Journal of Natural Products
Vol. 50, No. 1, pp. 75-83, Jan-Feb 1987

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ANALOGS OF FECAPENTAENE-12[1]

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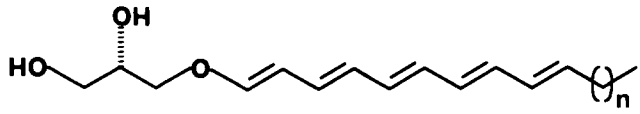
ABSTRACT.—Analogs of the potent fecal mutagen fecapentaene-12 [**1**] have been prepared and tested both for mutagenicity and for their ability to serve as biological precursors of **1**. It was found that mutagenicity in three different *Salmonella* tester strains TA96, TA100, and TA104, decreased rapidly as the number of conjugated double bonds was reduced. The aldehyde **8**, analogous to the hydrolysis product of **1**, showed only low mutagenicity, even in the aldehyde-sensitive strain TA104. None of the polyenes prepared was able to function as a direct biological precursor of of **1** under the conditions employed.

The occurrence of highly mutagenic ether-extractable substances in the feces of certain individuals was first reported by Bruce (1), and the structure of one of these substances was shown by us to be the unique ether lipid fecapentaene-12 [**1**] (2,3). Subsequent studies indicated that related pentaenes were also produced in some individuals (4), and a survey of numerous individuals indicated that fecapentaene-12 and fecapentaene-14 [**2**] are the major ether-extractable mutagens in human feces (5).



1 n=1
2 n=3

Fecapentaene-12 is known to be a microbial metabolite rather than a dietary component, since incubation of the feces of a mutagen-producing individual results in enhanced levels of the compound (6). Production of fecapentaene-12 can also be accomplished in the absence of feces, provided that an extract of feces from a mutagen-producer is incubated with any of five species of *Bacterioides* (*B. thetaiotaomicron, B. fragilis, B. ovaeus, B. uniformis,* and *Bacterioides* sp. 3452A) in the presence of culture media with bile added (7,8). These facts indicate that the feces of mutagen-producers contain a precursor or precursors which are acted on by certain *Bacterioides* species in the presence of bile to form fecapentaene-12.

The occurrence of fecapentaene-12 can be correlated with conditions known to be associated with a high risk of colon cancer. Thus, we have shown that there is a correlation between the excretion of mutagenic feces and populations at risk for colon cancer

[1]This article commemorates the 50th year of publication of the *Journal of Natural Products* (formerly *Lloydia*).

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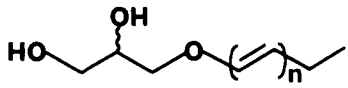
(9), and the bile required for mutagen production is also associated with groups at high risk for colon cancer (10).

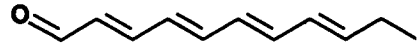
Because of the importance of fecapentaene-12 as a potential inducer of colon cancer, we have investigated two aspects of its nature. The first deals with structure-activity relationships of linear enol ether polyenes and also addresses the question of the importance of the chiral center in fecapentaene-12. The second aspect of our study asks whether fecapentaene-12 can be synthesized in vivo by desaturation of more highly reduced precursors.

RESULTS AND DISCUSSION

Our study of the structure-activity relationships of glyceryl enol ethers made use of the linear polyenes **3-7**. The synthesis of these compounds has been described (11). In addition to the racemic enol ethers **3-7**, natural (chiral) fecapentaene-12 [**1**] and the tetraenal **8** were also tested.



3 n=1
4 n=2
5 n=3
6 n=4
7 n=5



8

Mutagenicity testing was carried out using the revised standard plate incorporation method of the Ames' mutagenicity assay (12) and using tester strains TA98, TA100, and TA104 of *Salmonella typhimurium*. TA98 is used to detect frame-shift mutations, TA100 is more sensitive to base-pair substitutions, and TA104 is a relatively new strain that has been reported to be more sensitive than TA100 to mutagenesis by carbonyl compounds (13). The results of this testing are shown in Table 1; the triene **5**, diene **4**, and monoene **3** showed no mutagenicity at doses up to the very large dose of 250 μg/plate, and data for these compounds are, thus, not included in the table.

TABLE 1. Mutagenicity of Fecapentaene-12 and Analogs

Concentration (ng/plate)	Revertant Colonies[a]										
	TA98				TA100				TA104		
	1	**7**	**6**	**8**	**1**	**7**	**6**	**8**	**7**	**6**	**8**
250,000	NT[b]	NT	NT	25	NT	NT	NT	244	NT	NT	264
100,000	NT	NT	TOX	23	NT	NT	TOX	221	NT	TOX	181
50,000	NT	NT	TOX	24	NT	NT	TOX	187	NT	TOX	186
25,000	NT	NT	97	20	NT	NT	TOX	185	NT	TOX	165
10,000	TOX[c]	TOX	48	19	TOX	TOX	1258	163	TOX	1054	155
5,000	TOX	TOX	32	NT	TOX	TOX	714	NT	TOX	589	NT
2,500	207	215	21	NT	TOX	TOX	442	NT	TOX	388	NT
1,250	163	179	21	NT	1688	1955	204	NT	1488	162	NT
625	91	99	20	NT	1462	1258	180	NT	1380	158	NT
313	49	46	NT	NT	612	799	NT	NT	1039	NT	NT
156	34	23	NT	NT	459	561	NT	NT	698	NT	NT
78	25	23	NT	NT	306	204	NT	NT	202	NT	NT
36	22	18	NT	NT	187	184	NT	NT	174	NT	NT

[a]*Salmonella typhimurium* tester strains; spontaneous reversions: TA98=19, TA100=172, TA104=155.
[b]NT=not tested.
[c]TOX=Full or partial toxicity to background lawn observed.

Several conclusions emerge from the data of Table 1. In the first place, the racemic synthetic fecapentaene-12 [**7**] and natural (*S*)-fecapentaene-12 [**1**] show, within experimental error, the same mutagenicity. This suggests that the chiral nature of **1** plays no significant role in its mechanism of action, although this conclusion should be regarded as tentative inasmuch as we did not test synthetic (*R*)-fecapentaene-12. A similar conclusion can be deduced from a related study in which it was shown that a simple methyl pentaenyl ether had comparable mutagenicity to fecapentaene-12 (14).

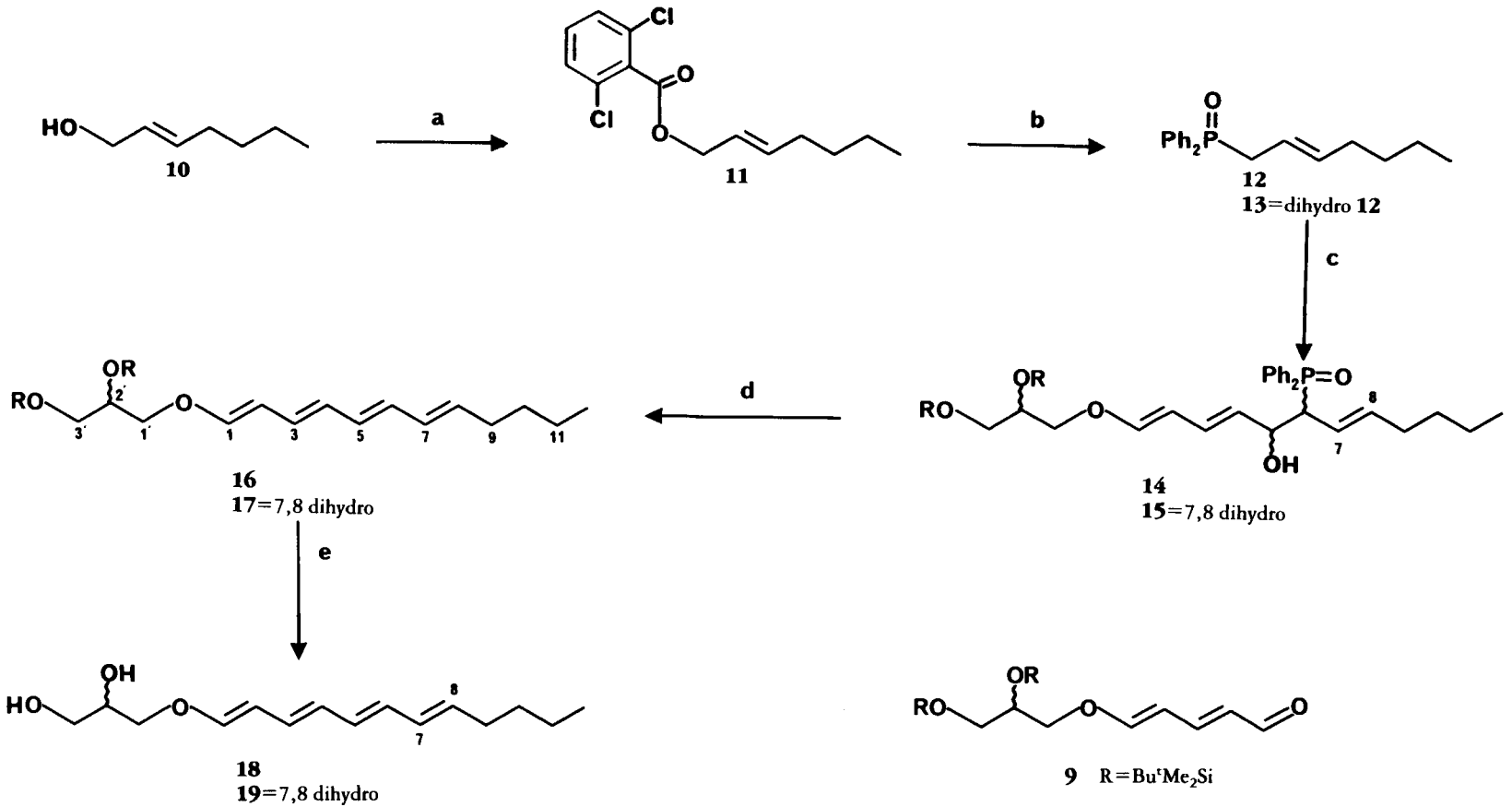
A second major conclusion is that mutagenicity depends critically on the presence of at least four double bonds in conjugation with the enol ether oxygen. Both the natural product **1** and synthetic fecapentaene-12 [**7**] show significant mutagenicity at doses as low as 300 ng/plate, while the tetraene **6** requires doses of 5,000-10,000 ng/plate, or 15-30 times larger, to achieve comparable mutation ratios. As already noted, the triene **5** failed to show significant mutagenicity at doses as high as 250,000 ng/plate and is, thus, at least 1000 times less mutagenic than the natural product.

A final point concerns the mutagenicity of the aldehyde **8** with the TA104 strain of *S. typhimurium*. Enol ethers are not notably electrophilic in nature, and it has been suggested that they may be activated towards DNA by, for example, protonation to generate an electrophilic cation (14). Another possibility that cannot be excluded a priori, however, is that the fecapentaenes serve as a protected aldehyde, which can enter the cell as the relatively unreactive enol ether and can then be transformed in vivo into a reactive electrophilic unsaturated aldehyde. Aldehydes are not notably mutagenic in the Ames' assay, presumably due in part to their reactivity with cell wall and other constituents of the testing medium, but the new strain TA104 is reported to be much more sensitive to aldehydes than TA98 and TA100 (13). If there is any substance to the hypothesis that the fecapentaenes serve as masked aldehydes for mutagenesis purposes, then we would expect an aldehyde such as **8**, which is analogous to the aldehyde hydrolysis product of fecapentaene-12, to show enhanced mutagenicity with TA104. In the event, aldehyde **8** shows only very slight mutagenicity in TA104, not significantly different from its effect in TA100 (Table 1). We, thus, conclude that an aldehyde analogous to **8** is probably not an intermediate in the mutagenic pathway of fecapentaene-12.

A second major aspect of our studies relates to our search for the biosynthetic precursor(s) of fecapentaene-12. As noted in the introduction, fecapentaene-12 is produced by various species of *Bacterioides* acting on an extract of feces of a mutagen-producer; no production is observed when feces of a non-producer are used as the source. This finding indicates that mutagen-producers have a mutagen precursor of unknown structure in their feces, and the question arose as to whether a fecatetraene-12 or a fecatriene-12 could serve as this mutagen precursor. We, thus, decided to synthesize these compounds to test this hypothesis.

Fecatetraene-12 [**18**] was prepared by a method analogous to those used in our synthesis of fecapentaene-12 (15) with the incorporation of some improvements from a related synthesis (16,17). The overall pathway used is shown in Scheme 1. 2-Heptenol [**10**] was converted via its 2,6-dichlorobenzoate **11** to the phosphine oxide **12**, which was condensed under Wittig-Horner conditions with the aldehyde **9** to give a mixture of diastereomeric alcohols **14**. Although this mixture could be separated, we elected to carry the mixture through to the tetraene **16** and, thence, after deprotection with fluoride ion, to the fecatetraenes-12 [**18**], which consisted of a mixture of *E* and *Z* isomers at the 5,6 double bond. A similar pathway from heptyl iodide proceeded through the phosphine oxide **13** to the adduct **15** and hence to the fecatriene-12 [**19**].

With compounds **18** and **19** in hand, possible incorporation into fecapentaene-12 was monitored using methods that we have developed for assaying for potential precursors (18). Compounds **18** and **19** were incubated with cultures of *B. thetaiotaomicron* in



SCHEME 1. (**a**) 2,6-Dichlorobenzoyl chloride, DMAP, pyridine. (**b**) Ph_2PLi, THF, then H_2O_2, (**c**) LDA, THF, $-78°$, then **9**. (**d**) KO^tBu, THF, $-20°$. (**e**) $Bu_4N^+F^-$, THF, 25°.

feces diluted with brain heart infusion and bile broth alone, with formation of fecapentaene-12 monitored by hplc. In no case was formation of fecapentaene-12 observed. This finding indicates either that simple lipids such as fecatetraene-12 and fecatriene-12 cannot serve as precursors to the natural product, or that our compounds were unable to penetrate the cell wall and reach the site of activity within the cell. We favor the first explanation, because the naturally occurring precursor is converted to fecapentaene-12 under the conditions of our experiment.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—All nonhydrolytic reactions were carried out in a nitrogen or argon atomsphere using standard techniques for the exclusion of air and moisture; light was also largely excluded in reactions involving polyenes. Glassware used for moisture-sensitive reactions was flame-dried with an internal inert gas sweep. THF, Et_2O, and C_6H_6 were distilled from potassium or sodium benzophenone ketyl; CH_2Cl_2 was distilled from CaH_2.

Ir spectra were recorded on neat samples unless otherwise specified, and ^{1}H-nmr spectra were determined in $CDCl_3$ on IBM WP-200 or WP-270 instruments. Chemical shifts are in ppm from internal TMS and coupling constants are in Hertz, with multiplicities expressed as s(singlet), d(doublet), t(triplet), q(quartet), m(multiplet), or br(broad). Mass spectra were obtained on a VG-7070E mass spectrometer.

Flash chromatography was performed by the published procedure (19). In separations involving enol ethers the silica gel was deactivated by stirring a suspension of it in Et_3N-hexane (1:20) for 1 h, filtering off the silica gel and drying in vacuo for 1 h. Tlc was performed on Merck silica gel PF 254 adsorbent on aluminum backing. Plates were deactivated as necessary by development in neat Et_3N and air drying prior to use. Hplc was performed using a Waters Radial Compression Module and 10μ silica cartridges.

1-(BENZYLOXY)-2,3-BIS[(*t*-BUTYLDIMETHYLSILYL)OXY]-PROPANE.—A mixture of 1-*O*-benzylglycerol (9.0 g, 49.5 mmol), *t*-butyldimethylsilyl chloride (18.0 g, 119 mmol), and imidazole (17.0 g, 250 mmol) in dry DMF (30 ml) was stirred for 20 h at 25° during which two layers separated. The mixture was transferred into a separatory funnel, and 10 ml each of H_2O and Et_2O were added. The organic phase was washed with H_2O, dried ($MgSO_4$), and evaporated to yield a colorless oil, homogeneous by tlc (hexane-Et_2O, 10:1) [(20.1 g, 99%], which was used directly in the next step. Distillation gave bp 148-150° (3 mm); ^{1}H nmr δ 0.00 (6H, s, $SiMe_2$), 0.03 (6H, s, $SiMe_2$), 0.87 (18H, s, $SiCMe_3$, 3.37-3.65 (4H, m, CH_2O), 3.83 (1H, pentet, CHOSi), 4.50 (2H, s, $PhCH_2O$), and 7.17-7.43 (5H, m, C_6H_5). Calcd. for $C_{22}H_{42}Si_2O_3$:C 64.33, H 10.31. Found: C 64.35, H 10.19%.

2,3-BIS[*t*-BUTYLDIMETHYLSILYL)OXY]-1-PROPANOL.—The benzyloxy derivative described above (6.0 g, 14.6 mmol) in hexanes (100 ml) containing 5% Pd-C catalyst (0.5 g) and *t*-butyldimethylsilyl chloride (30 mg) was hydrogenated for 1.5 h. The catalyst was filtered off, washed with hexanes, and the combined hexanes solution evaporated in vacuo to obtain 2,3-bis[(*t*-butyldimethylsilyl)oxy]-1-propanol as a colorless liquid (4.47 g, 98%); ^{1}H nmr δ 0.00 (6H, s, $SiMe_2$), 0.03 (6H, s, $SiMe_2$) 0.87 (18H, s, $SiCMe_3$), and 3.43-3.77 (5H, m, -*CH*O- and -CH_2O). Acetylation of a sample of the product gave the 1-*O*-acetyl derivative whose ^{1}H-nmr spectrum indicated that no isomerization to the 1,3-isomer had occurred.

2,3-BIS[(*t*-BUTYLDIMETHYLSILYL)OXY]-1-PROPANOL 4-METHYLBENZENESULFONATE.—A mixture of 2,3-bis[(*t*-butyldimethylsilyl)oxy]-1-propanol (0.64 g, 2 mmol) and recrystallized *p*-toluenesulfonyl chloride (0.51 g, 3 mmol) in dry pyridine (2 ml) was stirred at 20° for 16 h. The reaction mixture was diluted with Et_2O and washed with brine, and the aqueous layer was extracted with Et_2O. The combined Et_2O extracts were washed with dilute HCl and brine, dried ($MgSO_4$), and evaporated to yield a pale yellow oil. Purification by flash chromatography (1-1.5% Et_2O in hexanes) gave the tosylate (0.67 g, 71%); ^{1}H nmr δ 0.00 (6H, s, $SiMe_2$), 0.03 (6H, s, $SiMe_2$), 0.87 (18H, s, $SiCMe_3$), 2.47 (3H, s, $ArCH_3$), 3.40-3.53 (2H, m, CH_2OTs), 3.70-4.20 (3H, m, CHOR and CH_2OR), 7.40 (2H, d, J=10 ArH) 7.87 (2H, d, J=10 ArH).

(*E,E*)-5-(2,3-BIS[(*t*-BUTYLDIMETHYLSILYL)OXY]-PROPOXY)-2,4-PENTADIENAL [**9**].—The tosylate described above (4.0 g, 8.4 mmol) in DMF (40 ml) was treated with potassium glutaconate (20) (8.0 g, 147 mmol) at 80° under argon for 3 h. The product from four such reactions was combined and partitioned between Et_2O (1500 ml) and H_2O (400 ml). The Et_2O layer was washed (H_2O, brine), dried (K_2CO_3), and evaporated, and the product was purified by flash chromatography with elution by Et_2O-hexane (5:95 to 25:75). Unreacted starting tosylate eluted first, followed by a small amount of a *cis*-isomer of **9** followed by **9**. Yields varied to some extent from run to run, but in a typical run, 16 g of starting tosylate gave 8.25 g recovered starting material, 3.78 g aldehyde **9** (58% based on unrecovered starting material), and 0.7 g

cis-aldehyde. Aldehyde **9**: Rf 0.34 (Et_2O hexane, 25:75); ^1H nmr δ 0.09 (6H, s, SiMe), 0.10 (6H, s, SiMe), 0.90 (9H, s, $SiCMe_3$), 0.91 (9H, s, $SiCMe_3$), 3.51 (2H, m, CH_2OSi), 3.85 (2H, m, CH_2O), 3.98 (1H, bd d, *CH*OSi), 5.76 (1H, t, J=11, H-4), 5.98 (1H, dd, J=8, 15, H-2), 6.94 (1H, d, J=12, H-5), 6.99 (1H, dd, J=11, 15, H-3), 9.38 (1H, d, J=8, CHO) ppm.

1-(2,6-DICHLOROBENZOYLOXY)-*E*-2-HEPTENE [**11**].—*E*-2-Heptenal (Aldrich, 5 g, 44.6 mmol) was dissolved in MeOH (30 ml) and stirred at 0° under argon. $NaBH_4$ (0.5 g, 13.2 mmol) was added in portions over 15 min. The solution was stirred for a further 45 min at 0° and was then treated with H_2O (1 ml) and evaporated to a syrupy mass which was partitioned between Et_2O and H_2O. The aqueous portion was extracted with Et_2O (3 times), and the combined Et_2O extracts were washed with brine and dried ($MgSO_4$). Evaporation of the Et_2O gave crude *E*-2-hepten-1-ol [**10**] (4.82 g) which was used directly in the next step; ^1H nmr δ 0.89 (3H, t, J=7, CH_3), 1.22-1.40 (4H, m, 2×CH_2), 1.72 (1H, br s, OH), 2.05 (2H, br q, J=6.5, CH_2C=), 4.07 (2H, br t, J=4.1, CH_2O), 5.56-5.76 (2H, m, CH=CH); ir νmax 3360 cm^{-1}.

(*E*)-2-Hepten-1-ol [**10**] (2.05 g, 18.0 mmol) in anhydrous CH_2Cl_2 (40 ml) was treated at 0° with 2,6-dichlorobenzoyl chloride (4.36 g, 20.9 mmol), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 0.11 g), and pyridine (1.5 ml) in that order. Stirring was continued 18 h during which time the temperature was allowed to rise at room temperature. H_2O (15 ml) and Et_2O (200 ml) were added; the organic layer separated, washed (aqueous $CuSO_4$, H_2O, brine), and dried ($MgSO_4$). Evaporation of Et_2O gave crude product which was purified by flash column chromatography (Et_2O-hexane, 5:95) to give 3.88 g (75.2%) if **11** as a colorless oil; ^1H nmr δ 0.87 (3H, t, J=6.6, CH_3), 1.20-1.40 (4H, m, 2×CH_2), 2.09 (2H, br q, J=6.6, -CH_2C=), 4.83 (2H, m br d, J=7.4, -CH_2O), 5.70 (1H, m, -CH=), 5.90 (1H, m, -CH=), 7.20-7.32 (3H, m, ArH); ir 1840 (w), 1790 (s), 1621, 1608 cm^{-1}; ms *m/z* 286 (M^+, 3), 191 (4), 177 (11), 175 (64), 173 (100). Calcd. for $C_{14}H_{16}O_2Cl_2$, 286.0527; found, 286.0492.

(*E*)-2-HEPTENYLDIPHENYLPHOSPHINE OXIDE [**12**].—1-(2,6-Dichlorobenzoyloxy)-*E*-2-heptene (3.59 g, 12.50 mmol) was dissolved in anhydrous THF (40 ml) and cooled to −78°. A solution of lithium diphenylphosphide in THF, generated from 3.00 g (16.12 mmol) of diphenylphosphine in THF (80 ml) and *n*-butyllithium (16.12 mmol) at −78°, was transferred via a double-tipped needle to the first solution. Stirring was continued 18 h and the bath temperature allowed to rise to 10°. Aqueous NH_4Cl (10 ml) was added and the slurry extracted with Et_2O (700 ml). The ethereal layer was washed four times with 40 ml 10% aqueous H_2O_2, then with 20 ml H_2O, then five times with saturated aqueous $NaHCO_3$, and then dried ($MgSO_4$) and evaporated. Purification by flash chromatography [Et_2O-hexane (1:1), Et_2O, and MeOH-Et_2O(3:97)] led to the phosphine oxide **12** as a colorless oil, 1.40 g (37.5%). A second run gave a yield of 48.8%; ^1H nmr δ 0.80 (3H, t, J=7, CH_3), 1.08-1.25 (4H, m, 2×CH_2), 1.95 (2H, m, -CH_2-C=), 3.08 (2H, dd, J=5.5, 12.5, -CH_2-P), 5.35-5.56 (2H, m, -CH=), 7.43-7.55 (6H, m, ArH), 7.70-7.78 (4H, m, ArH); ^{13}C nmr δ 3.6(CH_3), 21.6 (CH_2), 30.8(CH_2), 31.8(CH_2), 34.5 (d, J=70, CH_2-P) 117.7, 128.0, 128.2, 130.6, 130.8, 131.2, 136.9, 137.0 ppm; ms *m/z* 201 (100, Ph_2P^+O), 175(22), 173(35), 77(17); M^+ not detected.

COUPLING OF ALDEHYDE **9** AND PHOSPHINE OXIDE **12**.—The phosphine oxide **12** (1.55 g, 5.20 mmol) in THF (50 ml) was cooled to −78° and treated with freshly prepared lithium diisopropyl amide (LDA) (10 mmol in 10 ml THF). The resultant red solution was stirred at −78° and treated with the aldehyde **9** (1.79 g, 4.48 mmol) in 25 ml THF. Stirring was continued at −78° for 45 min, when brine (60 ml) was added and the mixture extracted with Et_2O (800 ml). The Et_2O extract was dried (K_2CO_3) and evaporated to give a crude product which was used directly in the next step. On one occasion the product was purified by flash chromatography [Et_2O-hexane (1:1), Et_2O] to give the *erythro* and *threo* isomers of the adduct **14**. Isomer A (slower moving isomer): ^1H nmr δ 0.05 (6H, s, $SiMe_2$), 0.06 (6H, s, $SiMe_2$), 0.86 (9H, s, $SiCMe_3$), 0.87 (9H, s, $SiCMe_3$), 1.90 (2H, m, CH_2C=), 3.28 (1H, ddd, J=8.4, 10, 10, -CHP), 3.53-3.68 (3H, m, CHO and CH_2O), 3.78-3.88 (2H, m, CH_2O), 4.48 (1H, m, *CH*OH) 5.00 (1H, q, J=5), 5.35-5.46 (3H, m, -CH=), 6.12 (1H, dd, J=10, 15, -CH=), 6.48 (1H, d, OCH=), 7.43-7.54 (6H, m, ArH), 7.73-7.83 (4H, m, ArH). Isomer B (faster moving isomer): ^1H nmr δ 0.02 (6H, s, $SiMe_2$), 0.88 (18H, s, $SiCMe_3$), 1.90 (2H, m, CH_2C=), 3.04 (br dd, 1H, J=9, CHP), 3.54-3.83 (5H, m, -CHO- and -CH_2O-), 4.60 (1H, br t, J=9, *CH*OH), 5.38-5.48 (3H, m, -CH=), 5.65 (1H, ddd, J=3.7, 7.4, 14.7), 6.0 (1H, dd, J=11, 14.7, -CH=), 6.50 (1H, d, J=12.8, OCH=), 7.40-7.90 (10H, m, ArH).

ENOL ETHER **16**.—The crude product **14** of previous reaction (3.3 g, less than 4.7 mmol) containing excess phosphine oxide **12** was azeotroped with C_6H_6 (2×20 ml), dissolved in dry THF, and cooled to −20° under argon. Potassium *t*-butoxide (0.75 g, 6.7 mmol) was added in one lot and stirring continued at −20° for 30 min. Cold H_2O (30 ml) was added; the mixture was treated with Et_2O (500 ml). The Et_2O extract was washed, dried (K_2CO_3), and evaporated. The residue was purified by flash chromatography using deactivated silica gel with elution by Et_2O-hexane (1:1) to give enol ethers **16**, 0.64 g (30% from **9**),

as a 2:1 mixture of *E* and *Z* stereoisomers at the newly formed double bond. Compounds **16**: pale yellow oil; ^{1}H nmr δ 0.04, 0.06 (12H, s, $SiMe_2$), 0.83-0.90 (21H, $SiCMe_3$ and -CH_2CH_3), 1.20-1.39 (4H, m, CH_2CH_2), 2.06 and 2.14 (2H total, CH_2C= for major and minor isomers), 3.53 (2H, m, CH_2O), 3.66 (1H, m, CHO-), 3.84 (2H, m, CH_2OC=), 5.60 (2H, m, CH=), 5.98-6.13 (5H, m, CH=), 6.56 (1H, d, J=12, -OCH=); ^{13}C nmr δ 13.9 (C-1), 22.2(C-2), 31.6(C-3), 32.5(C-4), 128.8, 130.5, 130.8, 131.2, 134.0 (C-8-C-4, unassigned), 128.0 (C-3), 107.5(C-2), 151.1(C-1), 72.4(C-1'), 72.1(C-2'), 64.6(C-3'), 25.8($SiCMe_3$), 18.2, 18.4($SiCMe_3$); the following peaks for the minor isomer were detected separately: δ 151.5, 133.2, 131.4, 129.5, 125.9 (all -*CH*=); ms *m/z* (rel. int.) 423 (M-57, 0.2), 369(2), 291(8), 289(4), 245(12), 217(9), 189(10), 171(34), 147(40), 131(100), 115(13), 105(19), 101(21), 89(37), 75(29), 73(65).

3'-(DODECA-1,3,5,7-TETRAENYLOXY)PROPAN-1,2-DIOL [**18**].—The disilyl ethers **16** (0.56 g, 1.2 mmol) were dissolved in THF (40 ml) and stirred with 2.2 ml 1 *M* $Bu_4N^+F^-$ in THF 25° under argon. After 1 h Et_3N (2 drops) was added and the mixture diluted with Et_2O (500 ml). The Et_2O extract was washed (H_2O, then brine) and dried (K_2CO_3). The residue after evaporation was purified by flash chromatography to yield the tetraene **18** as a mixture of *E* and *Z* isomers in 2:1 ratio as a yellowish gum; ^{1}H nmr δ 0.89 (t, major isomer, J=6.5) and 0.90 (t, minor isomer, J=6.5) (3H total area), 1.26-1.43 (4H, m, CH_2CH_2), 1.90 (1H, br s, OH), 2.44 (1H, br s, OH), 2.10 (q, major isomer, J=6.5) and 2.19 (q, minor isomer, J=6.5) (2H total, CH_2C=), 3.62-3.84 (4H, m, -CH_2OH and -CH_2OR), 3.98 (br q, 1H, CHO-), 5.60-5.74 (2H, m, -CH=), 6.02-6.20 (5H, m, -CH=), 6.57 (d, major isomer), and 6.59 (d, minor isomer), (1H total, J=12.7, -OC*H*=); ^{13}C nmr δ 13.9(C-1), 22.3(C-2), 31.5, 31.9 (C-3*H* major and minor isomers), 32.5(C-4), 126.4, 128.8, 129.0, 130.6, 130.9, 131.2, 132.1, 132.9 (C-8 to C-4, major and minor isomers), 128.0(C-3), 108.4 (C-2), 150.3, 150.0 (C-1 major and minor isomers), 71.2(C-11), 70.4(C-2'), 63.5(C-3'); ms *m/z* 252 (M+, 100), 219(10), 195(3), 135(6), 121(10), 103(10), 90(8), 77(9). Calcd. for $C_{15}H_{24}O_3$: *m/z* 252.1725, observed: *m/z* 252.1707; ir ($CHCl_3$) 3425 cm^{-1} (OH); uv λ max (ε) 330 (26,700), 316 (28,600), 305 (inf., 20,400) nm.

(1-HEPTYL)DIPHENYLPHOSPHINE OXIDE [**13**].—Diphenylphosphine (3.03 g, 16.25 mmol) in dry THF was treated at −78° with *n*-butyllithium (6.3 ml of 2.5M solution in hexane, 15.75 mmol) for 15 min, and the orange solution of lithium diphenylphosphide was then transferred to a solution of iodoheptane (2.83 g, 12.5 mmol) in 20 ml dry THF at −78°. The reaction mixture was stirred at −78° for 1 h and then worked up as described for the phosphine oxide **12**. Yield of chromatographically pure product (colorless oil) 3.23 g (89.2%); ^{1}H nmr δ 0.80 (3H, t, J=8, CH_3), 1.10-1.25 (4H, m, CH_2CH_2), 1.35 (2H, m, CH_2), 1.60 (2H, m, CH_2), 2.32 (2H, m, CH_2), 7.4-7.5 (6H, m, ArH), 7.7-7.8 (4H, m, ArH); ^{13}C nmr δ 14.0(CH_3), 21.2, 22.4, 28.6, 30.6, 31.5, (CH_2), 30.2 (d, J=80 Hz, CH_2-P), 128.7(2C), 128.8, 130.8(2C), 132.0 ppm; ms *m/z* 300.1612 (M+, 26; calcd. for $C_{19}H_{25}OP$ 300.1637), 215(100), 202(64), 125(13).

ENOL ETHER **17**.—(1-Heptyl)diphenylphosphine oxide **13** (1g, 3.3 mmol) was condensed with aldehyde **9** (1.15 g, 2.88 mmol) and the crude adduct **15** was treated with potassium *t*-butoxide as described above to yield enol ether **17** after purification by flash chromatography; yield 0.38 g (27% from **9**); ^{13}C nmr δ 14.1(C-12), 22.7(C-11), 31.8(C-10), 29.5(C-9), 28.9(C-8), 32.8(C-7) 128.1, 130.8, 132.3 (C-4, C-5, C-6), 127.0(C-3), 107.3(C-2), 150.6(C-1), 63.6(C-1'), 72.3(C-2'), 64.9(C-3'), 18.2($SiCMe_3$), 18.4($SiCMe_3$), 25.9($SiCMe_3$), −4.6 ($SiMe_2$), −5.4 ($SiMe_2$).

3-(DODECA-1,3,5-TRIENYLOXY)PROPAN-1,2-DIOL [**10**].—The enol ether **13** (0.23 g, 0.47 mmol) in THF (20 ml) was treated with 1.0M $Bu_4N^+F^-$ in THF (0.9 ml) at 25° for 1 h. Work-up as previously described yielded the triene **10**, 32 mg (27%), as a mixture of *E* and *Z* isomers, and as a pale yellow solid; ^{1}H nmr δ 0.86 (3H, t, J=6.5, CH_3), 1.22-1.38 (8H, m, $CH_2CH_2CH_2CH_2$) 2.06 (2H, q, J=7, CH_2CH=), 3.66(1H, OH), 3.76-3.85 (4H, m, -CH_2O-), 3.98 (1H, m, C*H*-O) 5.56-5.68 (2H, m, -CH=), 5.92-6.12 (3H, m, -CH=), 6.57 (1H, d, J=12.4, -OC*H*=) ppm; ^{13}C nmr δ 14.0(C-12), 22.6(C-11), 31.7(C-10), 29.4(C-9), 28.9(C-8), 32.8(C-7), 129.1, 130.5, 133.1, (C-6, C-5, C-4), 126.2(C-3), 108.1(C-2), 149.4(C-1), 71.1(C-1'), 70.4(C-2'), 64.5(C-3') ppm; ms *m/z* 254.1890 (M$^+$, 100; calcd. for $C_{15}H_{26}O_3$, 254.1882), 183(19), 169(9), 144(11), 119(23), 109(63), 103(36), 94(50), 79(47); ir($CHCl_3$) 3325 cm^{-1}; uv λ max 290, 284 nm.

MUTAGENICITY ASSAYS.—Natural fecapentaene-12 [**1**], the racemic synthetic analogs **3-7**, and the aldehyde **8** were tested for mutagenicity using the revised standard plate incorporation method of the Ames' mutagenicity assay (12). Tester strains TA98, TA100, and TA104 of *Salmonella typhimurium* were obtained from Dr. Ames and were grown from liquid nitrogen stocks in 20 ml nutrient broth (Oxoid #2) for 10 h at 37°. The concentrations of each compound tested (ng/plate) were: **1** and **7**, 36 to 10,000; **6**, 625 to 100,000; **5**, 100 to 100,000; **4**, 1000 to 250,000; **3**, 1000 to 250,000. The concentrations of natural fecapentaene-12 [**1**] were calculated from the extinction coefficient; the concentrations of the synthetic

samples **3-7** were determined by weight. Test samples were diluted in DMSO and 100 μl of each dilution was mixed in the soft agar overlay with 100 μl of each tester strain and immediately poured onto 2% basal agar assay plates. The assay plates were incubated at 37° for 48 h prior to counting the revertant colonies. Results were expressed as the average number of revertant colonies at each sample concentration from three assays performed on separate days. The average spontaneous reversion rate for each tester strain was as follows: TA98, 19; TA100, 172: TA104, 155.

PRECURSOR BIOASSAY.—Stock cultures of *B. thetaiotaomicron* 5482A from the Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, were grown at 37° for 18-24 h in chopped-meat broth and maintained at room temperature. The culture used to inoculate feces or fecal extract broths were grown at 37° for 18 h in prereduced anaeorobically sterilized brain heart infusion (BHI) broth.

Feces were collected in plastic bags, homogenized under argon, and 5 g aliquots dispensed into 50 ml tubes. Samples were frozen at −80° until supplemented and inoculated. Prior to inoculation and supplementation with the triene and tetraene, the feces were diluted with an equal volume of BHI/bile broth (BBB) to provide the necessary nutrient and cofactors for fecapentaene production by *B. thetaiotaomicron*.

Fecal extract broths (FEB) were prepared by extracting freeze-dried feces three times (30 min each) with 10 volumes of hplc-grade MeOH. The extracts were pooled, vacuum filtered through a Whatman No. 1 filter, and flash evaporated at 50°. The extract was then anaerobically resuspended in BHI broth supplemented with 10mg/ml Oxgall bile to a concentration of 0.5 g feces per ml broth. Aliquots (2 ml) were dispersed into 8 ml culture tubes, and the tubes stoppered under argon and stored at 80° until used for in vitro incubations.

Separate incubations were carried out using feces diluted with BBB, fecal extract broths, and BHI/bile broth alone as media. Each medium was supplemented with 100 μg/ml or 1 mg/ml of the tetraene **18** or the trienes **19** and was then inoculated with 10% (v/v) of the 18 h culture of *B. thetaiotaomicron* and incubated at 37° for 3 days for FEB and BBB and 5 days for the feces. Controls consisted of unincubated feces, FEB, and BBB, with and without analog supplementation and incubated feces, FEB, and BBB with DMSO alone.

After incubation, the inoculated feces were frozen to −80°, lyophilized, extracted twice with 10 volumes of Me_2CO, and filtered through Whatman No. 1 paper. The pooled Me_2CO extracts were evaporated in vacuo at 50°, resuspended in 0.5 ml cold CH_2Cl_2 containing 200 μg butylated hydroxytoluene (BHT), filtered through a 0.45 μ membrane filter, and sealed under argon. The incubated BBB and FEB cultures were extracted by vortexing each culture with 4 ml methyl *t*-butyl ether (MTBE) for 1 min. The tubes were centrifuged, and the MTBE layer was removed and evaporated under a stream of N_2. The extracts were resuspended in CH_2Cl_2 and filtered as described above.

The production of fecapentanes was monitored by hplc as previously described (18) using a 10μ silica cartridge and $CHCl_3$-iPrOH (95:5) containing 50 μl/ml BHT as solvent. The system was calibrated with dilutions of known concentration of synthetic fecapentaene-12 using uv absorption at 280, 313, and 365 nm. No production of fecapentaene-12 over control levels was observed in any of the incubates. In order to control for false negatives due to degradation of the analogs on incubation in the BBB, FEB, and feces, we compared the relative amounts of each analog in unincubated samples with that in the incubated ones by hplc. In all cases, greater than 75% of each analog was recovered.

ACKNOWLEDGMENTS

Financial support for this work was provided by Grant CA23857 from the National Cancer Institute. We thank Mr. Clare Zaronsky for assistance in some experiments.

LITERATURE CITED

1. W.R. Bruce, A.J. Varghese, R. Furrer, and P.C. Land, *Cold Spring Harbor Conf. Cell Proliferation*, **3**, 1641 (1977).
2. N. Hirai, D.G.I. Kingston, R.L. van Tassell, and T.D. Wilkins, *J. Am. Chem. Soc.*, **104**, 6149 (1982).
3. N. Hirai, D.G.I. Kingston, R.L. van Tassell, and T.D. Wilkins, *J. Nat. Prod.*, **48**, 622 (1985).
4. I. Gupta, J. Baptista, W.R. Bruce, C.T. Che, R. Ferrer, J.S. Gingerich, A.A. Grey, L. Marai, and P. Yates, *Biochemistry*, **22**, 241 (1983).
5. J. Baptista, W.R. Bruce, I. Gupta, J.J.F. Krepinsky, R.L. van Tassell, and T.D. Wilkins, *Cancer Lett.*, **22**, 299 (1984).
6. M. Lederman, R.L. van Tassell, S.E.H. West, M.F. Ehrich, and T.D. Wilkins, *Mutat. Res.*, **79**, 115 (1980).
7. R.L. van Tassell, D.K. MacDonald, and T.D. Wilkins, *Mutat. Res.*, **103**, 233 (1982).

8. R.L. van Tassell, D.K. MacDonald, and T.D. Wilkins, *Infect. Immun.*, **37**, 975 (1982).
9. M.F. Ehrich, J.E. Aswell, R.L. van Tassell, T.D. Wilkins, A.R.P. Walker, and N.J. Richardson, *Mutat. Res.*, **64**, 231 (1979).
10. B.S. Reddy and E.H. Wynder, *J. Nat. Cancer Inst.*, **50**, 1437 (1973).
11. P.P. de Wit, T.A.M. van Schaik, and A. van der Gen, *Recl. Trav. Chim. Pays-Bas*, **103**, 369 (1984).
12. D.M. Maron and B.N. Ames, *Mutat. Res.*, **113**, 173 (1983).
13. J.L. Marnett, H.K. Hurd, M.C. Hollstein, D.E. Levin, H. Esterbauer, and B.N. Ames, *Mutat. Res.*, **148**, 25 (1985).
14. I. Gupta, K. Suzuki, W.R. Bruce, J.J. Krepinsky, and P. Yates, *Science*, **225**, 521 (1984).
15. A.A.L. Gunatilaka, N. Hirai, and D.G.I. Kingston, *Tetrahedron Lett.*, **24**, 5457 (1983).
16. K.C. Nicolaou, R. Zipkin, and D. Tanner, *J. Chem. Soc. Chem. Commun.*, 349 (1984).
17. R. Zipkin, "Synthesis of Several Highly Unsaturated Natural Products," Ph.D. Thesis, University of Pennsylvania, Philadelphia, 1984.
18. R.L. van Tassell, R.L. Schram, and T.D. Wilkins, in: "Progress in Clinical and Biological Research, vol. 206, Genetic Toxicology of the Diet." Ed. by I. Knudsen, Alan R. Liss, Inc., New York, 1985, pp. 199-212.
19. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
20. J. Becker, *Org. Synth.*, **59**, 79 (1981).

Received 21 July 1986