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Formation of Isoprostane-like Compounds (Neuroprostanes) *in Vivo* from Docosahexaenoic Acid*

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F_2-isoprostanes are prostaglandin F_2-like compounds that are formed nonenzymatically by free radical-induced oxidation of arachidonic acid. We explored whether oxidation of docosahexaenoic acid (C22:6ω3), which is highly enriched in the brain, led to the formation of F_2-isoprostane-like compounds, which we term F_4-neuroprostanes. Oxidation of docosahexaenoic acid *in vitro* yielded a series of compounds that were structurally established to be F_4-neuroprostanes using a number of mass spectrometric approaches. The amounts formed exceeded levels of F_2-isoprostanes generated from arachidonic acid by 3.4-fold. F_4-neuroprostanes were detected esterified in normal whole rat brain and newborn pig cortex at a level of 7.0 ± 1.4 ng/g and 13.1 ± 8 ng/g, respectively. Furthermore, F_4-neuroprostanes could be detected in normal human cerebrospinal fluid and levels in patients with Alzheimer's disease (110 ± 12 pg/ml) were significantly higher than age-matched controls (64 ± 8 pg/ml) ($p < 0.05$). F_4-neuroprostanes may provide a unique marker of oxidative injury to the brain and could potentially exert biological activity. Furthermore, the formation of F_4-neuroprostane-containing aminophospholipids might adversely effect neuronal function as a result of alterations they induce in the biophysical properties of neuronal membranes.

Isoprostanes (IsoPs)[1] are prostaglandin (PG)-like compounds that are formed nonenzymatically *in vivo* by free radical-induced peroxidation of arachidonic acid (AA). Their formation proceeds through bicyclic endoperoxide PGH_2-like intermediates. The endoperoxide intermediates are reduced to form PGF_2-like compounds (F_2-IsoPs) (1) or undergo rearrangement to form E-ring and D-ring compounds (E_2/D_2-IsoPs) (2) and thromboxane-like compounds (isothromboxanes) (3). A novel aspect of the formation of IsoPs is that, unlike cyclooxygenase-derived prostaglandins, IsoPs are formed *in situ* esterified to phospholipids and subsequently released (4). Quantification of F_2-IsoPs has emerged as one of the most accurate approaches to assess oxidant injury *in vivo* (5, 6). Furthermore, IsoPs are capable of exerting potent biological activity (5, 6).

Docosahexaenoic acid (C22:6ω3) (DHA) has been the subject of considerable interest owing to the fact that it is highly enriched in the brain, particularly in gray matter, where it comprises approximately 25–35% of the total fatty acids in aminophospholipids (7, 8). Although DHA is present in high concentrations in neurons, neurons are incapable of elongating and desaturating essential fatty acids to form DHA. Rather, DHA is synthesized primarily by astrocytes after which it is secreted and taken up by neurons (9). Although the precise function of DHA in the brain is not well understood, deficiency of DHA is associated with abnormalities in brain function (10). We considered the possibility that IsoP-like compounds could be formed by free radical-induced peroxidation of DHA. Because such compounds would be two carbons longer in length than IsoPs, it would be inappropriate to term these compounds IsoPs. Since DHA is highly enriched in neurons in the brain, we therefore propose to term these compounds "neuroprostanes" (NPs).

One of our interests in the possibility that IsoP-like compounds could be formed from DHA derives from the fact that a role for free radicals in the pathogenesis of a number of neurodegenerative diseases, *e.g.* Alzheimer's diesease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, has been suggested (11–13). Thus, quantification of such compounds might provide a unique marker of oxidative injury in the brain. Furthermore, these compounds, like IsoPs, could potentially exert biological activity. This possibility is supported by the finding that $PGF_{4\alpha}$, the four series F-prostaglandin corresponding to the structure expected from cyclooxygenase action on C22:6, is approximately equipotent with cyclooxygenase-derived $PGF_{2\alpha}$ in contracting gerbil colonic smooth muscle strips (13). In addition, the formation of NPs esterified in lipids might be expected to have significant effects on the biophysical properties of neuronal membranes, which might impair normal neuronal function. This may be particularly relevant, since it has been suggested that one of the physiological functions of DHA may be to maintain a certain state of membrane fluidity and promote interactions with membrane proteins that are optimum for neuronal function (14, 15).

The mechanism by which F_4-NPs could be formed is outlined in Fig. 1, *A–C*. As noted, five DHA radicals are initially generated, which following addition of molecular oxygen, results in the formation of eight peroxyl radicals. These peroxyl radicals then undergo endocyclization followed by further addition of molecular oxygen to form eight bicylic endoperoxide intermediate regioisomers (not shown), which are then reduced to form eight F-ring NP regioisomers. Each regioisomer is theoretically

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[1] The abbreviations used are: IsoP, isoprostane; NP, neuroprostane; PG, prostaglandin; DHA, docosahexaenoic acid; AA, arachidonic acid.

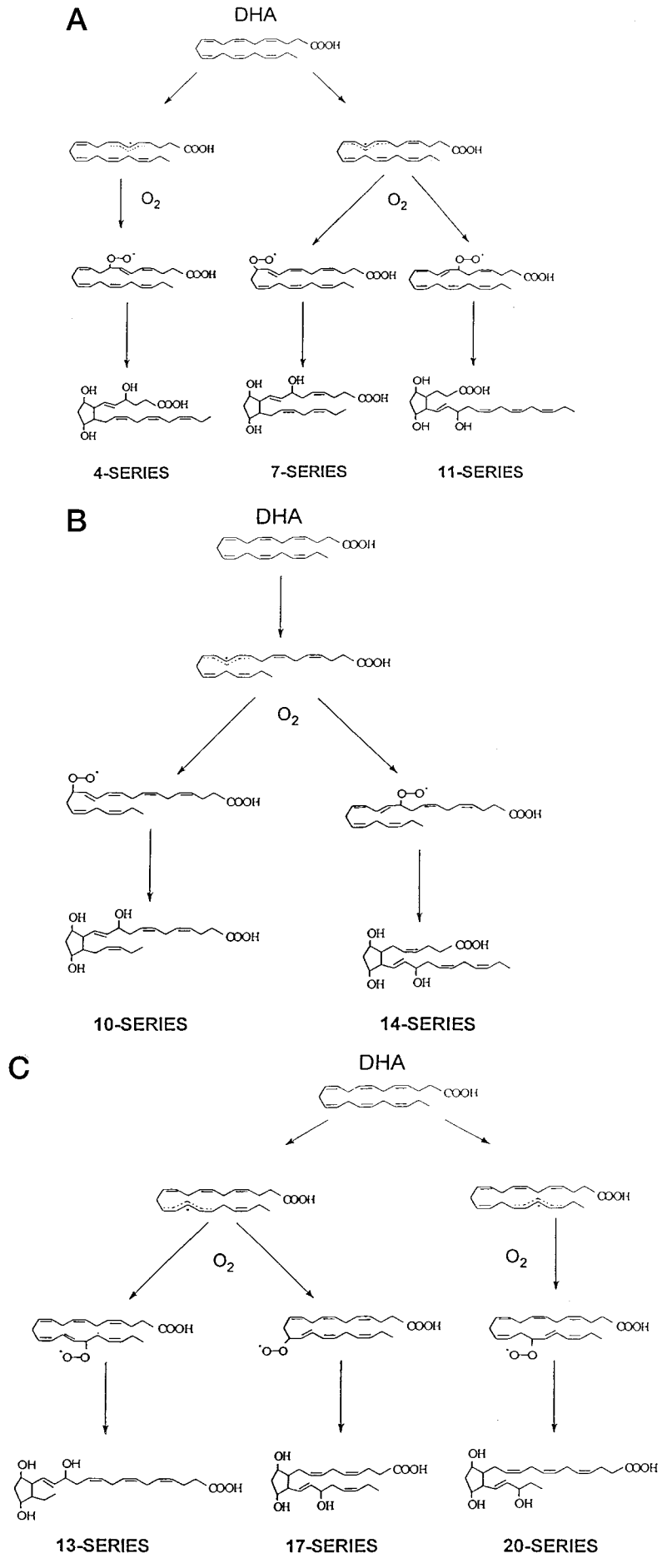

FIG. 1. **Pathway for the formation of F_4-NPs by nonenzymatic peroxidation of DHA (*A–C*).**

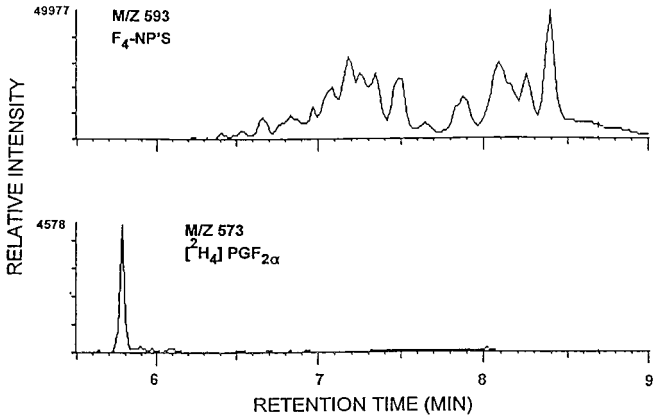

FIG. 2. **Selected ion current chromatograms obtained from the analysis of F_4-NPs generated during iron/ADP/ascorbate-induced oxidation of DHA *in vitro*.** The series of peaks in the m/z 593 ion current chromatogram represent putative F_4-NPs, and the single peak in the m/z 573 ion current chromatogram represents the [$^2H_4$]$PGF_{2\alpha}$ internal standard.

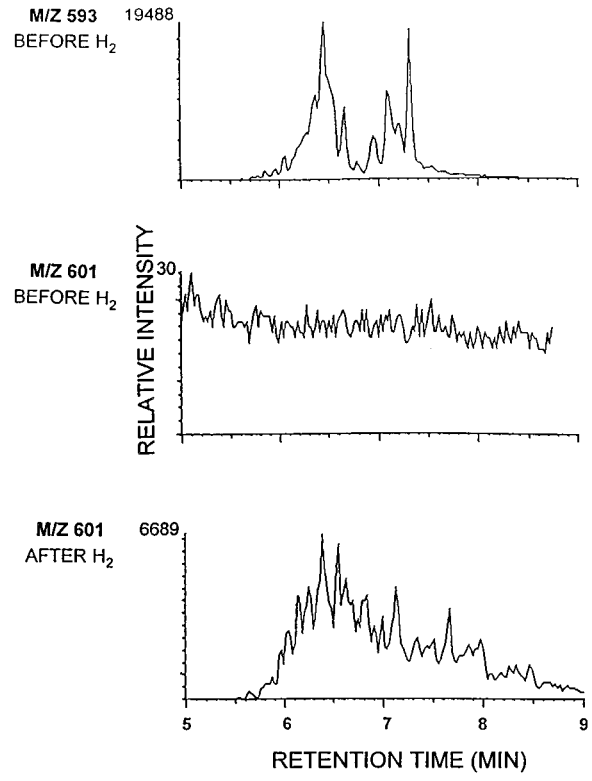

FIG. 3. **Analysis of putative F_4-NPs before and after catalytic hydrogenation.** In the absence of hydrogenation, intense peaks are present in the m/z 593 ion current chromatogram representing F_4-NPs and absent are peaks of significant intensity 8 atomic mass units higher at m/z 601. Following catalytic hydrogenation, intense peaks appear at m/z 601, indicating that the m/z 593 compounds have four double bonds.

comprised of eight racemic diastereomers for a total of 128 compounds. A nomenclature system for the IsoPs has been established and approved by the Eicosanoid Nomenclature Committee in which the different regioisomer classes are designated by the carbon number on which the side chain hydroxyl is located with the carboxyl carbon designated as C-1 (16). Thus, in accordance with this nomenclature system, the F-ring NP regioisomers are similarly designated as 4-series F_4-NPs, 7-series F_4-NPs, etc.

EXPERIMENTAL PROCEDURES

Materials—Docosahexaenoic acid, pentafluorobenzyl bromide, and diisopropylethylamine were purchased from Sigma; dimethylformamide, undecane, and 1-butaneboronic acid from Aldrich; *N,O*-bis(trimethylsilyl)trifluoroacetamide from Supelco (Bellefonte, PA); [$^2H_9$]*N,O*-bis(trimethysilyl)trifluoroacetamide from Regis Chemical (Morton Grove, Il); organic solvents from Baxter Healthcare (Burdick and Jackson Brand, McGaw Park, Il); C-18 Sep-Paks from Waters Associates (Milford, MA); 60ALK6D TLC plates from Whatman (Maidstone, UK); and [$^2H_4$]$PGF_{2\alpha}$ from Cayman Chemical (Ann Arbor, MI).

Oxidation of DHA—DHA and AA were oxidized *in vitro* using iron/ADP/ascorbate as described (17).

Purification and Analysis of F_4-NPs—Free and esterified F_4-NPs were extracted using a C-18 Sep-Pak cartridge, converted to a pentafluorobenzyl ester, purified by TLC, converted to a trimethylsilyl ether derivative, and quantified by stable isotope dilution negative ion chemical ionization gas chromatography mass spectrometry using [$^2H_4$]$PGF_{2\alpha}$ as an internal standard using a modification of the method described for the quantification of F_2-IsoPs (18). Instead of scraping 1 cm below to 1 cm above where $PGF_{2\alpha}$ methyl ester migrates on TLC for analysis of F_2-IsoPs, the area scraped was extended to 3 cm above where $PGF_{2\alpha}$ methyl ester migrates. This extended area of the TLC plate was determined to contain F_4-NPs by analyzing small 5-mm cuts using approaches for their identification described below. The M-·$CH_2C_6F_5$ ions were monitored for quantification (m/z 593 for F_4-NPs and m/z 573 for [$^2H_4$]$PGF_{2\alpha}$). Quantification of the total amount of F_4-NPs and F_2-IsoPs was determined by integrating peak areas. Formation of cyclic boronate derivatives and hydrogenation were performed as described (19). Electron ionization mass spectra were obtained using a Finnigan Incos 50B quadropole instrument as described (19).

Analysis of F_4-NPs in Human Cerebrospinal Fluid—Cerebrospinal fluid was obtained from seven subjects following informed consent. Subjects with Alzheimer's disease (n = 4) had been diagnosed with probable Alzheimer's disease during life. Control subjects (n = 3) were age-matched individuals without clinical evidence of dementia or other neurological disease; each had annual neuropsychological testing with all test scores within the normal range. Ventricular cerebrospinal fluid was collected as part of a rapid autopsy protocol. Mean post-mortem intervals were 2.9 ± 0.3 h in control subjects and 2.7 ± 0.2 h in Alzheimer's patients. Brains were evaluated using standard criteria for Alzheimer's disease (20, 21). Patients with brainstem or cortical Lewy body formation, or significant cerebrovascular disease, were excluded. Control subjects demonstrated only age-associated alterations. Statistical analysis of data was performed using the unpaired t test.

Molecular Modeling of NP-containing Phosphatidylserine—Molecular modeling was performed with Macspartan computer software.

RESULTS

A representative selected ion current chromatogram obtained from the analysis for F_4-NPs following oxidation of DHA *in vitro* with iron/ADP/ascorbate is shown in Fig. 2. A series of m/z 593 peaks eluted over approximately a 90-s period beginning approximately 30 s after the elution of the [$^2H_4$]$PGF_{2\alpha}$ internal standard. F_4-NPs would be expected to have a longer GC retention time than $PGF_{2\alpha}$ because their C-value is two units higher. It should be pointed out to avoid confusion that the time scales of some of the chromatograms obtained from the analysis of F_4-NPs shown in subsequent figures are compressed or expanded compared with that in Fig. 2; this may give the impression that the relative abundances/pattern of the different isomers detected differs. Furthermore, the retention times over which the F_4-NPs elute may differ somewhat in the different figures, because these analyses were performed on different days using different columns that vary somewhat in length.

Analysis of these compounds as a [$^2H_9$]trimethylsilyl ether derivative resulted in a shift in the m/z 593 peaks to m/z 620, indicating the presence of three hydroxyl groups (not shown). Analysis following catalytic hydrogenation is shown in Fig. 3. Prior to hydrogenation, no peaks were present 8 Da above m/z 593 at m/z 601. However, following hydrogenation, intense peaks appear at m/z 601, indicating the presence of four double bonds. The pattern of the hydrogenated compounds differs significantly from that of the nonhydrogenated compounds,

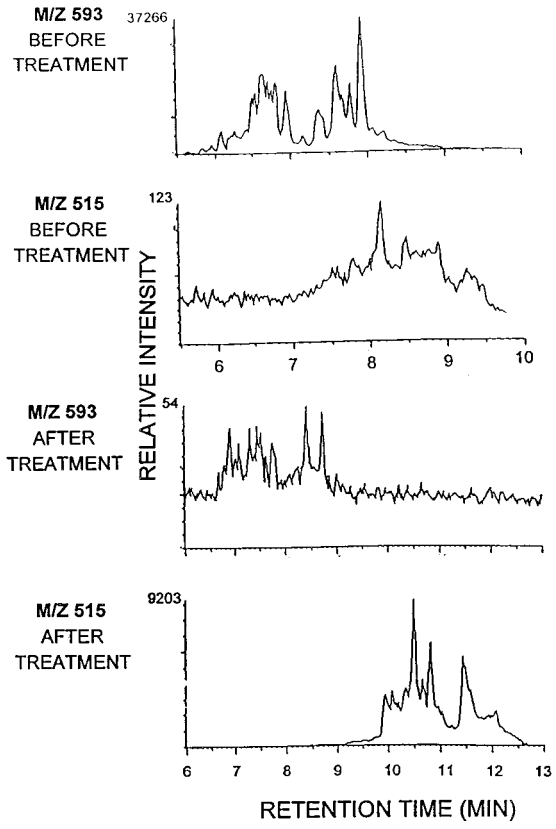

FIG. 4. **Formation of a cyclic butylboronate derivative of putative F_4-NPs.** The M-·$CH_2C_6F_5$ ion for the pentafluorobenzyl ester, cyclic butylboronate, trimethylsilyl ether derivative is *m/z* 515. In the absence of treatment of the compounds with 1-butaneboronic acid, the peaks representing the putative F_4-NPs are present in the *m/z* 593 ion current chromatogram, and no peaks of significant intensity are present in the *m/z* 515 ion current chromatogram. However, analysis of compounds treated with 1-butaneboronic acid revealed a disappearance of the *m/z* 593 peaks and the appearance of intense peaks at *m/z* 515.

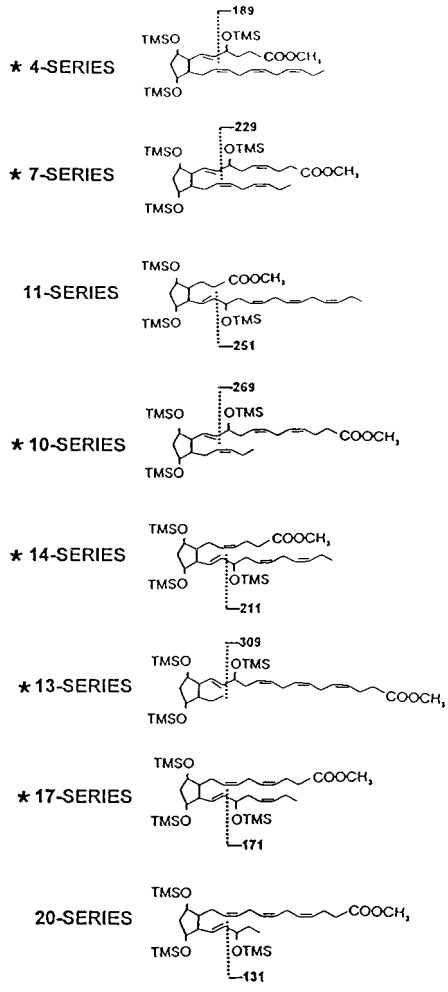

FIG. 5. **Predicted specific α-cleavage ions of the trimethylsiloxy substituents on the side chains of the different F_4-NP regioisomer series.** The α-cleavage ions for the regioisomer series designated by *asterisks* were prominent ions in the mass spectrum shown in Fig. 6.

because hydrogenation converts the compounds into new compounds that are resolved differently than the nonhydrogenated compounds.

F_4-NPs are formed by reduction of endoperoxide intermediates (Fig. 1). Thus, the cyclopentane ring hydroxyls must be oriented *cis*, but they can be either α,α or β,β. Evidence that these compounds contained a cyclopentane (prostane) ring with *cis*-oriented hydroxyls was obtained by analyzing the compounds as a cyclic boronate derivative (Fig. 4). PGF_2 compounds with *cis*-oriented prostane ring hydroxyls will form a cyclic boronate derivative bridging the ring hydroxyls (22). The M-·$CH_2C_6F_5$ ion for the cyclic boronate derivative is *m/z* 515. When the compounds were analyzed as a pentafluorobenzyl ester, trimethysilyl ether derivative, no intense peaks were present at *m/z* 515. However, when the pentafluorobenzyl ester derivatives were treated with 1-butaneboronic acid and then converted to a trimethylsilyl ether derivative, the intense peaks at *m/z* 593 were no longer present and intense peaks appeared at *m/z* 515. Again, the pattern of the *m/z* 515 peaks differs from that of compounds that were not treated with 1-butaneboronic acid because of differences in resolution of the individual compounds as a cyclic boronate derivative.

Finally, these compounds were subjected to analysis by electron ionization mass spectrometry as a methyl ester, trimethylsilyl ether derivative. Multiple mass spectra consistent with compounds representing the different regioisomers of F_4-NPs eluted from the GC column over approximately 45 s. This elution time differs from that of the pentafluorobenzyl ester derivatives used for negative ion chemical ionization, because methyl esters elute from the GC column much earlier and thus the duration over which they elute is compressed. When analyzed by electron impact mass spectrometry, the different F_4-NP regioisomers would be expected to give characteristic α-cleavage ions of the trimethylsiloxy substituents on the side chains (Fig. 5). One of the mass spectra obtained is shown in Fig. 6. The ions designated with "A" are ions that would be generated from all of the different regioisomers. These include, in addition to the molecular ion at *m/z* 608, *m/z* 593 (M − 15, loss of ·CH_3), *m/z* 539 (M - 90, loss of Me_3SiOH), *m/z* 518 (M − 2 × 90)), *m/z* 501 (M − (90 + 15)), *m/z* 487 (M − 121, loss of ·OCH_3 + 90), *m/z* 217 ($Me_3SiO–CH=CH=O^+SiMe_3$), a characteristic ion of F-ring prostanoids (23), and *m/z* 191 ($Me_3SiO^+=CH–OSiMe_3$), a rearrangement ion characteristic of F-ring prostanoids (23). The ions designated (17-S), (4-S), etc. indicate ions generated specifically from 17-series, 4-series, etc. regioisomers. These include the following: (*a*) 10-series regioisomer ions *m/z* 539, (M − 69, loss ·$CH_2(CH_2)_2CH_3$), *m/z* 449 (M − (69 + 90), *m/z* 359 (M − (69 + 2 × 90)), (*b*) 17-series regioisomer ion *m/z* 437 (M − 171, loss of ·$CH(Me_3SiOH)CH_2CH=CHCH_2CH_3$), (*c*) 7-series regioisomer ion *m/z* 409 (M − (109 + 90), loss of ·$CH_2CH=CHCH_2CH=CHCH_2CH_3$ + 90), (*d*) 13-series regioisomer ions *m/z* 401 (M − 207, loss of ·$CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOCH_3$), *m/z* 311, (M − (207 + 90)), *m/z* 219 (M − (309 + 90), loss of ·$CH(Me_3SiOH)CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2CO$

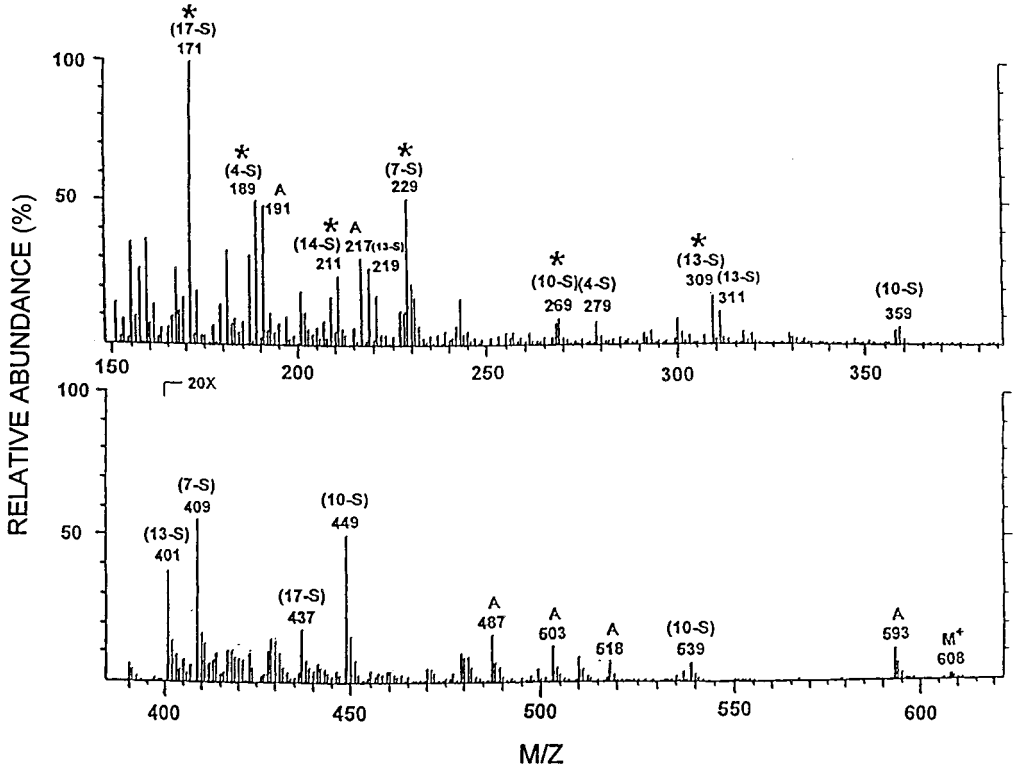

FIG. 6. **Electron ionization mass spectrum obtained of putative F_4-NPs as a methyl ester, trimethylsilyl ether derivative.** An intense molecular ion is present at *m/z* 608. The ions designated with an "*A*" are common ions generated from all regioisomers (see text for explanation). The designations (17-S), (4-S), etc. indicate ions specifically generated by compounds in the 17-series, 4-series regioisomers, etc. (see text for explanation). Ions further designated with an *asterisk* are specific α-cleavage ions of the trimethylsiloxy substituents for the different regioisomer classes as indicated in Fig. 5.

OCH_3 + 90), and (*e*) 4-series regioisomer ion *m/z* 279 [M − (149 + 2 × 90), loss of $^{\cdot}CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH_3$ + 2 × 90). The six ions further designated with an asterisk represent specific α-cleavage ions of the trimethylsiloxy substituents of different regioisomers as shown in Fig. 5. These data indicated that this was a mass spectrum of a mixture of six of the eight regioisomers co-eluting simultaneously from the GC column. This evidence for the presence of predicted six out of eight regioisomers supports the proposed mechanism of formation of these compounds outlined in Fig. 1.

The time course of formation of F_4-NPs during oxidation of DHA using iron/ADP/ascorbate was rapid, reaching a maximum level of approximately 5 μg/mg DHA at 50 min (Fig. 7). We then compared the amounts of F_2-IsoPs formed from oxidation of AA with the amounts of F_4-NPs formed from DHA. In these experiments, equal molar amounts of AA and DHA were co-oxidized with Fe/ADP/ascorbate and the total amounts of F_2-IsoPs and F_4-NPs generated quantified. Interestingly, the relative amounts of F_4-NPs formed exceeded that of F_2-IsoPs by a mean of 3.4-fold (Fig. 8).

We then undertook experiments to determine whether F_4-NPs are present esterified in brain lipids *in vivo* (Table I). Both F_2-IsoPs and F_4-NPs were present at readily detectable levels esterified in lipids of normal whole rat brain at levels of 10.3 ± 3.1 and 7.0 ± 1.4 ng/g, respectively (n = 4). A selected ion current chromatogram obtained from one of these analyses is shown in Fig. 9. Although the levels of F_2-IsoPs were slightly higher than the levels of F_4-NPs, these differences were not significant ($p > 0.05$). However, levels of F_4-NPs esterified in the cortex of newborn pig brain (13.1 ± 0.8 ng/g) greatly exceeded levels of F_2-IsoPs (2.9 ± 0.4 ng/g) by a mean of 4.5-fold (n = 3) ($p < 0.0001$). Note that the pattern of F_4-NP peaks detected esterified in brain differs somewhat than that of compounds formed by oxidation of DHA *in vitro*. We have also

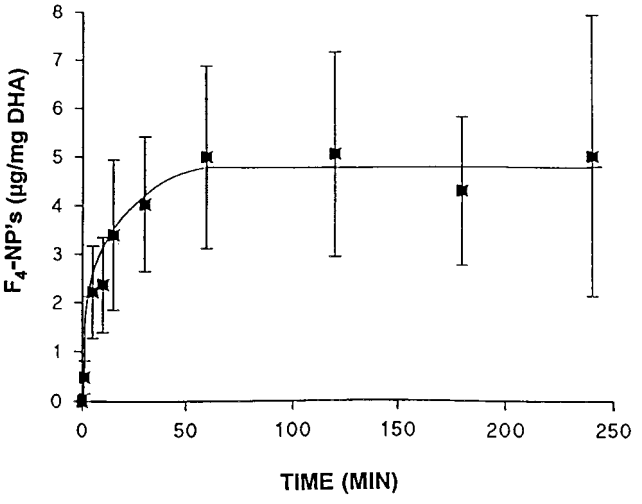

FIG. 7. **Time course of formation of F_4-NPs during oxidation of DHA *in vitro* by iron/ADP/ascorbate.**

observed slight differences in the pattern of F_2-IsoPs formed from oxidation of arachidonic acid *in vitro* compared with that of compounds present esterified in tissue lipids. Although the reason for these differences has not been firmly established, a reasonable explanation for this is that there may be steric influences of phospholipids on the formation of different isomers from esterified substrate.

As a measure of specificity of the assay to detect esterified F_4-NPs in tissues, we analyzed for F_4-NPs esterified in lipids in 1 ml of human plasma, which contains only very small amounts of DHA (Fig. 10) (7). Intense peaks were present in the *m/z* 569 ion current chromatogram representing F_2-IsoPs but absent were peaks of significant intensity in the *m/z* 593 ion current

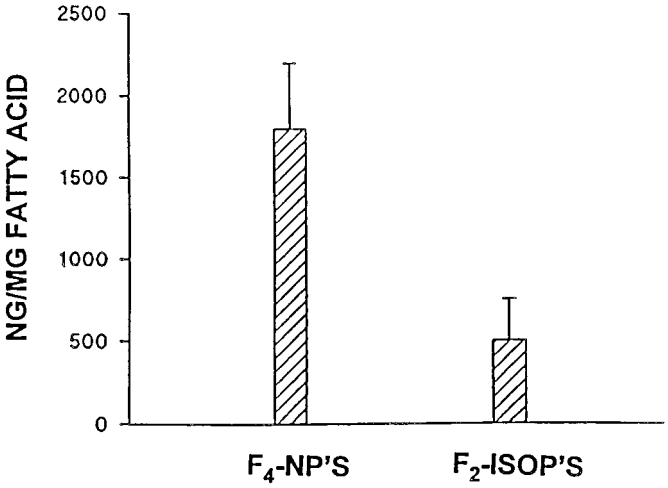

FIG. 8. **Relative amounts of F_4-NPs and F_2-IsoPs formed during co-oxidation of equal amounts of DHA and AA *in vitro*.**

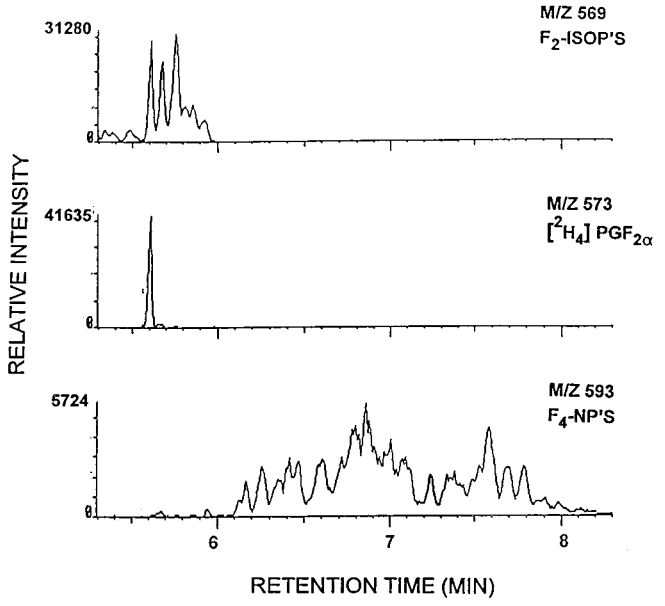

FIG. 9. **Selected ion current chromatogram obtained from the analysis for F_2-IsoPs and F_4-NPs esterified in whole rat brain.** The peaks in the m/z 569 ion current chromatogram represent F_2-IsoPs; the peak in the m/z 573 ion current chromatogram is $[^2H_4]PGF_{2\alpha}$; the peaks in the m/z 593 ion current chromatogram represent F_4-NPs. The total amounts of F_2-IsoPs and F_4-NPs present were 7.9 and 6.3 ng/g brain tissue, respectively.

TABLE I

Levels of F_2-IsoPs and F_4-NPs measured esterified to lipids in whole normal rat brain (n = 4) and in brain cortex from newborn pig (n = 3)

F_2-IsoPs and F_4-NPs were measured as free compounds following base hydrolysis of a Folch lipid extract of brain tissue as described under "Experimental Procedures." The data are expressed as nanograms of F_2-IsoPs and F_4-NPs measured per g of wet weight of tissue.

	F_2-IsoPs	F_4-NPs	p value (F_2-IsoP *vs* F_4-NP)
	ng/g		
Whole brain from normal rat	10.3 ± 3.1	7.0 ± 1.4	>0.05
Brain cortex from newborn pig	2.9 ± 0.4	13.1 ± 0.8	<0.0001

chromatogram that would indicate the presence of F_4-NPs at levels above the lower limits of detection (~5 pg/ml).

Although F_4-NPs can be readily detected esterified in the brain, the utility of such measurements to assess oxidative injury would primarily be restricted to animal models of neurological disorders or brain samples obtained post-mortem from humans. We therefore examined whether F_4-NPs could be detected in cerebrospinal fluid obtained from four patients with Alzheimer's disease and three age-matched control subjects. F_4-NPs were detected in 1–2 ml of cerebrospinal fluid from the control subjects at a level of 64 ± 8 pg/ml. Of considerable interest was the finding that the concentrations measured in the patients with Alzheimer's disease were significantly higher (110 ± 12 pg/ml) ($p < 0.05$). A selected ion current chromatogram obtained from the analysis of F_4-NPs in cerebrospinal fluid from a patient with Alzheimer's disease is shown in Fig. 11. The pattern of F_4-NP peaks detected in free form in cerebrospinal fluid differs somewhat from the pattern peaks detected esterified in tissue phospholipids (Fig. 9). Similar differences have been observed for the pattern of F_2-IsoP peaks detected in free form in plasma and urine compared with the pattern of peaks detected esterified in tissue phospholipids as free compounds following base hydrolysis of a tissue lipid extract. Although the reason for these differences has not been established, this may be explained by differences in the efficacy of phospholipases to hydrolyze different isomers from phospholipids. Cerebrospinal fluid concentrations of F_2-IsoPs were similarly increased in patients with Alzheimer's disease but were lower than the levels of F_4-NPs in both control subjects and Alzheimer's patients (46 ± 4 and 72 ± 7 pg/ml, respectively).

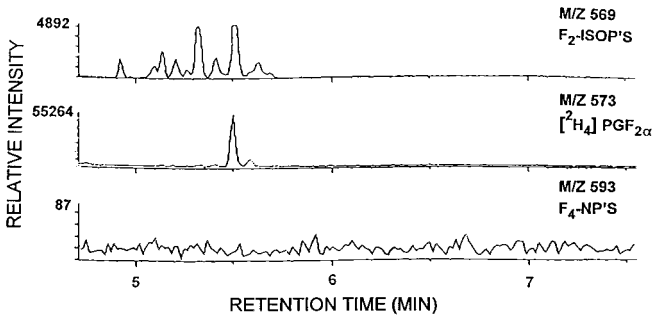

FIG. 10. **Selected ion current chromatogram obtained from the analysis for F_2-IsoPs and F_4-NPs esterified in lipids in 1 ml of plasma.** The intense peaks present in the m/z 569 ion current chromatogram represent F_2-IsoPs. The peak in the m/z 573 ion current chromatogram represents the $[^2H_4]PGF_{2\alpha}$ internal standard. Absence are peaks in the m/z 593 ion current chromatogram representing F_4-NPs at a level above the lower limit of detection (~5 pg/ml).

DISCUSSION

These studies have elucidated a new class of F_2-IsoP-like compounds formed *in vivo* by free radical-induced peroxidation of DHA. Free radical-induced peroxidation of AA results not only in the formation of F-ring IsoPs but also D-ring and E-ring IsoPs and thromboxane-like compounds (isothromboxanes) (2, 3). Thus, although it remains the subject of future studies, it is likely that analogous compounds in addition to F-ring NPs are also formed as products of nonenzymatic peroxidation of DHA.

One of our motivations for determining whether IsoP-like compounds could be formed as peroxidation products of DHA involves the possibility that quantification of these compounds might provide a unique marker of oxidative injury in the brain that could be exploited to investigate the role of free radicals in the pathogenesis of neurological disease. The ultimate hope would be that quantification of F_4-NPs could be utilized in studies of neurological disease in humans during life. Our findings indicate that these compounds are readily detected esterified in lipids in the brain. However, the application of such measurements in humans would be limited to samples of

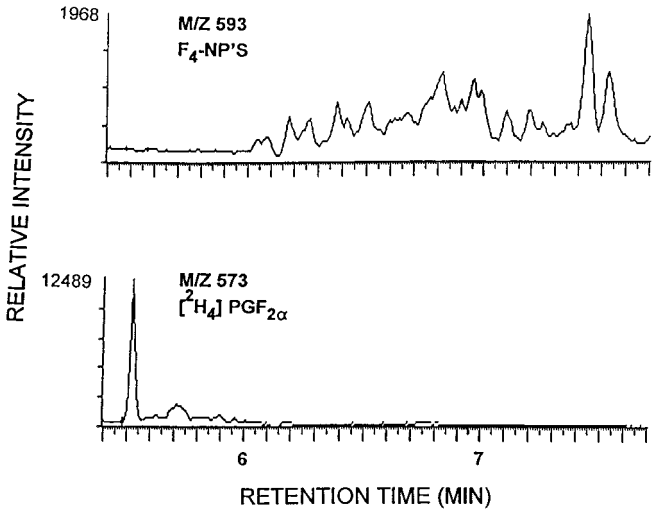

FIG. 11. **Selected ion current chromatogram obtained from the analysis for F_4-NPs in cerebrospinal fluid from a patient with Alzheimer's disease.**

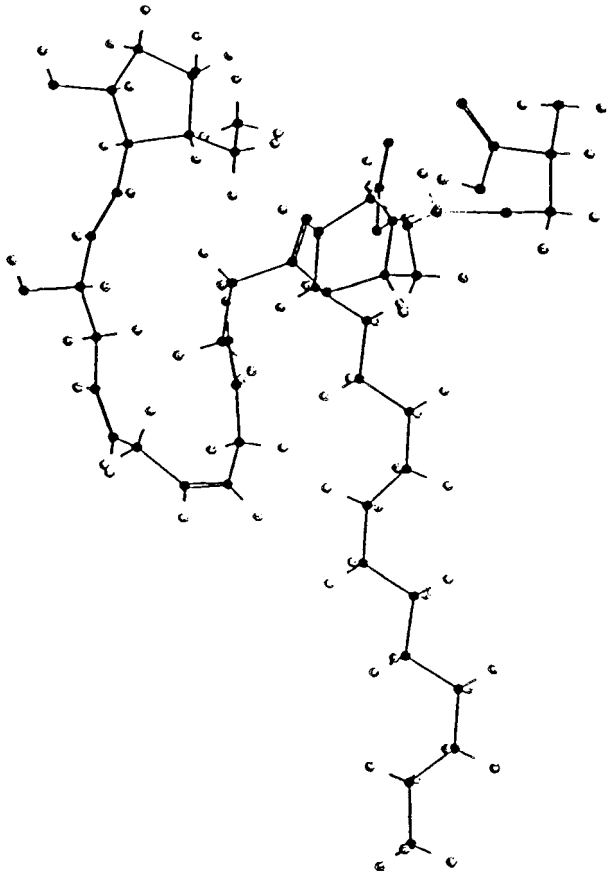

FIG. 12. **Ball and wire molecular model of phosphatidylserine containing palmitate esterified in the *sn*-1 position and a 13-series NP (13-F_{4t}-NP) esterified in the *sn*-2 position.** Trailing downward on the *right* from the polar head group above is palmitic acid. Trailing downward and then curving sharply upward on the *left* is the NP molecule in which the cyclopentane ring is seen at the *top*.

brain removed surgically or post-mortem samples of human brain. Measurements of F_4-NPs made in human brain samples obtained after death could be quite problematic because of the possibility of artifactual generation of NPs by autoxidation of DHA during the time interval between death and sample procurement. This is not a unfounded concern as we previously demonstrated the occurrence of the generation of significant quantities of IsoPs by autoxidation of arachidonic acid in plasma, even when stored at −20 °C (19).

Although invasive, cerebrospinal fluid is frequently obtained for diagnostic purposes in patients with suspected neurological disorders. Thus, the availability of a marker of oxidative injury in the brain that could be measured in cerebrospinal fluid *intra vitam* would be an important advance. Thus, the finding that F_4-NPs could be detected in human cerebrospinal fluid clearly has potentially important clinical applications. Recently we have shown that markers of lipid peroxidation are increased in cerebrospinal fluid of patients with Alzheimer's disease (24, 25). However, these assays have shortcomings related to measurement of reactive molecules, *i.e.* 4-hydroxynonenal, and require large volumes of fluid. However, F_4-NPs were detectable using negative ion chemical ionization mass spectrometry in 1–2 ml of cerebrospinal fluid from normal subjects, an amount that can usually be obtained safely from patients for diagnostic purposes. Although it was a limited study, the finding that F_4-NP concentrations in cerebrospinal fluid from patients with Alzheimer's disease were significantly higher than levels in age-matched control subjects highlights the potential of this approach to provide insights into the role of free radicals in the pathogenesis of neurological disorders. Another potentially important aspect of this finding is that serial measurements of F_4-NPs in cerebrospinal fluid might provide a biochemical assessment of disease progression as well as a means to monitor efficacy of therapeutic intervention, *e.g.* with antioxidants, during life. To our knowledge, no other method has proven to be reliable to obtain such information.

One question that arises is whether there is a distinct advantage of measuring either IsoPs or NPs to assess oxidative injury in the brain. The answer to this question is not forthcoming from the results of the studies reported herein. However, it is of interest that the relative amounts of F_4-NPs formed during oxidation of DHA *in vitro* exceeded the amounts of F_2-IsoPs generated from an equivalent amount of AA by as much as 3.4-fold (Fig. 8). This is consistent with the fact that of the naturally occurring fatty acids, DHA is the most easily oxidizable (15). This suggests that measurement of F_4-NPs in some situations may provide a more sensitive index of oxidative injury in the brain than measurement of F_2-IsoPs. The ratio of levels of AA and DHA, and thus the capacity to form IsoPs and NPs, respectively, varies significantly between different regions of the brain (white matter, gray matter), different cell types (neurons, astrocytes, oligodendrocytes), and subcellular fractions (myelin, synaptosomes) (7, 8, 26). In this regard, we found that levels of F_4-NPs and F_2-IsoPs esterified in whole rat brain were similar, whereas levels of F_4-NPs were higher than levels of F_2-IsoPs in the cortex of newborn pigs and in human cerebrospinal fluid. Therefore, there may be distinct advantages associated with measuring either IsoPs or NPs to assess oxidant injury in the brain depending on the site of oxidant injury and the predominant cell types involved. Thus, the best approach at this time, which will provide valuable insight into this question, would be to quantify both IsoPs and NPs in a variety of situations involving different types of oxidative insults to the brain both in experimental animals and in human neurological disorders. The practicality of this approach will be facilitated by the fact that the method of assay we developed allows simultaneous measurement of both F_4-NPs and F_2-IsoPs in the same sample.

There are additional ramifications that are potentially relevant to neuropathobiology that emerge from this discovery. Two of the IsoPs, previously referred to as 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2, now termed 15-F_{2t}-IsoP and 15-E_{2t}-IsoP according to the approved nomenclature for IsoPs (16), have been found to

possess potent biological activity ranging from effects on vascular and bronchial smooth muscle, endothelin release, platelet function, to cellular proliferation (5, 6). Of interest has been the evidence obtained which suggests that these IsoPs may exert their vascular effects by interacting with a unique receptor (5, 6). Thus, the possibility exists that NPs might also be found to possess important biological actions that may be relevant to the pathophysiology of oxidant injury to the brain. As mentioned, this possibility is greatly supported by the finding that C22-$PGF_{4\alpha}$ is bioactive (7). This compound is one of the F_4-NPs that would be formed, although, analogous to IsoPs, compounds in which the side chains are oriented *cis* likely predominate over compounds in which the side chains are oriented *trans* in relation to the cyclopentane ring (19). However, in the case of the IsoPs, inversion of the stereochemistry of the upper side chain of $PGF_{2\alpha}$ and PGE_2 affords different and/or more potent biological actions (5, 6).

In addition, phospholipids containing esterified NPs are very unnatural and unusual molecules. Shown in Fig. 12 is a molecular model of phosphatidylserine with palmitate esterified at the *sn*-1 position and a 13-series NP (13-F_{4t}-NP) esterified at the *sn*-2 position. Mass spectral evidence for the formation of 13-series F_4-NPs during oxidation of DHA was presented in Fig. 6. Trailing downward on the right from the polar head group above is palmitic acid. Trailing downward and then curving sharply upward on the left is the NP molecule in which the cyclopentane ring is seen at the top. Unmistakably, this is a remarkably distorted molecule. Thus, enhanced formation of these unusual phospholipids in neuronal membranes in settings of oxidant injury to the brain might lead to profound alterations in the biophysical properties of the membrane, *e.g.* degree of fluidity, which in turn might greatly impair normal neuronal function. Future studies using synthetic NP-containing aminophospholipids in model membranes to assess the extent to which these unique phospholipids alter membrane properties should provide valuable insight into the potential relevance of the formation of these phospholipids in settings of oxidative neuronal injury.

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CARBOHYDRATES, LIPIDS, AND OTHER NATURAL PRODUCTS:

Formation of Isoprostane-like Compounds (Neuroprostanes) *in Vivo* from Docosahexaenoic Acid

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