

Arachidonic and eicosapentaenoic acids in Araucariaceae, a unique feature among seed plants

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Résumé : Il est généralement admis que les plantes à graines (spermaphytes) ne sont pas capables de synthétiser les acides arachidonique ou éicosapentaénoïque (AA et AEP). Nous montrons ici, à l'aide de techniques chromatographiques et spectrométriques, que des espèces de la famille primitive Araucariaceae (gymnospermes) sont capables de synthétiser en faibles quantités ces acides dans leurs graines et feuilles. *Agathis robusta*, en particulier, contient à la fois l'AA et l'AEP dans ses graines, mais est dépourvu d'acides D5-insaturés polyméthylène-interrompus (D5-IPMI) à 18 atomes de carbone, alors que les espèces d'*Araucaria* étudiées en contiennent. En revanche, les deux genres contiennent des acides D5-IPMI, caractéristiques de tous les Coniférophytes. Tous les intermédiaires métaboliques de l'AA et/ou de l'AEP ont été caractérisés dans les graines d'Araucariaceae. L'intérêt de ces observations est discuté dans le cadre de la phylogénie des Coniférophytes.

Summary : It is generally admitted that seed plants (spermaphytes) are unable to synthesize either arachidonic or eicosapentaenoic acids (AA and EPA), the classic essential fatty acids in animals. We give here chromatographic and spectrometric data showing that species from the primitive family Araucariaceae (gymnosperms) are able to synthesize AA and/or EPA in their seeds and leaves. *Agathis robusta*, in particular, contains AA and EPA in small amounts in its seeds, with no D5-unsaturated polymethylene-interrupted fatty acids (D5-UPIFA) with 18 carbon atoms, whereas *Araucaria* spp. contain both AA and C18 D5-UPIFA. In both species, D5-UPIFA with 20 carbon atoms are present as in all other Coniferophytes. All metabolic intermediates necessary for the biosynthesis of AA and/or EPA have been characterized in Araucariaceae seeds. The relevance of these observations is discussed with regard to the phylogeny of Coniferophytes.

Mots-clés : *Agathis* spp., acide arachidonique, acide éicosapentaénoïque, *Araucaria* spp., Araucariaceae, chromatographie en phase gazeuse, chromatographie d'argentation sur couche mince, composition en acides gras, Coniférophytes, phylogénie, spectrométrie de masse.

Keywords : *Agathis* spp., arachidonic acid, *Araucaria* spp., Araucariaceae, argentation thin-layer-chromatography, Coniferophytes, eicosapentaenoic acid, fatty acid composition, gas-liquid chromatography, mass spectrometry, phylogeny.

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Arachidonic (5,8,11,14-20:4) and eicosapentaenoic (5,8,11,14,17-20:5) acids are the classic essential fatty acids in animals that otherwise have not yet been characterized with certainty in spermatophytes (seed plants) [1]. At most, a few angiosperm species can synthesize their more immediate precursors gamma-linolenic (6,9,12-18:3) and stearidonic (6,9,12,15-18:4) acids from linoleic (9,12-18:2) and alpha-linolenic (9,12,15-18:3) acids, respectively, but they cannot further process these fatty acids. In contrast, plants of lower phyletic rank (microalgae, algae, bryophytes, pteridophytes) have the capability to synthesize arachidonic and eicosapentaenoic acids. In the course of systematic studies of gymnosperm lipids, we observed that a species (*Agathis robusta*) of the Araucariaceae family (gymnosperms of the division Coniferophytina) contains in low amounts arachidonic and eicosapentaenoic acids in its seeds, as well as all intermediate metabolites that are necessary for their biosynthesis [2]. We give here evidence that the presence of arachidonic acid is not limited to *A. robusta* seeds among Araucariaceae, as it also occurs in *Araucaria* spp. seeds, and *Agathis* and *Araucaria* leaves.

Lipids from the seeds [3-6], leaves [7], and wood [8, 9] of a great number of gymnosperm species are characterized by a particular class of fatty acids, the DELTA5-unsaturated polymethylene-interrupted fatty acids (DELTA5-UPIFA) (*figure 1*). These fatty acids occur in the seeds from all Coniferophyte families studied so far, encompassing approximately 180 species [3-6] (almost one-fourth of extant species). Known exceptions that do not contain DELTA5-UPIFA belong to the Cycadophytes [10, 11]. Sciadonic acid (a C20 DELTA5-UPIFA), in particular, is a constant lipid component in the seed and leaf lipids of gymnosperms containing DELTA5-UPIFA [12], whereas the presence of other DELTA5-UPIFA (in particular C18 DELTA5-UPIFA) is much more variable. Outside this subphylum, among land plants, some DELTA5-UPIFA similar to those described in gymnosperms only occur in the distant-related *Equisetum* spp. [13] and in a handful of angiosperm species [14].

We recently examined the seed and leaf total lipids from three supplementary species belonging to the Araucariaceae, a family that had remained largely unexplored to date as regards to its fatty acid constituents. In addition to the usual linoleic and alpha-linolenic acids, and the C18 and C20 DELTA5-UPIFA usually encountered in gymnosperms (*table 1*), the seeds from *Araucaria bidwillii*, *Araucaria cunninghamii*, and *Araucaria araucana*, like those of *A. robusta*, contain arachidonic acid in small amounts, though easily observable under routine analytical conditions. The identity of arachidonic acid was established by capillary gas-liquid chromatography (GLC) coupled to mass spectrometry of appropriate derivatives [15, 16] prepared from *A. bidwillii* seed lipids (*figure 2*), and comparison with authentic standards. The main diagnostic fragmentation points are indicated on the figure. All other chromatographic data supported these structural characterizations [GLC on capillary columns with different polarities, *i.e.*, DB-Wax, CP-Sil 88, and Silar 5CP, fractionation by argentation thin-layer chromatography (Ag-TLC), and by reversed-phase high-performance liquid chromatography]. Experimental details are given elsewhere [2]. As an example, we show in *figure 3* the fractionation of *A. araucana* seed fatty acids by Ag-TLC and analyses of the fractions by GLC on a 30-m DB-Wax column. Eico-sapentaenoic acid, characterized in the same way in *A. robusta* seeds (*figure 2*), was not observed in any *Araucaria* spp. seeds. Moreover, all necessary metabolic precursors of arachidonic acid (and of eicosapentaenoic acids in the case of *A. robusta* seeds), gamma-linolenic and dihom-

gamma-linolenic (8,11,14-20:3) acids [stearidonic and dihomo-stearidonic (8,11,14,17-20:4) acids in the case of *A. robusta*], were characterized similarly. Arachidonic acid was also detected in lipids prepared from fresh *A. araucana* and *Agathis dammara* leaves (table 1).

Arachidonic and eicosapentaenoic acids are known to occur, among higher plants, in mosses, liverworts, and ferns, as well as in lower plant forms, microalgae, algae, and some moulds. No examples of the presence of arachidonic or eicosapentaenoic acid in the seeds of any gymnosperm or angiosperm species are known with certainty, and even in those few unconfirmed reports claiming the finding of these acids in other parts than seeds in spermatophytes, their metabolic precursors were not characterized [1, 17]. With regard to fatty acid biosynthesis, there thus existed an apparent hiatus between plants able to synthesize arachidonic and eicosapentaenoic acids (pteridophytes, in particular), and plants unable to effect these biosyntheses (spermatophytes), apparently filled by *A. robusta* and particularly *Araucaria* species.

The difference in the C20 DELTA5-polyunsaturated fatty acid patterns between the two plant groups can be explained by differences in the desaturation mode of the initial precursors, linoleic and alpha-linolenic acids. The first step in the biosynthesis of arachidonic and eicosapentaenoic acids is a DELTA6-desaturation of linoleic and alpha-linolenic acids, followed by a two-carbon atom elongation, and a further DELTA5-desaturation of the resulting eicosapolyenoic acids. With regard to the initial step, *A. robusta* is still able to introduce a DELTA6-desaturation in linoleic and alpha-linolenic acids, but not a DELTA5-desaturation (presence of gamma-linolenic and stearidonic acids, with no pinolenic or coniferonic acids), whereas *Araucaria* species still DELTA6-desaturate but also DELTA5-desaturate linoleic acid (deduced from the presence of both gamma-linolenic and pinolenic acids), only the latter reaction occurring in other Coniferophytes.

The *A. robusta* status thus resembles that found in plants of lower phyletic rank, mosses and ferns, whereas the status in *Araucaria* spp. would be intermediary between that in *Agathis* and that in several other gymnosperm families, in particular Pinaceae, that still DELTA5-desaturate, but do no more DELTA6-desaturate linoleic and alpha-linolenic acids. However, a point common to bryophytes, pteridophytes, Araucariaceae, all Coniferophyte families, and some Cycadophyte families, is the chain elongation of C18 unsaturated acids followed by a DELTA5-desaturation of the resulting C20 acids [12]. The principal difference resides in the C18 precursors that are elongated, linoleic acid and alpha-linolenic in gymnosperms, and their DELTA6-desaturation products in bryophytes and pteridophytes, with the genera *Agathis* and *Araucaria* showing intermediary positions.

The fatty acid metabolism in extinct Araucariaceae species may not have been similar to that present in extant species, but it is generally recognized that many characters of this family are especially primitive. Our observations do not forcefully mean that extant Araucariaceae represent relict intermediaries between gymnosperm ancestors and extant gymnosperms. However, it is quite strange to observe that a change in desaturase activities and/or specificities has apparently accompanied the emergence, radiation, and expansion of most gymnosperm families, as can be deduced from the wide distribution of DELTA5-UIPFA in this subphylum. This possibly occurred through a transition status such as that observed in Araucariaceae, that finally led to the complete loss of arachidonic and eicosapentaenoic acids in all other gymnosperms, where these acids are replaced by the structural analogs sciadonic and juniperonic acids, respectively, that just differ from the former acids by the absence of a DELTA8-ethylenic bond (figure 1), crucial for the biosynthesis of

prostaglandins in animals. In all angiosperm families analysed so far, except the family Ranunculaceae [14], some rare members of which contain the gymnospermous DELTA5-UIFA, the capability to biosynthesize DELTA5-polyunsaturated C20 acids (arachidonic acid, eicosapentaenoic acid, or DELTA5-UIFA), has been apparently irremediably lost, at least through the normal C18 DELTA6-desaturation/C18-C20 elongation/C20 DELTA5-desaturation pathway. Unfortunately, no biological roles for DELTA5-UIFA in gymnosperms are known and discussing any evolutionary advantages of these peculiar fatty acids would be premature. A possibility however could be that (pro)gymnosperms escaped herbivores just because they were no more a source of arachidonic and eicosapentaenoic acids for these animals.

The apparently unique intermediate and possibly primeval lipidic status of Araucariaceae among gymnosperms and more generally spermatophytes obviously need further investigations. This family of approximately 40 species is limited to three genera, *Agathis* and *Araucaria* analysed here, and the newly discovered monotypic *Wollemia* genus, that may help to better trace the phylogeny of gymnosperms. However, fossil records suggest that *Araucaria* spp. would be of greater antiquity than *Agathis* spp. [18]. Then, the intermediate C18 DELTA5/DELTA6-desaturation status in *Araucaria* spp. might correspond to a reversal of a primitive (pro)gymnospermous DELTA5-desaturation status, that has further evolved to a simple DELTA6-desaturation status in *Agathis* spp. Consequently, our present knowledge does not allow making a decision concerning the real direction of evolution of the C18-desaturation in Araucariaceae: DELTA6 --> DELTA5, or DELTA5 --> DELTA6.

CONCLUSION

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Illustrations

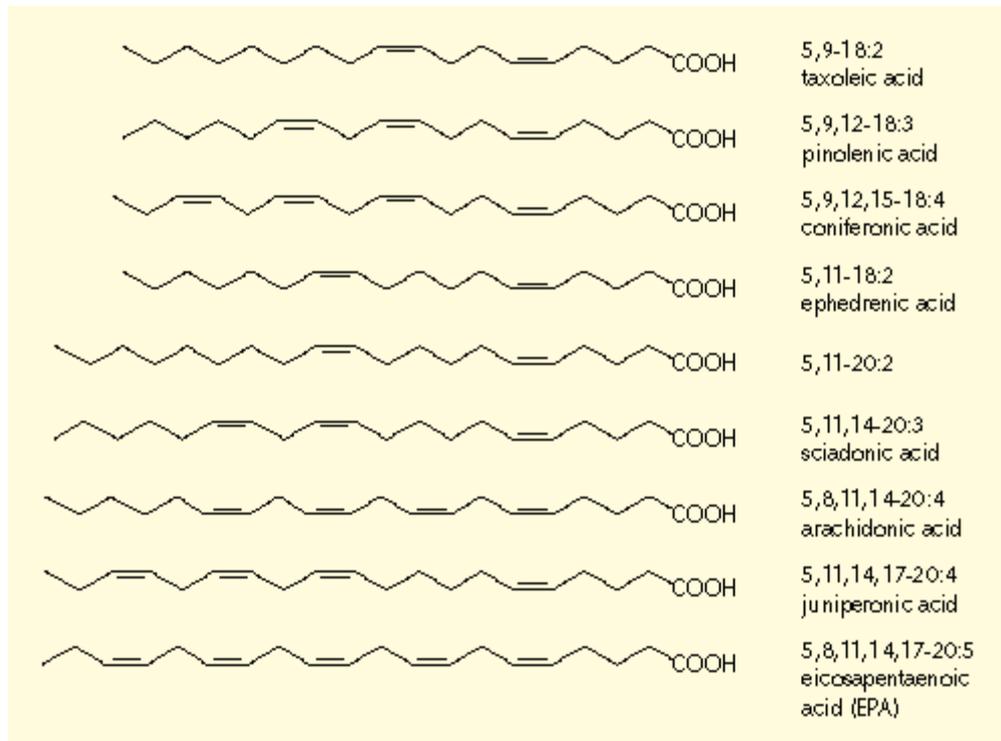


Figure 1. Structures, formulas, and trivial names of DELTA5-unsaturated polymethylene-interrupted fatty acids present in gymnosperm lipids, and of arachidonic and eicosapentaenoic acids.

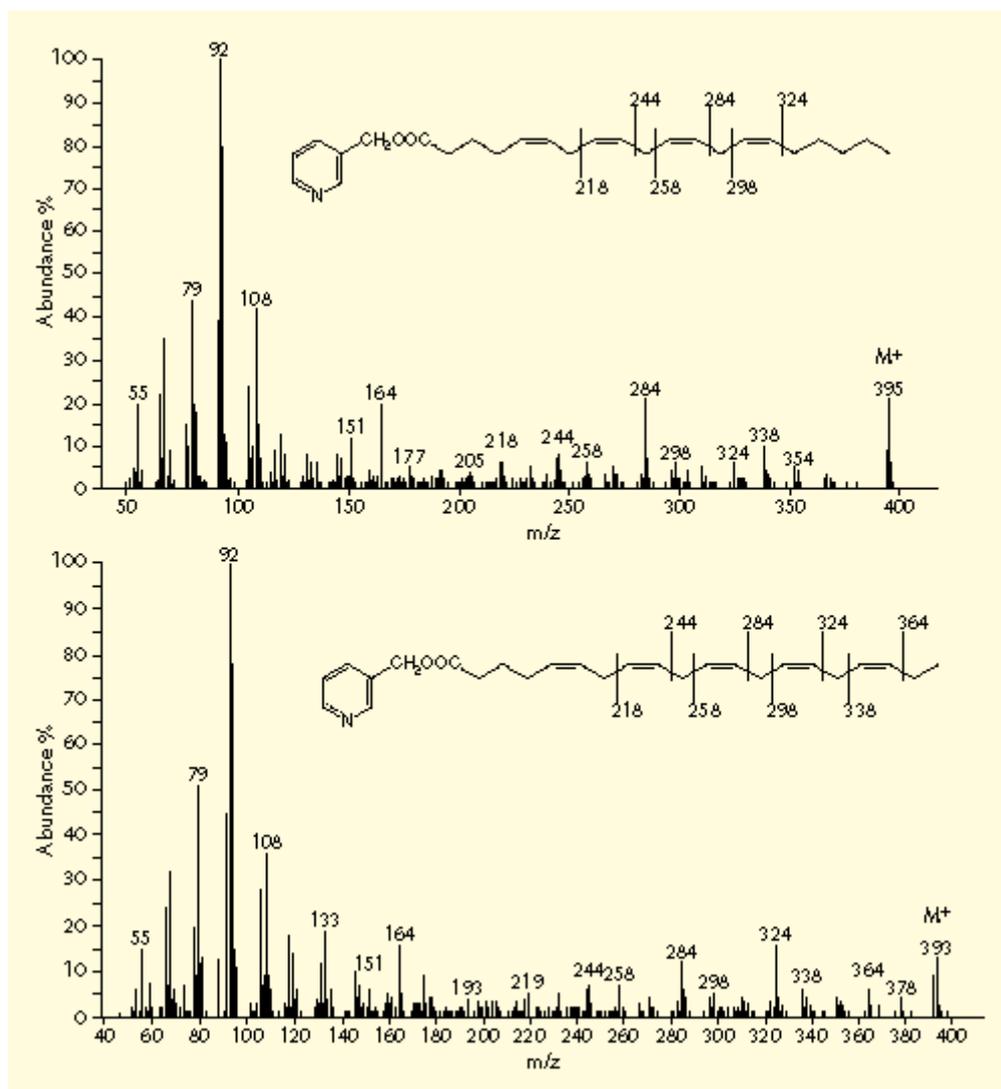


Figure 2. Mass spectra and fragmentation points of the picolinyl esters of arachidonic and eicosapentaenoic acids prepared from the seed lipids of *Agathis robusta*.

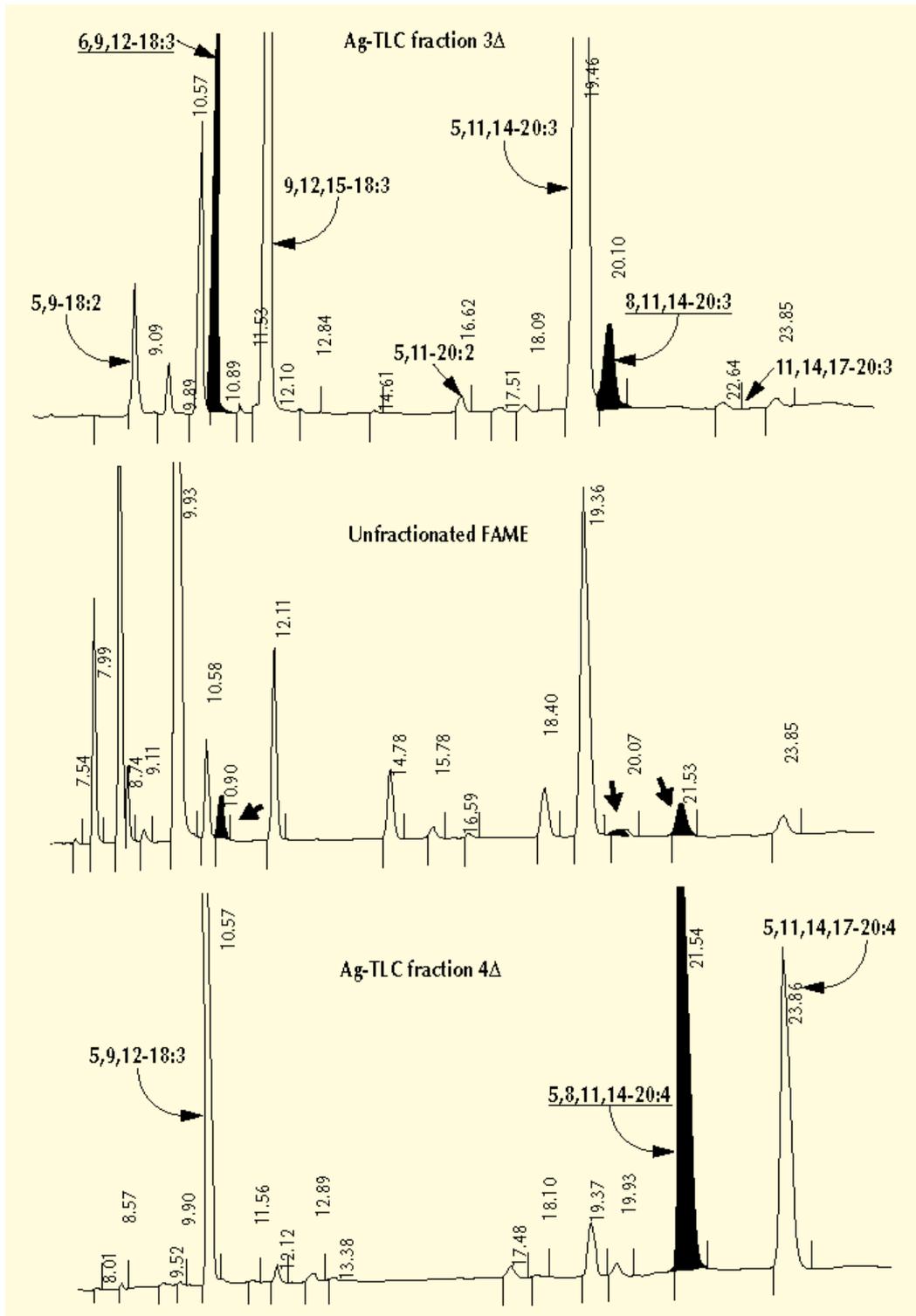


Figure 3. Chromatograms of fractions of fatty acid methyl esters prepared from *A. araucana* seed lipids separated by argentation thin-layer chromatography.

Fatty acid structure	Seeds				Leaves	
	<i>A. robusta</i>	<i>A. bidwillii</i>	<i>A. cunninghamii</i>	<i>A. araucana</i>	<i>A. araucana</i>	<i>A. dammara</i>
5,9-18:2	– ^b	0.8	0.5	2.0	2.4	0.3
5,9,12-18:3	–	3.4	13.8	13.2	23.5	0.9
6,9,12-18:3^c	4.4	5.9	12.7	5.5	–	1.1
6,9,12,15-18:4	2.3	–	–	–	–	–
5,11-20:2	0.5	2.0	1.5	1.0	1.4	0.5
11,14-20:2	3.2	1.9	4.0	12.4	5.2	7.4
5,11,14-20:3	0.6	13.1	14.2	89.6	43.3	3.0
8,11,14-20:3	1.9	0.2	1.0	1.6	trace^d	trace
5,8,11,14-20:4	2.0	0.6	4.6	8.7	0.5	5.2
11,14,17-20:3	0.4	–	0.3	–	6.3	0.8
5,11,14,17-20:4	1.7	1.3	0.6	6.5	37.0	1.4
8,11,14,17-20:4	0.6	–	–	–	–	–
5,8,11,14,17-20:5	0.5	–	–	–	–	–

Table 1. *DELTA5*-, *DELTA6*-Unsaturated, and other related polyunsaturated fatty acids in *Agathis robusta*, *Araucaria bidwillii*, *Araucaria cunninghamii*, and *Araucaria araucana* seeds, and in *A. araucana* and *Agathis dammara* leaves^a. Results determined by capillary gas-liquid chromatography and expressed as mg per g of total fatty acids.

^a Seeds were purchased from tree-seed sellers or obtained from botanic gardens.

Fresh leaves were collected from botanic gardens.

^b Not detected.

^c Fatty acids derived from a *DELTA6*-desaturation are bold faced.

^d Trace amounts.