Phytochemistry, Vol. 26, No. 5, pp. 1441–1445, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

FATTY ACIDS OF SOME CORNACEAE, HYDRANGEACEAE, AQUIFOLIACEAE, HAMAMELIDACEAE AND STYRACACEAE

BEATE BREUER, THOMAS STUHLFAUTH, HEINRICH FOCK and HERBERT HUBER

Fachbereich Biologie der Universität, Postfach 3049, D-675 Kaiserslautern, F.R.G.

(*Revised received* 16 *September* 1986)

Key Word Index—*Curtisia*; *Corokia*; *Mastixia*; *Aucuba*; *Griselinia*; *Nyssa*; *Davidia*; *Halesia*; *Ilex*; seed oils; fruit oils; petroselinic acid.

Abstract—Seed, kernel or fruit oils of the Cornaceae (nine species), *Hydrangea*, *Hamamelis*, *Ilex* (Aquifoliaceae) and the Styracaceae (two species) were analysed for fatty acid composition. Special attention was paid to the occurrence of petroselinic acid (18:1Δ6c). In the species investigated, C_{18} acids were always present in greater quantities than C_{16} fatty acids; C_{20} and C_{22} acids were only minor components. The Cornaceae show differing fatty acid patterns which correlate well with anatomical, morphological and other chemical data. In *Cornus*, *Curtisia*, *Mastixia* and *Corokia* linoleic acid predominates, whereas linoleic and linolenic acid form the major components in *Davidia* and *Nyssa*. 18:1Δ6c, an aralioid type, occurs in large amounts in *Aucuba* and *Griselinia*. *Hamamelis*, *Hydrangea* and *Ilex* show a common fatty acid pattern with linoleic acid as the dominant component in all cases. Classification currently based on morphological and anatomical differences between the two species of Styracaceae which were investigated here should include their different fatty acid compositions: in *Halesia* linoleic acid predominates over oleic acid, whereas in *Styrax* equal amounts of these two acids are found.

INTRODUCTION

The Cornaceae, usually recognized as a rather heterogeneous group, caused Gibbs [1] to characterize the family as a typical example of "chaos in taxonomy". Apart from the uncertain position of the whole group, the number of genera belonging to the Cornaceae is also the subject of dispute. For example, Harms [2] combined 15 genera in his broadly defined Cornaceae, whereas in other systems many of the genera are elevated to family rank, or even included in other taxa. Traditionally, the Cornaceae have been associated with the Araliales based on morphological and anatomical features [2–4]. This is currently accepted by several taxonomists, e.g. Thorne [5] and Takhtajan [6]. By contrast, Dahlgren [7], Jensen *et al.* [8], Bate-Smith *et al.* [9], Hegnauer [10] and Cronquist [11] deny any relationship to the Araliales, an opinion supported by several chemical data. The Araliales contain polyacetylenes; they accumulate essential oils and resins in schizogenous cavities and synthesize oleanene and ursene-type triterpene saponins [12]. These features seem to be absent in the Cornaceae though only few chemical data are available [12–14]. Secondary metabolism in the Cornaceae is mainly characterized by the occurrence of iridoids [8–10].

In contrast to the Cornaceae, extended information about seed oil composition of the Araliales is available. Petroselinic acid occurs as the major component [15]. The accumulation of this unusual acid is restricted to the Araliales and several genera of the Simarubaceae [16–19]. Based on the inconsistent chemical data of the Araliales and Cornaceae, the absence of petroselinic acid in the latter family was expected [20]. Surprisingly, Kleiman and Spencer [21] reported the occurrence of petroselinic acid in fruit oils of *Aucuba japonica* and species of *Garrya*, genera usually recognized as members of the Cornaceae. On the other hand, this unusual acid has not been found in *Cornus*, *Davidia*, *Nyssa*, *Camptotheca* or *Alangium*.

On the basis of this confusion it seemed that an investigation into the fatty acid compositions of the Cornaceae might also help to enlighten the natural relationships within this group, as it has in the Pittosporaceae [18] and Proteaceae [22]. In order to discover corresponding fatty acid patterns, we also analysed the seed oils of groups usually placed near the Cornaceae, e.g. species of the Styracaceae [23, 24] and Aquifoliaceae [7, 23, 24], *Hamamelis* [24, 25] and the iridoid containing *Hydrangea* [7, 23, 24, 26].

RESULTS AND DISCUSSION

The lipid content (dry wt basis) varied between 2–35% depending on the species investigated and the material used. The fatty acids form 20–80% of the total lipids (Table 1). Tables 2 and 3 summarize the results of the fatty acid analyses. GC analyses revealed no fatty acids lower than C_{16} or greater than C_{22}, which indicates the maturity of the material by virtue of the fact that during development the fatty acids with lower carbon chain lengths disappear [unpublished results]. Neither tariric (18:1Δ6a) nor elaidic (18:1Δ9t) nor odd-numbered acids could be detected.

From our analyses it appears that in all species investigated, the unsaturated C_{18} fatty acids constituted the greatest proportion of the total. 18:0, C_{20} and C_{22} acids were of minor importance.

The uniform fatty acid patterns in some genera of the Cornaceae are as follows: (A) linoleic acid (18:2) rich oils are found in *Cornus* (all species so far investigated

Table 1. Total lipids and fatty acids of the analysed species

Species	Origin	Component analysed	Lipids (%)	Fatty acids* (%)
Cornaceae				
Cornus mas I	Japan	Kernel	5.89	4.23
II			4.99	3.32
Cornus officinalis I	Japan	Kernel	6.36	†
II			7.60	3.56
Curtisia dentata I	South Africa	Kernel	4.50	3.64
II			4.63	3.53
Corokia macrocarpa I	New Zealand	Fruit	11.26	2.65
II			12.25	2.46
Mastixia sp. I (Huber 500)	Ceylon	Seed fragments	10.62	2.56
Aucuba japonica I	Japan	Fruit	2.30	†
II			2.75	0.45
Griselinia littoralis I	New Zealand	Seed	†	17.30
Nyssa sylvatica I	U.S.A.	Kernel	7.46	6.12
II			7.67	5.27
Davidia involucrata I	Germany	Kernel	1.81	0.90
Hydrangeaceae				
Hydrangea aspera I	Germany	Seed	34.98	28.10
Styracaceae				
Styrax japonica I	Japan	Kernel	†	17.69
Halesia carolina I	U.S.A.	Fruit	3.71	3.58
II			4.72	2.65
Aquifoliaceae				
Ilex verticillata I	U.S.A.	Kernel	15.93	5.31
Hamamelidaceae				
Hamamelis virginiana I	Germany	Seed	9.92	7.47

*Values were recorded as wt % (dry wt basis of seeds, stones or fruits).

†Not estimated.

I Lipids were transesterified by acid-catalysed methanolysis.

II Lipids were transesterified by base-catalysed methanolysis.

[21, 27]), *Curtisia dentata*, *Mastixia* sp., *Corokia macrocarpa* (position uncertain); (B) 18:2 and linolenic (18:3) acid rich oils are found in *Davidia involucrata*, *Nyssa* (all species so far investigated [21, 27]), *Camptotheca* [21], *Alangium* [21]; (C) petroselinic acid rich oils are found in *Garrya* (all species so far investigated [21]), *Aucuba japonica*, *Griselinia littoralis*.

The A-group probably represent an ancestral type of fatty acid pattern, whereas the shift to 18:3 as the dominating component in the B-group and the occurrence of the unusual petroselinic acid in the C-group indicates different evolutionary trends within the Cornaceae. The classification of this family on the basis of fatty acid patterns in seed oils correlates well with other chemical features, e.g. the distribution of phenolic constituents [9, 10, 28]. The phenolic metabolism of *Cornus*, *Corokia* and *Curtisia* shows a simple pattern (occurrence of trihydroxylated phenolics and proanthocyanidins), whereas *Mastixia* and the B-group are more advanced following the loss of proanthocyanidins [9, 16, 28]. Chlorogenic acid occurs in the C-group as the main phenolic constituent [10]. The lack of trihydroxylated phenolics and proanthocyanidins in *Aucuba* and *Garrya* indicate an 'advanced' status [9]. The (seco)iridoids, a common feature of the Cornaceae, show great diversity [8, 9]. Within the A-group this heterogeneity is typical in *Cornus* [29]. Interestingly, iridoid alkaloids have been developed in the B-group, probably independently. In the C-group only iridoids with simple structures occur.

Similarities in the fatty acid characteristics of the species investigated reflect previously proposed relationships, based on morphological and anatomical data. Similar pollen morphology keeps *Cornus*, *Mastixia* and *Curtisia* together [30]. Titman [31], Eyde [32] and Cronquist [11] emphasize close affinities between *Davidia*, *Nyssa* and *Camptotheca*. In contrast to the other Cornaceae, knotted thickenings in the trichomes appear in *Davidia*, *Nyssa* and *Camptotheca*. It is suggested that these genera should be combined in a family of their own (Nyssaceae). Serological results confirm the relationships between *Davidia*, *Nyssa*, *Cornus* and *Garrya* [33, 34]. There is a noteworthy shift from 18:2 to 18:3 rich seed oils as one moves from the primitive *Davidia* to *Nyssa* and finally to *Camptotheca* [27]. This correlates well with the degree of evolution indicated by both floral and wood anatomy [31, 32] and chemical constituents [9]. The presence of petroselinic acid unites the cornaceous genera *Griselinia*, *Aucuba* and *Garrya*. Eyde [35], Hegnauer [10] and Rodriguez [36] postulated that these three genera have been derived from a common ancestral stock. Anatomy and morphology of the ovary [35] as well as chemical features (occurrence of ellagitannins [9]) in-

Table 2. Fatty acid composition of the Cornaceae recorded as wt %

Species	Fatty acids according to carbon chain length* (%)				C_{18}-Fraction† (%)				
	C_{16}	C_{18}	C_{20}	C_{22}	18:0	18:1Δ9	18:1Δ6	18:2	18:3
Cornus mas I	6.96	93.04	tr	tr	1.75	21.89	—	74.47	1.89
II	7.02	92.98	tr	tr	2.01	21.57	—	74.44	1.98
Cornus officinalis I	6.28	91.51	2.21	—	2.90	21.00	—	76.10	—
II	6.72	92.12	1.16	—	3.13	17.79	—	79.09	—
Curtisia dentata I	10.27	89.73	tr	—	3.29	19.80	—	76.90	tr
II	9.52	90.48	tr	—	3.01	19.92	—	77.07	tr
Corokia macrocarpa I	18.79	81.21	—	—	1.36	34.70	—	48.45	15.50
II	12.86	87.14	—	—	3.00	28.26	—	47.67	21.06
Mastixia sp. I	24.55	73.66	1.79	—	9.55	18.03	—	68.17	4.25
Aucuba japonica I	‡				7.02	25.72	33.78	13.94	19.54
II	26.37	71.02	2.62	—	2.38	26.99	28.55	17.98	24.09
Griselinia littoralis I	15.94	82.62	tr	1.44	1.64	17.71	52.37	28.28	tr
						19.27§	50.81		
Nyssa sylvatica I	7.27	92.73	tr	—	2.56	15.96	—	33.80	47.68
II	6.96	93.04	tr	—	2.41	14.57	—	30.53	52.49
Davidia involucrata I	8.82	89.83	1.35	—	1.64	19.36	—	49.48	29.53

*Values are recorded as wt % of total fatty acids.
†Values are recorded as wt % of the C_{18} fraction.
‡Not estimated.
§Repetition of the argentation chromatography.
C18:0, stearic acid; C18:1Δ9, oleic acid; C18:1Δ6, petroselinic acid; C18:2, linoleic acid; C18:3, linolenic acid.
—Not detected (for minimum detection limits see Experimental).
tr—traces (<1% of total fatty acids of C_{18} fraction, respectively).
I, II see Table 1.
All data are from a single analysis.

Table 3. Fatty acid composition of the Hydrangeaceae, Styracaceae, Aquifoliaceae and Hamamelidaceae recorded as wt %

Species	Fatty acids according to carbon chain length* (%)				C_{18}-Fraction† (%)				
	C_{16}	C_{18}	C_{20}	C_{22}	18:0	18:1Δ9	18:1Δ6	18:2	18:3
Hydrangeaceae									
Hydrangea aspera I	5.83	94.17	tr	—	tr	14.11	—	84.67	1.22
Styracaceae									
Styrax japonica II	9.16	90.84	tr	—	2.06	48.6	—	45.78	3.56
Halesia carolina I	6.31	93.69	tr	—	1.13	26.66	—	71.08	1.13
II	6.13	93.87	tr	—	tr	28.03	—	70.75	1.21
Aquifoliaceae									
Ilex verticillata I	8.30	91.70	tr	—	3.25	34.97	—	61.77	tr
Hamamelidaceae									
Hamamelis virginiana I	8.03	91.97	tr	—	2.82	28.92	—	63.48	4.78

*, †, —, tr, I, II: see Table 2.

dicate a primitive condition in *Griselinia* compared to *Aucuba* and *Garrya*.

We consider that the group members are closely related. Small quantitative differences in the fatty acids occur in members falling into the same group, resembling differences at the generic level. For example, *Corokia macrocarpa* has a higher 18:3 content, which cannot be caused by the interference of fruit coat oils with seed oils. It is noteworthy that the C_{16}:C_{18} ratio varied in the Cornaceae. This ratio was significantly higher in *Corokia*, *Aucuba*, *Griselinia* and *Mastixia* compared to *Cornus*, *Curtisia*, *Nyssa* and *Davidia* (Table 2).

A capacity to synthesize petroselinic acid represents a common inherited feature of the Araliales, Simarubaceae and Cornales. This renews interest in the discussion about a mono- or polyphylogenetic origin of the unitegmic sympetalae. Due to the appearance of a common acid pattern, a connexion between several Cornaceae species and the Hamamelidaceae, Hydrangeaceae and Aquifoliaceae seems possible, but the chemosystematic

relevance is minor due to the appearance of a common acid pattern. The occurrence of the iridoids [8, 9], the anatomy of wood and ovules [23], the difference in phenolic metabolism [12] and the serology of seed proteins [26] of the Hydrangeaceae and Escalloniaceae distinguish them from the Saxifragaceae and suggests the inclusion of the former families in the Cornales. The fatty acid composition also suggests that a considerable difference may exist between the two investigated genera of the Styracaceae, namely *Halesia* (unitegmic, capsel fruit) and *Styrax* (bitegmic, stone fruit).

Further chemical investigations are required to strengthen the relationships between the Cornaceae and their allies; for example the seed oils of the Symplocaceae and Escalloniaceae have not yet been adequately studied. More genera should be investigated to complete the classification of the Cornaceae. Especially interesting results are expected for *Helwingia* or *Melanophylla* and *Aralidium*, which fit well chemically with the C-group members.

EXPERIMENTAL

All plant material was stored at 9°. The time between reception and analysis did not exceed 1 year. Lipids were extracted and quantitatively determined according to ref. [18]. Transesterification using acid-catalysed methanolysis [37] yielded the Me esters. If sufficient material was available an additional analysis was carried out using the tetramethylammonium hydroxide esterification method of ref. [38]. The synthesis of tariric acid has previously been described [18].

C_{18} esters were isolated by prep. GC (5% OV 17 on Chromosorb W/HP, 2 m × 4 mm i.d.) using a carrier-gas flow-split of 1:9 (FID: trap with liquid N_2). Under the same conditions the fatty acid Me esters with a different carbon number (C_8–C_{24}) were quantified with Me nonadecanoate as int. standard. Analysis of the C_{18} fraction was carried out under the following conditions: 10% Silar 10C on Chromosorb W/AW-DCMS (2 m × 2 mm i.d.), 30 ml/min N_2, injection port temp. 255°, FID temp. 260°, temp. programme: 5 min at 170°, then 12°/min to 220°. In addition the Me esters were analysed by capillary GC on a chromatograph fitted with a split-system (1:30). The fused silica column (30 m × 0.26 mm i.d.) was coated with OV 351. The He flow was 1.2 ml/min. Injection port temp. was maintained at 240° and FID temp. at 250°. The temp. programme was 5 min at 150° then 8°/min to 175°.

In the GC analyses, ester concns lower than 0.5% of the greatest peak were not evaluated. The positional and geometrical C_{18} isomers were separated by alumina argentation TLC. A good separation of the TLC bands was achieved with a an ester mixture containing Me oleate with Me petroselinate, up to a ratio of 1:80 or with Me tariricate and Me oleate 1:60 (but not greater). The individual isomers were scraped from the plates. After adding Me nonadecanoate as int. standard for the subsequent GC quantification (see above), the esters were extracted from TLC adsorbent using the method of ref. [39].

Acknowledgement—We wish to thank Professor G. Bate for helpful discussion and correcting the manuscript.

REFERENCES

1. Gibbs, R. D. (1974) *Chemotaxonomy of Flowering Plants*, Vol. 1, p. 30. McGill-Queen's University Press, Montreal.
2. Harms, H. (1898) in *Die natürlichen Pflanzenfamilien* (Engler, A. and Prantl, K., eds) III. Teil, 8. Abteilung, p. 250. Engelmann, Leipzig.
3. Lindley, J. (1853) *The Vegetable Kingdom*, 3rd edn. Bradbury & Evans, London.
4. Hooker, J. D. (1867) in *Genera Plantarum* (Bentham, G. and Hooker, J. D., eds) Volumen I, Pars III. London.
5. Thorne, R. F. (1981) in *Phytochemistry and Angiosperm Phylogeny* (Young, D. A. and Seigler, D. S., eds) p. 233. Praeger, New York.
6. Takhtajan, A. L. (1980) *Bot. Rev.* **46**, 225.
7. Dahlgren, R. (1983) *Nord. J. Bot.* **3**, 119.
8. Jensen, S. R., Nielson, B. J. and Dahlgren, R. (1975) *Bot. Not.* **128**, 148.
9. Bate-Smith, E. C., Ferguson, I. K., Hutson, K., Jensen, S. R., Nielson, B. J. and Swain, T. (1975) *Biochem. Syst. Ecol.* **3**, 79.
10. Hegnauer, R. (1969) in *Perspectives in Phytochemistry* (Harborne, J. B. and Swain, T., eds) p. 121. Academic Press, New York.
11. Cronquist, A. (1981) *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.
12. Hegnauer, R. (1964–1973) *Chemotaxonomie der Pflanzen*, Vols 3–6. Birkhäuser, Basel.
13. Hegnauer, R. (1965) in *Beiträge zur Biochemie und Physiologie von Naturstoffen* (Gröger, D., ed.) p. 235. VEB Gustav Fischer, Jena.
14. Yosioka, I., Kimura, T., Imagawa, H. and Takara, K. (1966) *J. Pharm. Soc. Japan* **86**, 1216.
15. Placek, L. L. (1963) *J. Am. Oil Chem. Soc.* **40**, 319.
16. Tsujimoto, M. and Koyanagi, H. (1933) *Bull. Chem. Soc.* **8**, 161.
17. Spencer, G. F., Kleiman, R., Earle, E. F. and Wolff, I. A. (1970) *Lipids* **5**, 285.
18. Stuhlfauth, T., Fock, H., Huber, H. and Klug, K. (1985) *Biochem. Syst. Ecol.* **13**, 447.
19. Pearl, M. B., Kleiman, R. and Earle, F. R. (1983) *Lipids* **8**, 627.
20. Gershenzon, J. and Mabry, T. J. (1983) *Nord. J. Bot.* **3**, 5.
21. Kleiman, R. and Spencer, G. F. (1982) *J. Am. Oil Chem. Soc.* **59**, 29.
22. Vickery, J. R. (1971) *Phytochemistry* **10**, 123.
23. Huber, H. (1963) *Mitt. Bot. Staatssamml. München* **5**, 1.
24. Huber, H. (1985) *Leitfaden durch die Familien und Ordnungen der Angiospermen*. Universität Kaiserslautern, Fachbereich Biologie.
25. Jensen, U. and Greven, B. (1984) *Taxon* **33**, 563.
26. Grund, C. and Jensen, U. (1981) *Pl. Syst. Evol.* **137**, 1.
27. Hohn, M. E. D. and Meinschein, W. G. (1976) *Biochem. Syst. Ecol.* **4**, 193.
28. Bate-Smith, E. C. (1962) *J. Linn. Soc.* **58**, 95.
29. Jensen, S. R., Kjaer, A. and Nielson, B. J. (1975) *Biochem. Syst. Ecol.* **3**, 75.
30. Ferguson, I. M. (1977) in *World Pollen and Spore Flora* (Nielson, S., ed.) Vol. 6, p. 1. Almqvist & Wiksell, Stockholm.
31. Titman, P. W. (1949) *J. Elisha Mitch. Sci. Soc.* **65**, 245.
32. Eyde, R. H. (1963) *J. Arnold Arboretum* **44**, 1.
33. Fairbrothers, D. E. (1966) *Serol. Museum Bull.* **35**, 2.
34. Fairbrothers, D. E. and Johnson, M. A. (1964) in *Taxonomic Biochemistry and Serology*. International conference on taxonomic biochemistry, physiology and serology 1962, Lawrence, Can. p. 305.
35. Eyde, R. H. (1964) *Am. J. Botany* **51**, 1083.
36. Rodriguez, R. L. (1971) in *The Biology and Chemistry of the Umbelliferae. Bot. J. Linn. Soc.*, Suppl. 1 (Heywood, V. L., ed.) p. 64. Academic Press, London.

37. Chalvardjian, A. (1964) *Biochem. J.* **90**, 518.
38. Metcalfe, L. D. and Wang, C. N. (1981) *J. Chromatogr. Sci.* **19**, 530.
39. Myher, J. J. (1978) in *Handbook of Lipid Research* 1, *Fatty Acids and Glycerides* (Kuksis, A., ed.) p. 133. Plenum Press, New York.