Articles

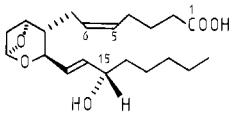
Synthesis of Prostanoids with Bicyclo[2.2.1]heptane, Bicyclo[3.1.1]heptane, and Bicyclo[2.2.2]octane Ring Systems. Activities of 15-Hydroxy Epimers on Human Platelets

Norman H. Wilson,* Venkateswarlu Peesapati, Robert L. Jones, and Kenneth Hamilton

Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ, Scotland. *Received July 16, 1981*

A number of prostanoids with bicyclo[2.2.1]heptane, bicyclo[3.1.1]heptane, and bicyclo[2.2.2]octane ring systems have been prepared by routes which allow the introduction of the ω chain after the α chain. The introduction of a 16-*p*-halophenoxy substituent confers platelet aggregation activity on both 15α- and 15β-hydroxy epimers. In the case of the pinane thromboxane ring system, the natural ω-chain compound is an inhibitor of aggregation, whereas the 16-*p*-fluorophenoxy analogue is a potent aggregation agent.

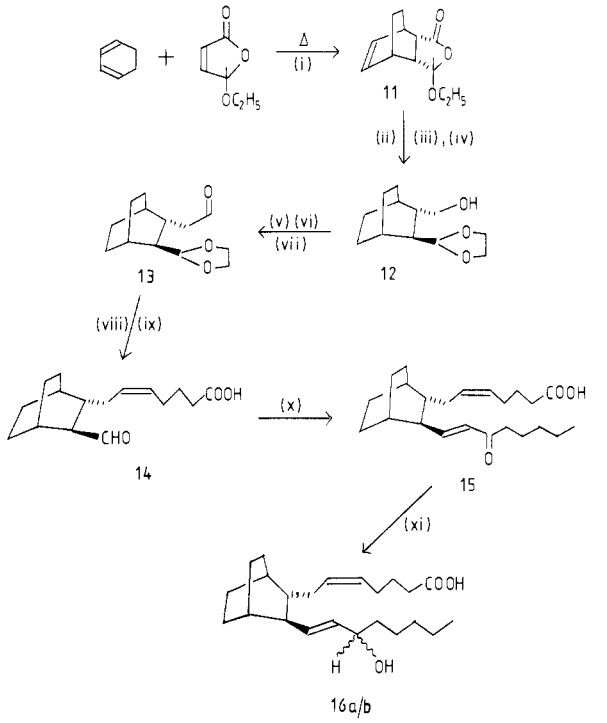
The substitution of a *p*-fluorophenoxymethyl group for the terminal *n*-pentyl moiety in natural prostaglandins, such as PGD_2 and $PGF_{2\alpha}$, increases markedly their thromboxane A_2 like activity.[1] Even greater potency is achieved when this type of aryloxy ω chain is coupled with a bicyclo[2.2.1]heptene or bicyclo[2.2.1]heptane ring system.[2,3] These compounds produce vasoconstriction, bronchoconstriction, and platelet aggregation and are lethal to some laboratory animals. We decided to investigate how other alterations in the ω chain and the ring system influence thromboxane-like activity.[4] It soon became apparent from these studies that whereas the 15β-hydroxy (unnatural) epimers of compounds with an *n*-pentyl terminus show very weak activity relative to the corresponding 15α-hydroxy epimers, the 15β-hydroxy-16-*p*-halophenoxy epimers are potent agonists.



TXA_2

Chemistry. The 15α-hydroxy-9,11-epoxymethano-PGH_2 analogue (**3b**) was prepared from natural PGA_2 as described by Bundy.[5] Oxidation to the 15-ketone (**2**) with Jones reagent, followed by reduction with aluminum isopropoxide in toluene (Meerwein–Ponndorf–Verley), yielded a mixture of C15 epimers. These were completely separated by liquid–gel partition chromatography (see Experimental Section). The more polar isomer cochromatographed with the starting alcohol (**3b**). The less polar isomer was then assumed to have a 15β-hydroxyl (**3a**). This order of elution (15β-OH before 15α-OH) is found for a large number of 15-hydroxyprostaglandins with 8α,12β side chains in this type of straight-phase partition system.[6]

Scheme I



16a/b

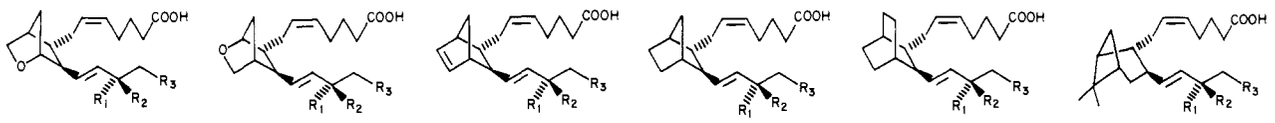
[a] (i) 120 °C; (ii) glycol/H^+; (iii) $LiAlH_4$; (iv) H_2, Pd/C; (v) TsCl/pyridine; (vi) CN^-/Me_2SO; (vii) DIBAL; (viii) Wittig reaction; (ix) H^+/H_2O; (x) Horner reaction; (xi) Meerwein-Ponndorf-Verley reduction; (xii) 9-BBN/$LiAlH(OMe)_3$/CO; (xiii) liquid NH_3/Na; (xiv) PDC/CH_2Cl_2.

The formyl precursors for the bicyclo[2.2.1]heptane and bicyclo[2.2.1]heptene analogues were prepared by methods previously described.[7] The ω chains were added by standard steps,[8] and the aryl Wittig reagents were prepared as detailed by the ICI group.[9] Sodium borohydride reduction of the enones (e.g., **4**) yielded about equal proportions of the 15α- and 15β-hydroxy epimers, together

(1) R. L. Jones and C. G. Marr, *Br. J. Pharmacol.*, 61, 694 (1977).
(2) R. L. Jones, N. H. Wilson, and C. G. Marr, In "Chemistry, Biochemistry, and Pharmacological Activity of Prostanoids", S. M. Roberts and F. Scheinmann, Eds.; Pergamon Press, Oxford, 1979, pp 210–220.
(3) R. L. Jones and N. H. Wilson, *Adv. Prostaglandin Thromboxane Res.*, 6, 467 (1980).
(4) The 11,9-epoxymethano analogue of PGH_2 (U 44619) (1) has been employed in this and other studies as a stable thromboxane mimic.
(5) G. L. Bundy, *Tetrahedron Lett.*, 1957 (1975).
(6) A. R. Brash and R. L. Jones, *Prostaglandins*, 5, 441 (1974).
(7) T. J. Leeney, P. R. Marsham, G. A. F. Ritchie, and M. W. Senior, *Prostaglandins*, 11, 953 (1976).
(8) J. S. Bindra and R. Bindra, in "Prostaglandin Synthesis", Academic Press, New York, 1977, p 201.
(9) D. Binder, J. Bowler, E. D. Brown, N. S. Crossley, J. Hutton, M. Senior, L. Slater, P. Wilkinson, and N. C. A. Wright, *Prostaglandins*, 6, 87 (1974).

0022-2623/82/1825-0495$01.25/0

Table I. Activities of Some Bicyclic Prostaglandin Analogues on Human Platelets



1 2, 3a,b 4, 5a,b, 6a,b 7a,b–10a,b 16a,b 23a,b, 24a,b

no.	R_1	R_2	R_3	aggregation response	concn,[a] μM
1[b]	OH	H	n-C_4H_9	irreversible	0.23
2	O		n-C_4H_9	no effect	(20)
3a	H	OH	n-C_4H_9	shape change only	(20)
3b	OH	H	n-C_4H_9	irreversible	0.26
4 (racemic)	O		O-Ph-F	no effect	(10)
5a (racemic)	H	OH	O-Ph-F	irreversible	1.7
5b (racemic)	OH	H	O-Ph-F	irreversible	0.19
6a (racemic)	H	OH	CH_2-Ph-Cl	no effect	(80)
6b (racemic)	OH	H	CH_2-Ph-Cl	shape change only	(80)
7a (racemic)	H	OH	n-C_4H_9	inhibition	10/1.5
7b (racemic)	OH	H	n-C_4H_9	reversible	15
8a (racemic)	CH_3	OH	n-C_4H_9	inhibition	10/1.3
8b (racemic)	OH	CH_3	n-C_4H_9	irreversible	6.2
9a (racemic)	H	OH	O-Ph-F	irreversible	1.3
9b (racemic)	OH	H	O-Ph-F	irreversible	0.14
10a (racemic)	H	OH	O-Ph-Cl	irreversible	3.5
10b (racemic)	OH	H	O-Ph-Cl	irreversible	0.72
16a (racemic)	H	OH	n-C_4H_9	inhibition	10/2.9
16b (racemic)	OH	H	n-C_4H_9	irreversible	1.3
23a	H	OH	n-C_4H_9	inhibition	10/1.4
23b	OH	H	n-C_4H_9	inhibition	2.5/4.8; 10/25
24a	H	OH	O-Ph-F	reversible	5.0
24b	OH	H	O-Ph-F	irreversible	0.31

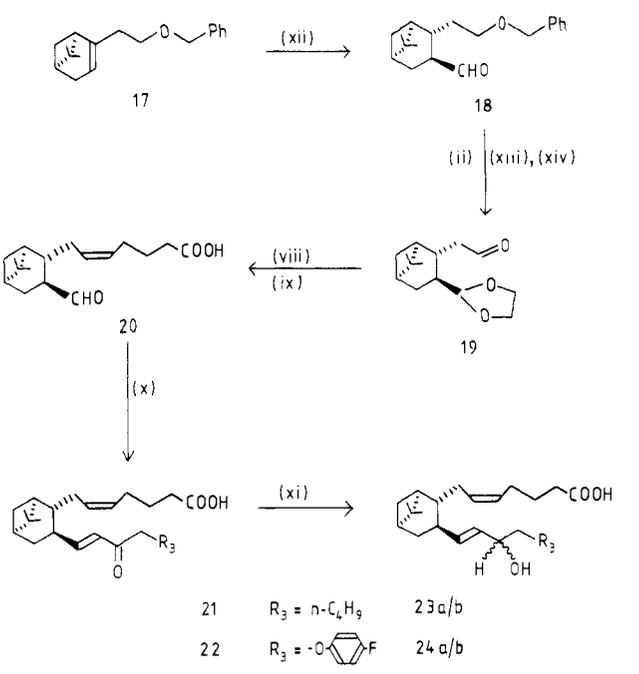
[a] For a compound producing either irreversible or reversible aggregation, the stated concentration corresponds to a primary aggregation response of 20 units on the chart recorder: irreversible aggregation corresponds to a response of 80–90 units. For compounds showing inhibitory activity toward 11,9-epoxymethano-PGH_2 (1), the concentration(s) employed, together with the corresponding dose ratio(s), are given. Dose ratio is defined as the ratio of the concentrations of the agonist producing recorder unit responses in the presence and in the absence of the inhibitor. Each value is the mean of single determinations on platelet-rich plasma obtained from at least three different individuals. Figures in parentheses are the highest concentrations tested. [b] Standard agonist: U 46619.

with small quantities (2–5%) of the corresponding 13,14-dihydro analogues arising from 1,4 reduction. All these products had similar GC retention times (methyl ester–trimethylsilyl ether derivatives). However, the dihydro contaminants could be readily detected in the crude mixture by gas chromatography–mass spectrometry using a multiple ion detection facility. Essentially, the technique involved the continuous recording of the intensities of the major fragment ions arising from the desired compound and of the corresponding ions 2 mass units higher originating from the dihydro form. Partition chromatography afforded the pure 15α- and 15β-allylic alcohols. The assignment of configuration at C15 is based on the earlier elution of the 15β isomer. Compounds **4–7a,b**, **9a,b**, and **10a,b** were prepared by these methods.

The bicyclo[2.2.2]octane analogues were prepared, as shown in Scheme I, from cyclohexa-1,3-diene and maleinaldehyde ethyl pseudoester.[10] It is essential to employ the cyclic acetal protecting group to avoid internal transacetalization in compound **12**. The trans stereochemistry in **12** was inferred by comparison with the bicycloheptene case (loc. cit.); the acetalization conditions are known to also epimerize the aldehydic group. The Meerwein–Ponndorf–Verley reduction in the last step avoided the 13,14-dihydro contamination problem.

The bicyclo[3.3.1]heptane analogues were synthesized from (–)-nopol[11] as depicted in Scheme II. Again the cyclic

Scheme II



[a] Reaction conditions as outlined in Scheme I.

acetal protecting group proved superior to the diethyl acetal. The benzyl protecting group could be removed by hydrogenolysis, but liquid ammonia/sodium cleavage gave better results. Only one aldehyde isomer of **18** and **20** was observed (TLC, GC, and NMR). The formyl group stereochemistry has been assumed to be β owing to the attack

(10) For details of the photoaddition process, see S. H. Schroeter, R. Appel, R. Brammer, and G. O. Schenck, *Justus Liebigs Ann. Chem.*, **697**, 42 (1966).
(11) K. C. Nicolaou, R. I. Magolda, and D. A. Claremon, *J. Am. Chem. Soc.*, **102**, 1404 (1980), and references contained therein.

of the 9-BBN reagent at the unhindered face of **17**. The steric properties of 9-BBN are well documented.[12]

Biological Activities. On human platelets a full agonist produces with increasing dosage a shape change, a primary reversible aggregation wave, a primary wave followed by secondary irreversible aggregation, and a rapid, smooth irreversible aggregation. The concentrations quoted in Table I give a measure of the ability of compounds to produce a primary aggregation wave. The endoperoxide synthetase inhibitor indomethacin (3 μM) has no effect on these values, and thus we appear to be dealing with the direct agonist actions of these compounds.

Of the compounds with the natural 15α-hydroxy ω-chain, the epoxymethano analogues **1** and **3b** and the bicyclo[2.2.2]octane analogue **16b** are full agonists. The bicyclo[2.2.1]heptane analogue **7b** is a partial agonist, producing reversible aggregation only and able to antagonize the action of the standard agonist when both compounds are added simultaneously to the platelet suspension. The pinane analogue **23b** at concentrations between 1 and 10 μM inhibits the action of **1**; this has been reported previously by Aharony et al.[13] and has been presumed to be an antagonistic action at the receptor level. The substitution of a 16-*p*-halophenoxy unit in the latter two molecules removes their antagonistic activity and results in a potent irreversible aggregatory action (compounds **9b**, **10b**, and **24b**).

The importance of the aryloxy grouping for agonist activity is very apparent when we consider the weak activity of the 16-*p*-chlorobenzyl compound **6b**; there is only weak inhibition of the aggregation elicited by the standard agonist **1**.

The 15β-hydroxy epimers of the natural ω-chain compounds have either weak agonist activity (**3a**) or very weak blocking activity (**7a**, **16a**, and **23a**). On the other hand, the 15β-hydroxy-16-*p*-halophenoxy compounds (**5a**, **9a**, **10a**, and **24a**) are potent agonists, though still less active (5–20 times) than the corresponding 15α-hydroxy epimers. To be quite certain of the activities quoted for the 15β-hydroxy analogues, we have taken great care to exclude the corresponding 13,14-dihydro-15α-hydroxy compounds. The latter compounds are slightly less polar than the 13,14-ene-15α-hydroxy compounds and can contaminate the 15β-hydroxy analogues. Neither of the 15-oxo analogues (**2** and **4**) showed any activity.

Discussion

In 15α-hydroxy compounds with a variety of ring systems, the introduction of 16-*p*-halophenoxy group always confers a potent, full agonist action in the platelet-aggregation assay. The nature of this effect at the molecular level is unknown. It is possible, however, that the electron-deficient 17-oxa function is involved in hydrogen bonding to an agonist form of the receptor. Such an interaction could make the configuration of the C15 secondary alcohol less crucial for efficient binding to the receptor site and thus account for the high potency of the 15β-hydroxy-16-*p*-halophenoxy analogues. In the 16-*p*-chlorobenzyl analogues, this type of interaction is not possible.

We were surprised to find that the bicyclo[2.2.2]octane analogue (**16b**), a compound not previously described in the literature, showed considerably higher agonist activity than the corresponding bicyclo[2.2.1]heptane analogue (**7b**). This raises the possibility that replacement of one of the α-orientated methylene groups in the bicyclo octane ring with an ether oxygen might lead to an extremely active compound on thromboxane-sensitive preparations.

Although a considerable number of bicyclic endoperoxide and thromboxane analogues have been prepared,[14] there is limited information on the relative activities of the 15α and 15β epimers on human platelets. The 15β epimer of the highly active thromboxane mimic 9,11-azo-PGH_2 is reported to have minimal activity on human platelets;[15] in our system, 9,11-azo-PGH_2 (Upjohn) gives the primary aggregation response (20 chart units) at a concentration of 0.05 μM and is the most active aggregating agent we have tested. A related analogue, 9,11-azo-13-oxa-15-hydroxyprostanoic acid, inhibits the aggregating action of 9,11-epoxymethano-PGH_2 (receptor blockade) and also inhibits the biosynthesis of TXA_2 from PGH_2.[16] A hydroxy group at C15 is not, however, obligatory for high affinity at the thromboxane receptor, since substitution of the complete ω-chain in 9,11-ethano-PGH_2 (**7b**) with a *N*-(phenylcarbamoyl)hydrazonomethyl group results in a potent competitive antagonist.[17]

The norpinane analogue of TXA_2, in contrast to the dimethylnorpinane analogue (**23b**), shows reversible aggregatory activity on human platelets at high concentrations (100 μM); its 15β-hydroxy epimer is inactive.[18] However, Nicolaou and co-workers have reported potent thromboxane antagonist activity for the 15α-norpinane analogue.[11,19] Syntheses of other thromboxane analogues in which one of the ring oxygens has been replaced by either a methylene group[20] or a sulfur atom[21] have been described.

Experimental Section

^{1}H NMR spectra were obtained on a Perkin-Elmer R32 90 MHz instrument, using tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer 237 spectrometer. MS and GC–MS data were obtained on a VG Micromass 70-70F system, using a 3% OV1 on Supelcoport column in the GC. Carbon values were calculated with respect to the retention times of straight-chain fatty acid methyl esters.

Chromatography Systems. For preparative separation of C15 epimers, a liquid–gel partition system was used. The gel stationary phase was made by treating Sephadex LH-20 (Pharmacia) with 1,2-epoxytetradecane, using BF_3 etherate as catalyst, to give a 20% incorporation of substituent by weight.[22] Columns (70–200 mL) of the gel were eluted with hexane/1,2-dichloroethane/ethanol (100:100:5) with 0.1% acetic acid added. All compounds were eluted between 1 and 3 bed volumes of eluant. Final products were checked for purity by TLC on Merck silica

(12) H. C. Brown, in "Organic Synthesis via Boranes", Wiley, New York, 1975.
(13) D. Aharony, J. B. Smith, E. F. Smith, A. M. Lefer, R. L. Magolda, and K. D. Nicolaou, *Adv. Prostaglandin Thromboxane Res.*, **6**, 489 (1980).
(14) K. C. Nicolaou, G. P. Gasic, and W. E. Barnette, *Angew. Chem., Int. Ed. Engl.*, **17**, 293 (1978).
(15) E. J. Corey, K. Narasaka, and M. Shibasaki, *J. Am. Chem. Soc.*, **98**, 6417 (1976).
(16) S. Kam, P. S. Portoghese, J. W. Gerrard, and E. W. Dunham, *J. Med. Chem.*, **22**, 1402 (1979).
(17) R. L. Jones and N. H. Wilson, *Br. J. Pharmacol.*, **73**, 220P (1981).
(18) S. Ohuchida, N. Hamanaka, and N. Hayashi, *Tetrahedron Lett.*, 3661 (1979).
(19) A. M. Lefer, E. F. Smith III, H. Araki, J. B. Smith, D. Aharony, D. A. Claremon, R. L. Magolda, and K. C. Nicolaou, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 1706 (1980).
(20) E. J. Corey, J. W. Ponder, and P. Ulrich, Tetrahedron *Lett.*, **21**, 137 (1980); K. M. Maxey and G. L. Bundy, *ibid.*, **21**, 445 (1980).
(21) S. Kosuye, N. Hamanaka, and M. Hayashi, *Tetrahedron Lett.*, **22**, 1345 (1981); S. Ohuchida, N. Hamanaka, and M. Hayashi, *ibid.*, **22**, 1349 (1980).
(22) J. Ellingboe, E. Nyström, and J. Sjövall, *J. Lipid Res.*, 11, 266 (1970).

Table II. Chromatographic Data for Prostaglandin Analogues

compd	R_f value on TLC[a] system I	R_f value on TLC[a] system II	elution time on HPLC, min
1	0.42	0.53	
2	0.48	0.57	
3a,b	0.46, 0.43	0.55, 0.53	
4	0.64	0.71	44.5
5a,b	0.625, 0.62	0.675, 0.665	43.0, 37.5
6a,b	0.61, 0.605	0.69, 0.67	95.0, 79.0
7a,b	0.63, 0.62	0.715, 0.695	
8a,b	0.66, 0.66	0.735, 0.730	
9a,b	0.63, 0.62	0.68, 0.67	55.0, 49.0
10a,b	0.63, 0.62	0.70, 0.69	79.5, 67.5
16a,b	0.63, 0.62	0.725, 0.705	
23a,b	0.64, 0.63	0.73, 0.72	
24a,b	0.635, 0.63	0.725, 0.72	115, 94.5
PGB_2[b]	0.43	0.50	11.0

[a] Solvent systems are described under Experimental Section. [b] PGB_2 is 15(*S*)-hydroxy-9-oxoprosta-5(*Z*),-8(12),13(*E*)-trienoic acid [λ_{max} (MeOH) 278 nm].

gel 60 plates using two solvent systems: system I, 1% acetic acid in ethyl acetate; system II, the organic layer after equilibration of 2,2,4-trimethylpentane–ethyl acetate–water–acetic acid (30:110:100:20). Compounds were visualized by spraying with 5% phosphomolybdic acid in ethanol and heating to 110 °C for 15 min: each compound gave a single spot in both systems (Table II). Compounds with strong UV chromophores were also examined by reversed-phase HPLC on a Perkin-Elmer series II instrument using 4.6 × 250 mm Partisil ODS 10 columns eluted with acetonitrile–water–acetic acid (500:500:1). Each compound gave a single symmetrical peak (Table II).

Human Platelet Aggregation. Platelet assays were performed using citrated human platelet-rich plasma in a Born-type aggregometer. PRP (1 mL), Kreb's solution (1 mL), and 0.9% NaCl solution (0.4 mL) were mixed and incubated at 37 °C for 2 min. The test compound was added in 0.1 mL of 0.9% NaCl solution, and the aggregation response was recorded.

5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-oxo-4-(*p*-fluorophenoxy)but-1(*E*)-enyl]bicyclo[2.2.1]hept-2-ene (4). Reaction of 550 mg of 5-*endo*-[6-carboxyhex-2(*Z*)-enyl]-6-*exo*-formylbicyclo[2.2.1]hept-2-ene with the ylide generated from dimethyl 2-oxo-3-(*p*-fluorophenoxy)propanephosphonate yielded, after chromatography on both silica gel and the liquid–gel partition system, 210 mg of enone 4: UV (MeOH) λ_{max} 248 nm (ϵ_{max} 11 840); NMR ($CDCl_3$) δ 8.8 (1 H, br, COOH), 6.7–7.3 (5 H, m, aromatic and CH=CCO), 6.39 (1 H, d, J = 16 Hz, C=CHCO), 6.10 (2 H, m, ring olefinic), 5.33 (2 H, m, α-chain olefinic), 4.67 (2 H, s, $COCH_2O$), 1.0–3.9 (14 H, m, aliphatic); GC–MS for methyl ester *O*-methyl oxime trimethylsilyl ether derivative, single peak with carbon value of 26.6; mass spectrum, m/e 441 (M^+), 410 (M − 31), 375 (M − 66, retro-Diels–Alder), 344 (M − 31 − 66), 316 (M − 125), 264 (M − 66 − 111).

5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-hydroxy-4-(*p*-fluorophenoxy)but-1(*E*)-enyl]bicyclo[2.2.1]hept-2-ene (5a,b). Reduction of 4 (200 mg) with sodium borohydride in ethanol at −20 °C, followed by liquid–gel partition chromatography, gave two isomers, less polar **5a** (83 mg) and more polar **5b** (85 mg): UV (MeOH) λ_{max} 279 nm [ϵ_{max} 2250 (less polar) and 2450 (more polar)]; NMR ($CDCl_3$)[15] for less polar isomer (15β), δ 6.9 (4 H, m, aromatic), 6.2 (1 H, m, ring olefinic), 6.0 (1 H, m, ring olefinic), 5.85 (1 H, dd, trans olefinic), 5.5 (1 H, dd, trans olefinic), 5.45 (1 H, m, cis olefinic), 5.35 (1 H, cis olefinic), 4.5 (1 H, m, CHOH), 3.9 (2 H, m, CH_2OAr), 2.8–1.4 (14 H, m, aliphatic); for more polar isomer (15α), δ 6.9 (4 H, m, aromatic), 6.2 (1 H, m, ring olefinic), 6.0 (1 H, m, ring olefinic), 5.85 (1 H, dd, trans olefinic), 5.5 (1 H, dd, trans olefinic) 5.45 (1 H, m, cis olefinic), 5.3 (1 H, m, cis olefinic), 4.5 (1 H, m, CHOH), 4.0 (1 H, dd, CH_2OAr), 3.9 (1 H, dd, CH_2OAr), 2.8–1.4 (14 H, m, aliphatic); GC–MS carbon value for methyl ester trimethylsilyl ether derivatives of both isomers = 26.0; mass spectra, identical with major ions at m/e 420 (M − 66, retro-Diels–Alder) and 295 (M − 66 − 125). High-resolution MS calcd for $C_{23}H_{33}O_4SiF$: m/e 420.213. Found: 420.210 (more polar).

Compounds **9a,b** and **10a,b** were prepared by essentially the same sequence, and each had physical data consistent with the proposed structure.

5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-hydroxy-5-(*p*-chlorophenyl)pent-1(*E*)-enyl]bicyclo[2.2.1]hept-2-ene (6a,b). 5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-oxo-5-(*p*-chlorophenyl)pent-1(*E*)-enyl]bicyclo[2.2.1]hept-2-ene was purified by silica gel chromatography (220 mg): UV (MeOH) λ_{max} 220 and 242 nm (ϵ_{max} 12 900 and 10 200). Reduction with sodium borohydride in ethanol at −20 °C, followed by liquid–gel partition chromatography, gave two isomers, less polar **6a** (56 mg) and more polar **6b** (62 mg): UV (MeOH) for less polar isomer, λ_{max} 220 and 268 nm (ϵ_{max} 8200 and 480); more polar isomer, λ_{max} 220 and 268 nm (ϵ_{max} 7850 and 450); NMR ($CDCl_3$), identical for both isomers, δ 7.0–7.4 (4 H, m, aromatic), 6.10 (2 H, m, ring olefinic), 5.55 (2 H, m, trans olefinic), 5.35 (2 H, m, cis olefinic), 4.7 (2 H, br, OH and COOH), 4.05 (1 H, m, CHOH), 0.8–2.8 (18 H, m, aliphatic); GC–MS carbon values of methyl ester trimethylsilyl ether derivatives, less polar = 28.0; more polar = 27.85. Prominent ions occur at m/e 434/436 (M − 66) and 293/295 (M − 66 − 141, retro-Diels–Alder and loss of α chain). High-resolution MS calcd for $C_{24}H_{35}O_3SiCl$: m/e 434.204. Found: 434.203 (more polar).

5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-hydroxyoct-1(*E*)-enyl]bicyclo[2.2.1]heptane (7a,b). 5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-oxooct-1(*E*)-enyl]bicyclo[2.2.1]heptane was purified by silica gel chromatography: UV (MeOH) λ_{max} 232 nm (ϵ_{max} 9950); NMR ($CDCl_3$) δ 6.73 (1 H, dd, J = 8 and 16 Hz, CH=CCO), 6.02 (1 H, d, J = 16 Hz, C=CHCO), 5.35 (2 H, m, cis olefinic), 1.1–2.6 (29 H, m, aliphatic). Reduction of 100 mg of the enone was achieved with sodium borohydride in ethanol at −20 °C. Chromatography in the liquid–gel system yielded two major products, the less polar **7a** (49 mg) and more polar **7b** (36 mg): NMR ($CDCl_3$) δ 6.0 (2 H, br, OH and COOH), 5.2–5.55 (4 H, m, olefinic), 4.05 (1 H, m, CHOH), 0.8–2.5 (29 H, m, aliphatic); both isomers have almost identical spectra. Each compound gave a single peak (carbon values for **7a**, 22.6; for **7b**, 22.5) as the methyl ester trimethylsilyl ether derivatives on GC. The mass spectra were identical: major ions at m/e 434 (M^+), 363 (M − 71), 273 (M − 71 − 90) and 199 (ω chain). High-resolution MS calcd for $C_{26}H_{46}O_3Si$: m/e 434.322. Found: 434.319 (more polar).

5-*endo*-[6-Carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-hydroxy-3-methyloct-1(*E*)-enyl]bicyclo[2.2.1]heptane (8a,b). The methyl ester of 5-*endo*-[6-carboxyhex-2(*Z*)-enyl]-6-*exo*-[3-oxooct-1(*E*)-enyl]bicyclo[2.2.1]heptane (100 mg) was stirred in 5 mL of dry THF at −78 °C. Methyl magnesium iodide (0.5 mL of a 0.33 M solution in ether) was added, and the reaction mixture was stirred for 2 h at −78 °C. Aqueous ammonium chloride solution was added, and the product was extracted into ether. The residue after evaporation was treated with 0.05 M KOH in methanol/water (2:1) for 6 h at room temperature to remove the ester group. Chromatography in the liquid–gel system gave two major products, the less polar **8a** (25 mg) and the more polar **8b** (21 mg): NMR ($CDCl_3$) δ 6.3 (2 H, v br, COOH and OH), 5.75 (2 H, m, trans olefinic), 5.40 (2 H, m, cis olefinic), 1.32 [about 3 H, s, $C(OH)CH_3$], 0.8–2.5 (29 H, m, aliphatic); identical spectra for each isomer. GC–MS carbon values for methyl ester trimethylsilyl ether derivatives = 22.75; mass spectrum, M^+ absent, ions at m/e 433 (M − 15), 377 (M − 71), 358 (M − 90), 287 (M − 71 − 90), and 217 (M − 90 − 141). Some pyrolysis involving loss of Me_3SiOH was present, resulting in a broad peak of carbon value 22.4.

Adduct of Cyclohexa-1,3-diene and Maleinaldehyde Pseudoester (11). The diene (8.2 g) was mixed with the dienophile (12.8 g) and the mixture was heated in a sealed tube at 120 °C for 8 h. Yields of the adduct were about 90% by NMR: bp 95–98 °C (0.2 mm). Anal. ($C_{12}H_{16}O_3$) C, H.

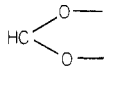
***trans*-2-(Hydroxymethyl)-3-[(dioxyethylene)methyl]bicyclo[2.2.2]octane (12).** The adduct 11 (10 g) was heated under a Dean–Stark apparatus with 12 mL of ethylene glycol in 100 mL of toluene containing a crystal of *p*-toluenesulfonic acid. After water had ceased to form, half of the solvent was distilled off, and the mixture was added to excess lithium aluminum hydride (3 g) in 200 mL of dry ether. The addition was performed at a rate which maintained a gentle boiling of the ether. After a further 1 h of heating, the excess hydride was discharged by addition of water (care), followed by aqueous 10% sodium hydroxide solution to precipitate aluminum salts. The mixture was dried over

magnesium sulfate and then filtered. The organic solvent was evaporated to give the crude product, which was hydrogenated directly in ethanol at atmospheric pressure over 10% palladium on charcoal. One molecular equivalent of hydrogen was adsorbed. The catalyst was filtered off, and the solvent was evaporated. The crude product, a colorless oil, was distilled under vacuum: yield 6.1 g (60%); bp 110–112 °C (0.15 mm). Anal. ($C_{12}H_{20}O_3$) C, H.

***trans*-2-(Formylmethyl)-3-[(dioxyethylene)methyl]bicyclo[2.2.2]octane (13).** The alcohol **12** (7.0 g) in 15 mL of dry pyridine was added to 7.5 g of *p*-toluenesulfonyl chloride in 45 mL of pyridine with stirring at 0 °C. After 20 h, the mixture was poured into ice/water, and after the mixture was stirred for 30 min, the product was isolated (86%) by ether extraction as a colorless oil.

The tosylate ester (12.0 g) in dimethyl sulfoxide (15 mL) was added to potassium cyanide (3.0 g) in dimethyl sulfoxide (20 mL). The mixture was stirred and heated at 100 °C under nitrogen for 6 h. The reaction mixture was poured into water, and the product was isolated by ether extraction. The product was an oil (7.2 g), which showed nitrile IR absorption at 2205 cm^{-1} and was purified by passage through a short Florisil column in toluene. The material was used directly in the next step.

The nitrile (7.0 g) was stirred in 100 mL of dry toluene under nitrogen at −15 °C. Diisobutylaluminum hydride (42.5 mL of 1 M solution in toluene) was added slowly over 25 min, and the mixture was slowly allowed to warm to room temperature. After 1 h, methanol (10 mL) was slowly added, followed by 200 mL of saturated aqueous sodium hydrogen tartrate. The mixture was stirred at 40 °C for 2 h. The upper organic layer was separated, and the aqueous phase was further extracted with ethyl acetate. The combined organic solutions were dried ($MgSO_4$) and evaporated. The yellow oil obtained was chromatographed on Florisil in toluene to give the title compound (**13**) in 83% yield (5.8 g): IR (film) 1720 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 9.75 (1 H, t, J = 2 Hz, CHO), 4.85 (1 H, d, J = 8 Hz, i), 3.9 (4 H, m, OCH_2CH_2O), 2.8–2.4 (2 H, m, CH_2C=O), 2.1–1.2 (12 H, m, aliphatic). Anal. ($C_{13}H_{20}O_3$) C, H.



i

***trans*-2-[6-Carboxyhex-2(*Z*)-enyl]-3-formylbicyclo[2.2.2]octane (14).** 4-Carboxyl-*n*-butyltriphenylphosphonium bromide (17.0 g) was dried at 75 °C for 3 h under vacuum. The solid was cooled and the vacuum released to argon. Dimethyl sulfoxide (50 mL) was added, and butyllithium (270 mL of 1.5 M solution in pentane) was added slowly over 1 h. The deep red ylide thus formed was stirred at room temperature for 15 min, and then the aldehyde (4.6 g) was added slowly over 15 min. The mixture was stirred overnight at room temperature, and then the solvent was removed at 50–60 °C under vacuum. The residue was dissolved in water and the aqueous phase was extracted with ether to remove nonacidic material. The water layer was acidified (pH 4) with 2 N HCl and then extracted with ether. The ethereal solution was dried and evaporated to give the acid acetal (3.5 g, 55%).

The acetal group was removed by stirring the material (3 g) with 100 mL of water/dioxane (1:1) containing 0.5 HCl at 40 °C. The aldehyde **6** was isolated by ether extraction. The product was purified by chromatography on silica gel in toluene/ethyl acetate (90:10): yield 2.3 g (48% overall); IR (film) 1725 (CHO), 1710 (COOH) cm^{-1}; NMR ($CDCl_3$) δ 9.73 (1 H, s, CHO), 5.3–5.5 (2 H, m, olefinic), 1.45–2.2 (21 H, m, aliphatic). Anal. ($C_{16}H_{24}O_3$) C, H.

***trans*-2-[6-Carboxyhex-2(*Z*)-enyl]-3-[3-oxooct-1(*E*)-enyl]bicyclo[2.2.2]octane (15).** To a stirred suspension of sodium hydride (0.065 g of 50% dispersion, 1.3 mmol) in 15 mL of dry THF cooled to 0 °C under nitrogen was added dropwise a solution of dimethyl 2-oxoheptanephosphonate (0.33 g, 1.5 mmol) in 10 mL of dry THF. Upon completion of the addition, the reaction was warmed to room temperature (25 °C) for 1 h. The phosphonate anion was cooled to 0 °C and treated with a solution of the aldehyde (0.295 g, 1.12 mmol) in 5 mL of dry THF. After 0.5 h at 0 °C and 2.5 h at room temperature, the reaction mixture was neutralized with glacial acetic acid. The product was extracted with ethyl acetate, washed with water, dried, and evaporated to give the crude enone. Chromatography using silica gel (ether/ethyl acetate, 9:1) gave the pure compound: yield 0.275 g (68%); IR (film) 1700 (COOH), 1660 (C=O), 1615 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 10.5 (1 H, broad, COOH), 6.9 (dd, J = 16 and 9 Hz, CH=CCO), 6.15 (d, J = 16 Hz, C=CHCO), 5.41 (2 H, m, olefinic), 2.6 (t, J = 7.5 Hz, $COCH_2$), 2.4 (t, J = 7.5 Hz, CH_2COOH), 0.95 (t, J = 6 Hz, CH_3). High-resolution MS calcd for the methyl ester $C_{24}H_{38}O_3$: m/e 374.282. Found: 374.301. GC–MS carbon value = 24.65 (methyl ester); major ions at m/e 374 (M^+), 275 (M − 99), 233 (M − α chain), 99 ($C_5H_{11}CO$) (base peak).

***trans*-2-[6-Carboxyhex-2(*Z*)-enyl]-3-[3-hydroxyoct-1-(*E*)-enyl]bicyclo[2.2.2]octane (16a,b).** The enone **15** (0.2 g) was dissolved in 30 mL of dry toluene and 4 mL of ~1 M aluminum isopropoxide in toluene was added along with 4 mL of dry 2-propanol. The mixture was boiled under a stream of nitrogen (which carried away the acetone formed) for 2.5 h. At this stage, another 4 mL of 2-propanol and 2 mL of isopropoxide solution were added, and the mixture was boiled a further 1 h with removal of 15 to 20 mL of solvent by slow distillation. The reaction mixture was cooled and saturated with a solution of sodium hydrogen tartrate to destroy excess reagent. Water was added and the product was extracted with ether after checking that the aqueous phase was at pH 3. Drying ($MgSO_4$) and evaporating the extracts gave 0.2 g (99% yield) of alcohols **16a,b**, which were separated by liquid–gel partition chromatography: IR (film) 3400 (OH), 1700 (COOH) cm^{-1}; NMR ($CDCl_3$) δ 6.98 (1 H, br, COOH), 5.25–5.75 (4 H, m, olefinic), 4.09 (1 H, m, CHOH), 2.39 (2 H, t, J = 7.5 Hz, CH_2COOH), 0.94 (3 H, t, J = 6 Hz, CH_3); both isomers have almost identical NMR; GC–MS data for both isomers were identical; methyl ester trimethyl silyl ether derivative, carbon value = 23.65, major ions at m/e 448 (M^+), 377 (M − 71), 287 (M − 90 − 71) and 199 (ω chain). High-resolution MS calcd for $C_{27}H_{48}O_3Si$: 448.33. Found: 448.334 (both isomers).

2-[2-(Benzyloxy)ethyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene (17). (−)-Nopol (33.0 g) was added slowly to 6.25 g of an 80% sodium hydride dispersion in oil, stirring in 150 mL of dimethylformamide at room temperature. After the addition was completed (~1 h), stirring was continued for 4–5 h until all hydrogen evolution had ceased. Benzyl chloride (26.0 g, 23 mL) was added over 1 h at room temperature; an exothermic reaction was observed. After the addition was completed, the mixture was heated at 80 °C for 4 h. The material was cooled and poured into water, and the product was isolated by ether extraction, followed by distillation under vacuum. A small early fraction consisted mainly of unchanged nopol: yield 35.5 g (70%); bp 128–131 °C (0.2 mm); NMR ($CDCl_3$) δ 7.3 (5 H, m, aromatic), 5.25 (1 H, m, olefinic), 4.45 (2 H, s, OCH_2O), 3.45 (2 H, t, OCH_2), 2.5–1.9 (7 H, m, aliphatic), 1.25 (3 H, s, Me), 1.2 (1 H, d, CH), 0.82 (3 H, s, Me); GC–MS carbon value 15.85, M^+ 236, and m/e 91 (base peak).

2-*exo*-[2-(Benzoyloxy)ethyl]-6,6-dimethyl-3-*endo*-formylbicyclo[3.1.1]heptane (18). Benzylated nopol (**17**; 10.2 g) was placed in a flask (1 L) with THF (30 mL) under argon. 9-BBN (90 mL of 0.5 M in THF) was added over 5–10 min at room temperature. The solution was boiled for 30 h, after which time most of the olefin had reacted. During this heating period, a THF solution of lithium trimethoxyaluminum hydride (0.7 M) was prepared from lithium aluminum hydride and methanol. The hydroborated nopol was cooled to 0 °C, while the argon atmosphere was replaced by carbon monoxide. The lithium trimethoxyaluminum hydride solution (62 mL of 0.7 M in THF) was then added over 30–60 min while vigorous stirring was employed, and a positive pressure of carbon monoxide was maintained. A vigorous uptake of gas was observed (~1 L) and after an additional 1 h of stirring, the argon atmosphere was reestablished and 82 mL of pH 7 aqueous saturated phosphate buffer was added (97.5 g of $NA_2H_2PO_4{\cdot}2H_2O$ and 108.75 g of K_2HPO_4 dissolved in 250 mL of water), with vigorous stirring. Finally, 30% hydrogen peroxide was carefully added while keeping the temperature of the mixture below 20 °C. The mixture was stirred an additional 10 min and then poured into water. The product was isolated by ether extraction and purified by chromatography on Florisil eluted with petrol/ether: yield 8.9 g (81%); IR 1718 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 9.5 (1 H, d, CHO), 7.25 (5 H, s, aromatic), 4.45 (2 H, s, OCH_2Ar), 3.4 (2 H, t, OCH_2), 2.7–1.5 (9

H, m, aliphatic), 1.4 (3 H, s, CH_3), 0.92 (3 H, s, CH_3), 0.65 (1 H, d, J = 9 Hz, CH); GC–MS carbon value 18.5, m/e 286 (M^+), 91 (C_7H_7 base peak). Anal. ($C_{19}H_{26}O_2$)C, H.

2-*exo*-(Formylmethyl)-6,6-dimethyl-3-*endo*-[(dioxyethylene)methyl]bicyclo[3.1.1]heptane (19). The acetal was formed on the aldehyde (18; 20 g) using glycol and toluenesulfonic acid in toluene as detailed in the first part of the preparation of 12. Material of bp 160–170 °C at 0.15 mm was obtained. Following this, the benzyl protecting group was removed.

The acetal (15 g) in 50 mL of dry ether was stirred, and 300 mL of liquid ammonia was added by distillation, using a carbon dioxide/acetone condenser. Small pieces of metallic sodium were added over 30 min until a blue color was persistent. The mixture was maintained a deep blue for an additional 1 h. Solid ammonium chloride was carefully added to quench the reaction, and the ammonia was allowed to evaporate. The product alcohol, a colorless oil (12.1 g), was isolated by ether extraction and oxidized directly to the aldehyde.

The alcohol (11.5 g) was added in 15 mL of methylene chloride to 28 g of pyridinium dichromate stirring at room temperature in 50 mL of methylene chloride. After 24 h the black solid was filtered off, and the methylene chloride solution was passed through a short Florisil column. The product was thus obtained in a reasonably pure form: IR 1720 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 9.7 (1 H, t, J = 1 Hz, CHO), 4.8 (1 H, d, J = 5 Hz, i), 3.9 (4 H, m, OCH_2CH_2O), 2.7 (2 H, s, broad, CH_2CO), 2.6–0.9 (8 H, m, aliphatic), 1.25 (3 H, s, CH_3), 1.05 (3 H, s, CH_3).

2-*exo*-[6-Carboxyhex-2(*Z*)-enyl]-6,6-dimethyl-3-*endo*-formylbicyclo[3.1.1]heptane (20). The Wittig procedure used in the synthesis of 14 was adopted. The ylide obtained from butyllithium treatment of 31 g of phosphonium salt was treated with 8.8 g of aldehyde. The yield of crude material was 7.6 g. Aliquots of the crude material were deacetalized as required by the process given previously for 14. The product was purified by column chromatography using Unisil (toluene/5% ether): total yield was around 70% from 19; IR (film) 1700 (broad, C=O) cm^{-1}; NMR ($CDCl_3$) δ 10.15 (1 H, br, COOH), 9.64 (1 H, d, CHO), 5.4 (2 H, m, olefinic), 2.6–0.8 (16 H, aliphatic), 1.24 (3 H, s, CH_3), 1.06 (3 H, s, CH_3). Anal. ($C_{17}H_{26}O_3$)C, H.

2-*exo*-[6-Carboxyhex-2(*Z*)-enyl]-6,6-dimethyl-3-*endo*-[3-oxooct-1(*E*)-enyl]bicyclo[3.1.1]heptane (21). The Horner reaction of the aldehyde 20 (0.31 g, 1.12 mmol) was carried out with dimethyl 2-oxo-heptanephosphonate (1.5 mmol), following the procedure described for the synthesis of 15. The crude product (0.305 g) was chromatographed on silica gel (ether/ethyl acetate, 9:1), yielding 0.72 g of enone 21: IR (film) 1710 (COOH), 1665 (C=O), 1620 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 9.9–9.7 (1 H, broad, COOH), 6.68 (dd, J = 16 and 9 Hz, CH=CCO), 5.9 (d, J = 16 Hz, C=CHCO), 5.40 (2 H, m, olefinic), 2.55 (t, J = 7.5 Hz, CH_2COOH), 1.26 (3 H, s, ring CH_3), 1.11 (3 H, s, ring CH_3), 0.95 (3 H, t, J = 6 Hz, CH_3); high-resolution MS calcd for methyl ester $C_{25}H_{40}O_3$: m/e 388.297. Found: 388.294. GC–MS carbon value 24.55, major ions at m/e 317 (M – 71), 247 (M – 141), 298 (M – 99), 99 (base peak).

2-*exo*-[6-Carboxyhex-2(*Z*)-enyl]-6,6-dimethyl-3-*endo*-[3-hydroxyoct-1(*E*)-enyl]bicyclo[3.1.1]heptane (23a,b). Enone 21 (0.2 g) was reduced with aluminum isopropoxide as described for 16a,b to give 0.190 g of a mixture of alcohols, which were separated by liquid–gel partition chromatography [less polar isomer 23a (56 mg) and more polar isomer 23b (55 mg)]: IR (film) 3350 (br, OH) 1705 (COOH) cm^{-1}; NMR ($CDCl_3$) δ 6.5 (2 H, br, OH and COOH), 5.25–5.55 (4 H, m, olefinic), 4.10 (1 H, m, CHOH), 1.20 and 1.07 (6 H, both s, ring methyls), 0.90 (3 H, t, J = 6 Hz, CH_3), both isomers almost identical NMR; GC–MS, both isomers identical as methyl ester trimethylsilyl ether derivatives, carbon value = 23.7. High-resolution MS calcd for $C_{28}H_{50}O_3Si$: m/e 462.352. Found: 462.350 (less polar). Major ions at m/e 391 (M – 71), 301 (M – 71 – 90) (base), 231 (M – 141 – 90), 173 and 199 (ω chain).

2-*exo*-[6-Carboxyhex-2(*Z*)-enyl]-6,6-dimethyl-3-*endo*-[3-hydroxy-4-(*p*-fluorophenoxy)but-1(*E*)-enyl]bicyclo[3.1.1]-heptane (24a,b). Horner reaction of 20 with the ylide from dimethyl 2-oxo-3-(*p*-fluorophenoxy)propanephosphonate yielded the enone 22, which was partially purified by silica gel chromatography. Reduction of about 200 mg of the enone with aluminum isopropoxide as described earlier gave 24a and 24b, which were separated by liquid–gel partition chromatography [less polar (32 mg) and more polar (48 mg)]: UV (MeOH) λ_{max} 280 nm [ϵ_{max} 2640 (less polar) and 1960 (more polar)]; NMR ($CDCl_3$)[23] for less polar isomer (15β), δ 6.9 (4 H, m, aromatic), 5.7 (1 H, dd, trans olefinic), 5.45 (1 H, dd, trans olefinic), 5.45 (1 H, m, cis olefinic), 5.35 (1 H, m, cis olefinic), 4.5 (1 H, m, CHOH), 3.9 (2 H, m, CH_2OAr), 2.95 (2 H, br, OH, COOH), 2.45–0.75 (16 H, m, aliphatic), 1.2 (3 H, s, CH_3), 1.05 (3 H, s, CH_3); more polar isomer (15α), δ 6.9 (4 H, m, aromatic), 5.8 (1 H, dd, trans olefinic), 5.45 (1 H, dd, trans olefinic), 5.45 (1 H, m, cis olefinic), 5.35 (1 H, m, cis olefinic), 4.55 (1 H, m, CHOH), 3.95 (1 H, dd, CH_2OAr), 3.85 (1 H, dd, CH_2OAr), 3.5 (2 H, br, OH, COOH), 2.45–0.75 (16 H, m, aliphatic), 1.2 (3 H, s, CH_3), 1.05 (3 H, s, CH_3). GC–MS methyl ester trimethylsilyl derivatives, carbon value for less polar isomer = 27.40, and carbon value for more polar isomer = 27.30. Mass spectra were identical with major ions at m/e 391 [M – (CH_2O-Ph-F)], 301 [M – CH_2O-Ph-F and Me_3SiOH], and 269 [M – CH_2O-Ph-F and Me_3SiOH and CH_3OH]. High-resolution MS calcd for $C_{23}H_{39}O_3Si$: 391.267. Found: 391.265 (less polar).

Acknowledgment. This work was supported by Grants from the British Medical Research Council and the National Research Development Corp. We thank the Upjohn Co. for gifts of 11,9-epoxymethano-PGH_2 and 9,11-azo-PGH_2.

(23) These spectra were obtained at 350 MHz by courtesy of Dr. A. S. F. Boyd of the Edinburgh University Chemistry Department using a Bruker WM 300 instrument. At these high-field strengths, the methylene protons at C16 adjacent to the asymmetric center at C15 show distinctive patters reflecting the *R* or *S* configuration at the asymmetric center.