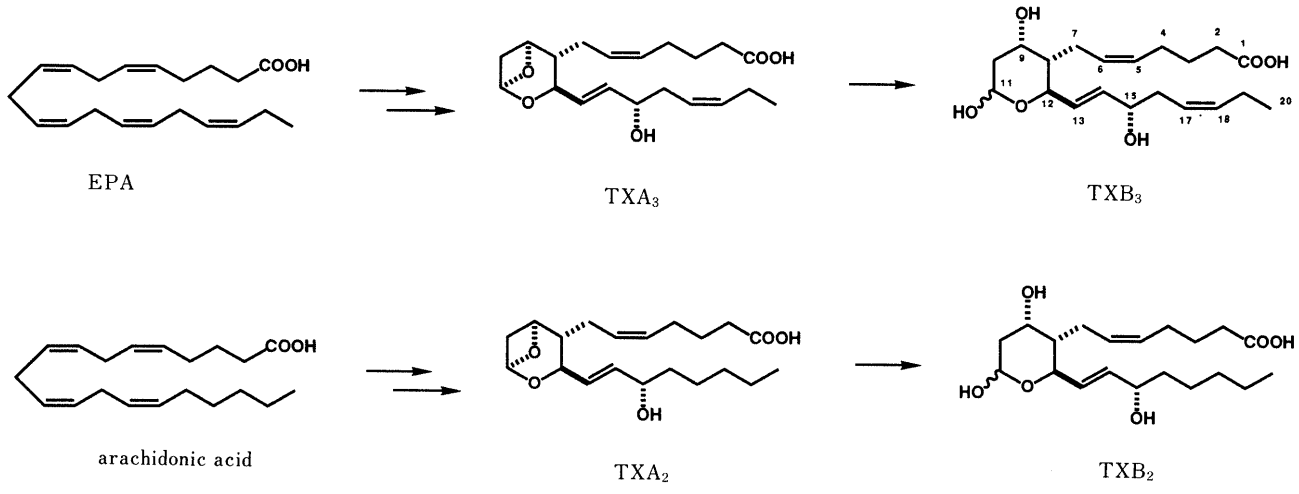
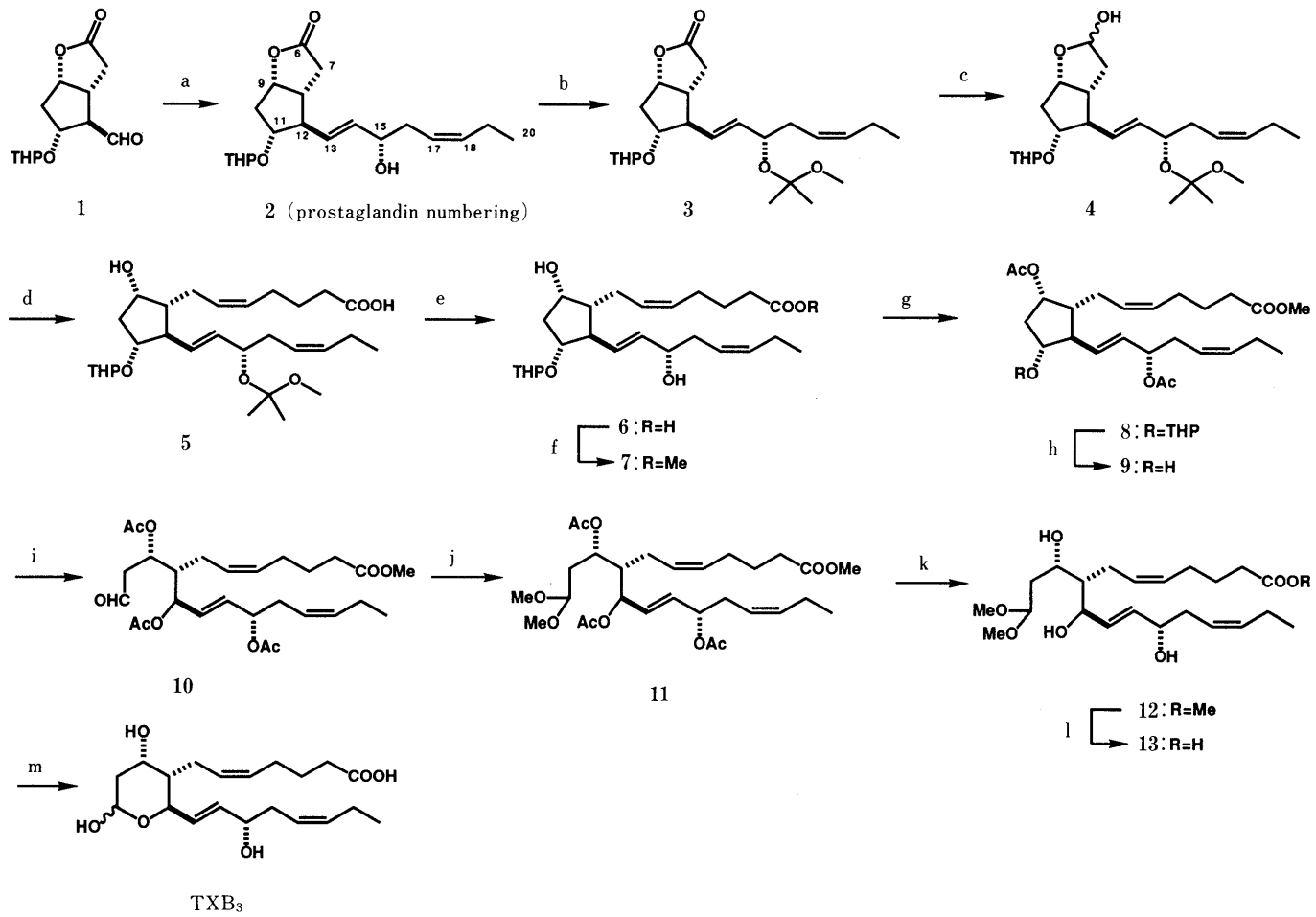


Chart 1. Metabolism of Eicosapentaenoic Acid and Arachidonic Acid



a : (2S-hydroxy-5-heptenyl)triphenylphosphonium iodide/MeLi/THF; b : methoxypropene/*p*-TsOH/CH_2Cl_2; c : diisobutylaluminum hydride/toluene; d : (4-carbonyl-butyl)triphenylphosphonium bromide/dimsyl anion/DMSO; e : 0.5M HCl/THF; f : K_2CO_3/MeI/acetone; g : acetic anhydride/pyridine; h : *p*-TsOH/MeOH; i : lead tetraacetate/$CaCO_3$/benzene; j : methyl orthoformate/pyridine hydrochloride/MeOH; k : K_2CO_3/MeOH; l : 5% aq KOH/EtOH; m : 85% H_3PO_4-H_2O-THF (1:10:12)

Chart 2. Synthetic Scheme for TXB_3

weak acid, the lactone **3** was reduced to the lactol **4** with diisobutylaluminum hydride in anhydrous toluene at −78 °C. The Wittig reaction of the lactol **4** with 4-carboxybutylidene triphenylphosphorane in dimethyl sulfoxide at room temperature furnished the acid **5**, which was treated with cold 0.5 N HCl–AcOEt, resulting in selective cleavage of the (1-methoxy-1-methyl)ethyl ether to afford 11-THP-prostaglandin $F_{3\alpha}$ (**6**). The methyl ester **7** was obtained by the reaction of **6** with excess methyl iodide in the presence of anhydrous potassium carbonate in acetone in 46% yield from **2**. Two hydroxy groups were acetylated with acetic anhydride in pyridine, and the THP group was removed with *p*-toluenesulfonic acid in methanol at room temperature to produce the diacetate **9** in 73% yield. The subsequent ring-opening reaction was the crucial step in this synthesis. Treatment of **9** with lead tetraacetate in the presence of calcium carbonate in benzene at 50 °C led to the rather unstable aldehyde **10**, which was directly converted to its dimethyl acetal **11** using trimethyl orthoformate and pyridine hydrochloride in methanol in 45% yield. Deacetylation with anhydrous potassium carbonate in methanol and then saponification with 5%

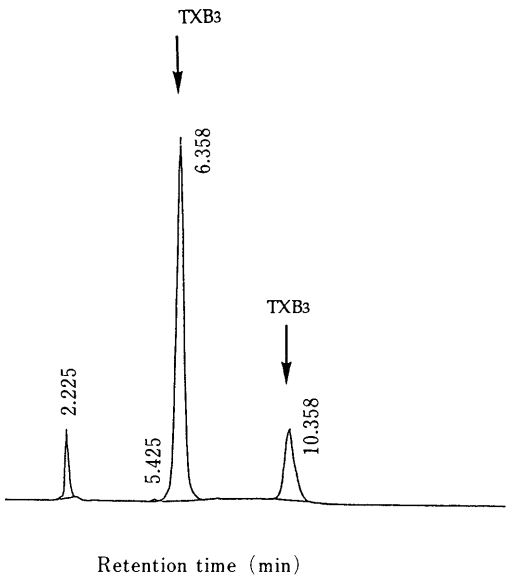


Fig. 1. HPLC Chromatogram of Thromboxane B_3
Chart speed 5 mm/min, attenuation 256 mV F.S.

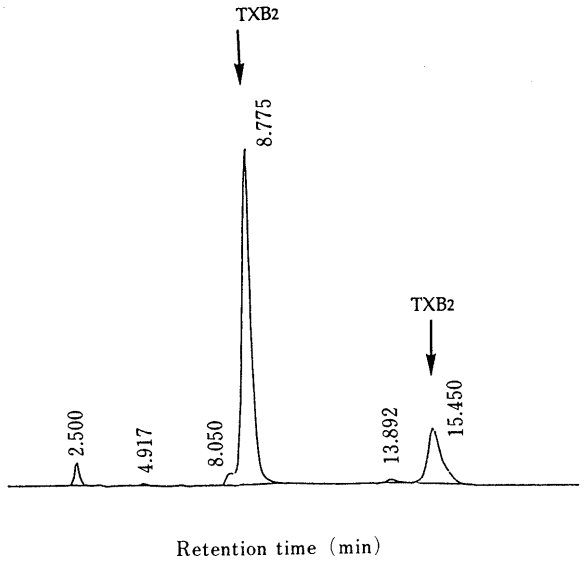


Fig. 2. HPLC Chromatogram of Thromboxane B_2
Chart speed 5 mm/min, attenuation 256 mV F.S.

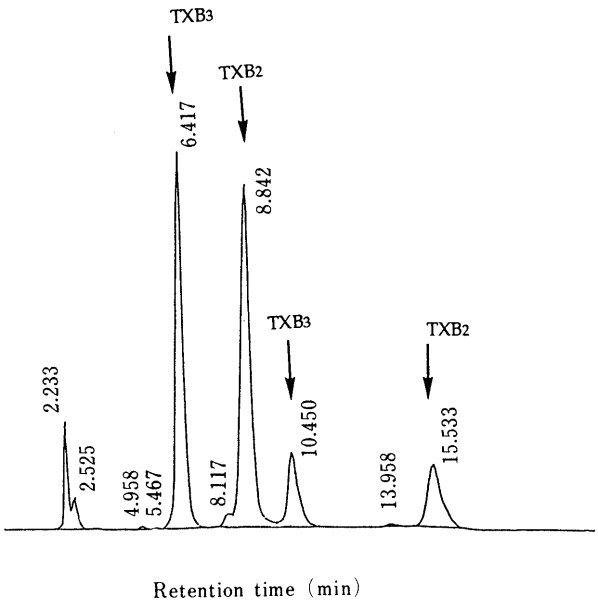


Fig. 3. HPLC Chromatogram of a mixture of Thromboxanes B_3 and B_2
Chart speed 5 mm/min, attenuation 256 mV F.S.

aqueous potassium hydroxide in ethanol provided the hydroxy acetal **13** in 88% yield. Direct aqueous hydrolysis of both the acetyl and the ester groups gave **13** in much lower yield. Finally, **13** was transformed to TXB_3 by treatment with 85% H_3PO_4–H_2O–THF (1 : 10 : 12) at 50 °C for 2 h in 64% yield.[14] No elimination of the C_{15} hydroxy function occurred under this condition, in accord with the model study using **2** as described above.

HPLC Analysis of TXB_3 and TXB_2 In TLC analysis, TXB_3 and TXB_2 showed the same *Rf* values in all solvent systems we examined, including $AgNO_3$-impregnated silica TLC plates. The direct detection of TXB_3 in the presence of TXB_2 by HPLC analysis should allow differentiation of their biochemical and biological roles. Previously TXB_3 and TXB_2 have been distinguished by HPLC analysis after precolumn derivatization of the mixture with 9-anthryldiazomethane.[15] After extensive trials, we have succeeded in the direct determination of TXB_3 in the presence of TXB_2 by reversed-phase HPLC without derivatization. The best conditions found were: column, YMC-Pack A-312 ODS; sample size, 4 μg/2 μl; eluent, 20 mM KH_2PO_4/CH_3CN 2 : 1; flow rate, 1.2 ml/min; column temperature, 0 °C; and detection, UV 205 nm. Both TXB_3 and TXB_2 were separated into two peaks due to the epimers at C_{11}. The t_R (min) values of TXB_3 are 6.35 and 10.35 min (ratio of 2 : 1) (Fig. 1), and those of TXB_2 are 8.77 and 15.45 min (ratio 2 : 1) (Fig. 2). The chromatogram of a mixture of TXB_3 and TXB_2 shows that complete separation is achieved by this straighforward method (Fig. 3).

Experimental

General ^{1}H- and ^{13}C-NMR spectra were taken on a Varian VXR500S, VXR200S, or JEOL FX90Q FT spectrometer in $CDCl_3$. Chemical shifts are reported as parts per million relative to tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 1760X spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-DX303HF for electron impact (EI)-, fast atom bombardment (FAB)-, and exact MS. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. All reactions were monitored by TLC, and for TLC analysis throughout this work, Merck TLC plates (Kiesel gel $60F_{254}$, precoated, layer thickness 0.25 mm) were used with UV light and sprayed with a solution of 7% ethanolic phosphomolybdic acid and then heated until the spots became clearly visible. Column chromatography was carried out on silica gel (YMC gel, particle size 70/230 mesh, Mallinckrodt CC-7 gel, or Wako gel C-200). Unless otherwise specified, all reactions were conducted under an atmosphere of argon. THF was distilled from the sodium benzophenone ketyl under argon. CH_2Cl_2, toluene, and dimethyl sulfoxide (DMSO) were distilled from calcium hydride.

15(*S*)-Hydroxy-11-THP-lactone 2 (Prostaglandin Numbering) (2*S*-Hydroxy-5-heptenyl)triphenylphosphonium iodide[13] (8.0 g, 15.9 mmol) was azeotropically dried with a mixture of anhydrous THF (20 ml) and anhydrous toluene (100 ml) four times. To a solution of the above phosphonium salt in anhydrous THF (150 ml) at −75 °C was added 1.4 M methyllithium in ether (22.7 ml, 31.8 mmol) dropwise while maintaining the temperature below −50 °C. The mixture was stirred at −78 °C for 5 min, and then at −40 °C for 30 min. The solution turned orange. After the resulting β-oxido ylide solution was cooled again to −78 °C, a solution of the Corey aldehyde **1** (5.25 g, 20.7 mmol) in anhydrous THF (50 ml) was added dropwise, maintaining the temperature below −50 °C. The mixture was stirred at −78 °C for 5 min, and then at −30 °C for 40 min. The reaction was quenched by addition of aqueous NH_4Cl, and the product was extracted with AcOEt (×2). The combined extracts were washed with water and brine successively, dried on $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (150 g) with hexane–AcOEt (1 : 2) to afford the pure desired alcohol **2** (2.20 g, 40% yield). TLC (hexane–AcOEt 1 : 1, twice developed) homogeneous: *Rf* 0.45 for the aldehyde **1** and *Rf* 0.40 for the alcohol **2**. ^{1}H-NMR ($CDCl_3$) δ: 5.61 [1H, dd, J=15.5, 5.5 Hz, H_{14} (prostaglandin numbering)], 5.52 (1H, dd, J=15.5, 7.3 Hz, H_{13}), 5.6—5.4 (1H, m, H_{18}), 5.30 (1H, m, H_{17}), 4.97 (1H, m, H_9), 4.67, 4.61 (total 1H, each t-like, –O–CH–O in THP group), 4.2—3.9 (2H, m, H_{15} and H_{11}), 3.80 (1H, m, –O–CH in THP group), 3.45 (1H, m, –O–CH in THP group), 2.76 (2H, m, H_8 and H_7), 2.67 (1H, m, H_7), 2.50 (1H, m, H_{12}), 2.40 (1H, m, H_{10}), 2.28 (1H, m, H_{16}), 2.15 (1H, m, H_{10}), 2.07 (1H, q, J=7.5 Hz, H_{19}), 0.98 (3H, t, J=7.5 Hz, H_{20}). MS *m/z*: 350 (M^+), 281 [M^+−69 (CH_2–CH=CH–CH_2–CH_3)], 248 (M^+−THPOH). IR (film): 3450, 3030, 2950, 2850, 1780, 1180, 980, 720 cm^{-1}. $[\alpha]_D$ −34° (*c*=1.67, MeOH). Treatment of **2** with 65% aqueous acetic acid to remove the THP function afforded the diol in 80% yield. ^{1}H-NMR spectrum ($CDCl_3$): δ: 5.60 (1H, dd, J=15.5, 5.5 Hz, H_{14}), 5.45 (1H, dd, J=15.5, 7.3 Hz, H_{13}), 5.6—5.4 (1H, m, H_{18}), 5.30 (1H, m, H_{17}), 4.88 (1H, dt, J=6.6, 3.0 Hz, H_9), 4.08 (1H, q, J=6.6 Hz, H_{15}), 3.91 (1H, q, J=7.7 Hz, H_{11}).

15-(1-Methoxy-1-methyl)ethyl-11-THP-lactone 3 A mixture of the alcohol **2** (2.82 g, 8.05 mmol), methoxypropene (2.30 ml, 24.1 mmol), *p*-TsOH (27 mg, 0.142 mmol), and anhydrous CH_2Cl_2 (40 ml) was stirred at room temperature for 10 min. The reaction was quenched by addition of Et_3N (1 ml). The mixture was concentrated *in vacuo* to leave crude **3** (2.85 g, 100% yield), which was used for the next reaction without

purification. TLC (AcOEt): *Rf* 0.75 for the alcohol **2** and *Rf* 0.90 for the desired **3**. In the ^{1}H-NMR ($CDCl_3$) spectrum, the signals at δ: 3.19 (3H, s, OMe) and 1.32 (6H, s, Me × 2) were characteristic for **3**.

11-THP-prostaglandin $F_{3\alpha}$ Methyl Ester (6) To a solution of the lactone **3** (2.85 g, 8.05 mmol) in anhydrous toluene (50 ml) at −78 °C was added a solution of diisobutylaluminum hydride in toluene (25 g/100 ml, 10 ml, 17.7 mmol) dropwise while the temperature was maintained below −60 °C. The mixture was stirred at −78 °C for 15 min, and the reaction was quenched by careful and slow addition of methanol (17 ml) until hydrogen evolution ceased. The mixture was allowed to warm up to −30 °C, and water (6 ml) was added. The mixture was stirred at room temperature for 1 h. The solids were filtered and the filtrate was washed with brine, dried on $MgSO_4$, and concentrated *in vacuo* to give the crude lactol **4** (2.88 g, 100% yield), which was pure enough to be used in the next reaction without purification. TLC (hexane–AcOEt 1 : 1): *Rf* 0.76 for **3** and *Rf* 0.58 for **4**. (4-Carbohydroxybutyl)triphenylphosphonium bromide (8.35 g, 18.9 mmol) was azeotropically dried with a mixture of anhydrous THF (30 ml) and toluene (80 ml) three times. To a solution of this salt in anhydrous DMSO (80 ml) was added at room temperature dimsyl anion dropwise prepared from sodium hydride (60%, 1.32 g, 37.7 mmol) and anhydrous DMSO (20 ml) by heating at 60—70 °C for 1 h. After the resulting red-colored ylide solution was stirred at room temperature for 10 min, a solution of the lactol **4** (1.62 g, 3.77 mmol) in anhydrous DMSO (5 ml) was added in one portion. The mixture was stirred at 35 °C for 1 h, and then poured into cold aqueous potassium carbonate solution (300 ml). The neutral materials were extracted with a mixture of ether and AcOEt (1 : 1). The aqueous layer was acidified with oxalic acid to pH 3, and the product was extracted twice with a mixture of ether and pentane (1 : 1). The combined extracts were washed with water and brine successively, dried on $MgSO_4$, and concentrated *in vacuo*. The residue was treated with a mixture of 0.5 M HCl (20 ml) and THF (40 ml) at 4—5 °C for 10 min to cleave selectively the (1-methoxy-1-methyl)ethyl ether function. Dilution with AcOEt (200 ml), washing with water, drying on $MgSO_4$, and concentration *in vacuo* afforded 11-THP-prostaglandin $F_{3\alpha}$ (**6**) (1.50 g). TLC (MeOH–CH_2Cl_2 1 : 10) *Rf* 0.50. A mixture of **6** (3.02 g, 6.92 mmol), anhydrous potassium carbonate (2.86 g, 20.7 mmol), methyl iodide (4.3 ml, 69.2 mmol), and anhydrous acetone was stirred at room temperature for 15 h. The mixture was filtered, and the filtrate was concentrated *in vacuo*. Column chromatography on silica gel (90 g, hexane–AcOEt 1 : 1) gave the pure ester **7** (1.69 g, 46% yield from the lactone **2**). TLC (hexane–AcOEt 1 : 1): *Rf* 0.42 for **7**. ^{1}H-NMR ($CDCl_3$) δ: 5.65—5.20 (6H, m, olefinic protons), 4.63 (1H, br, O–CH–O in THP group), 4.20—3.92 (3H, m, H_9, H_{11}, and H_{15}), 3.80 (1H, m, O–CH in THP group), 3.65 (3H, s, COOMe), 3.40 (1H, m, O–CH in THP group), 0.95 (3H, t, J=7.5 Hz, H_{20}). MS *m/z*: 450 (M^+), 381 [M^+ − 69 (CH_2–CH=CH–CH_2–CH_3)], 348 (M^+ − THPOH), 330 (348 − H_2O), 312 (330 − H_2O). IR (film): 3330, 3080, 2950, 2840, 1735, 1260, 980, 720 cm^{-1}. $[\alpha]_D$ +15° (c=1.02, MeOH).

9,15-Diacetylprostaglandin $F_{3\alpha}$ Methyl Ester (9) A mixture of the diol **7** (1.69 g, 3.75 mmol), acetic anhydride (0.88 ml, 9.39 mmol), and anhydrous pyridine (20 ml) was stirred at room temperature for 15 h. The mixture was concentrated *in vacuo*, and excess pyridine was removed by azeotropic evaporation with toluene three times. The residue was dissolved in AcOEt (50 ml), and the solution was washed with water and then brine, dried on $MgSO_4$, and concentrated *in vacuo* to leave the diacetate **8** (1.88 g, 94% yield). TLC (hexane–AcOEt 1 : 1): *Rf* 0.32 for the diol **7** and *Rf* 0.89 for the diacetate **8**. A mixture of **8** (1.88 g), *p*-TsOH (a catalytic amount), and methanol (20 ml) was stirred at room temperature for 1 h. The reaction was quenched by addition of Et_3N (three drops), and the mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt (60 ml), and the solution was washed with water and brine in succession, dried on $MgSO_4$, and concentrated *in vacuo*. Purification by column chromatography on silica gel (40 g, hexane–AcOEt 1 : 1) afforded the 9,15-diacetylprostaglandin $F_{3\alpha}$ methyl ester (**9**) (1.38 g, 82% yield in two steps). TLC (AcOEt–hexane 1 : 1): *Rf* 0.89 for **8** and *Rf* 0.41 for **9**. ^{1}H-NMR ($CDCl_3$) δ: 5.55—5.20 (7H, m, olefinic protons and H_{15}), 5.10 (1H, m, H_9), 3.95 (1H, m, H_{11}), 3.65 (3H, s, COOMe), 2.05 (6H, s × 2, AcO), 0.94 (3H, t, J=7.5 Hz, H_{20}). MS *m/z*: 503 (M^+ − OMe), 474 (M^+ − AcOH), 390 (474 − THP), 372 (474 − THPOH), 330 (M^+ − 2AcOH − THP). IR (film): 3300, 3050, 2960, 2850, 1735, 1260, 980, 710 cm^{-1}.

The Triacetyl Acetal 11 To a stirred mixture of lead tetraacetate (138 mg, 0.26 mmol, recrystallized from AcOH before use) and calcium carbonate (115 mg, 1.14 mmol) in anhydrous benzene (20 ml) was added a solution of **9** (100 mg, 0.223 mmol) in anhydrous benzene (1 ml) at room temperature. Then, the resulting mixture was warmed at 50 °C with stirring for 2 h. The mixture was filtered through a pad of silica gel, and the silica gel was washed with a mixture of benzene and AcOEt (1 : 1). The filtrate was washed with aqueous sodium bicarbonate and brine successively, dried on $MgSO_4$, and concentrated *in vacuo* to leave the oily aldehyde **10** (108 mg). The aldehyde was so unstable that it was used immediately for the next reaction. TLC (AcOEt–hexane 1 : 1): *Rf* 0.41 for the alcohol **9** and *Rf* 0.68 for the aldehyde **10**. A mixture of **10** (108 mg, 0.223 mmol), methyl orthoformate (120 mg, 1.13 mmol), pyridine hydrochloride (3 mg), and methanol (2 ml) was stirred at room temperature for 15 h. The mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt (30 ml). The solution was washed with aqueous sodium bicarbonate and brine in succession, dried on $MgSO_4$, and concentrated *in vacuo*. Column chromatography on silica gel (5 g, AcOEt–hexane 1 : 2) afforded the acetal **11** (55 mg, 45% yield in two steps). TLC (hexane–AcOEt 2 : 1): *Rf* 0.35 for the aldehyde **10** and *Rf* 0.56 for the acetal **11**. ^{1}H-NMR ($CDCl_3$) δ: 5.80—5.00 (9H, olefinic protons, H_9, H_{12} and H_{15}), 4.00 (1H, m, H_{11}), 3.65 (3H, s, COOMe), 3.25 (6H, s × 2, acetal OMe), 2.10 (2H, t, J=7.5 Hz, H_2), 2.06—2.02 (9H, s × 3, AcO), 1.68 (1H, q, J=7.5 Hz, H_3), 0.95 (3H, t, J=7.5 Hz, H_{20}). MS *m/z*: 523 (M^+ − MeOH), 403 (523 − 2AcOH), 343 (403 − AcOH).

The Acetal Triol 12 A mixture of the triacetate **11** (0.895 g, 1.61 mmol), anhydrous potassium carbonate (1.33 g, 9.69 mmol), and methanol (10 ml) was stirred at 40 °C for 5 h. The mixture was cooled to 4—5 °C in an ice-water bath, and the reaction was quenched by slow addition of acetic acid (1.0 ml) to pH 3—4. The mixture was diluted with AcOEt (50 ml), and the solution was washed with aqeuous sodium bicarbonate and brine successively, dried on $MgSO_4$, and concentrated *in vacuo*. Column chromatography on silica gel (20 g, AcOEt–hexane 7 : 3) afforded the triol **12** (0.518 g, 75% yield). TLC (AcOEt): *Rf* 0.96 for triacetate **11** and *Rf* 0.42 for the triol **12**. ^{1}H-NMR ($CDCl_3$) δ: 5.58 (1H, dd, J=15.5, 5.5 Hz, H_{14}), 5.55 (1H, dd, J=15.5, 7.3 Hz, H_{13}), 5.51 (1H, m, H_{18}), 5.40 (3H, m, H_5, H_6, and H_{17}), 4.55 (1H, t-like J=7.5 Hz, H_{11}), 4.30, 4.15, 3.97 (each 1H, each m, H_{15}, H_{12}, and H_9, respectively), 3.65 (3H, s, COOMe), 3.38, 3.34 (each 3H, each s, acetal OMe), 2.30 (2H, t, J=7.5 Hz, H_2), 2.15 (2H, q, J=7.5 Hz, H_{19}), 1.62 (2H, quintet, J=7.5 Hz, H_3), 0.95 (3H, t, J=7.5 Hz, H_{20}). MS *m/z*: 410 (M^+ − H_2O), 397 (M^+ − OMe). IR (film): 3350, 3030, 2960, 2850, 1735, 1720 cm^{-1}.

Thromboxane B_3 A mixture of the ester **12** (0.314 g, 0.733 mmol), 5% aqueous KOH (3.2 ml, 2.93 mmol), and ethanol (3.2 ml) was stirred at room temperature for 1 h. Cooled to 4—5 °C, the mixture was acidified by slow addition of 1 M HCl to pH 4—5, and immediately the product was extracted with AcOEt (× 2). The combined extracts were washed with water and brine successively, dried on $MgSO_4$, and concentrated *in vacuo* to leave the acid **13** (0.278 g). TLC (AcOEt–HCOOH 400 : 5) *Rf* 0.98 for the ester **12** and *Rf* 0.43 for the acid **13**. A solution of **13** (0.228 g) in 85% H_3PO_4–H_2O–THF (1 : 10 : 12, 4 ml) was stirred at 50 °C for 3.5 h. After dilution with cold water (20 ml), the product was extracted with AcOEt (× 2). The combined extracts were washed with water and then brine, dried on $MgSO_4$, and concentrated *in vacuo*. Column chromatography on silical gel (8 g, AcOEt–AcOH 400 : 1) afforded the final product, TXB_3 (0.130 g, 48% yield in two steps). TLC (AcOEt–HCOOH 400 : 5): *Rf* 0.49 for **13** and *Rf* 0.56 for TXB_3. TLC ($CHCl_3$–THF–AcOH 10 : 2 : 1): *Rf* 0.27 for TXB_3. TXB_3 could not be distinguished from TXB_2 by TLC analysis, even with $AgNO_3$-impregnated TLC plates. ^{1}H-NMR ($CDCl_3$) δ: 5.85 (1H, dd, J=17.5, 6.3 Hz, H_{14}), 5.71 (1H, dd, J=17.5, 7.5 Hz, H_{13}), 5.55 (1H, td, J=20.0, 12.5 Hz, H_{18}), 5.48—5.31 (4H, m, H_5, H_6, H_{11}, and H_{17}), 4.41 (1H, dd, J=12.5, 7.5 Hz, H_{12}), 4.23 (1H, dt, J=12.5, 6.3 Hz, H_{15}), 4.08 (1H, m, H_9), 2.40—2.24 (4H, m, H_4 and H_{16}), 2.18—1.96 (7H, m, H_2, H_7, H_{19}, and H_{10}), 1.81 (1H, dt, J=13.8, 3.8 Hz, H_{10}), 1.74—1.63 (2H, m, H_3), 1.45 (1H, tdd, J=8.8, 5.0, 5.0 Hz, H_8), 0.96 (3H, t, J=7.5 Hz, H_{20}). ^{13}C-NMR ($CDCl_3$) δ: 177.25, 136.52, 135.22, 130.75, 129.22, 127.49, 123.66, 123.59, 92.56, 71.58, 69.20, 64.95, 44.99, 36.01, 34.72, 32.89, 26.31, 24.80, 24.58, 24.58, 20.76, 14.21. IR (film): 3392, 3010, 2932, 1713, 1407, 1363, 1231, 1154, 1104, 1024, 973, 895 cm^{-1}. MS (FAB) *m/z*: 351 (M^+ + 1 − H_2O), 333 (351 − H_2O), 315 (333 − H_2O), 307 (M^+ + 1 − AcOH). Exact MS Calcd for $C_{20}H_{31}O_5$ (dehydration peak from M^+ + 1): 351.2171. Found: 351.2188. $[\alpha]_D$ +56° (c=1.0, EtOH).

HPLC Apparatus The chromatographic system consisted of a Model PU-980, a Model UV-970 detector, and a Model 807-IT integrator (all from JASCO, Tokyo, Japan).

HPLC Conditions The mobile phase was 20 mM KH_2PO_4–CH_3CN (2 : 1), which had been filtered through a 0.45 μm membrane filter and degassed under vacuum. Samples were chromatogtaphed at 0 °C on a YMC-Pack A-312 ODS (6.0 mm i.d. × 150 mm, YMC Co., Ltd., Kyoto, Japan). The flow rate was 1.2 ml/min, and the UV absorbance was measured at 205 nm.

References and Notes

1) S. Murota, S. Yamamoto (eds.), "Kouza Prostaglandin," Vol. 8, Tokyo Kagaku Dojin, Tokyo, 1988, pp. 138—177.
2) B. J. Weaver, B. J. Holob, *Prog. Food Nutr. Sci.*, **12**, 111 (1988).
3) *a*) J. Dyerberg, H. O. Bang, E. Stoffersen, S. Moncada, J. R. Vane, *Lancet*, **80**, 117, (1978); *b*) J. Dyerberg, *J. Nutrition Reviews*, **44**, 125 (1986).
4) J. M. Cremer, D. A. Lawrence, W. Jubiz, R. Digiacomo, R. Rynes, L. E. Bartholomew, M. Sherman, *Arthritis Rheum.*, **33**, 810 (1990).
5) V. Kher, U. Barcelli, M. Weiss, L. Gallon, P. Pajel, P. Laskarzewski, V. E. Pollâk, *PG LT Med.*, **22**, 323 (1986).
6) J. A. Salmon, T. Terano, *NATO ASI Ser., Ser A*, **131**, 131 (1987).
7) E. L. Rhodes, *Br. J. Clin. Pract.*, **38**, 115 (1984).
8) U. N. Das, *Nutrition*, **6**, 429 (1990).
9) S. Fisher, P. C. Weber, *Biochem. Biophys. Res. Commun.*, **116**, 1091 (1983).
10) P. Needleman, M. Minkes, A. Raz, *Science*, **193**, 163 (1976).
11) *a*) N. A. Nelson, R. W. Jackson, *Tetrahedron Lett.*, **1976**, 3275; *b*) R. C. Kelly, I. Schletter, S. J. Sten, *ibid.*, **1976**, 3279; *c*) W. P. Schneider, R. A. Morge, *ibid.*, **1976**, 3283.
12) *a*) E. J. Corey, T. K. Schaaf, W. Huber, U. Kolliker, N. M. Weinshenker, *J. Am. Chem. Soc.*, **92**, 397 (1970); *b*) E. J. Corey, X.-M Cheng, "The Logic of Chemical Synthesis," John Wiley and Sons, Inc., New York, 1989, pp. 255—296.
13) E. J. Corey, H. Shirahama, H. Yamamoto, S. Terashima, A. Venkatesuwarlu, T. K. Schaaf, *J. Am. Chem. Soc.*, **93**, 1490 (1971).
14) Unfortunately we could not compare our TXB_3 with the naturally occurring one in NMR, IR, MS, and TLC analyses, since we could not obtain a natural sample. We could not find any report giving the spectra data of natural TXB_3. However, our spectral data of synthetic TXB_3 are consistent with the expected structure of TXB_3: its NMR spectrum is similar to that of TXB_2 except for the existence of the *cis* double bond at the lower side chain; its IR spectrum is reasonably similar to that of TXB_2; the MS fragments are reduced by two mass units from those of TXB_2; and it has the same *Rf* value on TLC.
15) A. Hirai, T. Terano, M. Takenaga, S. Kobayashi, H. Makuta, A. Ozawa, T. Fujita, Y. Tamura, H. Kitagawa, A. Kumagai, S. Yoshida, *Adv. PG TX LT Res.*, **17**, 838 (1987).