Synthesis of Thromboxane B₃ and Its Direct Separation from Thromboxane B₂ by Reversed-Phase High Performance Liquid Chromatography

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The first chemical synthesis of thromboxane B₃ 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 B₃ in the presence of thromboxane B₂.

Keywords thromboxane B₃; thromboxane B₂; 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 A₃ (TXA₃), 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 H₃ (PGH₃) to TXA₃, and immediately to the final product TXB₃, while arachidonic acid containing the four *cis* double bonds in a molecule is metabolized *via* PGH₂ in a similar way to TXA₃, and then to TXB₂.^{3a,9)} TXA₃ is reported to possess different biological activities from those of TXA₂: *e.g.*, TXA₃ showed the less potent platelet aggregating activity than TXB₂.¹⁰⁾ The biological roles of EPA and its metabolite TXA₃ are not yet fully understood.

The both arachidonic acid (the precursor of TXB₂) and EPA (the precursor of TXB₃) exist in the body of mammals, and the extracts obtained in *in vivo* biological experiments contain both TXB₂ and TXB₃. It would be very useful to develop an analytical method for TXB₃ in the presence of TXB₂. 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 TXB₂ and various other metabolites but also TXB₃ by HPLC analysis. Therefore, we have conducted the first chemical synthesis of TXB₃, and developed an HPLC separation of TXB₃ and TXB₂.

Synthesis TXB₂ was synthesized according to the reported synthetic method,¹¹⁾ and TXB₃ was synthesized by means of a modification of TXB₂ preparation, starting with the Corey lactone aldehyde **1**,¹²⁾ as shown in Chart 2. In the literature so far, the chemical synthesis of TXB₃ is not previously reported. The optically active aldehyde **1** was converted to the alcohol **2** by Wittig coupling with the β -oxido ylide¹³⁾ derived from (2*S*-hydroxy-5-heptenyl)-triphenylphosphonium iodide and methyllithium in tetrahydrofuran (THF) at -78 to -30°C in 40% yield. In general, the linkage from C₁₃ through C₁₈ including the hydroxy function at C₁₅ 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% H₃PO₄-H₂O-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 C₁₅ was stable, and only the THP (tetrahydropyranyl) group at C₁₁ was cleaved to produce the hydroxy function. After the hydroxy group at C₁₅ 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/CH₂Cl₂; c : diisobutylaluminum hydride/toluene; d : (4-carboxyl-butyl)triphenylphosphonium bromide/dimsyl anion/DMSO; e : 0.5M HCl/THF; f : K₂CO₃/MeI/acetone; g : acetic anhydride/pyridine; h : *p*-TsOH/MeOH; i : lead tetraacetate/CaCO₃/benzene; j : methyl orthoformate/pyridine hydrochloride/MeOH; k : K₂CO₃/MeOH; l : 5% aq KOH/EtOH; m : 85% H₃PO₄-H₂O-THF (1:10:12)

Chart 2. Synthetic Scheme for TXB₃

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 0.5N HCl-AcOEt, resulting in selective cleavage of the (1-methoxy-1-methyl)ethyl ether to afford 11-THP-prostaglandin F_{3 α} (**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 B₃
Chart speed 5 mm/min, attenuation 256 mV F.S.

Fig. 2. HPLC Chromatogram of Thromboxane B₂
Chart speed 5 mm/min, attenuation 256 mV F.S.

Fig. 3. HPLC Chromatogram of a mixture of Thromboxanes B₃ and B₂
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 TXB₃ by treatment with 85% H₃PO₄-H₂O-THF (1 : 10 : 12) at 50 °C for 2 h in 64% yield.¹⁴ No elimination of the C₁₅ hydroxy function occurred under this condition, in accord with the model study using **2** as described above.

HPLC Analysis of TXB₃ and TXB₂ In TLC analysis, TXB₃ and TXB₂ showed the same *R_f* values in all solvent systems we examined, including AgNO₃-impregnated silica TLC plates. The direct detection of TXB₃ in the presence of TXB₂ by HPLC analysis should allow differentiation of their biochemical and biological roles. Previously TXB₃ and TXB₂ have been distinguished by HPLC analysis after precolumn derivatization of the mixture with 9-anthryl-

diazomethane.¹⁵ After extensive trials, we have succeeded in the direct determination of TXB₃ in the presence of TXB₂ 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 KH₂PO₄/CH₃CN 2 : 1; flow rate, 1.2 ml/min; column temperature, 0 °C; and detection, UV 205 nm. Both TXB₃ and TXB₂ were separated into two peaks due to the epimers at C₁₁. The *t_R* (min) values of TXB₃ are 6.35 and 10.35 min (ratio of 2 : 1) (Fig. 1), and those of TXB₂ are 8.77 and 15.45 min (ratio 2 : 1) (Fig. 2). The chromatogram of a mixture of TXB₃ and TXB₂ shows that complete separation is achieved by this straightforward method (Fig. 3).

Experimental

General ¹H- and ¹³C-NMR spectra were taken on a Varian VXR500S, VXR200S, or JEOL FX90Q FT spectrometer in CDCl₃. 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 60F₂₅₄, 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. CH₂Cl₂, toluene, and dimethyl sulfoxide (DMSO) were distilled from calcium hydride.

15(S)-Hydroxy-11-THP-lactone 2 (Prostaglandin Numbering) (2S-Hydroxy-5-heptenyl)triphenylphosphonium iodide¹³ (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 NH₄Cl, and the product was extracted with AcOEt (× 2). The combined extracts were washed with water and brine successively, dried on MgSO₄, 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: *R_f* 0.45 for the aldehyde **1** and *R_f* 0.40 for the alcohol **2**. ¹H-NMR (CDCl₃) δ: 5.61 [1H, dd, *J* = 15.5, 5.5 Hz, H₁₄ (prostaglandin numbering)], 5.52 [1H, dd, *J* = 15.5, 7.3 Hz, H₁₃], 5.6–5.4 (1H, m, H₁₈), 5.30 (1H, m, H₁₇), 4.97 (1H, m, H₉), 4.67, 4.61 (total 1H, each t-like, -O-CH-O in THP group), 4.2–3.9 (2H, m, H₁₅ and H₁₁), 3.80 (1H, m, -O-CH in THP group), 3.45 (1H, m, -O-CH in THP group), 2.76 (2H, m, H₈ and H₇), 2.67 (1H, m, H₇), 2.50 (1H, m, H₁₂), 2.40 (1H, m, H₁₀), 2.28 (1H, m, H₁₆), 2.15 (1H, m, H₁₀), 2.07 (1H, q, *J* = 7.5 Hz, H₁₉), 0.98 (3H, t, *J* = 7.5 Hz, H₂₀). MS *m/z*: 350 (M⁺), 281 [M⁺ - 69 (CH₂-CH=CH-CH₂-CH₃)], 248 (M⁺ - THPOH). IR (film): 3450, 3030, 2950, 2850, 1780, 1180, 980, 720 cm⁻¹. [α]_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. ¹H-NMR spectrum (CDCl₃): δ: 5.60 (1H, dd, *J* = 15.5, 5.5 Hz, H₁₄), 5.45 (1H, dd, *J* = 15.5, 7.3 Hz, H₁₃), 5.6–5.4 (1H, m, H₁₈), 5.30 (1H, m, H₁₇), 4.88 (1H, dt, *J* = 6.6, 3.0 Hz, H₉), 4.08 (1H, q, *J* = 6.6 Hz, H₁₅), 3.91 (1H, q, *J* = 7.7 Hz, H₁₁).

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 CH₂Cl₂ (40 ml) was stirred at room temperature for 10 min. The reaction was quenched by addition of Et₃N (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): *R_f* 0.75 for the alcohol **2** and *R_f* 0.90 for the desired **3**. In the ¹H-NMR (CDCl₃) 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_{3α} 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 MgSO₄, 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): *R_f* 0.76 for **3** and *R_f* 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 dimethyl 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 MgSO₄, 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 MgSO₄, and concentration *in vacuo* afforded 11-THP-prostaglandin F_{3α} (**6**) (1.50 g). TLC (MeOH–CH₂Cl₂ 1:10): *R_f* 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): *R_f* 0.42 for **7**. ¹H-NMR (CDCl₃) 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, H₉, H₁₁, and H₁₃), 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, H₂₀). MS *m/z*: 450 (M⁺), 381 [M⁺ – 69 (CH₂–CH=CH–CH₂–CH₃)], 348 (M⁺ – THPOH), 330 (348 – H₂O), 312 (330 – H₂O). IR (film): 3330, 3080, 2950, 2840, 1735, 1260, 980, 720 cm^{−1}. [α]_D²⁵ +15° (*c* = 1.02, MeOH).

9,15-Diacetylprostaglandin F_{3α} 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 MgSO₄, and concentrated *in vacuo* to leave the diacetate **8** (1.88 g, 94% yield). TLC (hexane–AcOEt 1:1): *R_f* 0.32 for the diol **7** and *R_f* 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 Et₃N (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 MgSO₄, and concentrated *in vacuo*. Purification by column chromatography on silica gel (40 g, hexane–AcOEt 1:1) afforded the 9,15-diacetylprostaglandin F_{3α} methyl ester (**9**) (1.38 g, 82% yield in two steps). TLC (AcOEt–hexane 1:1): *R_f* 0.89 for **8** and *R_f* 0.41 for **9**. ¹H-NMR (CDCl₃) δ: 5.55–5.20 (7H, m, olefinic protons and H₁₅), 5.10 (1H, m, H₉), 3.95 (1H, m, H₁₁), 3.65 (3H, s, COOMe), 2.05 (6H, s × 2, AcO), 0.94 (3H, t, *J* = 7.5 Hz, H₂₀). 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 MgSO₄, 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): *R_f* 0.41 for the alcohol **9** and *R_f* 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 MgSO₄, 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): *R_f* 0.35 for the aldehyde **10** and *R_f* 0.56 for the acetal **11**. ¹H-NMR (CDCl₃) δ: 5.80–5.00 (9H, olefinic protons, H₉, H₁₂ and H₁₅), 4.00 (1H, m, H₁₁), 3.65 (3H, s, COOMe), 3.25 (6H, s × 2, acetal OMe), 2.10 (2H, t, *J* = 7.5 Hz, H₂), 2.06–2.02 (9H, s × 3, AcO), 1.68 (1H, q, *J* = 7.5 Hz, H₃), 0.95 (3H, t, *J* = 7.5 Hz, H₂₀). 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 aqueous sodium bicarbonate and brine successively, dried on MgSO₄, 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): *R_f* 0.96 for triacetate **11** and *R_f* 0.42 for the triol **12**. ¹H-NMR (CDCl₃) δ: 5.58 (1H, dd, *J* = 15.5, 5.5 Hz, H₁₄), 5.55 (1H, dd, *J* = 15.5, 7.3 Hz, H₁₃), 5.51 (1H, m, H₁₈), 5.40 (3H, m, H₅, H₆, and H₁₇), 4.55 (1H, t-like *J* = 7.5 Hz, H₁₁), 4.30, 4.15, 3.97 (each 1H, each m, H₁₅, H₁₂, and H₉, respectively), 3.65 (3H, s, COOMe), 3.38, 3.34 (each 3H, each s, acetal OMe), 2.30 (2H, t, *J* = 7.5 Hz, H₂), 2.15 (2H, q, *J* = 7.5 Hz, H₃), 1.62 (2H, quintet, *J* = 7.5 Hz, H₃), 0.95 (3H, t, *J* = 7.5 Hz, H₂₀). MS *m/z*: 410 (M⁺ – H₂O), 397 (M⁺ – OMe). IR (film): 3350, 3030, 2960, 2850, 1735, 1720 cm^{−1}.

Thromboxane B₃ 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 MgSO₄, and concentrated *in vacuo* to leave the acid **13** (0.278 g). TLC (AcOEt–HCOOH 400:5): *R_f* 0.98 for the ester **12** and *R_f* 0.43 for the acid **13**. A solution of **13** (0.228 g) in 85% H₃PO₄–H₂O–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 MgSO₄, and concentrated *in vacuo*. Column chromatography on silica gel (8 g, AcOEt–AcOH 400:1) afforded the final product, TXB₃ (0.130 g, 48% yield in two steps). TLC (AcOEt–HCOOH 400:5): *R_f* 0.49 for **13** and *R_f* 0.56 for TXB₃. TLC (CHCl₃–THF–AcOH 10:2:1): *R_f* 0.27 for TXB₃. TXB₃ could not be distinguished from TXB₂ by TLC analysis, even with AgNO₃-impregnated TLC plates. ¹H-NMR (CDCl₃) δ: 5.85 (1H, dd, *J* = 17.5, 6.3 Hz, H₁₄), 5.71 (1H, dd, *J* = 17.5, 7.5 Hz, H₁₃), 5.55 (1H, td, *J* = 20.0, 12.5 Hz, H₁₈), 5.48–5.31 (4H, m, H₅, H₆, H₁₁, and H₁₇), 4.41 (1H, dd, *J* = 12.5, 7.5 Hz, H₁₂), 4.23 (1H, dt, *J* = 12.5, 6.3 Hz, H₁₅), 4.08 (1H, m, H₉), 2.40–2.24 (4H, m, H₄ and H₁₆), 2.18–1.96 (7H, m, H₂, H₇, H₁₉, and H₁₀), 1.81 (1H, dt, *J* = 13.8, 3.8 Hz, H₁₀), 1.74–1.63 (2H, m, H₃), 1.45 (1H, tdd, *J* = 8.8, 5.0, 5.0 Hz, H₈), 0.96 (3H, t, *J* = 7.5 Hz, H₂₀). ¹³C-NMR (CDCl₃) 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 – H₂O), 333 (351 – H₂O), 315 (333 – H₂O), 307 (M⁺ + 1 – AcOH). Exact MS Calcd for C₂₀H₃₁O₅ (dehydration peak from M⁺ + 1): 351.2171. Found: 351.2188. [α]_D²⁵ +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 KH₂PO₄–CH₃CN (2:1), which had been filtered through a 0.45 μm membrane filter and degassed under vacuum. Samples were chromatographed 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

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