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Identification and occurrence of a novel *cis*-4,7,10,*trans*-13-docosatetraenoic fatty acid in the scallop *Pecten maximus* (L.)

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Abstract

A new fatty acid isolated from the scallop *Pecten maximus* was determined to be a 22:4 geometrical isomer [Δ4?,7?,10?,13?] (X) using GC–MS analysis of the 2-alkenyl-4,4-dimethyloxazoline derivative. The number of *trans* double bonds was determined on silver ion HPLC by comparison with the mono-*trans* geometrical isomers of 22:4[Δ4*cis*,7*cis*,10*cis*,13*cis*] (S), obtained through partial hydrazine reduction of all-*cis*-22:6(*n*-3) followed by a *p*-toluenesulfinic isomerization of the derivatives. Comparing the trienes obtained through partial hydrazine reduction of (X) and (S) determined a *cis*-4,7,10,*trans*-13-docosatetraenoic fatty acid. This fatty acid has not been reported previously in any organisms and is believed to be specific to the pectinid family.

Keywords: *Pecten maximus*; Scallops; Fatty acids

1. Introduction

Bivalves have a limited, or total inability to biosynthesize polyunsaturated fatty acids (PUFAs) with 20 and 22 carbons and more than three double bonds. However, they are found in great quantities in molluscs and their diets, the phytoplankton [1–6]. Non-methylene-interrupted dienoic acids (NMIDs), that are not present in the phytoplankton, have been reported in many species of molluscs. Thus, they are presumably synthesized by molluscs [7,8]. A new 22-carbon PUFA (designated as C22? [9]) was first reported in *Pecten maximus* (L.) larvae; its hydrogenation gave a compound of 22:0. A preliminary fractionation using high-performance liquid chromatography (HPLC) had indicated that this compound has two or more double bonds [9]. This C22? was also found in the female and male gonads of the scallop *Pecten maximus* [6,10–12].

Previously, a sufficient quantity of C22? was isolated from female gonads of the scallop *P. maximus*. Its 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivative was analyzed using gas chromatography–mass spectrometry (GC–MS) and identified as being a 22:4(*n*-9) [13]. However, the equivalent chain length (ECL) value of this compound obtained by GC analysis did not correspond to an all-*cis*-22:4(*n*-9). The C22? is thus hypothesized to be a geometri-

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cal isomer of 22:4(*n*-9). Structural determinations of *trans*-PUFAs were previously conducted by several investigators. These determinations were based on hydrazine reduction and pre- and/or post-fractionation by $AgNO_3$ thin-layer chromatography (TLC), *p*-toluenesulfinic acid isomerization of *cis*-standards was required to resolve the identification of some *trans*-fatty acid methyl esters (FAMEs) [14–17].

The objective of the present work was to establish the geometry of the ethylenic bonds of compound C22? using silver ion HPLC and GC–MS.

2. Experimental

2.1. Standards, reagents and solvents

Methyl docosahexenoate (all-*cis*-22:6-[Δ4,7,10,13,16,19]) and a mixture of 18:3[9,12,15] *cis–trans* isomers were obtained from Supelco (Bellefonte, PA, USA) and used as standards. Hydrazine monohydrate 98%, *p*-toluenesulfinic sodium hydrate and the solvent dioxane were obtained from Sigma (St. Quentin Fallavier, France). Ten percent (w/w) boron trifluoride (BF_3) in methanol and 2-amino-2-methylpropanol were obtained from Supelco and Fluka (Buchs, Switzerland), respectively. Isopropanol, hexane, chloroform, dichloromethane, acetonitrile (ACN) were obtained from Fisons (Loughborough, UK).

2.2. Sample preparation and extraction of lipids

Adult *P. maximus* scallops were collected from the Bay of Brest. The gonads were removed from the scallops and homogenized with a Dangoumeau homogenizer at −180°C. The tissue was homogenated and extracted with a mixture of chloroform–methanol (2:1, v/v). Then, 0.01% (w/w) butylated hydroxytoluene (BHT) (antioxidant) was added to the extract which was stored at −20°C under nitrogen.

To determine whether other bivalve species contain the C22?, the whole body of dog-cockle (*Glycymeris glycymeris*), mussel (*Mytilus edulis*), flat oyster (*Ostrea edulis*), Pacific oyster (*Crassostrea gigas*), clam (*Tapes decussatus*) and cockle (*Cerastoderma edule*) and the gonads of a scallop (*Argopecten purpuratus*) were extracted for total lipid and analyzed as described below.

2.3. Isolation of unknown compound C22?

An aliquot of above the lipid extract was evaporated to dryness and transesterified by boron trifluoride–methanol (10:90, w/w) [18], as previously described [9]. The FAMEs were fractionated according to their unsaturation using silver nitrate liquid column chromatography [50×5 mm I.D. Kieselgel, 70–230 mesh (Merck), impregnated with 10% $AgNO_3$] and eluted first with 5 ml of CH_2Cl_2–ACN (99:1, v/v) and then 5 ml of CH_2Cl_2–ACN (98:2, v/v) as eluent. The first 3 ml of the second solvent mixture containing the C22? was evaporated to dryness and conserved in hexane. This fraction of FAMEs was further fractionated by pre-separative HPLC (see Section 2.6). Then pure C22? was obtained from the adequate fraction by silver ion HPLC (see Section 2.7). Only two FAMEs co-elute with the C22?. These FAMEs (located between the 16 and 18:0 on a gas chromatogram) do not interfere with the further analysis of C22?.

2.4. Hydrazine reduction

The C22? compound and standards were reduced with hydrazine using a method modified from Conway et al. [19]. Briefly, about 100 μg of their FAMEs were evaporated to dryness under nitrogen in a 6-ml Wheaton vial, 2.5 ml of 10.5% (v/v.) hydrazine in ethanol added and kept (open) in a dry bath (Thermoline) at 35°C for 3 h. (The hydrazine concentration was reduced to 6% for low quantities of FAMEs such as a few micrograms). After cooling, 3 ml of distilled water was added and the derivatives extracted with 1 ml of hexane. The hexane extract was washed twice with 3 ml of distilled water and the remaining traces of water were frozen out. The organic phase was then fractionated by pre-separative HPLC and the derivatives isolated from the adequate fraction by silver ion HPLC.

2.5. p-Toluenesulfinic acid-catalyzed isomerization

All the *cis* and *trans* isomers derived from the all-*cis* precursor or from the C22? compound were

prepared using *p*-toluenesulfinic in dioxane [20]. This procedure does not affect bond position [20]. Briefly, about 10 to 100 μg of FAMEs were dried under nitrogen in a 6-ml Wheaton vial, 1 ml dioxane and 500 μg of *p*-toluenesulfinic acid (prepared as Ref. [21]) were added and the sample was heated in a dry bath at 100°C for 15 min. After cooling, 2.5 ml of 0.25 *M* NaOH was added and the FAMEs extracted as previously described (see Section 2.4).

2.6. Fractionation of FAMEs by pre-separative HPLC

FAMEs were separated in a Merck–Hitachi (Darmstadt, Germany) L6200 gradient HPLC system and detected with a UV detector at 206 nm (Pye Unicam 871LC detector). To fractionate the FAMEs based on their degree of unsaturation, two columns (250 mm×4 mm I.D., 5 μm) (aligned in a series) were used, [an OH-bound silica gel column (Diol) and a silica gel column (Si60) (Merck)]. A binary mobile phase composed of solvent A (hexane) and solvent B (hexane–isopropanol, 90:10, v/v) was used at a flow-rate of 1 ml/min. (100% A, 0–2 min; 85% A, 2–12 min; 70% A, 12–22 min; 100% A, 22–50 min).

2.7. trans Double bond determination by silver ion HPLC

The HPLC system described above was used. A ChromSpher Lipids column coated with silver ion (250 mm×4.6 mm I.D., 5 μm) (Chrompack, Middelburg, The Netherlands) was used with an isocratic solvent system (0.35% acetonitrile in hexane at 1 ml/min) [22].

2.8. Gas chromatography

A Chrompack 9002 gas chromatograph equipped with two on-column injectors and two flame ionization detectors was used to monitor each step of the purification, derivation and identification of the C22? compound. Two capillary columns programmed from 150 to 250°C at 3°C/min were employed: a CP-Wax-52 (50 m×0.25 mm I.D., 0.25 μm film thickness), and a CP-Sil 8 (30 m×0.25 mm I.D., 0.25 μm film thickness) (Chrompack). Hydrogen (1.3 ml/min) was used as the carrier gas.

The FAMEs were converted to the DMOX derivatives for GC–MS analysis [23]. The mass spectra of the DMOX derivatives was obtained on a 5995C GC–MS system (Hewlett-Packard).

Samples were chromatographed in hexane. FAMEs of 18:0, 20:0, 22:0 and 24:0 were added as reference compounds to allow the calculation of ECLs [24].

3. Results and discussion

3.1. Identification of the cis-4,7,10,trans-13-docosatetraenoic fatty acid

A gas chromatogram of FAMEs derived from *P. maximus* lipid extract showed a compound arising just after the 22:5(*n*-3) on a non polar column (Fig. 1a) whereas in the carbowax polar column this compound came after the 22:4(*n*-6) (Fig. 1b). This compound was hydrogenated to a 22:0 FAME. To ascertain the number and position of double bonds, the FAME of this compound was subjected to a DMOX derivation and a subsequent GC–MS analysis. This compound was subsequently identified as a 22:4(*n*-9)[Δ4,7,10,13] [13]. However, the retention times obtained from both polar and apolar column did not coincide with that of an all-*cis*-[Δ4*c*,7*c*,10*c*,13*c*]. Therefore, the compound was hypothesized as a 22:4 geometrical isomer [Δ4?,7?,10?,13?]. Further identification was carried out in two steps, (i) determination of the number of *trans* double bonds and (ii) determination of the position of *trans* double bond.

3.1.1. Number of trans double bonds in the 22:4[Δ4?,7?,10?,13?] "C22?"

The methodology employed to identify the number of *trans* double bonds was based on Adlof [22]. This method employed the silver ion HPLC with a UV-compatible solvent system and allows the separation of FAMEs by the number of *trans* double bonds, more than on specific location of the *cis* and *trans* double bonds. Adlof [22] resolved 15 of the 16 *cis–trans* isomer combinations of 20:4(*n*-6) with an elution pattern of (A) four *trans*, (B) three *trans*–one

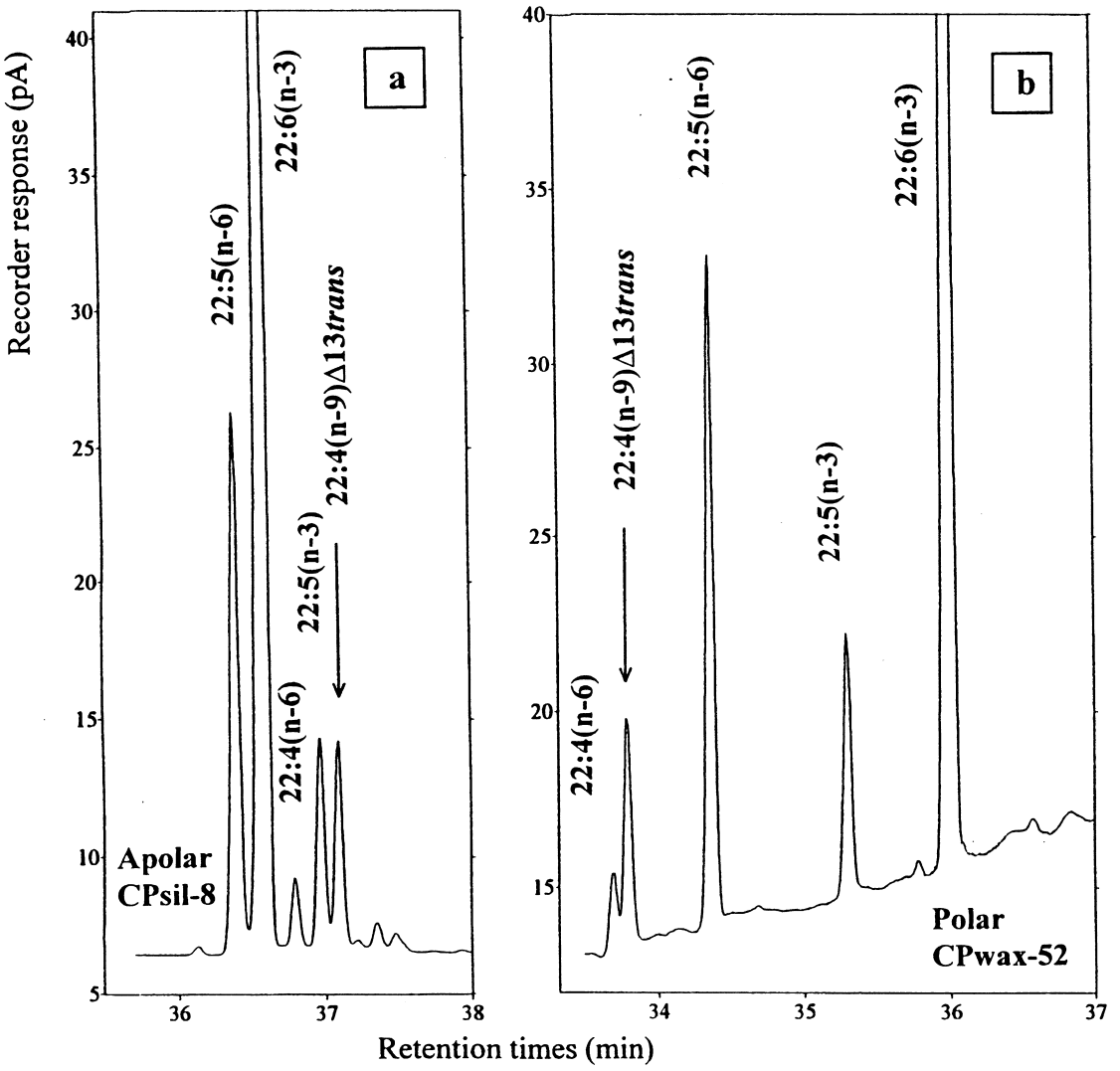

Fig. 1. Partial chromatograms of FAMEs prepared from a *P. maximus* female gonad sample. (a) The C_{21} to C_{22} region, apolar column CP-Sil 8CB. (b) The C_{23} to C_{24} region, polar column CP-Wax-52CB.

cis, (C) two *trans*–two *cis*, (D) one *trans*–three *cis* and (E) four *cis*.

Unfortunately, all-*cis*-22:4[Δ4*c*,7*c*,10*c*,13*c*] (a fortiori, all its geometrical *cis–trans* isomers) was not available to be used as a standard. Thus, preliminary chemical transformation and purification were required. The all-*cis*-22:4[Δ4*c*,7*c*,10*c*,13*c*] was prepared from all-*cis*-22:6[Δ4*c*,7*c*,10*c*,13*c*,16*c*,19*c*] through partial hydrazine reduction. The hydrazine reduction of a hexaenoic gives a mixture of pentaenes, tetraenes, trienes, dienes, monoenes and saturated compounds. However only pentaenes, tetraenes and trienes were obtained in substantial amounts under the above described experimental conditions. Thus, the mixture was subsequently fractionated by pre-separative HPLC. The all-*cis*-22:4-[Δ4*c*,7*c*,10*c*,13*c*], designated as compound S, was further isolated from the adequate fraction by silver ion HPLC. The identity of the compound S was confirmed using GC–MS of the DMOX derivative (data not shown).

All geometrical *cis–trans* isomers (from A to E

indicated above) of the compound S were then obtained by geometrical isomerisation with *p*-toluenesulfinic acid and analyzed using silver ion HPLC (Fig. 2a). The 22:4[Δ4?,7?,10?,13?] was designated as compound X. To verify the elution times, the compound X was co-chromatographed with the two of the isomers, A* and E** previously collected. Fig. 2b shows that compound X belongs to the [1-*trans*; 3-*cis*] group and coincide with the peak named T (Fig. 2a and b). The compounds T and X, were collected and analyzed on polar and apolar columns. They are identical and their MS spectrum indicated that they are 22:4[4,7,10,13] isomers (Fig. 3, only compound X presented).

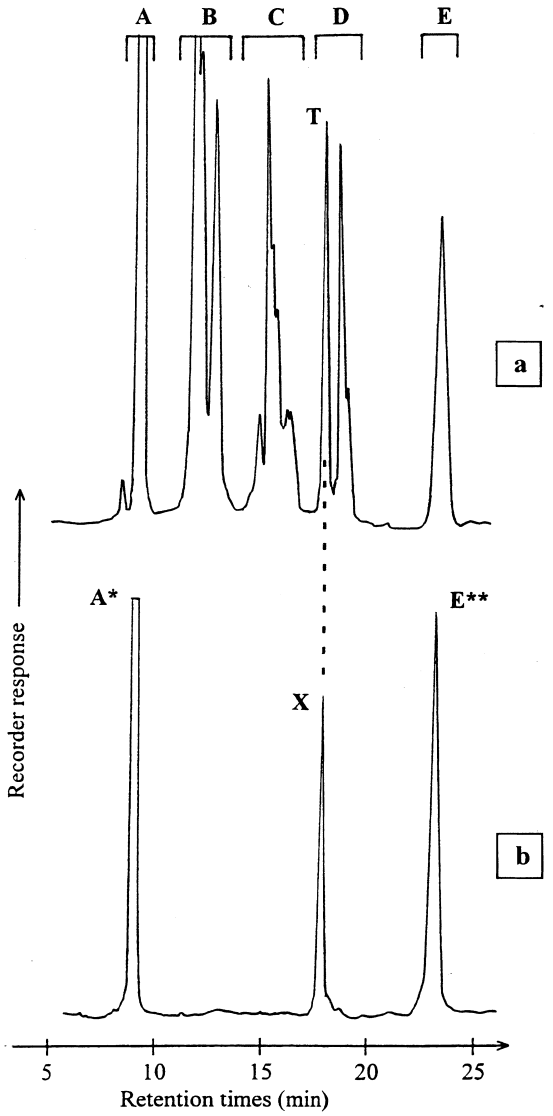

Fig. 2. Partial chromatograms on silver ion HPLC of: (a) *p*-toluene isomerization of compound S [all-*cis*22:4(*n*-9)], A=[four *trans*], B=[three *trans*–one *cis*] group, C=[two *trans*–two *cis*] group, D=[one *trans*–four *cis*] group, E=[four *cis*]; (b) compound X [22:4(*n*-9)?]; peaks A* and E** (*22:4[Δ4*t*,7*t*,10*t*,13*t*] and ** 22:4[Δ4*c*,7*c*,10*c*,13*c*]) were coinjected to check the elution times.

3.1.2. Position of the trans double bond

To identified the position of *trans* double bond, the hydrazine reduction of compounds S and X was performed. The four trienes derivatives, 22:3[Δ4,7,10], 22:3[Δ7,10,13] and the two NMI 22:3[Δ4,7,13] and 22:3[Δ4,10,13] of compound S and compound X (named S1, S2, S3, S4 and X1, X2, X3, X4, respectively), were purified using pre-separative HPLC and separated from each other using silver ion HPLC (Fig. 4). The isolated and purified trienes were further analyzed on polar and non polar GC columns. their structures were verified by analysis of DMOX derivatives with GC–MS. The comparison of the ECLs, relative retention times (Table 1) and MS spectra of these trienes (Fig. 3, only compound X1 presented) revealed that S1 and X1 were identical 22:3(*n*-12) all-*cis*. Then, the Δ4, Δ7, Δ10 double bonds of X1 and X are of *cis* configuration. By deduction, the Δ13? double bond of the compound C22? (X) was identified as a *trans* configuration. The X compound was characterized as 22:4[Δ4*cis*,7 *cis*,10 *cis*,13*trans*].

3.2. Occurrence of the cis-4,7,10,trans-13-docosatetraenoic fatty acid

To the best of our knowledge, the newly identified 22:4[Δ4*c*,7*c*,10*c*,13*trans*] discovered in *P. maximus* has not been previously reported in any organisms. This fatty acid is absent from the diet and appears to be of endogenous origin, even though the biosynthesis of all-*cis*-methylene-interrupted long chain polyunsaturated fatty acids is considered to be non-existent or limited in molluscs as in most marine animals [1–6].

Nevertheless, the capacity to biosynthesize PUFAs, NMIDs, has been demonstrated in molluscs [7]. For example, the NMIDs (20:2[Δ5,13];

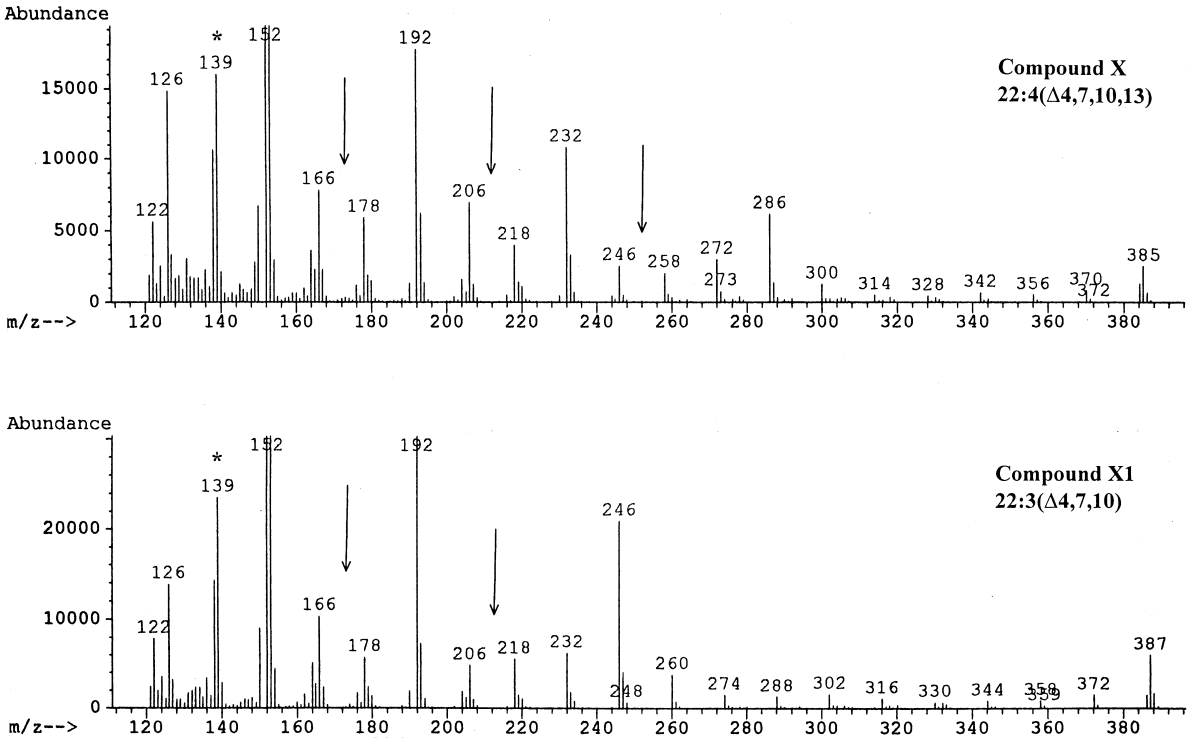

Fig. 3. Mass spectrum of the DMOX derivative of 22:4[Δ4*c*,7*c*,10*c*,13*t*] (compound X) and of 22:3[Δ4*c*,7*c*,10*c*] (compound X1). Arrows indicate the positions that differ by 12 mass units corresponding to Δ7, Δ10, Δ13 unsaturation; * *m*/*z* 139 corresponding to Δ4 unsaturation.

22:2[Δ7,15] and 20:2[Δ5,11]; 22:2[Δ7,13]) were synthesized, respectively, from 16:1(*n*-7) and 18:1(*n*-9) through elongations and a Δ5 desaturation [8]. In members of the bivalve class levels of NMIDs can be significant (*Ostrea edulis* 11%, *Mytilus planatus* 9% of total lipids [25,26]) although they are only found as traces in pectinids [27,28].

The newly identified 22:4(*n*-9)Δ13*trans* found in female gonads and larvae accounts for about 3 to 4% of the polar lipid fraction (Table 2). The presence of 22:4(*n*-9)Δ13*trans* was also found in *A. pupuratus* in the similar amounts. An analysis of the bivalves species *G. glycymeris, M. edulis, O. edulis, C. gigas, T. decussatus* and *C. edule* did not reveal the presence of 22:4(*n*-9)Δ13*trans* (data not shown). Thus, this 22:4(*n*-9)Δ13*trans* may be present only in the pectinid family and could play a similar role to NMIDs.

The newly identified 22:4(*n*-9)Δ13*trans* showed an apparent association with the phosphatidylserine (PS). It accounted for up to 14% of total fatty acids of PS [10]. Similar amounts of an all-*cis*-22:4(*n*-9) was found in the PS of the Topminnow cell line [29]. In contrast, the all *cis*22:4(*n*-9) in rat sperm was specifically located in the plasmenylcholine fraction [30,31]. These reported high percentages of (*n*-9) 22 carbon-PUFA were quite unusual in animals, with diets which were not deficient of essential fatty acids (EFAs). The end product of desaturation–elongation of the oleic acid is the *cis*20:3(n-9) (mead acid), which accumulates in mammals [32], in salmonids and freshwater fish fed a diet deficient of EFAs [33,34]. To date there is no any report of significant metabolism of 20:3(*n*-9) acid to longer-chain higher unsaturated fatty acids in mammals [35]. However, the presence of all-*cis*-22:4[Δ4,7,10,13] was reported in an octopod (2.1% of total fatty acids) [36].

Unsaturated fatty acids in plants and animals typically have a *cis* configuration. However, the presence of a *trans* double bond is not so rare in living organisms, as pointed out by Ackman in a recent review [37]. The same author compiled a list

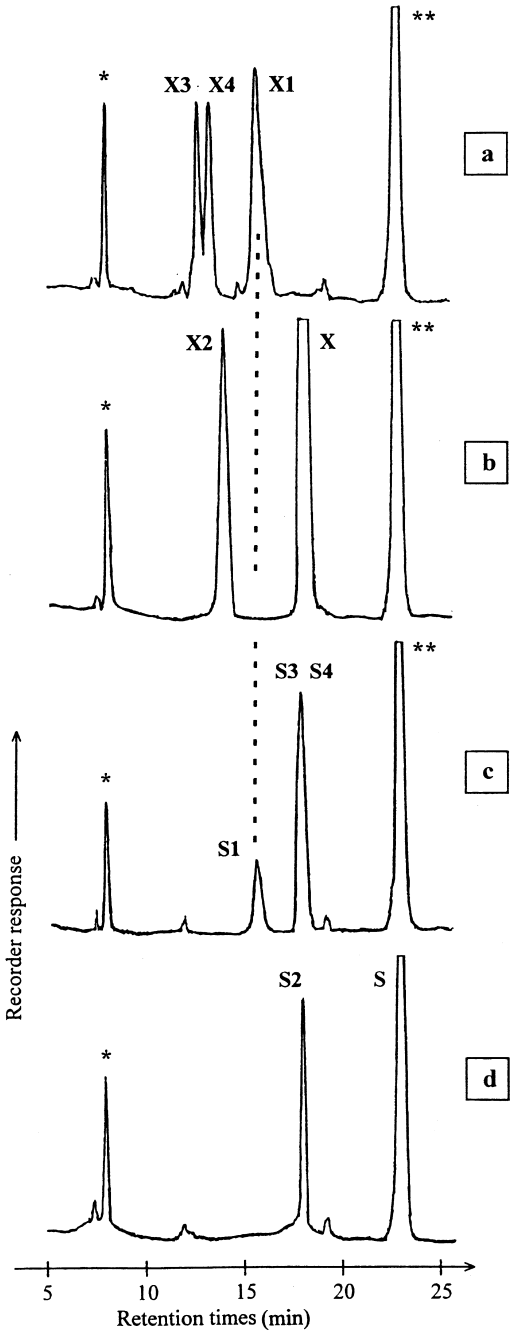

Fig. 4. Partial chromatograms on silver ion HPLC of: (a and b): fractions 1 and 2, respectively, of preseparation HPLC of hydrazine reduction of compound X [22:4(*n*-9)?], (c and d) fractions 1 and 2, respectively, of preseparation HPLC of hydrazine reduction of compound S [all-*cis*22:4(*n*-9)]. Identification of peaks, see Table 1. *=22:3[Δ7*t*,10*t*,13*t*] and **= 22:4[Δ4*c*,7*c*,10*c*,13*c*] (S), were coinjected to check the elution times.

of natural *trans* fatty acids [38]. The heat treatment of edible oils, during deodorization or hydrogenation processes, is a non-natural source of *trans* fatty acids [39,40]. A recent review by Sébédio and Christie [41] discussed the importance of natural or non natural *trans* fatty acids in human nutrition.

One of the hypothetical pathways in the formation of 22:4(*n*-9)Δ13 *trans,* is successive elongation and Δ6,5,4 desaturation, involving the possible presence of a mono-*trans* (*n*-9) precursor. The *trans*16:1(Δ3) was found initially in higher plants and later in some marine phytoplankton [26,42,43] and seaweeds [44] The latter was also found to contain *trans*14:1(Δ3). Gurr and Harwood [45] showed also that the *trans*12:1(*n*-9)Δ3*t* is an intermediate in metabolic cycles. Bacteria, such as intestinal microflora have the ability to synthesize a large variety of *trans* monoethylenic fatty acids [46] either from acetyl CoA or bioconversion of *cis*-polyethylenic fatty acids. *trans* Fatty acids were observed to be accumulated in fat depots in ruminants [47] and beaver [48]. Ackman [36] hypothesized that presence of *trans*-fatty acids in molluscan tissues could be due to indigenous bacterial production. Mono-*trans* (*n*-9) fatty acids from ingested microalgae or from indigenous bacteria de novo synthesis could then be possible precursors in the synthesis of 22:4(*n*-9)Δ13*trans*. Abilities to elongate and desaturate a *trans* precursor to 20–22C-PUFAs have been also demonstrated in mammals [49]. However, complete synthesis of 22:4(*n*-9)Δ13*trans* requires Δ4, Δ5, Δ6 desaturase activities which have been demonstrated to be absent or very low in molluscan tissues. The resulting desaturase intermediate products have not been previously reported in *P. maximus*. Nevertheless, the synthesis of 22:4(*n*-9)Δ13*trans* by indigenous bacteria is still a possibility since some marine bacteria have been shown to produce all-*cis* eicosapentaenoic acid [50] or to possess geometric isomerase [46]. A more complete screening for *trans* precursor or *trans* intermediate products in the scallop diet and in the scallop itself should be undertaken.

Acknowledgements

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Table 1
Equivalent chain lengths (ECLs)[a] and relative retention times (RRTs) of compounds X [22:4(*n*-9)?] and S [all-*cis*22:4(*n*-9)] and their respective triene derivatives obtained by hydrazine reduction

Fatty acids	Compound	ECL[a] on CP-52 (FAME)	ECL[a] on CP-Sil 8 (FAME)	RRT on silver ion HPLC[b]	GC–MS DMOX Δ position[c]
22:4(*n*-9)?	X	23.24	21.42	0.78	Δ4, 7, 10, 13
22:3(*n*-12)?	X1	22.73	21.41	0.68	Δ4, 7, 10
22:3(*n*-9)?	X2	22.96	21.56	0.60	Δ7, 10, 13
22:3(*n*-9)?NMI1	X3	22.78	21.49	0.54	Δ4, 7, 13
22:3(*n*-9)?NMI2	X4	22.89	21.55	0.56	Δ4, 10, 13
22:4(*n*-9)all-*cis*	S	23.03	21.23	1.00	Δ4, 7, 10, 13
22:3(*n*-12)all-*cis*	S1	22.73	21.41	0.68	Δ4, 7, 10
22:3(*n*-9)all-*cis*	S2	22.75	21.38	0.78	Δ7, 10, 13
22:3(*n*-9)all-*cis*NMI1	S3	22.71	21.39	0.80	Δ4, 7, 13
22:3(*n*-9)all-*cis*NMI2	S4	22.75	21.40	0.80	Δ4, 10, 13

[a] GC conditions as described in Section 2.8.
[b] Referenced to peak S.
[c] Determined as indicated in Fig. 3.

Table 2
22:4(*n*-9)Δ13*trans* content in two pectinid species (expressed as molar percentage of the total FAMEs)

	Pectinid species		
	P. maximus female gonad	*P. maximus* 7-day-old larva	*A. purpuratus* female gonad
22:4(n-9) Δ13*trans*	2.9	3.6	5.2
Ref.	[10]	[9]	[51]

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References

[1] J.E.A. De Moreno, V.J. De Moreno, R.R. Brenner, Lipids 11 (1976) 561–566.
[2] D.J. Trider, J.D. Castell, J. Nutr. 110 (1980) 1303–1309.
[3] C.J. Langdon, M.J. Waldock, J. Mar. Biol. Assoc. UK 62 (1981) 431–448.
[4] M.J. Waldock, D.L. Holland, Lipids 19 (1984) 332–336.
[5] N. Uki, M. Sugiura, T. Watanabe, Nippon Suisan Gakkaaishi 52 (1986) 1013–1023.
[6] F. Delaunay, Y. Marty, J. Moal, J.F. Samain, J. Exp. Mar. Biol. Ecol. 173 (1993) 163–179.
[7] J.D. Joseph, Prog. Lipid Res. 21 (1982) 109–153.
[8] N.V. Zhukova, Biochim. Biophys. Acta 878 (1986) 131–133.
[9] Y. Marty, F. Delaunay, J. Moal, J.F. Samain, J. Exp. Mar. Ecol. 163 (1992) 221–234.
[10] P. Soudant, J. Moal, Y. Marty, J.F. Samain, J. Chromatogr. B 673 (1995) 15–26.
[11] P. Soudant, J. Moal, Y. Marty, J.F. Samain, J. Exp. Mar. Biol. Ecol. 205 (1996) 149–163.
[12] P. Soudant, J. Moal, Y. Marty, J.F. Samain, J. Exp. Mar. Biol. Ecol. 215 (1997) 103–114.
[13] P. Soudant, Thèse de Doctorat, Université de Bretagne Occidentale, France, 1995, p. 293.
[14] A. Grandgirard, A. Piconneaux, J.L. Sébédio, S.F. O'Keefe, E. Sémon, J.L. Le Quéré, Lipids 24 (1989) 799–804.
[15] W.M.N. Ratnayake, Z.Y. Chen, G. Pelletier, D. Weber, Lipids 29 (1994) 707–714.
[16] J.L. Sébédio, J.L. Le Quéré, E. Sémon, O. Morin, J. Prévost, A. Grandgirard, J. Am. Oil Chem. Soc. 64 (1987) 1324–1333.
[17] R.C. Wijesundera, W.M.N. Ratnayake, R.G. Ackman, J. Am. Oil Chem. Soc. 66 (1990) 1822–1830.
[18] L.D. Metcalfe, A.A. Schmitz, Anal. Chem. 33 (1961) 363–364.
[19] J. Conway, W.M.N. Ratnayake, R.G. Ackman, J. Am. Oil Chem. Soc. 62 (1985) 1340–1343.

[20] J.M. Snyder, C.R. Scholfield, J. Am. Oil Chem. Soc. 59 (1982) 469–470.
[21] J.L. Kice, G. Guaraldi, C.G. Venier, J. Org. Chem. 31 (1966) 3561–3567.
[22] R.O. Adlof, J. Chromatogr. A 659 (1994) 95–99.
[23] L. Fay, U. Richli, J. Chromatogr. 541 (1991) 89–98.
[24] R.G. Ackman, in: R.T. Holman (Ed.), Progress in the Chemistry of Fats and Others Lipids, Pergamon Press, Oxford, 1972, p. 165, Vol. XII.
[25] G.E. Napolitano, W.M.N. Ratnayake, R.G. Ackman, Comp. Biochem. Physiol. 90B (1988) 875–883.
[26] G.J. Perry, Ph.D. Thesis, University of Melbourne, Melbourne, 1977, pp. 103 and 173–176
[27] G.E. Napolitano, R.G. Ackman, J. Shellfish Res. 12 (1993) 267–277.
[28] R.J. Pollero, M.R. Re, R.R. Brenner, Comp. Biochem. Physiol. 64A (1979) 257–263.
[29] D. Tocher, J.R. Dick, J.R. Sargent, Lipids 30 (1995) 555–565.
[30] M.I. Aveldano, N.P. Rodstein, N.T. Vermouth, Lipids 27 (1992) 676–680.
[31] M.I. Aveldano, N.P. Rodstein, N.T. Vermouth, Biochem. J. 283 (1992) 235–241.
[32] B.L. Walker, Arch. Biochem. Biophys. 114 (1966) 465–471.
[33] T. Watanabe, Comp. Biochem. Physiol. 73B (1982) 3–15.
[34] R.J. Henderson, D. Tocher, Prog. Lipid Res. 26 (1987) 281–347.
[35] R.T. Holman, Prog. Lipid Res. 25 (1986) 29–39.
[36] F. Culkin, R.J. Morris, Deep Sea Res. 17 (1970) 171–174.
[37] R.G. Ackman, J. Food Lipids 4 (1997) 295–318.
[38] R.G. Ackman, in: J.L. Sébédio, W.W. Christie (Eds.), Trans Fatty Acids in Human Nutrition, The Oily Press, Dundee, 1998, pp. 303–311.
[39] R.L. Wolff, J.L. Sebedio, J. Am. Oil Chem. Soc. 68 (1991) 719–725.
[40] R.L. Wolff, J. Am. Oil Chem. Soc. 69 (1992) 106–110.
[41] J.L. Sébédio, W.W. Christie (Eds.), Trans Fatty Acids in Human Nutrition, The Oily Press, Dundee, 1998.
[42] J.L. Harwood, A.L. Jones, Advances in Botanical Research, Academic Press, New York, 1989, Vol. 16, p. 22.
[43] J.K. Volkman, S.W. Jeffre, P.D. Nichols, G.I. Rogers, C.D. Garland, J. Exp. Mar. Biol. Ecol. 128 (1989) 219–240.
[44] M. Lamberto, R.G. Ackman, Lipids 29 (1994) 441–444.
[45] M.I. Gurr, J.L. Harwood, Lipid Biochemistry, 4th ed, Chapman and Hall, London, 1991.
[46] H. Keweloh, H.J. Heipieper, Lipids 31 (1996) 129–137.
[47] J. Pettersen, J. Opstvedt, Lipids 26 (1991) 711–717.
[48] R. Käkelä, H. Hyvärinen, P. Vainiotalo, Comp. Biochem. Physiol. 113B (1996) 625–629.
[49] A. Grandgirard, J.M. Bourre, F. Julliard, P. Homayoun, O. Dumont, M. Piciotti, J.L. Sebedio, Lipids 29 (1994) 251–258.
[50] R.J. Henderson, R.M. Millar, J.R. Sargent, Lipids 30 (1995) 181–185.
[51] Y. Marty, unpublished results.