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Lipid Composition of the Pineal Organ from Rainbow Trout (*Oncorhynchus mykiss*)

R.J. Henderson[a,*], M.V. Bell[a], M.T. Park[a], J.R. Sargent[a] and J. Falcon[b]

[a]N.E.R.C. Unit of Aquatic Biochemistry, Department of Biological and Molecular Science, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom and [b]Laboratoire de Neurobiologie et de Neuroendocrinologie Cellulaires, URA CNRS 290, Faculte des Sciences, 86022 Poitiers Cedex, France

**The lipid composition of the pineal organ from the rainbow trout (*Oncorhynchus mykiss*) was determined to establish whether the involvement of this organ in the control of circadian rhythms is reflected by specific adaptations of lipid composition. Lipid comprised 4.9% of the tissue wet weight and triacylglycerols were the major lipid class present (47% of total lipid). Phosphatidylcholine (PC) was the principal polar lipid, and smaller proportions of other phospholipids and cholesterol were also present. Plasmalogens contributed 11% of the ethanolamine glycerophospholipids (EGP). No cerebrosides were detected. The fatty acid composition of triacylglycerols was generally similar to that of total lipids in which saturated, monounsaturated and polyunsaturated fatty acids (PUFA) were present in almost equal proportions. Each of the polar lipid classes had a specific fatty acid composition. With the exception of phosphatidylinositol (PI), in which 20:4n-6 comprised 27.4% of the total fatty acids, 22:6n-3 was the principal PUFA in all lipid classes. The proportion of 20:5n-3 never exceeded 6.0% of the fatty acids in any lipid class. The predominant molecular species of PC were 16:0/22:6n-3 and 16:0/18:1, which accounted for 33.2 and 28.5%, respectively, of the total molecular species of this phospholipid. Phosphatidylethanolamine (PE) contained the highest level of di-22:6n-3 (13.0%) of any phospholipid. There was also 4.9% of this molecular species in phosphatidylserine (PS) and 4.1% in PC. In PE, the species 16:0/22:6, 18:1/22:6 and 18:0/22:6 totalled 45.1%, while in PS 18:0/22:6 accounted for 43.9% of the total molecular species. The most abundant molecular species of PI was 18:0/20:4n-6 (37.8%). The lipid composition of the pineal organ of trout, and particularly the molecular species composition of PI, is more similar to the composition of the retina than that of the brain.
Lipids 29, 311–317 (1994).**

In fish, as in all vertebrates, the retina of the eye and the pineal organ of the brain are essential components of the circadian system that measures the period and phase of the daily light–dark cycle and ultimately controls rhythmic processes (1,2). In keeping with this role, the pineal organ of fish contains photoreceptor cells that display close cyto-functional analogies with the photoreceptors of the retina (3,4). The photoreceptor cells from both tissues translate the light–dark information into a neural output of excitatory neurotransmitter (5) and a neurohormonal output in the form of melatonin (3). In addition to being multimessenger cells, the photoreceptor cells of the pineal organ are also multieffectors and can transduce information supplied by other eternal (such as temperature) or internal (catechol-amines, adenosine, steroids) factors. Whereas retinal melatonin acts preferentially in an autocrine/paracrine manner, the melatonin secreted by the pineal contributes largely to the circulating levels and may be involved in the control of seasonal events, particularly reproduction, in vertebrates (3,6–8).

The structural phospholipids of the retina and brain of vertebrates, including fish, are known to contain high levels of the polyunsaturated fatty acid (PUFA) 22:6n-3 (9,10). Within the retina, the 22:6n-3 is apparently concentrated in the membranous outer segments of the photoreceptor rod cells (11), and these cells isolated from frog retina exhibit a selective uptake of 22:6n-3 *in vitro* (12). A requirement for 22:6n-3 in the visual process has been demonstrated in studies with newborn primates and preterm human infants, which have shown that the visual acuity is affected by deprivation of 22:6n-3 during postnatal development of the infant (13). Peroxidation of 22:6n-3 is one of the primary events observed in inherited or traumatically induced photoreceptor degeneration (9,14).

Di-22:6n-3 phospholipids are known to be major components of rod outer segment membranes in frog and rat (15,16). Recent analyses of the phospholipids of the brain and retina of trout and cod have shown that di-22:6n-3 molecular species are major constituents of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) in these tissues and that this is particularly true of the retina, where the amounts are considerably higher than those found in terrestrial mammals (17,18). The importance of 22:6n-3 in the visual process of fish is also indicated by the observation that the proportion of di-22:6n-3 in the phospholipid of the developing eyes of herring larvae increases with age as the rods are recruited into the photoreceptor population (19). The results of these nutritional studies and the presence of large amounts of di-22:6 phospholipid in the photoreceptors of the retina suggest that 22:6n-3 has an essential and unique role in the primary events associated with the absorption and transduction of photons. This might be of crucial importance for the photoperiodic control of the production of messages, such as melatonin.

*To whom correspondence should be addressed.

Abbreviations: EGP, ethanolamine glycerophospholipids; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids; SM, sphingomyelin; TAG, triacylglycerols. Molecular species are abbreviated as follows: e.g., 16:0/22:6 PC is 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine.

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By analogy with the retina, it is probable that 22:6n-3 is a major PUFA of the lipids in the photoreceptor cells of the fish pineal organ. To examine this hypothesis, we determined in the present study the detailed lipid composition of the trout pineal with particular attention to the PUFA content and molecular species of phospholipids. As far as we are aware, the detailed lipid composition of the pineal organ from fish has not been reported previously and only a limited amount of information is available on the lipids of the organ from mammals (20,21). Information gained from the analysis of the trout pineal organ is of basic importance as mammalian pinealocytes are accepted as being phylogenetically derived from the fish pineal photoreceptor cells (2).

MATERIALS AND METHODS

Fish and pineal organs. Rainbow trout (*Oncorhynchus mykiss*) of average weight 800 g were obtained from a commercial fish farm (Pisciculture Bellet, Angouleme, France) where they had been maintained under natural conditions of water temperature and photoperiod. One hundred fish were killed by decapitation. Pineal organs were removed immediately from the fish, frozen in liquid nitrogen and stored at −80°C until taken for analysis.

Chemicals and solvents. Phospholipase C from *Bacillus cereus* was purchased from Boehringer Corporation (London) Ltd. (Lewes, East Sussex, England). Oxalyl chloride and anthracene 9-carboxylic acid were supplied by Aldrich Chemical Co. (Gillingham, Dorset, England). All other chemicals and biochemicals were purchased from Sigma (Poole, Dorset, England), and solvents of high-performance liquid chromatography (HPLC) grade were obtained from Rathburn Chemicals (Walkerburn, Peeblesshire, Scotland).

Lipid extraction and analysis. After thawing, the pineal organs were weighed and transferred to a Teflon-in-glass homogenizer. The organs were homogenized in 37 mL of chloroform/methanol (2:1, vol/vol) essentially as described by Christie (22) to extract lipids. Solvent was removed under a stream of nitrogen and the resulting lipid extract desiccated overnight under vacuum in a preweighed tube. The tube and contents were then reweighed to obtain the weight of the lipid extract which was redissolved in chloroform/methanol (2:1, vol/vol) and stored under an atmosphere of nitrogen at −70°C between analyses.

To establish the lipid class composition, aliquots of lipid extract were subjected to high-performance thin-layer chromatography (HPTLC) alongside authentic standards using hexane/diethyl ether/glacial acetic acid (80:20:2, by vol) as the developing solvent for the separation of neutral lipid classes and methyl acetate/propan-2-ol/chloroform/methanol/0.25% (wt/vol) aq. KCl (25:25:25:10:9, by vol) for the separation of polar lipids. To confirm which polar lipid classes were present, aliquots of total lipid were also subjected to two-dimensional HPTLC. The polar lipid developing solvent system described previously was used for development in the first dimension and chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1, by vol) was employed for development in the second dimension. Developed chromatograms were visualized with copper acetate in phosphoric acid (23). Lipid class composition was quantitated by double-development HPTLC coupled with scanning densitometry, as described elsewhere (24). Estimates of the relative amounts of the plasmalogen and the diacyl forms of ethanolamine glycerophospholipids (EGP) were obtained by acid hydrolysis of the isolated EGP *in situ* on an HPTLC plate followed by chromatography and quantitative phosphate staining as described by Bell and Dick (25).

For the analysis of fatty acid composition, individual lipid classes were separated by two-dimensional TLC on 20 × 20 cm glass plates coated with silica gel G 60 (0.25 mm thick) using the solvent systems described here. The separated classes were visualized by spraying the chromatogram with 0.1% 2′,7′-dichlorofluorescein in methanol containing 0.01% butylated hydroxytoluene and by viewing under ultraviolet light. Triacylglycerols (TAGs) were purified by redeveloping the chromatogram in the reverse direction of the second development using hexane/diethyl ether/glacial acetic acid (80:20:2, by vol) after removal of the individual polar lipid classes. The fatty acids of the separated lipid classes were converted to their methyl esters on the adsorbent by acid-catalyzed transesterification (22). An aliquot of total lipid was also subjected to the same procedure. The resulting fatty acid methyl esters were purified by HPTLC and recovered from the adsorbent with hexane/diethyl ether (1:1, vol/vol).

Fatty acid methyl esters were analyzed on a Packard 439 gas chromatograph equipped with a fused silica capillary column (50 m × 0.22 mm i.d.) coated with FFAP phase (S.G.E., Milton Keynes, United Kingdom). Sample application was by on-column injection, and hydrogen was used as the carrier gas. During the course of an analysis, the oven temperature was programmed to increase from 50 to 225°C. Samples were also analyzed using an Omegawax 250 fused silica column (30 m × 0.25 mm i.d., Supelchem U.K. Ltd., Essex, United Kingdom) with the oven temperature programmed from 50 to 260°C. Fatty acid components were identified by reference to a well-characterized fish oil fatty acid mixture, and the unsaturated nature of components was confirmed by re-analysis of samples after catalytic hydrogenation over PtO_2. The separated components were quantitated using a recording integrator linked to the chromatograph.

Analysis of molecular species. A 500-µg portion of total lipid was separated into the component phospholipids by HPTLC alongside 20 µg of a cod retina total lipid standard using methyl acetate/propan-2-ol/chloroform/methanol/0.25% (wt/vol) aq. KCl (25:25:25:10:9, by vol) as the developing solvent. The standard spots and the edge of the bands of pineal organ lipids were visualized by exposure to iodine vapor, and the bands of adsorbent, containing PC, PS, phosphatidylinositol (PI) and EGP, were scraped from the plate. The phospholipids were hydrolyzed on the silica with phospholipase C using a two-phase system of 1 mL diethyl ether and 1 mL of 0.1 M sodium borate buffer, pH 7.5 at room tem-

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perature under nitrogen for 2 h (26). At the end of the incubation period, 1,2-diradylglycerols were extracted, dried down under nitrogen and finally desiccated under vacuum for 1 h. 9-Anthroyl chloride was prepared from 9-anthracene carboxylic acid and oxalyl chloride as described by Goto *et al.* (27). The diradylglycerols were derivatized and purified by a modification of the method of Takamura and Kito (28) as described elsewhere (19). The 1-*O*-alk-1′-enyl-2-acyl derivatives were removed during the final HPTLC purification step. The 1,2-diacyl-3-anthrocyl-*sn*-glycerols were separated by HPLC at 19–20°C on an ODS column (25 × 0.46 cm, 5 μm particle size; Beckman Instruments U.K. Ltd., High Wycombe, Buckinghamshire, United Kingdom) using a Pye Unicam PU4010 pump (Pye Unicam Ltd., Cambridge, England) and two isocratic solvent systems, methanol/propan-2-ol (4:1, vol/vol) at a flow rate of 1.0 mL/min, and acetonitrile/propan-2-ol (7:3, vol/vol) at a flow rate of 1.0 mL/min as described by Takamura and Kito (28). Peaks were detected using a Waters 470 scanning fluorescence detector (Millipore UK Ltd., Edinburgh, Scotland) with excitation and emission wavelengths of 360 and 460 nm, respectively, and quantified using a Shimadzu CR3A recording integrator (Anachem, Luton, United Kingdom). Peaks were identified by their relative retention time using 16:0/22:6n-3 as a reference peak. Di-docosahexaenoylglycerol (di-22:6n-3; Nu-Chek-Prep, Elysian, MN) was also available for direct comparison of retention times, as were a range of samples of known composition from previous studies (17,18,29). Each sample was chromatographed three times in each solvent system and the standard deviations calculated. Where final peak areas were calculated by subtraction, the standard deviations of the contributing peaks were added to give the final error.

RESULTS

The lipid content and lipid class composition of trout pineal organ are presented in Table 1. Around 4.9% of the wet weight of the pineal organs was lipid, of which almost half (47%) was in the form of TAG. PC was the major polar lipid present (16.5% of total lipid), followed by EPG (9.9%). No choline plasmalogens were detected, whereas ethanolamine plasmalogens accounted for 11% of the total EPG fraction. PI and PS each accounted for less than 5% of the total lipid, and both phosphatidylglycerol (PG) and spingomyelin (SM) were present at less than 2%. No cardiolipin or cerebrosides were detected.

Palmitic acid (16:0) comprised 23.7% of the fatty acids in the total lipids and was the most abundant fatty acid in the pineal organ (Table 2). The monounsaturated 18:1n-9 and polyunsaturated 22:6n-3 accounted for 17.6 and 12.4%, respectively, of the total fatty acids. Overall, saturated, monounsaturated and polyunsaturated fatty acids accounted for similar proportions of the total lipid fatty acids. The fatty acid composition of TAGs (Table 2) was generally similar to that of the total lipid although the proportion of 18:2n-6 (12.8%) was notably higher, and that of 16:0 lower, than in total lipids.

TABLE 1

Lipids of Trout Pineal Organ: Lipid Class Composition[a]

Lipid class	% Total lipid
Cholesteryl esters	3.3 ± 0.2
Triacylglycerols	47.0 ± 2.1
Free fatty acids	3.4 ± 0.6
Cholesterol	8.7 ± 0.4
Diacylglycerols	1.5 ± 0.1
Ethanolamine glycerophospholipids	9.9 ± 0.6
Phosphatidylglycerol	1.4 ± 0.1
Phosphatidylinositol	3.2 ± 0.3
Phosphatidylserine	3.4 ± 0.3
Phosphatidylcholine	16.5 ± 0.8
Sphingomyelin	1.7 ± 0.1

TABLE 2

Fatty Acid Composition (wt%) of Total Lipid and Lipid Classes from Trout Pineal Organ[a]

Acyl chain	Total lipid	TAG	PC	EGP
14:0	2.9	3.5	0.6	0.2
15:0	0.9	0.3	0.3	0.9
16:ODMA	—	—	—	0.6
16:0	23.7	17.4	34.0	10.7
16:1n-9	—	—	0.6	—
16:1n-7	5.8	8.0	1.3	0.7
17:0	0.6	0.4	0.3	0.3
18:ODMA	—	—	—	0.4
18:1n-9DMA	—	—	—	2.2
18:1n-7DMA	—	—	—	0.7
18:0	7.2	4.2	5.4	10.2
18:1n-9	17.6	18.5	18.3	10.3
18:1n-7	4.0	3.7	2.4	3.9
18:2n-6	9.3	12.8	1.8	3.4
18:3n-3	1.2	2.0	—	0.3
20:1n-9	2.5	3.1	0.4	1.4
20:2n-6	0.6	1.2	0.4	0.9
20:3n-6	0.4	0.2	0.3	0.4
20:4n-6	1.6	0.6	2.0	7.2
20:3n-3	—	0.2	—	—
20:4n-3	0.9	1.4	0.3	0.6
20:5n-3	3.5	3.9	4.6	4.8
22:1n-11	1.7	2.0	—	—
22:1n-9	0.3	0.3	—	—
22:4n-6	0.1	0.3	—	—
22:5n-6	—	0.2	0.2	0.4
22:5n-3	1.2	1.8	1.3	2.2
22:6n-3	12.4	12.6	24.6	36.0
24:1n-9	0.7	0.1	—	0.4
Unidentified	0.9	1.4	0.9	0.7
Total saturated	35.3	25.8	40.6	23.3
Total monounsaturated	32.6	35.7	23.0	19.6
Total PUFA	31.2	37.1	35.5	56.2
Total n-3	19.2	21.9	30.8	43.9
Total n-6	12.0	15.2	4.7	12.3
n-3/n-6	1.60	1.44	6.55	3.57

[a]EGP, ethanolamine glycerophospholipids; PC, phosphatidylcholine; TAG, triacylglycerols; PUFA, polyunsaturated fatty acids;

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TABLE 3

Fatty Acid Composition (wt%) of Lipid Classes from Trout Pineal Organ[a]

Acyl chain	PI	PS	PG	SM
14:0	0.3	0.3	0.8	0.6
15:0	0.4	0.7	0.3	0.4
16:0	14.0	3.0	10.7	19.2
16:1n-9	—	—	—	—
16:1n-7	—	9.3	17.0	0.5
17:0	0.7	0.4	0.5	0.6
18:0	33.8	38.2	5.4	9.5
18:1n-9	5.3	5.5	15.5	8.9
18:1n-7	1.3	2.6	10.2	1.9
18:2n-6	1.5	1.4	8.8	2.2
18:3n-3	0.1	0.4	0.9	—
20:1n-9	0.2	1.2	1.4	0.4
20:2n-6	0.2	0.4	1.1	1.0
20:3n-6	0.3	—	1.0	1.0
20:4n-6	27.4	1.0	1.8	0.5
20:3n-3	—	—	—	—
20:4n-3	0.1	—	0.5	0.2
20:5n-3	5.7	1.3	1.6	0.9
22:1n-11	—	—	—	—
22:1n-9	—	—	—	0.6
22:4n-6	0.5	0.9	0.9	—
22:5n-6	—	0.4	—	—
22:5n-3	0.4	3.0	0.7	0.2
22:6n-3	7.0	29.4	19.8	2.9
24:0	—	—	—	0.8
24:1n-9	0.4	—	—	45.9
Unidentified	0.4	0.6	1.1	1.3
Total saturated	49.2	42.6	17.7	31.6
Total monounsaturated	7.2	18.6	44.1	58.2
Total PUFA	43.2	38.2	37.1	8.9
Total n-3	13.3	34.1	23.5	4.2
Total n-6	29.9	4.1	13.6	4.7
n-3/n-6	0.45	8.32	1.73	0.89

[a]PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; PUFA, polyunsaturated fatty acids.

Each of the polar lipid classes had a specific fatty acid composition. In PC, 16:0 and 22:6n-3 were the principal components and together accounted for nearly 60% of the total fatty acids present in this phospholipid (Table 2). In contrast, 16:0 comprised only 10.7% of the fatty acids in EGP, whereas the level of 22:6n-3 (36%) was the highest observed in any lipid class. As a consequence, the total content of PUFA in EGP (56.2%) was the highest of all lipid classes. Only the EGP fraction produced dimethyl acetals by transmethylation of 1-*O*-alk-1′-enyl linked ether chains.

PI was unique among the lipid classes in that 20:4n-6 was a major component and accounted for 27.4% of the fatty acids, whereas 22:6n-3 comprised only 7.0% (Table 3). As a consequence, the overall ratio of n-3 to n-6 PUFA in PI was the lowest of any lipid class. The saturated fatty acid 18:0 was also a major fatty acid in PI. PS was characterized by a high content of 18:0 and in monounsaturated fatty acids and PUFA which comprised 44.1 and 37.1%, respectively, of the total fatty acids in this phospholipid (Table 3). The levels of 16:1n-7 and 18:1n-7 (17.0 and 10.2%) in PG were higher than in any other lipid class, and, of all the phospholipids PG had the highest content of 18:2n-6. In SM more than half (58.2%) of the fatty acids were monounsaturated, mainly due to the presence of a very high proportion of 24:1 (45.9%). PUFA comprised only 8.9% of the SM fatty acids (Table 3).

In all lipid classes, 20:5n-3 was present in small amounts and never exceeded 6.0% of the component fatty acids. The long chain monoenoic fatty acid 22:1n-11 observed in total lipid was concentrated in TAG, where it accounted for around 2% of the fatty acids. Of all the lipid classes, TAG also contained the highest level of 14:0.

The principal molecular species of PC, PE, PS and PI are presented in Table 4. Two molecular species predominated in PC, namely 16:0/22:6n-3 and 16:0/18:1, which accounted for 33.2 and 28.5%, respectively, of the total molecular species of this phospholipid. Di-PUFA, disaturated and di-monounsaturated species each comprised less than 6% of the total PC and the content of monounsaturated-PUFA species totalled 4.9%, within which 18:1/22:6n-3 was the major component. Molecular species containing 22:6n-3 were particularly abundant in PE. Di-22:6n-3 comprised 13.0% of the total, and 16:0/22:6 and 18:1/22:6 were both present at levels of more than 15%. In PS, 18:0/22:6n-3 accounted for almost half (43.9%) of the total molecular species, and 16:0/22:6n-3 was the only other species present at a level of greater than 10%. The most abundant molecular species of PI was 18:0/20:4n-6 which accounted for 37.8%. Another species containing 20:4n-6, 16:0/20:4n-6, comprised 14.0% of the molecular species of PI and 18:0/20:5n-3 accounted for 10.8%, the highest level for a species containing 20:5n-3 observed in any of the phospholipids examined.

DISCUSSION

Although the pineal organ is an adjunct of the brain, it is known to have evolved from a well-differentiated photoreceptive organ that is frequently considered to be a functional third eye in lower vertebrates (3). In fact, to date, the properties established for the pineal photoreceptors have been extended to the retinal photoreceptors and *vice versa* (3). The major difference between the pineal and retina relates to the neuronal organization, which is simple in the pineal but complex in the retina. Thus, pineal photoreceptors make contact with second order neurons that send their axons to brain centers. Retinal photoreceptors, on the other hand, are in contact with bipolar cells that, in turn, communicate with ganglion cells and numerous interneurons that are present (horizontal, amacrine, interpexiform cells). Consequently, the ratio of photoreceptors to other neurons is much higher in the pineal than in the retina, and the lipid composition of the pineal organ can be expected to

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TABLE 4

Molecular Species of Phospholipids from Trout Pineal Organ[a]

Species	PC	PE	PS	PI
Di-PUFA				
20:5/22:6	0.6 ± 0.0	1.0 ± 0.0	0.3 ± 0.0	–
22:6/22:6	4.1 ± 0.2	13.0 ± 0.3	4.9 ± 0.0	0.3 ± 0.0
22:5/22:6	1.0 ± 0.1	4.6 ± 0.3	2.4 ± 0.1	0.1 ± 0.0
Saturated/PUFA				
14:0/22:6	1.2 ± 0.1	3.8 ± 0.1	0.7 ± 0.2	0.2 ± 0.1
16:0/20:5	6.7 ± 0.4	4.2 ± 0.1	1.8 ± 0.3	4.1 ± 0.2
16:0/22:6	33.2 ± 1.6	20.1 ± 0.1	15.0 ± 0.2	10.3 ± 0.5
16:0/20:4	1.5 ± 0.2	2.4 ± 0.1	1.8 ± 0.1	14.0 ± 0.3
16:0/22:5	2.1 ± 0.3	1.9 ± 0.1	1.1 ± 0.1	0.6 ± 0.2
18:0/20:5	1.2 ± 0.2	3.7 ± 0.1	3.7 ± 0.3	10.8 ± 0.1
18:0/22:6	3.7 ± 0.2	9.6 ± 0.3	43.9 ± 0.2	7.1 ± 0.5
18:0/20:4	0.5 ± 0.1	2.7 ± 0.1	0.6 ± 0.1	37.8 ± 1.0
18:0/22:5	1.6 ± 0.2	0.6 ± 0.2	4.7 ± 0.1	0.4 ± 0.1
Monounsaturated/PUFA				
16:1/22:6	0.5 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.0
18:1/20:5	0.9 ± 0.1	3.7 ± 0.1	–	0.7 ± 0.0
18:1/22:6	2.9 ± 1.7	15.4 ± 0.4	6.3 ± 1.0	0.6 ± 0.5
18:1/20:4	–	2.6 ± 0.1	1.1 ± 0.1	4.1 ± 0.2
18:1/22:5	0.6 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	–
Di-saturated				
16:0/16:0	0.9 ± 0.1	0.1 ± 0.0	1.1 ± 0.1	–
Saturated/monounsaturated				
16:0/18:1	28.6 ± 1.0	1.2 ± 0.1	2.8 ± 0.0	6.9 ± 0.3
18:0/18:1	2.5 ± 0.1	0.2 ± 0.1	0.9 ± 0.0	0.3 ± 0.0
Di-monounsaturated				
18:1/18:1	0.8 ± 0.1	0.6 ± 0.1	1.2 ± 0.1	0.6 ± 0.0
20:1/18:1	–	–	0.3 ± 0.0	–

[a]Values are mol% and are means of triplicate determinations ± 1 SD. It was assumed that the most saturated fatty acids were located on the *sn*-1 position. Molecular species containing minor fatty acids or fatty acid isomers were not resolved from the major components. Molecular species containing 18:2n-6 co-elute with those containing 22:5n-3 and 22:5n-6. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids.

The lipid content of the trout pineal (4.9% of wet weight) is almost exactly halfway between that found for the brain and retina (6.8 and 3.1%, respectively) of the same species (10). However, the level of TAG in the lipid of the pineal (47%) is considerably higher than the 8.4 and 30% found in the lipid of the brain and retina, but is nevertheless closer to the latter. The levels of TAG in brains and retina of trout are known to be higher than in the same tissues from cod (10). An influencing factor may be the fact that the trout examined were farmed fish. It is well known that farmed fish, including trout, have higher lipid contents in their flesh than their wild counterparts (30). Whether the lipid class content of neural tissues differs between wild and farmed fish or whether high TAG levels are a specific feature of the lipids of trout neural tissues remains to be established.

A notable feature of the brain of fish and animals in general is the high proportion of EGP in the total lipid (10,31). In both cod and trout, the EGP/PC ratio in brain lipid is approximately 1:1 (10). The level of EGP in the pineal lipid is notably lower than that of PC, the EGP/PC ratio being 0.6:1. This value is closer to the 0.7:1 observed for the ratio of these two phospholipids in the retina of both trout and cod (10). The ethanolamine plasmalogen content of the pineal is much lower than that of fish brain (36–38% of EGP) (25) and closer to that of retina (<6% of EGP; Bell, M.V., unpublished data). The absence of cerebrosides in the lipids of the pineal is also more typical of retina than brain, as is the low amount of ethanolamine plasmalogens (31). The absence of cardiolipin in significant amounts in pineal lipids is unusual as this lipid class is a common component of mitochondrial membranes and has been found in lipid extracted from both fish retina and brain (10).

The total lipid and component lipid classes of the pineal organs, with the exception of SM, were characterised by high contents of PUFA. Nevertheless, the content of PUFA in the total lipid of trout organ (31.2%) is lower than the corresponding values of 41.4 and 40.6% reported for the brain and retina, respectively, of the same species (10), and is considerably less than the PUFA content (43.6%) of total lipid from rat pineal (20). The distribution of fatty acids among the various lipid classes conforms to well established patterns. For example, the long-chain monoenoic fatty acid 22:1n-11 oc-

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