for $C_{14}H_{73}BO_{10}Na$). Anal. Calcd for $C_{14}H_{73}BO_{10}$: C, 66.85; H, 9.92. Found: C, 66.19; H, 10.15.

Reaction of 18 with Ethanolamine. Formation of 20. A solution of 18 (38 mg, 44 μmol) in CH_2Cl_2 (2 mL) was treated with excess ethanolamine (ca. 0.1 mL), and the resulting solution was maintained at 23 °C for 24 h. The solution was then diluted with ether and washed with water and brine. The organic material was dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexane (5:1)), giving 32 mg (80%) of **20** as a clear oil: ^1H NMR ($CDCl_3$, 490 MHz) δ 5.73 (m, 1 H), 5.40 (br s, 1 H), 5.14–4.92 (m, 6 H), 4.56 (br d, J = 13.5, Hz, 1 H), 4.03 (dd, J = 9.1, 4.9 Hz, 1 H), 3.83–3.70 (m, 4 H), 3.65–3.50 (m, 2 H), 3.45–3.20 (m, 3 H), 3.42 (s, 3 H), 3.39 (s, 3 H), 3.30 (s, 3 H), 3.02 (ddd, J = 4.2, 8.8, 11.3 Hz, 1 H), 2.71–2.63 (m, 2 H), 2.55 (m, 1 H), 2.40–1.95 (m, 8 H), 1.95–1.15 (m, 17 H), 1.68 (s, 3 H), 1.55 (s, 3 H), 1.15–0.80 (m, 20 H); ^{13}C NMR (63 MHz, $CDCl_3$) 171.1, 167.6, 165.5, 137.3, 136.7, 129.1, 125.5, 115.6, 95.8, 84.3, 76.7, 74.9, 74.0, 73.7, 72.6, 70.8, 69.5, 61.8, 57.6, 56.8, 56.7, 56.2, 55.8, 50.1, 42.9, 41.0, 39.6, 36.2, 35., 35.1, 33.7, 33.0, 31.4, 31.2, 30.8, 28.1, 26.3, 26.2, 26.2, 25.0, 22.1, 20.2, 16.4, 15.7, 15.3, 15.3, 14.6, 13.5, 9.8; IR (film) 3403, 2936, 1734, 1638, 1444, 1196 cm^{-1}; MS (FAB) m/e 915.6202 (915.6120 calcd for $C_{50}H_{84}N_2BO_{12}$), 897, 687, 309.

(22*S*)-Dihydro FK-506 (17).[19] A solution of 18 (9.5 mg, 11 μmol) in THF (2.0 mL) was treated with H_2O (3.0 mL), and the resulting solution was maintained at 23 °C for 2 days. This solution was then diluted with saturated aqueous sodium bicarbonate and extracted with EtOAc. The organic material was dried (K_2CO_3) and concentrated. The crude isolate was purified by chromatography (silica gel, 240–400 mesh, THF–hexanes (40:60)), giving 3.7 mg of 17 (42%) and 2.7 mg of recovered 18: ^1H NMR (490 MHz, $CDCl_3$) δ 5.78 (m, 1 H), 5.30 (br s, 0.5 H), 5.22 (br s, 0.5 H), 5.20–4.92 (m, 5 H), 4.65 (br s, 1 H), 4.43 (br d, J = 7.6 Hz, 1 H), 3.95–3.80 (m, 13 H), 3.78–3.65 (m, 1 H), 3.65–3.52 (m, 2 H), 3.50–3.20 (m, 5 H), 3.41, 3.38, 3.37, 3.32, 3.30 (s, 3 × OCH_3 for both major and minor rotamers), 3.01 (m, 1 H), 2.84 (m, 0.7 H), 2.70–1.95 (m, 9 H), 1.95–1.20 (m, 14 H), 1.66, 1.64 (s, CH=$CHCH_3$ for both major and minor rotamers), 1.54, 1.48 (s, CH=CH CH_3 for both major and minor rotamers) 1.20–0.80 (m, 13 H); ^{13}C NMR (63 MHz, $CDCl_3$; data given for both major and minor rotamers) δ 195.97, 195.82, 169.32, 169.21, 165.65, 165.06, 137.48, 136.42, 135.86, 132.72, 131.66, 128.95, 126.69, 126.22, 115.72, 90.49, 97.11, 84.31, 78.17, 76.81, 75.52, 73.96, 73.84, 73.69, 73.40, 72.90, 71.81, 71.02, 70.06, 70.55, 57.02, 56.64, 56.58, 56.26, 56.11, 52.61, 49.40, 48.97, 44.58, 44.11, 44.00, 40.85, 39.61, 39.50, 37.05, 36.85, 35.91, 35.11, 35.05, 34.82, 34.08, 33.97, 33.02, 32.71, 32.61, 34.41, 30.82, 29.70, 27.35, 26.85, 26.71, 26.05, 24.71, 24.53, 21.47, 20.67, 16.47, 16.29, 15.77, 15.38, 14.62, 14.30, 10.50, 9.44; IR (film) 3455, 2928, 1733, 1642, 1453, 1089 cm^{-1}; MS (FAB) m/e 828.4909 (828.4876 calcd for $C_{44}H_{71}O_{12}NNa$), 578.

Reductive Amination of (22*S*)-Dihydro FK-506. A solution of 18 (20 mg, 25 μmol) and dry methanol (0.5 mL) was treated with benzylamine (3.20 μL, 29 μmol), and $NaBH_3CN$ (ca 50 mg) at 23 °C for 24 h. At this time, the solution was diluted with water (10 mL) and extracted with EtOAc. The extracts were dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexanes (5:1)), giving 6.6 mg (40%) of **13** as a clear oil.

Reduction of FK-506 with $NaBH(OAc)_3$. A solution of FK-506 (0.117 g, 0.146 mmol) and THF (1.75 mL) was treated with $NaBH(OAc)_3$ (0.154 g, 0.730 mmol) and HOAc (0.35 mL) at 23 °C for 2 h. At this time, the solution was diluted with H_2O and extracted with EtOAc. The combined extracts were washed with saturated aqueous sodium bicarbonate and brine. The organic material was dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, 60:40 hexanes–THF), giving 9.0 mg of **20**[19] (7.7%) and 81 mg of 17 (69%). Prepared in this fashion, 17 contains ca. 10% (as evidenced by the appearance of unassignable signals at δ 5.95 (br s) and 5.50 (br s)) of an unknown impurity. Nevertheless, this material was used successfully in the transformations outlined in this paper.

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Supplementary Material Available: NMR spectra for compounds **4**, **9**, **17**, and **19–21** (6 pages). Ordering information is given on any current masthead page.

Notes

Prostaglandin 1,15-Lactones of the F Series from the Nudibranch Mollusc *Tethys fimbria*

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We have recently reported for the first time the natural occurrence of prostaglandin 1,15-lactones of the E series (**1–3**) in the nudibranch *Tethys fimbria*.[1] More recently,[2] we have found that these lactones are biosynthesized from free prostaglandins in the mantle of the mollusc and are converted back into the prostaglandins upon detachment of the cerata (body appendices) during the behavioral defense mechanism known as autotomy. We describe now the isolation and structure characterization of prostaglandin 1,15-lactones of the F series from the same mollusc and from its egg masses. In addition, **4**, which was not detected previously,[1] has also been isolated by HPLC and its structure established by comparison with standard PGE_2-1,15-lactone 11-acetate.

$PGF_{2\alpha}$-1,15-lactone 11-acetate (**5**) and $PGF_{3\alpha}$-1,15-lactone 11-acetate (**6**) were isolated from the mantles and cerata of the mollusc in the relative amounts reported in Table I. Comparison of their ^1H NMR spectra (Experimental Section) with those of **1–3**[1] and with the published spectrum of synthetic $PGF_{2\alpha}$-1,15-lactone[3] (**7**) suggested

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(1) Cimino, G.; Spinella, A.; Sodano, G. *Tetrahedron Lett.* **1989**, *30*, 3589.
(2) Cimino, G.; Crispino, A.; Di Marzo, V.; Sodano, G.; Spinella, A.; Villani, G. *Experientia* **1991**, *47*, 56.

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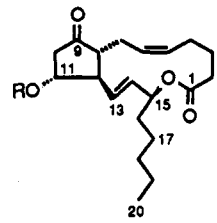
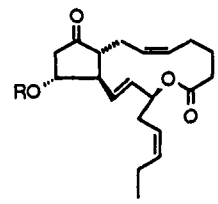


1 R = H
4 R = Ac



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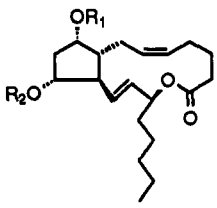
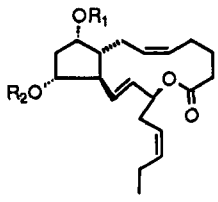


5 R_1 = H; R_2 = Ac 7 R_1 = R_2 = H
8 R_1 = R_2 = Ac 9 R_1 = H; R_2 = Ac; 9-*epi*



6 R_1 = H; R_2 = Ac
10 R_1 = H; R_2 = Ac; 9-*epi*

the gross structures **5** and **6** for the two compounds. In order to firmly establish the regio- and stereochemistry at C-9 and C-11, synthetic **4**[4] was reduced with $NaBH_4$ to afford two isomeric products in a relative ratio of ca. 5:1, the minor one being undistinguishable from **5** by TLC and ^1H NMR spectroscopy, thus assigning the position of the acetyl group and the stereochemistry at C-11. Furthermore, acetylation of **5** afforded a diacetyl derivative (**8**) that proved to be identical by TLC and ^1H NMR comparison to the diacetyl derivative prepared from synthetic **7**,[4] thus confirming the structure and stereochemistry of **5**. The major $NaBH_4$ reduction product of **4** is by inference 9-epi-$PGF_{2\alpha}$-1,15-lactone 11-acetate (**9**). Similarly, $NaBH_4$ reduction of **3** afforded **6** as the minor product and 9-epi-$PGF_{3\alpha}$-1,15-lactone 11-acetate (**10**) in the same ratio as the reaction products of **4**.

Egg masses of *T. fimbria* were collected either in the field or in a laboratory tank where *T. fimbria* specimens were preserved alive for several days. Extraction and usual purification procedures allowed the isolation from the egg masses of a complex mixture of $PGF_{2\alpha}$- and $PGF_{3\alpha}$-1,15-lactones fatty acid esters (PLFE), while the lactones isolated from the mantles and cerata were absent (Table I). A reversed-phase HPLC trace of the ester mixture is shown in Figure 1.

Part of the ester mixture was submitted to methanolysis (Na_2CO_3, MeOH), which resulted in the production of two compounds (ca. 1:1 ratio) coeluting in HPLC with standards of $PGF_{2\alpha}$- and $PGF_{3\alpha}$-methyl esters, $PGF_{2\alpha}$-1,15-lactone (**7**), and a mixture of fatty acid methyl esters. The structure of $PGF_{2\alpha}$-methyl ester was confirmed by TLC and ^1H NMR comparison with an authentic sample, while the structure of $PGF_{3\alpha}$-methyl ester was derived by comparison of its ^1H NMR and mass spectral data with those of $PGF_{2\alpha}$-methyl ester. Most of the fatty acid methyl esters were identified by gas chromatography (Experimental Section), the major components being palmitic, *cis*-5,8,11,14,17-eicosapentaenoic, and *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl esters.

Preliminary information on the constitution of the PLFE was gained from ^1H NMR spectra of the crude mixture. The presence of two carbinol protons (δ 4.19 and 3.97) in a ca. 1:4 ratio having the characteristic chemical shift and shape of H-9 and H-11 of $PGF_{2\alpha}$, together with the presence of a C-11-acylated proton (δ 4.90; 1:1 with the H-9), suggested that the mixture consisted of PGF-1,15-lactones acylated at C-9 (predominant) or C-11. A gross fractionation of the mixture has been achieved by preparative TLC, which yielded three main fractions (1–3, Table

Table I. Distribution and Amount[a] of the Various Prostaglandin 1,15-Lactones in the Anatomical Sections of *T. fimbria*

	anatomical section			
compd	mantle[b]	cerata[b]	ovotestis[c]	egg mass[d]
1	99	221		
2	89	245		
3	97	124		
4	2	10		
5	60	30		
6	199	139		
PLFE[e]			950	1600

[a] Micrograms per gram of dry weight tissue. See the Experimental Section for the quantitation method of compounds 1–6. The dry weights of the various sections of a medium-sized specimen were the following: mantle, 2 g; cerata, 2 g; ovotestis, 0.15 g. The dry weight of an egg mass is ca. 0.9 g. [b] Mean of three specimens. Most of **3** and **4** are transformed into PGA_3- and PGA_2-1,15-lactones, respectively, during the isolation and purification procedures.[1] [c] Mean of four specimens. [d] Mean of 15 egg masses. [e] Crude mixture of $PGF_{2\alpha}$- and $PGF_{3\alpha}$-1,15-lactone fatty acid esters.

Table II. Main Structural Features of the $PGF_{2\alpha}$- and $PGF_{3\alpha}$-1,15-lactone Fatty Acid Ester Fractions Recovered by TLC (1–3) and HPLC (A–H)

fraction	prostaglandin 1,15-lactone	main fatty acids
1	11-acyl-$PGF_{2\alpha}$/ 9-acyl-$PGF_{2\alpha}$	satd/monounsatd
2	9-acyl-$PGF_{3\alpha}$	satd/monounsatd
3	9-acyl-$PGF_{3\alpha}$	polyunsatd
A	9-acyl-$PGF_{3\alpha}$	$C_{20:5}$
B	9-acyl-$PGF_{3\alpha}$	$C_{14:0}$
C	9-acyl-$PGF_{3\alpha}$	polyunsatd
D	11-acyl-$PGF_{3\alpha}$/ 11-acyl-$PGF_{2\alpha}$	$C_{22:6}$ $C_{20:4}$
E	9-acyl-$PGF_{2\alpha}$	$C_{22:6}$
F	9-acyl-$PGF_{3\alpha}$	$C_{16:0}$
G	11-acyl-$PGF_{3\alpha}$/ 11-acyl-$PGF_{2\alpha}$/9-acyl-$PGF_{3\alpha}$/9-acyl-$PGF_{2\alpha}$	$C_{18:0}$ and/or $C_{18:1}$; $C_{16:0}$ and/or $C_{16:1}$
H	9-acyl-$PGF_{2\alpha}$	$C_{16:0}$

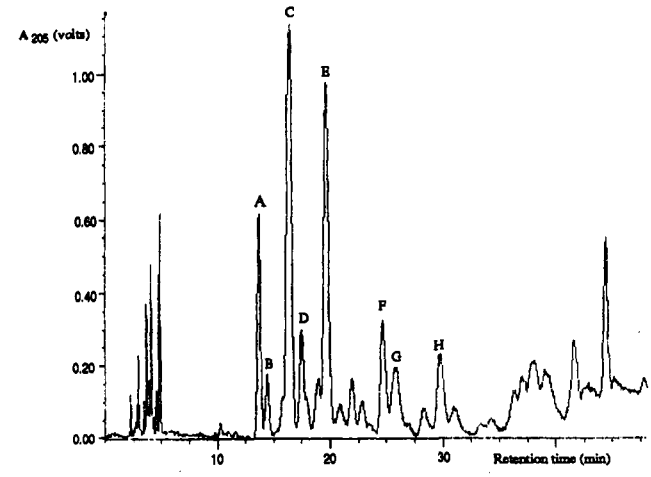


Figure 1. HPLC absorbance (205 nm) profile of the PLFE mixture from *T. fimbria* egg masses (Novopak C-18 4-μm analytical column; CH_3OH/H_2O, 94:6; 0.8 mL/min). Peak height is not representative of the actual relative abundance since polyunsaturated fatty acyl residues have a higher ϵ_{205} than saturated ones.

(3) Andersen, N. H.; Bor-Sheng Lin, *Biochemistry* **1985**, *24*, 2338.
(4) Bundy, G. L.; Peterson, D. C.; Cornette, J. C.; Miller, W. L.; Spilman, C. H., Wilks, J. W. *J. Med. Chem.* **1983**, *26*, 1089.

II), while several peaks of the reversed-phase HPLC shown in Figure 1 were isolated (A–H, Table II). Although several HPLC peaks were still mixtures, the main structural features of the major components of the fractions were inferred from their ^1H NMR and mass spectra with the aid of the criteria discussed below, which were derived by comparative analysis of the spectra and by comparison with the spectra of **5**, **6**, and **8**.

In the ^1H NMR spectra of the C-11 acylated derivatives (minor components of the mixture), H-9 resonates at δ 4.19 while H-11 resonates at δ 4.90; H-13 and H-14 resonate as two close double doublets at δ 5.88 (J 5.9 and 15.8 Hz) and 5.86 (J 8.2 and 15.8 Hz). These values are very close to those of **5** and **6**. In the C-9 acylated derivatives (major components of the mixture) the H-11 resonates at δ 3.97 while H-9 resonates at δ 5.20, overlapped with H-15; H-13 and H-14 resonate as well as separated double doublets at δ 5.78 (J 8.9 and 15.8 Hz) and 5.96 (J 6.5 and 15.8 Hz). The presence of polyunsaturated acyl residues was evidenced by large resonances at δ 5.38 (olefinic protons) and 2.84 (methylenes between two double bonds) in a ca. 1:1 ratio. In addition, the terminal methyl group of eicosapentaenoic and docosahexaenoic acyl residues resonates at ca. δ 0.97 and was distinguishable from that of the $PGF_{3\alpha}$ residue, which, when present, also absorbed at a similar chemical shift value in the 9-acyl derivatives, while in the 11-acyl derivatives this resonance was shifted to higher fields (δ 0.95).

In the EI mass spectra of several HPLC fractions, molecular ions were detected that, in conjunction with the presence of the large fragment arising by the loss of the fatty acid(s) from the molecular ion (m/z 318 and 316 for the $PGF_{2\alpha}$- and $PGF_{3\alpha}$-1,15-lactone derivatives, respectively) and with those originating by loss of water from these latter and from the molecular ion, aided in the identification of the fatty acyl residues linked to the PGF-lactones.

The combined evidence allows assignment of the structural features reported in Table II to the main components of the HPLC fractions. It is noteworthy that in several fractions the prostaglandin lactones have been found esterified by their fatty acid precursors, namely arachidonic or *cis*-5,8,11,14,17-eicosapentaenoic acid.

The PLFE's are absent in the mantle and cerata, where the other lactones have been found. In order to ascertain where these compounds are stored and then transferred to the eggs, anatomical sections of *T. fimbria* were made and analyzed by TLC. The PLFE mixture was found only in the ovotestis (hermaphrodite gland), a gland where the egg masses are produced (Table I). Interestingly, PLFE's were not found in immature *T. fimbria* specimens. This fact would suggest a specific role for PLFE's in the egg production, in their development, or in their protection, in relation either to fish predation or to pathogens.

A defensive role against fish predation seems unlikely since the PLFE mixture, as well as the PGF derivatives **5** and **6**, were not toxic in the *Gambusia affinis* bioassay, while **1**–**3** were found active.[2] A role, for example, as chemical release factor for ovulation appears more likely, since it has been reported that prostaglandins appear to stimulate egg production in several molluscs.[5] In this respect, the functions of PGE- and PGF-1,15-lactones in *T. fimbria* appear to be distinct, also because of their different anatomical location: for the former, a double role in chemical defense and precursor of PG free acids in the contraction of the cerata have been proposed;[2] for the latter, a role in mollusc reproduction seems reasonable.

(5) For a review see: Stanley-Samuelson, D. W. *Biol. Bull.* **1987**, *173*, 92.

Experimental Section

General Procedures. HPLC analyses were performed on a chromatograph equipped with two pumps and a UV–vis detector. NMR spectra were recorded at 500 MHz. GLC analyses were performed with capillary column FS SE-30-CB-0.5 (25-m length, 0.32-m i.d.).

Isolation of Prostaglandin Lactones from Mantles and Cerata. In an early extraction procedure[1] 26 *T. fimbria* specimens were deprived of the internal organs, and the resulting mantles and cerata were extracted with acetone (1 L × 3) over 18 h at room temperature. The acetone was removed under vacuum at 40 °C, and the remaining water was extracted with diethyl ether (100 mL × 3). After treatment with anhydrous Na_2SO_4, the solvent was evaporated to afford an extract (2.29 g) that was chromatographed on a Si gel column and eluted with $CHCl_3$ and increasing amounts of CH_3OH and then on μ-Porasil HPLC (*n*-hexane/EtOAc, 9:1) to afford **1**–**3** as previously described.[1] Acetate **4** (0.8 mg) was isolated as a faster moving peak during the HPLC purification of the fractions containing **3** and identified by comparison of its ^1H NMR spectrum with that of an authentic specimen.[1]

Fractions of the $CHCl_3/CH_3OH$ column containing **5** and **6** exhibited a characteristic red spot when sprayed with $Ce(SO_4)_2/H_2SO_4$ on Si gel TLC (R_f 0.35 and 0.30, respectively; C_6H_6/diethyl ether, 7:3). These fractions were pooled (431 mg), chromatographed on a Si gel column, and eluted with C_6H_6 and increasing amounts of diethyl ether to afford an enriched fraction (17 mg). Preparative HPLC of this fraction on μ-Porasil (*n*-hexane/EtOAc, 8:2) yielded 2 mg of **5** and 2 mg of **6**.

Prostaglandin $F_{2\alpha}$ 1,15-lactone 11-acetate (5): MS, m/z (%) 378 (M^+; 0.6), 318 (M^+ – AcOH, base peak), 300 (44), 231 (27), 191 (90), 173 (42); ^1H NMR δ ($CDCl_3$; assignments made by ^1H–^1H COSY) δ 5.88 (dd, J 6.2, 16.0 Hz; 1 H, H-14), 5.81 (dd, J 8.4, 16.0; 1 H, H-13), 5.53 (dt, J 4.5, 10.7; 1 H, H-6), 5.49 (m; 1 H, H-5), 5.18 (dt, J 6.1, 8.1; 1 H, H-15), 4.90 (m; 1 H, H-11), 4.20 (m; 1 H, H-9), 2.03 (s; 3 H, acetyl methyl), 0.88 (bt, J 6.2; 3 H, H-20).

Prostaglandin $F_{3\alpha}$ 1,15-lactone 11-acetate (6): MS, m/z (%) 376 (M^+, 3), 342 (5), 316 (M^+ – AcOH, 46), 307 (29), 298 (base peak), 247 (68), 229 (70), 163 (69); ^1H-NMR δ ($CDCl_3$; assignments made by ^1H–^1H COSY) 5.90 (dd, J 6.0, 15.8 Hz; 1 H, H-14), 5.84 (dd, J 8.0, 15.8; 1 H, H-13), 5.54 (dt, J 4.9, 10.1; 1 H, H-6), 5.49 (m; 1 H, H-18), 5.42 (m; 1 H, H-5), 5.30 (m; 1 H, H-17), 5.20 (dt, J 6.1, 7.7; 1 H, H-15), 4.90 (m; 1 H, H-11), 4.20 (m; 1 H, H-9), 2.04 (s; 3 H, acetyl methyl), 0.96 (t, J 7.6; 3 H, H-20).

An improved extraction procedure was used in more recent preparations. Typically, mantles and cerata were separately extracted by mixing the tissue in a mortar with sand (Merck; ca. 5 g/animal) and acetone (10 mL/animal). After the tissue was triturated, more acetone was added and the mixture was sonicated for 5 min. The acetone was decanted, and the sonication was repeated for five times with fresh acetone. The pooled acetone extracts were then concentrated under vacuum, and the aqueous residue was extracted with diethyl ether, dried on Na_2SO_4, and chromatographed on a Si gel column (C_6H_6 and increasing amounts of diethyl ether). Fractions were monitored by TLC and submitted to preparative HPLC on a μ-Porasil column eluted with *n*-hexane/EtOAc (85:15; flow rate 1 mL/min). The recovery of PG-lactones was higher than that of the previous extraction method and close to the values estimated by the quantitation method reported below.

Quantitation of the Prostaglandin Lactones in the Tissues. Each tissue was extracted with 5% AcOH in H_2O (1:1, v/v) containing 15 μM indomethacin, and the extracts were purified on Sep-pak cartridges (Waters), washed with 10% MeOH/85% H_2O/5% AcOH (5 mL), and eluted with MeOH (5 mL). The eluates were monitored by HPLC on a Spherisorb 5-μm column (ODS-2, 4.5 × 25 mm) and eluted with a 90-min gradient from 30 to 70% CH_3CN/0.1% CF_3COOH in H_2O/0.1% CF_3COOH. UV absorbance was monitored at 205 nm, and quantitation was made by using prostaglandin lactones previously purified from *T. fimbria* (detection limit 1 μg). Acquisition, integration, and calibration were performed with use of a Maxima 820 chromatography work station (Waters Associates).

$NaBH_4$ Reduction of 3 and 4. To 3.6 mg of synthetic[4,1] PGE_2-1,15-lactone 11-acetate (4) dissolved in 0.5 mL of MeOH was added 2 mg of $NaBH_4$. After 5 min at room temperature, 5 mL of CH_3COOH was added and the solvents were evaporated under N_2. The reaction mixture was loaded on an analytical Si gel TLC plate (C_6H_6/diethyl ether 8:2) and the mixture of 5 and 9 eluted together with diethyl ether (2.5 mg). The mixture was separated by HPLC on μ-Porasil (*n*-hexane/EtOAc, 85:15; flow rate 2 mL/min) to afford, in order of increasing polarity, 9 (1.5 mg) and 5 (ca. 0.3 mg).

9-Epiprostaglandin $F_{2\alpha}$ 1,15-lactone 11-acetate (9): MS, *m/z* 318 (M^+ – AcOH; 15), 300 (base peak), 289 (5), 274 (9), 243 (8), 213 (8), 191 (19); ^1H NMR δ ($CDCl_3$) 5.89 (dd, *J* 6.2, 16.0 Hz; 1 H, H-14), 5.79 (dd, *J* 8.5, 16.0; 1 H, H-13), 5.64 (dt, *J* 5.2, 10.8; 1 H, H-6), 5.40 (dt, *J* 6.1, 10.0; 1 H, H-5), 5.14 (dt, *J* 6.3, 8.1; 1 H, H-15), 4.98 (dt, *J* 6.0, 8.3; 1 H, H-11), 4.05 (m; 1 H, H-9), 2.01 (s; 3 H, acetyl methyl), 0.88 (t, 6.6; 3 H, H-20).

Reduction of 2.0 mg of natural PGE_3-1,15-lactone 11-acetate (3) under similar conditions with purification as above yielded 10 and 6 in a 5:1 ratio, as judged by HPLC.

9-Epiprostaglandin $F_{3\alpha}$ 1,15-lactone 11-acetate (10): MS, *m/z* 376 (M^+; 2.5), 316 (M^+ – AcOH; base peak), 307 (27), 298 (23), 247 (90), 229 (85), 211 (52), 171 (48); ^1H NMR δ ($CDCl_3$) 5.91 (dd, *J* 6.2, 15.8 Hz; 1 H, H-14), 5.83 (dd, *J* 8.2, 15.8; 1 H, H-13), 5.65 (dt, *J* 5.2, 10.8; 1 H, H-6), 5.48 (m; H-18), 5.41 (m; 1 H, H-5), 5.27 (m; 1 H, H-17), 5.16 (dt, *J* 6.2, 7.9; 1 H, H-15), 4.97 (dt, *J* 5.9, 8.2; 1 H, H-11), 4.05 (m; 1 H, H-9), 2.01 (s; 3 H, acetyl methyl), 0.95 (t, *J* 7.5; 3 H, H-20).

Acetylation of 5 and 7. Synthetic $PGF_{2\alpha}$-1,15-lactone (7; 2 mg) or natural $PGF_{2\alpha}$-1,15-lactone 11-acetate (5; 0.5 mg) was dissolved in 0.5 mL of anhydrous pyridine, and 0.2 mL of acetic anhydride was added. The mixture was kept at room temperature for 18 h, 1 mL of MeOH was added, and the solvents were removed under a N_2 stream to afford the same diacetate (8).

Prostaglandin $F_{2\alpha}$ 1,15-lactone 9,11-diacetate (8): MS, *m/z* (%) 420 (M^+, 0.5), 360 (M^+ – AcOH; 1), 318 (3), 300 (M^+ – 2 AcOH; base peak), 257 (3), 243 (5), 229 (6), 227 (5), 213 (10), 174 (9), 173 (8); ^1H NMR δ ($CDCl_3$) 5.90 (dd, *J* 6.6, 16.0 Hz; 1 H, H-14), 5.78 (dd, *J* 8.7, 16.0; 1 H, H-13), 5.46 (dt, *J* 4.4, 10.8; 1 H, H-6), 5.39 (dt, *J* 6.0, 10.0; 1 H, H-5), 5.19 (m; 1 H, H-9), 5.17 (dt, *J* 6.4, 8.1; 1 H, H-15), 4.89 (m; 1 H, H-11), 2.08 and 2.02 (two s; 3 H each, acetyl methyls), 0.88 (t, *J* 7.0; 3 H, H-20).

Isolation of Prostaglandin 1,15-Lactone Fatty Acid Esters (PLFE) from Egg Masses and Ovotestis. Thirty freshly laid egg masses from several specimens of *T. fimbria* were frozen at −80 °C and subsequently extracted with sand and acetone as described above. Aliquots of the ethereal extract (1.5 g) were submitted, when needed, to preparative Si gel TLC (C_6H_6/diethyl ether, 8:2), and the large band at R_f 0.6 ± 0.1 was eluted with diethyl ether (48 mg).

Part of the purified PLFE mixture (7 mg) was submitted to preparative Si gel TLC (*n*-hexane/EtOAc, 7:3) to give three bands (1–3; Table II) weighing 1.3, 1.3, and 0.5 mg, respectively, which were characterized by ^1H NMR spectroscopy ($CDCl_3$). Diagnostic resonances were as follows: fraction 1, δ 5.97 (dd, *J* 6.5, 15.8 Hz), 5.88 (dd, *J* 5.9, 15.8), 5.86 (dd, *J* 8.2, 15.8), 5.78 (dd, *J* 8.9, 15.8), 4.90 (m), 4.19 (m), 3.97 (m) (4.90:3.97 ≈ 1:1); fraction 2, δ 5.97 (dd, *J* 6.5, 15.8 Hz), 5.81 (dd, *J* 8.9, 15.8), 3.97 (m), 0.97 (t, *J* 7.4), 0.88 (t, *J* 6.9); fraction 3, δ 5.97 (dd, *J* 6.6, 15.9 Hz), 5.81 (dd, *J* 8.9, 15.9), 5.38 (m), 3.97 (m), 2.83 (m), 0.975 (t, *J* 7.3), 0.972 (t, *J* 7.3) (0.975:0.972 ≈ 1:1).

Part of the purified PLFE mixture (25 mg) was fractionated on a reversed-phase HPLC using repeated 1-mg runs on a Novopak 4-μm analytical column (Waters; CH_3OH/H_2O, 94:6; flow rate 0.8 mL/min) and monitoring the eluate at 205 nm. The eight major components (A–H, Figure 1) were collected to afford A, 1 mg; B, 2 mg; C, 3.7 mg; D, 0.5 mg; E, 1.1 mg; F, 1.2 mg; G, 0.8 mg; and H, 3.2 mg and analyzed by ^1H NMR spectra ($CDCl_3$) and EIMS (assignments, Table II). Fraction A: *m/z* 618 (M^+), 600, 316, 298; δ 5.97 (dd, *J* 6.5, 15.5 Hz), 5.81 (dd, *J* 8.5, 15.5), 5.38 (m), 3.97 (m), 2.82 (m), 0.974 (t, *J* 7.5), 0.970 (t, *J* 7.5). Fraction B: *m/z* 544 (M^+), 526, 316, 298; δ 5.97 (dd, *J* 6.5, 15.8 Hz), 5.81 (dd, *J* 8.9, 15.8), 3.96 (m), 0.97 (t, *J* 7.5), 0.88 (t, *J* 7.0). Fraction C: δ 5.97 (dd, *J* 6.5, 15.9 Hz), 5.81 (dd, *J* 8.9, 15.9), 5.38 (m), 3.97 (m), 2.82 (m), 0.974 (t, *J* 7.5), 0.970 (t, *J* 7.7); this sample decomposed before running the MS spectrum. Fraction D: *m/z* 644 (M^+), 626, 620 (M^+), 602, 316, 298; δ 5.88 and 5.86 (two poorly resolved dd's), 5.38 (m), 4.90 (m), 4.19 (m), 2.83 (m), 0.97 (t, *J* 7.4 Hz), 0.95 (t, *J* 7.4), 0.88 (distorted triplet). Fraction E: *m/z* 646 (M^+), 628, 318, 300; δ 5.96 (dd, *J* 6.5, 15.8 Hz), 5.78 (dd, *J* 8.9, 15.8), 5.38 (m), 3.97 (m), 2.83 (m), 0.97 (t, *J* 7.5), 0.89 (distorted triplet). Fraction F: *m/z* 572 (M^+) 554, 316, 298; δ 5.97 (dd, *J* 6.5, 15.9 Hz), 5.81 (dd, *J* 8.9, 15.9), 3.96 (m), 0.97 (t, *J* 7.5), 0.88 (t, *J* 6.6). Fraction G: *m/z* 598 (M^+), 580, 572 (M^+), 554, 318, 316, 300, 298; δ 5.88 (dd, *J* 5.9, 15.8 Hz), 5.86 (dd, *J* 8.2, 15.8), 4.90 (m), 4.19 (m), 3.97 (m) (4.90:3.97 ≈ 2:1), 0.97 (t, *J* 7.5), 0.95 (t, *J* 7.5). Fraction H: *m/z* 574 (M^+), 556, 318, 300; δ 5.96 (dd, *J* 6.6, 15.9 Hz), 5.78 (dd, *J* 8.9, 15.9), 3.97 (m), 0.88 (distorted triplet).

Anatomical sections of *T. fimbria* were obtained as follows. Mantles and cerata of four *T. fimbria* specimens were separated from the internal organs, and then the latter were subdivided in ovotestis, hepatopancreas, prostate, penis, and seminal receptacle. The anatomical sections were separately extracted and analyzed by TLC. The PLFE mixture was detected in the ovotestis only. The ethereal extract of the ovotestis (28 mg) was purified by TLC as above to give 0.6 mg of a PLFE mixture whose ^1H NMR spectrum was similar, where relevant, to that of the mixture extracted from the egg masses.

Methanolysis of the PLFE Mixture. Part of the PLFE mixture (3.5 mg) was dissolved in 1 mL of anhydrous MeOH, and 10 mg of solid Na_2CO_3 was added. After 20 h at room temperature, the MeOH solution was recovered by filtration and aliquots were analyzed by gas chromatography (25-m glass capillary column; SE-30 0.5% on CB; 110 °C). Identification of the fatty acid methyl esters was made by standard procedures, vs reference compounds (Sigma). The esters identified were the following (percent in the mixture): $C_{14:0}$ (7.4), $C_{16:1}$ (5.9), $C_{16:0}$ (12.1), $C_{17:1}$ (8.1), $C_{17:0}$ (3.4), $C_{18:1}$ (3.4), $C_{18:0}$ (9.5), $C_{20:4}$ (arachidonic; 1.0), $C_{20:5}$ (*cis*-5,8,11,14,17-eicosapentaenoic; 12.3), $C_{20:1}$ (5.1), $C_{22:6}$ (*cis*-4,7,10,13,16,19-docosahexaenoic; 14.6).

The remaining part of the MeOH solution was submitted to preparative HPLC on reversed-phase column (Spherisorb 5 μm as for the quantitation method; see above), resulting in the isolation, in order of increasing retention time, of $PGF_{3\alpha}$-methyl ester, $PGF_{2\alpha}$-methyl ester, and $PGF_{2\alpha}$-1,15-lactone. An authentic sample of $PGF_{2\alpha}$-methyl ester was prepared by CH_2N_2 methylation of commercially available $PGF_{2\alpha}$, while $PGF_{2\alpha}$-1,15-lactone was identified by comparison with a synthetic sample.[4]

Prostaglandin $F_{2\alpha}$ methyl ester: MS, *m/z* (%) 350 (M^+ – H_2O; 2.0), 332 (M^+ – 2 H_2O; 14), 314 (M^+ – 3 H_2O; 5), 278 (base peak); ^1H NMR δ ($CDCl_3$) 5.58 (dd, *J* 6.4, 15.2 Hz; 1 H, H-14), 5.51 (dd, *J* 8.5, 15.2; 1 H, H-13), 5.41 (m; 2 H, H-5 + H-6), 4.20 (m; 1 H, H-9), 4.08 (q, *J* 6.4; 1 H, H-15), 3.98 (m; 1 H, H-11), 3.67 (s; 3 H, OCH_3), 1.81 (bd, *J* 14.3; 1 H, H-10α), 0.89 (bt, *J* 6.6; 3 H, H-20).

Prostaglandin $F_{3\alpha}$ methyl ester: MS, *m/z* (%) 348 (M^+ – H_2O; 1.5), 330 (M^+ – 2 H_2O; 5), 312 (M^+ – 3 H_2O; 2), 297 (26), 279 (82), 261 (base peak); ^1H NMR δ ($CDCl_3$) 5.61 (dd, *J* 6.0, 15.3 Hz; 1 H, H-14), 5.56 (dd, *J* 9.0, 15.3; H-13, overlapped with another olefinic proton), 5.45–5.32 (three overlapped olefinic protons), 4.21 (m; 1 H, H-9), 4.16 (q, *J* 6.3; 1 H, H-15), 4.0 (m; 1 H, H-11), 3.67 (s; 3 H, OCH_3), 1.81 (bd, *J* 14.5; 1 H, H-10α), 0.97 (t, *J* 7.5; 3 H, H-20).

Ichthyotoxicity Test. Ichthyotoxicity assays were conducted on the mosquito fish *Gambusia affinis* as previously described.[6,7] In each test, six fishes were placed in distilled water (70 mL) and 0.5 mL of an acetone solution of appropriate concentration of the test compound was added. The toxicity ranking was defined according to ref 6. The results of the testing were the following: 1, toxic at 10 ppm; 2, toxic at 1 ppm; 3, toxic at 5 ppm; 4, not tested; 5, 6, and the PLFE mixture, nontoxic at 10 ppm.

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Registry No. 4, 62410-96-2; 5, 132541-84-5; 6, 132618-68-9; 7, 55314-49-3; 8, 132564-12-6; 9, 132541-85-6; 10, 132541-86-7; 9-acyl-$PGF_{3\alpha}$ $C_{20:5}$ fatty acid ester, 132564-41-1; 9-acyl-$PGF_{3\alpha}$ $C_{14:0}$ fatty acid ester, 132541-87-8; 11-acyl-$PGF_{3\alpha}$ $C_{22:6}$ fatty acid ester, 132541-88-9; 11-acyl-$PGF_{2\alpha}$ $C_{20:4}$ fatty acid ester, 132541-89-0; 9-acyl-$PGF_{2\alpha}$ $C_{22:6}$ fatty acid ester, 132541-90-3; 9-acyl-$PGF_{3\alpha}$ $C_{16:0}$ fatty acid ester, 132564-13-7; 11-acyl-$PGF_{3\alpha}$ $C_{a3:0}$ fatty acid ester, 132541-91-4; 11-acyl-$PGF_{2\alpha}$ $C_{18:1}$ fatty acid ester, 132541-92-5; 9-acyl-$PGF_{2\alpha}$ $C_{16:0}$ fatty acid ester, 132541-93-6; 11-acyl-$PGF_{3\alpha}$ $C_{18:1}$ fatty acid ester, 132541-94-7; 11-acyl-$PGF_{2\alpha}$ $C_{18:0}$ fatty acid ester, 132541-95-8; 9-acyl-$PGF_{3\alpha}$ $C_{16:1}$ fatty acid ester, 132541-96-9; 9-acyl-$PGF_{2\alpha}$ $C_{16:1}$ fatty acid ester, 132541-97-0; 3, 123314-21-6; 11-acyl-$PGF_{3\alpha}$ $C_{20:4}$ fatty acid ester, 132564-42-2; 11-acyl-$PGF_{2\alpha}$ $C_{22:6}$ fatty acid ester, 132541-98-1.

Use of *N,N'*-Dimethoxy-*N,N'*-dimethylurea as a Carbonyl Dication Equivalent in Organometallic Addition Reactions. Synthesis of Unsymmetrical Ketones

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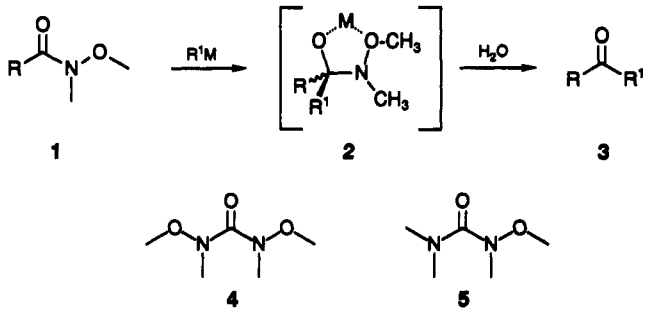
Many methods for the formation of ketones by the addition of organometallic reagents to CO_2 equivalents appear in the literature.[2] These include the addition of the organometallic reagent to carboxylic acid derivatives,[3-8] direct addition to CO_2, and reactions employing a transition-metal catalyst.[4] Even though many of these approaches produced the desired ketones, yields were often low and the reaction conditions were specific and not broadly applicable. A common complicating factor is the concomitant addition of the organometallic reagent to the ketone to produce a tertiary alcohol.

Several research groups have introduced ester or amide derivatives in which the formation of tertiary alcohols is not a problem.[5-8] These derivatives (e.g., 1) contain a ligand that stabilizes by chelation the tetrahedral intermediate 2 formed by addition of the organometallic reagent. The most effective of these appear to be amides



(1)[7] or ureas (4, 5),[9] which use the *N*-methoxy-*N*-methyl ligand. Nahm and Weinreb discovered that several *N*-methoxy-*N*-methylamides reacted cleanly with Grignard and organolithium reagents to produce ketones.[7] For these compounds, the intermediate adduct (2, M = Li, Mg) is stable up to room temperature. The ketone 3 is formed only during the hydrolysis, and the intermediate 2 can actually be submitted to additional chemical manipulation (e.g., deprotonation and alkylation of a remote *N,N*-dimethylhydrazone present in the molecule[10]).

Results and Discussion

We report the synthesis of *N,N'*-dimethoxy-*N,N'*-dimethylurea (4) and its use as a carbonyl dication equivalent. This reagent was also independently developed by Hlasta and Court.[9] The urea 4 was formed in 78% yield from the addition of a solution of bis(trichloromethyl) carbonate (triphosgene) in THF to *N*-methoxy-*N*-methylamine. (Trichloromethyl)chloroformate (diphosgene) also works well.

The addition of 4 to solutions of a variety of aryl, alkyl, alkenyl, and alkynyl organometallic reagents in THF readily produced the amide 1. The reactions were clean and occurred in high yield in all cases except where the formation of the organolithium reagent was difficult (1e and 1f, Table I). The reactions proceeded slowly at −78 °C, but rapidly as the reaction mixtures were warmed to 22 °C. A reaction mixture containing 2-lithio-5-methylthiophene and 4 at −78 °C was quenched with a 1 M aqueous/MeOH solution of NH_4Cl after 10 min and gave only 2% of 1a, whereas an analogous reaction mixture that was warmed to room temperature and then quenched gave a near quantitative conversion. The formation of the ketone 3 from the addition of RLi or RMgBr reagents to the amide 1 is well precedented[7] but for completeness is illustrated for compounds 1a, 1d, and 1e.

The urea 4 provides an excellent route to ketones and should prove useful in many synthetic applications. It is easily prepared and purified, is stable for long periods of time, and reacts cleanly with RLi and RMgBr reagents. It should be particularly useful when the intermediate amide 1 cannot be synthesized by traditional methods because of the instability of the parent carboxylic acid.

Experimental Section

General Procedures. Unless otherwise state, all ^1H NMR spectra were measured with reference to $CHCl_3$ (δ 7.24) or TMS (δ 0.0). The $CDCl_3$ triplet (δ 77.0) was used as reference for ^{13}C NMR. Infrared (IR) spectra were taken of neat liquids, unless otherwise stated, between NaCl plates. All elemental analyses were performed by Galbraith Laboratories.

Kugelrohr distillation refers to bulb-to-bulb distillation (bath temperatures are reported). Diethyl ether and tetrahydrofuran (THF) were freshly distilled from sodium benzophenone ketyl.

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