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ISOLATION AND STRUCTURE ELUCIDATION OF FECAPENTAENES-12, POTENT MUTAGENS FROM HUMAN FECES[1]

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ABSTRACT.—The isolation and structure elucidation of the novel mutagens fecapentaenes-12, which were isolated from human feces, are described.

Colon cancer is one of the most common human tumors in the United States (2,3). Its occurrence has been correlated with diet in a number of studies, and the high-fat, low-fiber diet typical of the United States and other industrialized nations is thought to be a major contributor to the disease (3-9). The actual mechanism(s) by which different diets apparently cause different levels of incidence of colon cancer is unknown, and hypotheses have ranged from those placing the blame on the lack of fiber (5) to those putting more weight on the high fat content of western diets (8,9). A common belief is that both fat and fiber are important in colon carcinogenesis and that the etiology is much more complex than the single dietary cause postulated in the early theories (10-13). Most investigators also believe that a carcinogen must be involved, with fat and fiber modifying its action to elevate or reduce the rate of cancer formation.

With this background, the report by Bruce and his co-workers of the occurrence of an ether-extractable strongly mutagenic agent in human feces suggested that this substance could be a significant factor in the etiology of colon cancer (14). Bruce's original finding was soon confirmed by ourselves (15-17) and others (18), and mutagenicity was shown to be associated with a substance or substances having an intense uv absorption spectrum (16,17,19,20). In our fecal samples, obtained from a single donor, mutagenic activity was associated with two closely related uv-absorbing compounds, and we were able to show that these compounds are produced by bacterial action on feces within the colon (17), that certain species of *Bacteroides* are involved in mutagen production (21), and that production requires bile (22). Approximately 40% of the Caucasian population excretes feces containing detectable levels of mutagenic compounds of this type, and approximately 3% of the same population excretes feces containing relatively high levels of these mutagenic compounds.

In this communication we report results that led to complete structural elucidation of the major mutagenic compounds present in our donor's feces, including the determination of their absolute stereochemistry.[2]

RESULTS AND DISCUSSION

Isolation and structural elucidation of the mutagens was complicated by the small amounts available—typically a 100 μg from a week's collection of feces—and by the marked lability of the compounds in air and in acid. Fortunately, fairly early in our investigations we discovered that the addition of anti-oxidants such as butylated hydroxytoluene (BHT) to solvents stabilized the mutagens sufficiently for operations

[1]A preliminary account of this work has appeared (1).

[2]Similar results have been reported by Bruce (23,24).

such as chromatography to be carried out in the open laboratory (16), and thenceforth, all purifications except the final one of a series were carried out with BHT-treated solvents. We were also assisted by the previously mentioned finding that incubation of the feces prior to extraction dramatically increased the amount of mutagens present (17).

The mutagenic compounds were isolated from freeze-dried feces by extraction with hexane and then Et_2O. The Et_2O extracts were then subjected to chromatography on silica gel followed by three successive separations by hplc using normal phase, reversed phase, and finally normal phase columns to effect complete purification. The resultant compounds appeared as two overlapping peaks in our final hplc system; we attribute these two peaks to the presence of two geometric isomers, for reasons which are described below.

Initial studies with the mutagens served to define their basic nature and functionality. They are lipophilic compounds, soluble in $CHCl_3$, CH_2Cl_2, C_6H_6, and Et_2O, but insoluble in H_2O. Although they are reasonably stable in $CHCl_3$ containing EtOH as a stabilizer, they rapidly degrade in unstabilized $CHCl_3$ such as $CDCl_3$ used for ^{1}H-nmr spectroscopy. Acetylation and trimethylsilylation yield less polar compounds, as judged by tlc, and *n*-butylboronation also yields a less polar derivative, suggesting the presence of a diol function (25).

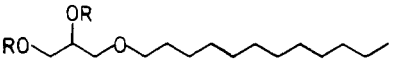
The uv absorption spectrum of the mutagens, with peaks at 325, 345, and 365 nm, and with large extinction coefficients as judged by the minute quantities of material required for a normal spectrum, is characteristic of a polyene. Simple pentaenes have their longest wavelength absorption at about 342 nm, while that for hexaenes is at about 380 nm (26), so the mutagens must be pentaenes with some additional conjugation.

Key structural information about the mutagens was obtained by ms.[3] Hydrogenation of the mutagens with hydrogen and platinum yielded a single major compound as judged by tlc, and derivatization by acetylation, trimethylsilylation, or methylation yielded derivatives that were essentially homogeneous by gc. These derivatives were then subjected to gc/ms in the chemical ionization mode, and yielded quasimolecular ions (MH^+) at *m/z* 345, 405, and 289, respectively. The hydrogenated mutagen itself gave a quasimolecular ion at *m/z* 261 on direct insertion, and deuteration of the mutagens with deuterium and platinum, followed by acetylation of the reduced substances, yielded an acetate that gave a molecular ion at *m/z* 354 on eims.[4] In confirmation of these results, we were finally able to obtain a chemical ionization mass spectrum of the mutagens, and this showed a quasimolecular ion at *m/z* 251. These data indicate conclusively that the mutagens have two derivatizable hydroxyl groups and five conjugated double bonds, and the fact that a single major compound was obtained on hydrogenation indicates that they occur as a mixture of *E-Z* isomers of one or more of the double bonds in the pentaene system. It may be noted that Bruce has also postulated the existence of *E-Z* isomers [compound **3**, in Gupta, *et al.* (24)].

The mass spectrum of the dimethyl ether of the hydrogenated mutagen indicated it to be the dimethyl ether of 3-dodecyloxy-1,2-propanediol (**4**). Major fragment ions were observed at *m/z* 257, 243, 199, 169, 133, 103, 89, and 45, and the formation of these ions can be rationalized as shown in Scheme 1. Although the 2-dodecyloxy isomer

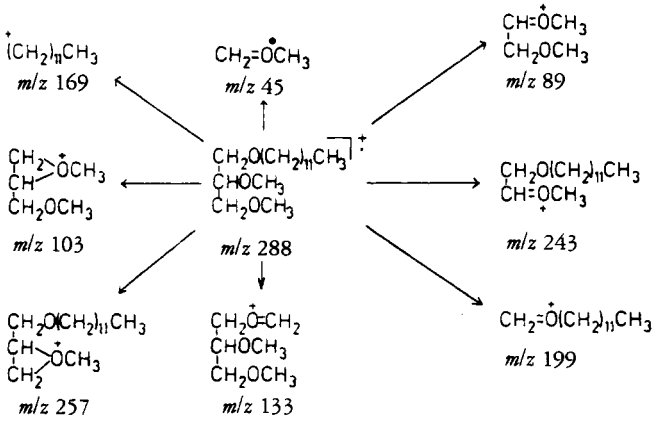
[3]Our initial studies in this area were hampered by the mutagen's instability and by the small quantities available, and the results obtained were highly variable and internally inconsistent. These results led us initially to believe that the mutagen had a molecular weight around 700 daltons, a view that appeared to be supported by the observation of a molecular ion at *m/z* 651 on plasma desorption mass spectrometry (25). Regrettably this result proved to be due to an artifact, and the first reliable mass spectral evidence was obtained from studies on reduced mutagen.

[4]Cims of this substance yielded a complex set of ions in the region *m/z* 345-355, due to hydrogen exchange between the ionized reagent gas and the sample.



1 R=H
2 R=$COCH_3$
3 R=$Si(CH_3)_3$
4 R=CH_3
5 R=$COC(OCH_3)(CF_3)C_6H_5$

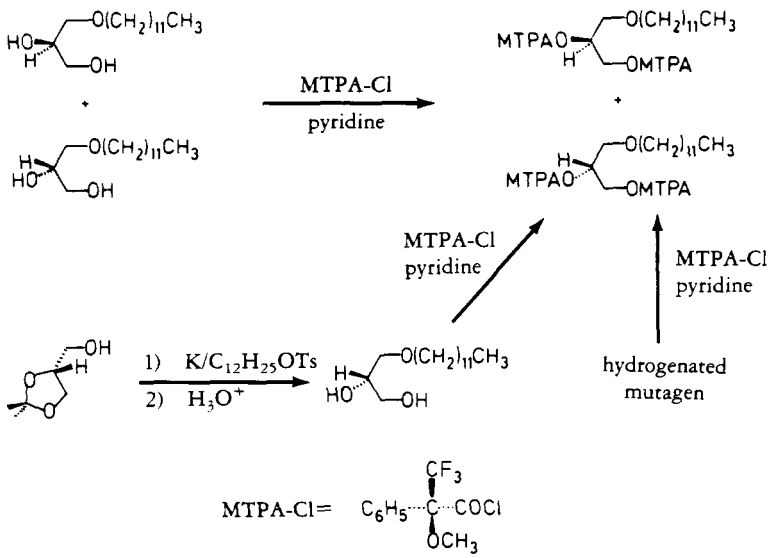
would show most of these same fragmentations, the occurrence of an intense peak at *m/z* 89 was only consistent with the 3-dodecyloxy formulation. Confirmation of this structural assignment was achieved by comparison with an authentic sample of **1**. The synthetic diol (**1**) and its diacetyl (**2**), bis(trimethylsilyl) (**3**), and dimethyl (**4**) derivatives showed chromatographic behavior identical with the hydrogenated mutagen and its corresponding derivatives. A synthetic sample of 2-dodecyloxy-1,2-propanediol had chromatographic properties very similar to those of **1**, but its dimethyl ether had a retention time on gc different from the dimethyl ether of **1** and from that of the hydrogenated mutagen. The mass spectrum of the dimethyl ether of synthetic **1** was identical with that of the methylated hydrogenated mutagen, while the mass spectrum of the dimethyl ether of 2-dodecyloxy-1,3-propanediol differed from both these spectra in lacking an intense ion at *m/z* 89 and in other respects.



SCHEME 1. Mass spectral fragmentation of the dimethyl ether of the hydrogenated mutagen.

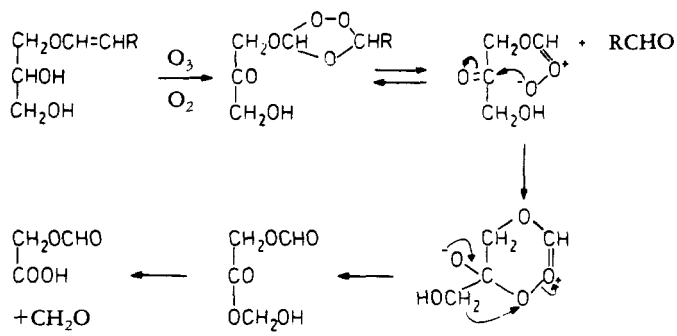
These data establish conclusively that the hydrogenated mutagen has the structure **1**, but the question of stereochemistry remained to be established. This was done by derivatization of synthetic racemic **1** with (+)-α-methoxy-α-(trifluoromethyl)-α-phenylacetyl chloride [(+)MTPA chloride] (27,28) to yield a mixture of diastereomeric esters **5** which could be separated by hplc. The bis(+)MTPA ester of the hydrogenated mutagen gave a single peak on hplc corresponding to the faster eluting peak of the diastereomeric mixture, and the bis(+)MTPA ester of (*S*)-1, prepared from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (Scheme 2) also gave a single peak which coincided with the first eluting peak on hplc. The natural mutagen thus has the *S* configuration, as do naturally occurring ether lipids such as batyl alcohol and chimyl alcohol (29).

These data establish the structure of the hydrogenated mutagen as *S*-**1**, but the question of the location of the double bonds in the dodecane chain remains to be established. The instability of the mutagen in acid is consistent with the presence of an enol ether linkage, and indeed mild acid hydrolysis of the mutagen did yield glycerol, identified as its tris(trimethylsilyl) derivative, and suggesting structure **6** or a geometric



SCHEME 2. Preparation of MTPA esters of racemic and chiral 3-dodecyloxy-1,2-propanediol.

isomer thereof for the mutagen. The position of the double bonds was further confirmed by microozonolysis, which yielded both propionaldehyde (identified as its 2,4-dinitrophenyl hydrazone) and, somewhat surprisingly, formaldehyde in approximately equal amounts as the only detectable carbonyl products.[5] The formation of formaldehyde can be rationalized by a pathway involving rearrangement of an intermediate in the ozonolysis reaction (Scheme 3), although other pathways are not excluded.[6]

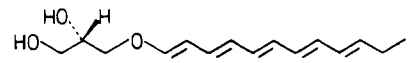


SCHEME 3. Possible mechanism for the production of formaldehyde on ozonolysis of the mutagen.

Final confirmation of the structure of the mutagens came from consideration of their ^{1}H-nmr spectrum (Figure 1), which showed signals at 6.56 (1H, d, J=12 Hz, OC*H*=) 5.9-6.3, 5.5-5.8, and 5.3-5.5 (~8H, *CH*=*CH*), 3.5-3.9 (~5H, m, CH_2O and *CH*(OH)), 2.05 (2H, m, CH_2-CH=C), and 0.94 (3H, t, J=8 Hz, CH_3CH_2). The coupling constant of the signal at 6.56 ppm indicates that the enolic ether double bond has the *E* configuration, but the configuration of the other double bonds remains to be established. It seems likely, however, that one isomer has the stable all *E* configuration

[5]Glyoxal, which would be expected to be formed from **2** by ozonolysis, was not detectable under our conditions.

[6]A referee has pointed out that the formation of formaldehyde could also be explained by oxidation-reduction involving MeOH and ozonides under palladium catalysis before H_2 is introduced.



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6. This conclusion is supported by our finding that synthetically prepared mutagens consisting of a mixture of two isomers in which four of the five double bonds are known to have the *E* configuration and the central double bond has both possible configurations, isomerized to a mixture indistinguishable from the natural product (30).

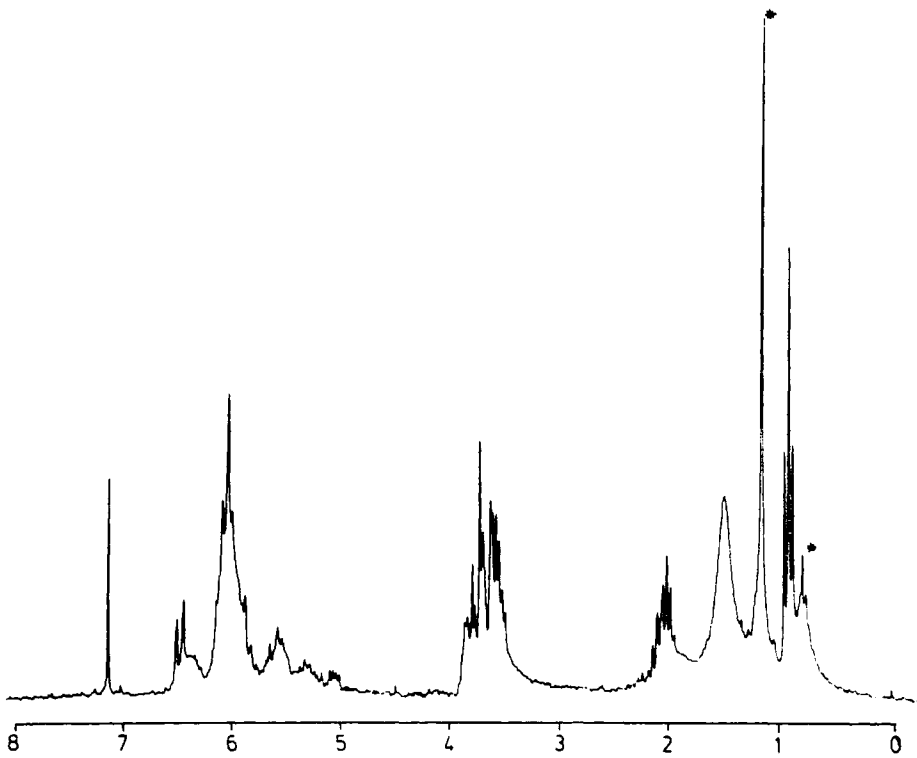

FIGURE 1. ^{1}H-nmr spectrum of the mutagen in C_6D_6. Peaks indicated with an asterisk are impurities which could not be completely removed and whose relative intensity varied from sample to sample.

These data thus support structure **6**, (*S*)-3-(1,3,5,7,9-dodecapentaenyloxy)-1,2-propanediol, for one isomer of the natural mutagen. Similar results have been obtained independently by Bruce (23,24), and he has suggested the general name "fecapentaene" for compounds of this type. On this system, mutagen **6** becomes fecapentaene-12, the subscript designating the number of carbon atoms in the side-chain. Bruce carried out his work with mixed feces from a number of donors, and found a mixture of fecapentaenes-12 and fecapentaenes-14 in his extracts (24). Our work was carried out with feces from one donor, and more recent work has shown that these feces contain fecapentaenes-12 and fecapentaenes-14 in a ratio of approximately 20:1 (31).

The observation that a glyceryl enol ether lipid has strongly mutagenic properties has potentially implications for the etiology of colon cancer. As we have already noted, fecapentaenes-12 are produced by colonic bacteria, and certain conditions are necessary for mutagen production. These conditions include the presence of a precursor of presently unknown structure and origin, the presence of a sufficiently high concentration of bile acids, a pH above 6.0, and the presence of lysed cells of *Bacteroides thetaiotaomicron*

or other related *Bacteroides* species (17,21,22). These conditions, with the exception of the presence of the unknown precursor, are those that would be expected in the colon of an individual on a high-fat, low-fiber diet. We have previously shown that there is a correlation between the excretion of this mutagen and populations at risk for colon cancer (15), and thus, the fecapentaenes may well be the missing link between a high-fat, low-fiber diet and the high incidence of colon cancer associated with it. If the fecapentaenes prove to be carcinogenic and to be involved in the initial lesion in colon cancer, then colon cancer may well turn out to be preventable by interfering with their biosynthesis by chemotherapy or by dietary methods.

EXPERIMENTAL

GENERAL.—Melting points were determined on a Kofler block and are uncorrected. ^{1}H-nmr spectra were taken on a JEOL FX-200 spectrometer at 46 kG or on a Varian EM-390 spectrometer. Mass spectra were obtained on a Finnigan MAT 112 gas chromatograph-mass spectrometer. Tlc was carried out on Merck silica gel PF 254 adsorbent. Hplc was carried out on μBondapak C_{18} and μPorasil columns (Waters). Solvents were all hplc grade (Fisher Scientific) and were treated with BHT as described.

All operations were carried out either in an anaerobic chamber or under an argon atmosphere and in such a way as to minimize, but not totally eliminate, illumination from the laboratory lights.

Gc was carried out on a Varian 2700 gas chromatograph, with 1 meter glass columns packed with 3% SP 2100 or 80/100 Supelcoport, and a carrier gas (N_2) flow rate of 30 ml/min.

ISOLATION OF FECAPENTAENES-12.—Feces were collected and immediately frozen prior to shipment to the laboratory. On receipt of feces in the laboratory (usually a week's collection) the samples were transferred to an anaerobic environment and incubated as previously described (17). The incubated feces were then freeze-dried until required for extraction. Freeze-dried feces were extracted once with four volumes (w/v) of hexane, and the hexane extract discarded. The residual sample was then extracted ten times with four volumes (w/v) of Et_2O. The Et_2O extracts were filtered, pooled, and evaporated to a volume of 2 ml/25 g of dried feces extracted.

The Et_2O extract was passed through a silica gel column (15×2.5 cm) using an Et_2O solvent system. After a dark brown nonpolar fraction had eluted from the column, the silica gel was removed from the column and extracted ten times with Et_2O. The extract was evaporated to a small volume, blown down to dryness under N_2, and redissolved in $CHCl_3$, 1 ml/25 g of dried feces extracted. The $CHCl_3$ solution was subjected to successive chromatography on three different hplc systems, with the systems monitored at 340 or 365 nm and all fractions being collected under argon. The three systems were (a) μPorasil; $CHCl_3$-iPrOH, 98:2, containing 50 mg/liter BHT; (b) μBondapak C_{18}; THF-H_2O, 45:55, containing 120 mg/liter BHT; and (c) μPorasil; CH_2Cl_2-iPrOH, 96:4. The uv-absorbing fraction collected from the final separation consisted of one major peak in this hplc system, but analysis in the system acetonitrile-H_2O-MeOH-THF, 36.2:32:25.4:6.4, showed it to consist of two overlapping peaks (31). The purified material was concentrated under N_2 and stored under argon at −70°.

MUTAGENICITY ASSAY.—Mutagenic activity was assayed by the Ames mutagenicity test using strain TA 100 as previously described (15). Dose responses were performed with triplicate plates at each dose; a sample of 1 ml of purified mutagen with an O.D. of 1 yielded 2000-3000 revertants.

FECAPENTAENE-12.—The isolated compounds showed uv (CH_2Cl_2) 325, 345, 365 nm. Ir (film) 3300, 3014, 2958, 2917, 1849, 1722, 1640, 1462, 996 cm^{-1}. ^{1}H nmr see text and Figure 1. Cims (isobutane) *m/z* 251 MH^+ (70), 250(100), 215(20), 185(45), 159(80), 100(85).

DECAHYDROFECAPENTAENE-12 (**1**).—Fecapentaene-12 (approximately 1 mg in 0.2 ml CH_2Cl_2) was dissolved in MeOH (3 ml) and treated with platinum oxide (1 mg) and H_2 for 2 h at 25°. The resulting solution was filtered and evaporated in vacuo to yield a single major product as judged by tlc ($CHCl_3$-iPrOH, 93:7, detection by H_2SO_4, Rf 0.4). The product **1** showed ^{1}H nmr ($CDCl_3$) 0.80 (3H, br t), 1.26 (~20 H, br s), 3.38-3.87 (7H, m). Cims (isobutane) *m/z* 261 MH^+ (100), 243(7), 227(4), 199(4), 169(6).

DIACETYLDECAHYDROFECAPENTAENE-12 (**2**).—Compound **1** (approximately 300 μg) was treated with Ac_2O (0.2 ml) at room temperature for 12 h. The resulting solution was treated with H_2O (1.0 ml) and extracted with $CHCl_3$ (5×1.0 ml). The $CHCl_3$ extract was divided into two portions. One portion (25%) was subjected to gc (100° to 250° at 10°/min) and showed only one peak under these conditions. This sample was then subjected to gc/cims (isobutane) and gave a spectrum showing *m/z* 345 MH^+ (7), 285(100), 241(7), 225(4), 199(5), 159(3), 149(77), 117(9). The remaining portion was purified by pre-

parative tlc (7×7 cm plate, CH_2Cl_2-EtOAc, 9:1). The plate was visualized by spraying one edge with H_2SO_4 to show one major band and three much less intense minor bands. The major band was eluted, and the eluate subjected to gc under the same conditions as described to yield a peak that co-eluted with the peak from the first run. This result confirms that the major peak observed by gc corresponds to the major reaction product and excludes the possibility that it is due to some minor artifact with the major product being involatile and therefore undetected. The isolated acetate **2** had ^{1}H nmr ($CDCl_3$) 0.88 (3H, br t), 1.26 (~20H, br s), 2.07 (3H, s), 2.09 (3H, s), 3.38-3.65 (4H, m), 4.17 (1H, dd, J=12 and 6 Hz), 4.33 (1H, dd, J=12 and 4 Hz), 5.19 (1H, m).

BIS(TRIMETHYLSILYL)DECAHYDROFECAPENTAENE-12 (**3**).—Compound **1** (approximately 200 μg) was treated with silylation reagent (Sigma-Sil-A, 40 μl), and the resulting solution was analyzed directly by gc (60° to 250° at 20°/min). A small peak was observed that co-eluted with tris(trimethylsilyl)glycerol, suggesting that some decomposition of fecapentaene-12 to glycerol had occurred before or during hydrogenation. The major peak was due to bis(trimethylsilyl)decahydrofecapentaene-12 (**3**), and on gc/cims (isobutane) it showed *m/z* 405 MH^+ (87), 389(11), 316(10), 258(16), 229(40), 206(94), 147(86), 131(100), 117(46), 103(56).

DIMETHYLDECAHYDROFECAPENTAENE-12 (**4**).—Sodium methylsulfinyl methide was prepared in a glove bag by a published procedure (32). A sample of **1** (approximately 300 μg) was dried in vacuo, and then treated in a glove bag with sodium methylsulfinyl methide in DMSO (100 μl). After 1 h the solution was treated with methyl iodide (10 μl), stirred for 1 h, and then treated with H_2O (1 ml) and extracted with $CHCl_3$ (3×1 ml). The $CHCl_3$ extract was evaporated in a stream of N_2, dried in vacuo, and dissolved in MeOH for analysis. Gc analysis (60° to 250° at 10°/min) showed one major peak in addition to peaks present in a control experiment carried out without **1**32 Gc/cims (isobutane) *m/z* 289 MH^+ (67), 288(100), 275(7), 257(18), 243(2), 229(2), 224(4), 199(2), 197(8), 183(3), 169(5), 166(5), 161(3), 133(4), 121(75), 103(40), 89(73).

DIACETYLDECADEUTEROFECAPENTAENE-12.—A sample of **6** (approximately 500 μg) was subjected to deuteration and then acetylation as described for the preparation of diacetyldecahydrofecapentaene-12. The resulting material showed one peak on gc (100° to 250° at 10°/min). Gc/eims *m/z* 354 M^+ (0.5), 285(3), 272(6), 242(67), 225(15), 200(50), 169(21), 159(78), 145(33), 117(94), 100(100).

3-DODECYLOXY-1,2-PROPANEDIOL.—This compound was prepared from 1,2-isopropylidene glycerol and lauryl tosylate as previously described (33). Mp 47.2-48°. Found: 69.3, H 12.3%. $C_{15}H_{32}O_3$ requires C 69.2, H 12.3%. Cims (isobutane) *m/z* 261 MH^+ (97), 243(33), 227(100).

3-DODECYLOXY-1,2-PROPANEDIOL DIMETHYL ETHER.—This compound was prepared from the alcohol as described earlier, and was homogeneous by gc. Gc-cims (isobutane) *m/z* 289 M^+ (100), 275(1), 257(2), 224(1), 197(1), 169(2), 161(1), 133(1), 121(20), 103 (10), 89(15).

2-DODECYLOXY-1,3-PROPANEDIOL.—This compound was prepared from 1,3-benzylideneglycerol and lauryl tosylate as previously described (32,33). Mp 35.8-36.2°. Found: C 69.3, H 12.5%. $C_{15}H_{32}O_3$ requires C 69.2, H 12.3%. Cims (isobutane) *m/z* 261 MH^+ (100), 243(3), 187(3), 170(7).

2-DODECYLOXY-1,3-PROPANEDIOL DIMETHYL ETHER.—This compound was prepared from the alcohol as described earlier, and was homogeneous by gc. Gc/cims (isobutane) *m/z* 289 MH^+ (48), 279(6), 257(14), 226(10), 212(15), 169(10), 161(12), 121(100), 103(23).

COMPARISON OF NATURAL AND SYNTHETIC MATERIALS.—A sample of dimethyldecahydrofecapentaene-12 was subjected to co-gas chromatography with 3-dodecyloxy-1,2-propanediol dimethyl ether and 2-dodecyloxy-1,3-propanediol dimethyl ether. The naturally derived material co-eluted with the 3-dodecyl isomer but did not co-elute with the 2-dodecyl isomer.

BIS(α-METHOXY-α-TRIFLUOROMETHYL-α-PHENYLACETYL) DECAHYDROFECAPENTAENE-12 (**5**).—Decahydrofecapentaene-12 (**1**, approximately 2 mg) was dissolved in CCl_4 and dry pyridine (50 μl each), and treated with *R*(+)-α-methoxy-α-trifluoromethyl-α-phenylacetyl chloride [(+)MTPA-Cl, 7 mg] for 12 h at 25°. The solution was then treated with 3-dimethylaminopropylamine (2 mg), diluted with Et_2O, and washed with dilute HCl followed by aqueous $NaHCO_3$. The Et_2O solution was dried and evaporated to yield the bis-MTPA derivative of **1** contaminated (tlc) with a small amount of unreacted **1**. Hplc (μ-Porasil, hexane-CH_2Cl_2, 1:1) showed that the product consisted of one major uv-absorbing compound.

BIS(α-METHOXY-α-TRIFLUOROMETHYL-α-PHENYLACETYL)-3-DODECYLOXY-1,2-PROPANEDIOL.—Synthetic racemic 3-dodecyloxy-1,2-propanediol (41 mg) in CCl_4 (1 ml) and dry pyridine (1 ml) was treated with (+)MTPA-Cl (76 mg) for 12 h at 25°. Work-up as described above yielded a product which

was purified by chromatography on silica gel (12 g) with elution by hexane-EtOAc, 1:1, to yield 70 mg of a diastereomeric mixture of bis MTPA derivatives. This mixture was separated by hplc (μPorasil, hexane-CH_2Cl_2, 1:1) to yield the individual diastereomers. Diastereomer A (first eluting): ^{1}H nmr 0.88 (3H, br t), 1.28 (c 20H, br s), 3.23-3.50 (10H, complex), 4.41 (1H, dd, J=12, 6 Hz), 4.66 (1H, dd, J=12, 4 Hz), 5.40 (1H, m), 7.36 (10H, br s). Diastereomer B (second eluting) ^{1}H nmr 0.88 (3H, br t), 1.27 (c 20H, br s), 3.29-3.59 (10H, complex), 4.37 (1H, dd, J=12, 6 Hz), 4.55 (1H, dd, J=12, 4 Hz), 5.43 (1H, m), 7.36 (10H, br s).

BIS(α-METHOXY-α-TRIFLUOROMETHYL-α-PHENYLACETYL)-(*S*)-3-DODECYLOXY-1,2-PROPANEDIOL.—(*S*)-3-dodecyloxy-1,2-propanediol was prepared from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (Fluka) by standard methods (Scheme 2) (29) and was converted to its bis(MTPA) ester as described above.

COMPARISON OF MTPA ESTERS.—Both the (+)MTPA ester **5** derived from fecapentaene-12 and the ester prepared from synthetic (*S*)-**1** coeluted with the first component of the diastereomeric (+)MTPA esters prepared from racemic **1** on hplc on μPorasil with elution with hexane-CH_2Cl_2, 1:1.

OZONOLYSIS OF FECAPENTAENE-12.—Fecapentaene-12 (approximately 500 μg) in CH_2Cl_2 (0.75 ml) was ozonized for 3 min at −70° in a micro-ozonizer (34). The solution was treated with MeOH (2 ml), reduced (H_2/Pt, 0°, 1 h), filtered, and treated with H_2O (25 ml) isooctane (10 ml), and 2,4-dinitrophenylhydrazine (0.2 ml of a solution of 0.25 g reagent in 100 ml 6N HCl). The biphasic mixture was stirred 1 h, the layers separated, and the aqueous layer extracted with isooctane (10 ml). The combined isooctane fractions were combined and extracted with acetonitrile to give an acetonitrile extract which was evaporated to small volume and analyzed by gc (175°). Two major peaks of approximately equal size were observed, corresponding to the 2,4-dinitrophenylhydrazones of formaldehyde and propanal (verified by co-gas chromatography with authentic samples) (35).

ACID HYDROLYSIS OF FECAPENTAENE-12.—Fecapentaene-12 (approximately 100 μg) was incubated at 38° with HOAc (0.2 ml). The resulting solution was evaporated and then treated with Sigma-Sil-A (100 μl) and analyzed by gc (60° to 200°, 10°/min). One major peak was observed which co-eluted with tris(trimethylsilyl)]glycerol.

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