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THE FATTY ACID COMPOSITION OF THE SEED OILS OF PROTEACEAE: A CHEMOTAXONOMIC STUDY

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Abstract—The fatty acid composition and the amounts of the positional isomers of the monoene acids have been determined in 26 Proteaceae sp. representing two sub families and seven tribes. Fatty acids containing from 12 to 24 carbon atoms were detected, the major components being monoene acids. The amounts of *cis*-hexadecenoic acid exceeded 10% in 13 species. Each of the four monoenes studied had several positional isomers of which Δ^9 and Δ^{11} predominated in hexadecenoic acid, Δ^9 in octadecenoic acid and Δ^{11} and Δ^{15} in eicosenoic acid. The regression of the concentration of *cis*-hexadec-9-enoic acid on that of *cis*-octadec-11-enoic in 14 species was not statistically significant. The two sub families, Grevilleoideae and Proteoideae, have several distinct differences, the former, for instance, having a much wider range of acids. Differences between tribes were reflected mainly in the differing patterns of the monoene and diene acids, whereas these patterns were rather uniform within genera. The data for *Placospermum* and *Bellendena* tend to support the claim that the former represents the primitive form of the Proteaceae.

INTRODUCTION

STUDIES ON the fatty acid composition of seed oils in the family Proteaceae have been limited to a few species. After the initial work of Bridge and Hilditch[1] on a species of *Macadamia*, Cattaneo *et al.*[2] published the detailed composition of *Gevuina avellana*, *Lomatia hirsuta* and *Embothrium coccineum*. Cattaneo's further studies on two *Grevillea* sp.,[3] several *Protea* sp.[4] and on *Roupala complicata* and *Hakea gibbosa*,[5] and those of Vickery on *Kermadecia sinuata*[6] extended the information but did not permit valid comparisons between sub-families, tribes and genera. The purpose of the present studies has been to provide data on a sufficiently large number of selected species to enable these comparisons to be made. Such comparisons may assist the development of a consistent taxonomy and may also provide some information on the evolutionary history of the family.

The classification of sub families and tribes used by Johnson and Briggs[7] in their study of evolution in the Proteaceae has been adopted. These authors have pointed out that, unlike the sub family Grevilleoideae, there is little to suggest that the sub-family, Proteoideae, represents a close-knit, monophyletic group. While convenience may govern the grouping of tribes in the latter sub family, chemical evidence, as reported below, lends some support to the grouping.

RESULTS AND DISCUSSION

The fatty acid composition (mole %) of the seed oils of 30 species representing two sub families and seven tribes is given in Table 1, and these data include the composition of

[1] R. E. BRIDGE and T. P. HILDITCH, *J. Chem. Soc.* 2396 (1950).
[2] P. CATTANEO, G. K. DE SUTTON, R. H. ARIAS, R. R. BRENNER and M. E. DE TOMAS, *Anales Asoc. Quim. Arg.* **50**, 31 (1962).
[3] P. CATTANEO, M. H. BERTONI and G. K. DE SUTTON, *Anales Asoc. Quim. Arg.* **54**, 117 (1966).
[4] P. CATTANEO, G. K. DE SUTTON and M. H. BERTONI, *Anales Asoc. Quim. Arg.* **54**, 123 (1966).
[5] P. CATTANEO, M. H. BERTONI and G. K. DE SUTTON, *Anales Asoc. Quim. Arg.* **55**, 95 (1967).
[6] J. R. VICKERY, *Australian J. Sci.* **31**, 334 (1969).
[7] L. A. S. JOHNSON and B. G. BRIGGS. *Australian J. Botany* **11**, 21 (1963).

four species obtained by Cattaneo *et al.*[2,3] The amounts of the various positional isomers of the monoene fatty acids are given in Table 2. Since the isolation of pure monoene esters from a complex mixture is difficult when they occur in minor amounts, the characterization of positional isomers is generally restricted to monoenes whose concentrations exceed 5% of the total fatty acids.

The oil contents of the seeds (dry-weight basis) varied from 1·5% (*Isopogon*) to 51·7% (*Beauprea neglecta*). The low values obtained for *Isopogon* and several other species do not necessarily reflect their true oil contents, but rather the difficulty of separating the seeds from their woody fruit coats.

General Relationships

The range of relative proportions of fatty acids found in Proteaceae are similar to those occurring in many other plant families, e.g. Betulaceae. They have carbon chain lengths of 12–24;* odd-numbered acids are absent; there is a predominance of monoenes, which, in many species exceed 80% of the total acids and are seldom less than 70%; there is a virtual absence of triene acids. The ratio of saturated to unsaturated acids is consequently low, varying from 0·046 (*Hicksbeachia*) to 0·50 (*Beauprea balansae*).

Chain lengths of the saturated acids extend from C12:0 to C24:0, but with the latter occurring in appreciable amounts only in *Hakea sericea* (1·2%) and *Grevillea floribunda* (1·3%). Palmitic acid varies in concentration from 2·0 to 25·6% of the total acids and generally predominates, except in three *Grevillea* sp.

Monoene acids from C14:1 to C24:1 were detected with C18:1 usually predominating (range 4·9–81·0%). Proteaceae seed oils are probably unique in the frequent occurrence of high concentrations of C16:1. In most seed oils and animal fats, its concentration rarely exceeds 10%. The concentrations were greater than 5% in 19 of the 30 species given in Table 1, exceeded 40% in four species and reached as high as 69% in *Kermadecia sinuata*. The only species approaching the latter is the seed oil of *Bignonia* (*Doxantha*) *unguiscati* (Bignoniaceae) which has 64% *cis*-hexadec-9-enoic acid.[8]

Linoleic acid was present in every species, but usually in relatively small amounts; it reached a maximum of 34·3% in *Persoonia lanceolata*. Triene acids (C18:3 only) were present in only one species (*Bellendena montana*), with traces (< 0·1%) in another (*Beauprea neglecta*).

The quantitative estimations of the positional isomers in the monoenes (Table 2) show an unusually large number of isomers. Nine isomers of C16:1 were identified in 15 species, with Δ† positions$^{5-11, 13, 15}$ in which Δ^9 and Δ^{11} usually predominated; attention is drawn, however, to the relatively large amount, 30%, of the unusual Δ^{15} in *Telopea truncata*. In the C18:1 acids, there were seven isomers at Δ positions$^{6-11, 13}$, with Δ^9 predominating. In seven species, seven isomers at $\Delta^{7-11, 13, 15}$ were also present in the C20:1 acids. As the isomers of C22:1 could be characterized in only four species, the occurrence of only five isomers ($\Delta^{9, 10, 13, 15, 17}$) must be accepted with some reservation. In C22:1, Δ^{13} and Δ^{17} appeared to predominate.

The concentrations of the positional isomers do not fit into any orderly pattern. It might

* The usual symbols for fatty acids are used; e.g. C18:0, C18:1, C18:2, C18:3 for saturated, monoene, diene and triene acids having 18 carbon atoms.

† Δ^5 etc. signify the number of carbon atoms from the carboxyl end of the molecule where the double bond occurs, while ω^5 etc. signify the number of carbon atoms from the methyl end.

[8] M. J. CHISHOLM and C. Y. HOPKINS. *J. Am. Oil Chem. Soc.* **42**, 49 (1965).

TABLE 1. PROTEACEAE SEED OILS. FATTY ACID COMPOSITION, MOLES %

			Saturated							Unsaturated										Total	
Sub family	Tribe	Genus and species	12:0	14:0	16:0	18:0	20:0	22:0	24:0	14:1	16:1	18:1	18:2	18:3	20:1	20:2	22:1	22:2	24:1	Satd.	Unsatd.
Grevilleoideae	Banksieae	*Banksia integrifolia*	—	0·2	5·7	3·5	3·8	0·8	—	—	0·7	71·5	0·6	—	13·2	—	—	—	—	14·0	86·0
		B. collina	—	0·2	10·4	1·9	2·5	0·4	—	—	2·0	68·1	1·3	—	13·2	—	trace*	—	—	15·4	84·6
		B. ericifolia	—	0·2	10·6	2·2	2·5	—	—	—	0·8	81·0	0·4	—	2·3	—	trace	—	—	15·5	84·5
	Grevilleae	*Grevillea floribunda* (1) (2)		0·3	2·3	7·2	5·9	3·3	1·3	0·2	6·6	45·0	6·2	1·0	5·6	9·3	1·5	3·3	0·4	20·3	79·7
		G. robusta	—	5·0	3·4	3·4	2·2	1·5	trace	0·6	14·9	62·7	0·7	—	2·0	1·8	0·5	1·3	trace	15·5	84·5
		G. banksii	—	0·1	2·0	5·6	4·3	1·2	—	—	7·8	64·3	2·6	—	10·4	—	1·7	—	—	13·2	86·8
		Hakea salicifolia	1·1	1·3	2·5	1·9	1·5	0·8	—	0·7	14·5	60·3	4·7	—	5·4	—	1·6	—	3·6	9·1	90·9
		H. sericea	0·8	1·9	3·3	1·8	2·5	3·1	1·2	trace	6·8	62·1	1·1	—	8·6	—	6·8	—	trace	14·6	85·4
	Oriteae	*Orites revoluta*	—	—	6·4	5·1	0·6	—	—	—	37·9	27·1	22·0	—	0·9	—	—	—	—	12·1	87·9
		O. diversifolia	—	0·1	4·5	0·9	0·7	—	—	—	40·4	37·5	14·6	—	1·3	—	—	—	—	6·2	93·8
	Macadamieae	*Gevuina avellana* (1)	0·1	0·4	4·0	0·8	1·5	1·6	0·2	—	24·3	37·2	11·3	—	10·5	—	7·8	—	0·8	8·6	91·4
		Macadamia integrifolia	—	1·0	9·0	3·8	2·0	trace	—	—	29·3	50·8	2·4	—	1·7	trace	trace	—	trace	15·8	84·2
		Hicksbeachia pinnatifolia	—	0·5	2·4	0·4	0·5	0·6	trace	trace	40·5	24·3	13·4	—	6·3	—	11·1	—	trace	4·4	95·6
		Xylomelum pyriforme	—	—	21·0	2·1	trace	—	—	—	2·4	69·8	2·7	—	2·0	—	trace	—	—	23·1	76·9
		Kermadecia sinuata	—	0·2	12·6	1·3	0·6	1·1	—	0·5	69·4	4·9	2·6	—	2·4	trace	4·4	—	trace	15·8	84·2
	Embothrieae	*Lomatia hirsuta* (1)	0·3	0·2	12·2	1·5	0·6	—	—	—	24·1	49·1	11·1	—	0·9	—	—	—	—	14·8	85·2
		Stenocarpus sinuatus	—	trace	8·1	7·9	0·9	0·7	—	0·4	1·4	80·1	0·5	—	—	—	—	—	—	17·3	82·7
		Embothrium coccineum (1) (3)	0·5	0·3	8·3	0·9	0·1	—	—	2·7	24·3	43·6	11·4	—	2·2	—	—	—	—	12·4	87·6
		Telopea truncata	—	1·0	6·6	trace	—	—	—	trace	45·1	34·6	12·4	—	0·3	—	—	—	—	7·6	92·4
		T. speciosissima	—	0·2	5·2	0·6	trace	—	—	—	33·1	53·8	7·1	—	trace	—	—	—	—	6·0	94·0
	?	*Cardwellia sublimis*	—	—	2·0	1·6	1·7	4·0	—	—	19·4	55·5	4·9	—	2·7	—	8·2	—	—	9·3	90·7
Proteoideae	Proteeae	*Isopogon anemonifolius*	1·3	1·5	15·3	4·0	2·5	—	—	—	10·8	57·9	6·7	—	trace	—	—	—	—	24·6	75·4
		Protea compacta	—	2·0	12·7	6·6	0·9	—	—	—	0·3	70·7	5·7	—	1·1	—	—	—	—	22·2	77·8
		P. longiflora	—	1·0	10·4	3·8	1·8	trace	—	—	0·5	73·3	7·0	—	2·2	—	—	—	—	17·0	83·0
	Persoonieae	*Bellendena montana*	—	0·2	13·0	0·7	—	—	—	—	0·3	52·1	30·5	3·1	—	—	—	—	—	14·0	86·0
		Persoonia lanceolata	—	0·4	11·4	1·0	—	—	—	—	0·5	52·3	34·3	—	—	—	—	—	—	12·8	87·2
		Cenarrhenes nitida	—	trace	7·5	0·7	—	—	—	—	32·9	51·7	7·2	—	trace	—	trace	—	—	8·2	91·8
		Beauprea balansae	—	0·6	25·6	5·3	1·8	—	—	—	5·7	33·8	27·2	—	—	—	—	—	—	33·3	66·7
		Beauprea neglecta	—	0·2	15·0	3·4	trace	—	—	0·1	10·5	43·3	27·5	trace	—	—	—	—	—	18·6	81·4
?	?	*Placospermum coriaceum*	—	0·2	21·0	0·8	trace	—	—	—	0·9	75·5	1·6	—	—	—	—	—	—	22·0	78·0

* Trace occurrence (< 0·1 %).
(1) Data of Cattaneo *et al.*[1–4]
(2) Plus 0·2% C17:1 and 0·4% C24:2.
(3) Plus 3·4% hydroxy acids and 2·3% acids < C12:0.

TABLE 2. PROTEACEAE MONOENE ACID POSITIONAL ISOMERS, % OF TOTAL MONOENE

Genus and species	Hexadecenoic acids (C16:1)									Octadecenoic acids (C18:1)							Eicosenoic acids (C20:1)							Docosenoic acids (C22:1)				
	Δ^5	Δ^6	Δ^7	Δ^8	Δ^9	Δ^{10}	Δ^{11}	Δ^{13}	Δ^{15}	Δ^6	Δ^7	Δ^8	Δ^9	Δ^{10}	Δ^{11}	Δ^{13}	Δ^7	Δ^8	Δ^9	Δ^{10}	Δ^{11}	Δ^{13}	Δ^{15}	Δ^9	Δ^{10}	Δ^{13}	Δ^{15}	Δ^{17}
Banksia integrifolia										—	—	1	74	—	2	23	2	—	6	—	74	6	12					
B. collina										—	1	—	98	—	1	—	—	45	4	—	51	—	—					
B. ericifolia										—	2	3	95	—	—	—												
Grevillea robusta	—	—	—	—	11	—	89	—	—	—	—	—	96	—	4	—												
G. banksii	—	—	—	—	11	—	89	—	—	—	—	—	96	—	4	—	—	—	—	—	78	—	22					
Hakea salicifolia	—	—	—	—	22	—	78	—	—	—	—	—	91	—	9	—	—	—	—	—	100	—	—	—	—	100	—	—
Orites revoluta	—	—	—	—	6	—	94	—	—	—	—	19	76	—	3	2												
O. diversifolia	13	—	—	—	56	—	31	—	—	—	—	2	90	—	2	6												
Macadamia integrifolia	—	—	—	—	99	—	1	—	—	—	—	—	91	—	9	—												
Hicksbeachia pinnatifolia	—	—	18	2	4	4	66	6	—	—	—	—	16	36	48	—	—	—	4	34	16	2	44	30	3	13	—	54
Kermadecia sinuata	—	—	9	—	33	—	58	—	—	—	3	—	73	—	6	18	—	—	16	—	4	25	55	—	—	13	4	83
Xylomelum pyriforme										—	1	18	60	—	—	21												
Telopea truncata	—	—	2	—	45	4	19	—	30	—	—	34	56	1	6	3												
T. speciosissima	—	—	1	—	97	—	2	—	—	1	—	9	82	—	8	—												
Stenocarpus sinuatus										—	—	6	93	—	1	—												
Cardwellia sublimis	—	—	—	—	12	—	88	—	—	—	—	—	60	—	—	40	—	—	—	—	45	—	55	—	—	38	—	62
Isopogon anemonifolius	—	—	—	—	100	—	—	—	—	—	—	—	86	—	2	12												
Protea compacta										—	—	—	100	—	—	—												
P. longiflora										—	—	38	60	—	2	—												
Bellendena montana										—	—	5	81	1	2	11												
Persoonia lanceolata										—	—	—	90	—	5	5												
Cenarrhenes nitida	—	—	23	—	77	—	—	—	—	—	—	2	87	1	5	5												
Beauprea balansae	2	3	27	1	57	—	10	—	—	—	14	—	86	—	—	—												
Beauprea neglecta	—	—	—	—	92	—	8			—	—	—	89	—	—	11												
Placospermum coriaceum										—	11	—	56	—	6	27												

Note—Isomers were characterized usually when the monoenes occurred in amounts greater than 5%.

be expected, for instance, that a relationship would exist between the concentrations of *cis*-hexadec-9-enoic acid and *cis*-octadec-11-enoic acid through direct chain elongation, such as occurs in the lipids of rats[9] and bacteria.[10] Kuemmel and Chapman[11] state that an approximate straight line relationship obtains when a semi-log plot is made of *cis*-hexadec-9-enoic acid contents of the monoenes of 30 plant and animal lipids against their *cis*-octadec-11-enoic acid contents. Comparable data from 14 species in the present study have been similarly analysed; the data were examined by linear regression which was found not to be significant. It should be pointed out, however, that a re-examination of the data of Kuemmel and Chapman for plant lipids alone shows a similar lack of significance in the linear regression, although it is highly significant for the animal lipids. Nevertheless, some biosynthetic patterns are discernible. In all monoenes, except C18:1, the ω^5 isomers frequently occur in substantial concentrations, thus indicating considerable chain elongation. Attention is drawn particularly to the high concentrations of Δ^{11} C16:1 (mean 49%), which may have arisen from the elongation of Δ^9 C14:1.

Considerable error in the quantitative estimation of the isomers can occur if certain procedures in argentation column chromatography are not adhered to. In particular it is necessary to sample virtually the whole of the eluate containing two monoenes. If this is not done, there will be reduced or increased proportions of one or the other isomer, depending on the position of the "cut". For example, if the fractions containing C16:1 and C18:1 esters of *Cardwellia sublimis* are taken from the rear of the elution peak, the percentage of Δ^9 C18:1 relative to Δ^{13} C18:1 falls from 60 to about 40. Even so, some error is unavoidable, because some longer-chain monoene esters will emerge with the saturated esters before it is expedient to collect the samples for subsequent purification.

The i.r. spectra of 10 pure monoene methyl esters from six species—*Grevillea robusta* (C16:1, C18:1), *Telopea truncata* (C16:1, C18:1), *Banksia collina* (C20:1), *Hicksbeachia pinnatifolia* (C18:1), *Cardwellia sublimis* (C18:1, C22:1) and *Orites diversifolia* (C16:1, C18:1), were examined in order to detect the presence of *trans*-acids; no absorption due to *trans* double bonds was found, indicating that only *cis*-acids occur in the monoenes of Proteaceae.

Sub Family Differences

Palmitic acid tends to be in higher concentrations in Proteoideae (mean 13·8%) than in Grevilleoideae (mean 6·9%). In contrast to Grevilleoideae, there was no C22:0 or C24:0 in Proteoideae, and only minor, occasional amounts of 20:0. In Proteoideae, C22:1 and C24:1 acids were absent and only small, occasional amounts of 20:1 occurred. Linoleic acid tended to be much higher in Proteoideae (mean 18·2%) than in Grevilleoideae (mean 5·4%). The concentrations of C16:1 varied widely in both sub families, the range in Grevilleoideae being 0·7–69% and in Proteoideae 0·3–33%.

Data on positional isomers of C16:1 are available in only four species of Proteoideae compared with 11 species of Grevilleoideae, and conclusions must therefore be tentative. The Δ^{11} and Δ^9 isomers appear to predominate in Grevilleoideae and in Proteoideae respectively. While the Δ^9 isomer of C18:1 predominates in both sub families, Δ^8, Δ^{11}, and Δ^{13} may occur in substantial amounts in several species of Grevilleoideae, but only minor concentrations in Proteoideae.

[9] P. W. Holloway and S. J. Wakil, *J. Biol. Chem.* **239**, 2489 (1964).
[10] G. Scheurbrandt and K. Bloch, *J. Biol. Chem.* **237**, 2064 (1962).
[11] D. F. Kuemmel and L. R. Chapman, *Lipids.* **3**, 313 (1968).

Characteristics of Tribes

While there were no important differences in the saturated acids, a number of consistent differences between tribes occurred in the unsaturated acids. Docosenoic and tetracosenoic acids occurred in Grevilleae and Macadamieae, whereas they are virtually absent in Banksieae, Embothrieae and Oriteae. The latter appears to resemble Persoonieae more closely than it does the other tribes within the sub-family Grevilleoideae, particularly in the absence of acids having chain lengths greater than 20 carbons, and also in having much higher concentrations of C18:2.

Characteristics of Genera

The patterns of occurrence and concentrations of fatty acids are fairly uniform from species to species within the genera *Banksia*, *Grevillea*, *Hakea*, *Orites*, *Telopea*, *Protea* and *Beauprea*. The patterns of the double-bond positions and the concentrations of the isomers are uniform within *Banksia* and *Grevillea* but not within *Orites*, *Telopea*, *Protea* and *Beauprea*.

For a given species, the relative amount of unsaturated fatty acids may be expected to increase with a decrease in mean daily temperature at the sites of growth,[12] such as may occur at increasing latitudes and altitudes. With the exception of *Telopea*, all species within a given genus were grown at approximately the same latitudes and altitudes. Within each genus, therefore, the results were not affected by climatic factors. Within tribes, the degree of unsaturation does not appear to be related to climatic factors. For instance, *Cenarrhenes nitida*, growing at low altitude at lat. 42°S in Tasmania, has a much lower content of C18:2 than *Persoonia lanceolata* (New South Wales coastal site; lat. 34°S) or *Beauprea* sp. (New Caledonia; lat. 21°S). In *Telopea* sp., there was little difference between *T. truncata* (grown in Tasmania, lat. 42°S) and *T. speciosissima* (grown in New South Wales, lat. 33°S) except that the former had a slightly higher concentration of C18:2.

Position of Placospermum coriaceum *and* Bellendena montana

Johnson and Briggs[7] consider that *Placospermum*, which is endemic in the tropical rain forests of northern Queensland, combines many primitive morphological and cytological features and that it is excluded from both existing sub families. They regard the genus as being derived from a very early stage in the evolution of the family. On the other hand, Venkata Rao[13] believes that *Bellendena*, a Tasmanian shrub growing on subalpine heaths, more truly represents the primitive form of the Proteaceae. If simplicity of chemical structure in the seed oil is a primitive character, then *Placospermum*, with C16:0 and C18:1 aggregating >96% of the total acids and only minor amounts of four other fatty acids, conforms to the ideas of Johnson and Briggs. The composition of the seed oil of *Bellendena*, on the other hand, conforms very closely to those of other members of the tribe Persoonieae which are not considered especially primitive.

The fatty acid pattern and concentrations in the seed oil of *Cardwellia*, a plant of doubtful affinities, closely resemble those of the Grevilleae and Macadamieae tribes, but the chemical data do not permit an unequivocal classification of this taxon. Chemical data for other genera and species support the current classification.

[12] T. P. HILDITCH and P. W. WILLIAMS, *The Chemical Constitution of Natural Fats*, pp. 207–212, Chapman & Hall, London (1964).
[13] C. VENKATA RAO, *Proc. Linnean Soc. N.S.W.* **82**, 257 (1957).

EXPERIMENTAL

Material

The author collected most of the seeds indigenous to New South Wales, and their authenticity was checked by submission of samples of plant material to the National Herbarium, Sydney. Various collectors provided seeds from Queensland, Tasmania, New Caledonia and South Africa.

Treatment of Oils

After drying in a desiccator at room temp. over P_2O_5, the seeds were disintegrated and extracted with hexane (b.p. 67–70°) in an "Omni-Mixer". The methyl esters were prepared by refluxing the oil for 1–1·5 hr in methanol containing 1·5% H_2SO_4. The esters were purified by dissolving them in hexane and placing about 250 mg on a column containing 15 g Florisil containing 7% water, and eluting with 20 ml hexane. The esters were eluted with 60–70 ml (5%, v/v) diethyl ether in hexane.

Gas Chromatography

The GLC analyses of the methyl esters were carried out on a Packard Gas Chromatograph fitted with flame ionization detectors. The columns used were either 25% diethylene glycol succinate or ethylene glycol succinate containing 2% phosphoric acid on Gas-Chrom P packed in 6 ft × 0·125 in. i.d. glass tubing. The GLC runs were always isothermal at temperatures in the range 165°–185°. Identification of peaks was assisted by semi-log plots of retention times relative to C18:0 against chain lengths separately for saturated, monoene and diene esters. When some uncertainty in the identification of unsaturated esters existed, the sample was fully hydrogenated, using Adams catalyst, and a GLC analysis of the hydrogenated material conducted.

Separation of Monoenes

The first step in separation of the monoenes was by argentation column chromatography using a load of up to 500 mg methyl esters on a column containing about 40 g "Absorbosil-CABN" (25% $AgNO_3$) (Applied Science Laboratories Inc., State College, Penn., U.S.A.), and using an automatic fraction collector. The initial eluant was benzene in hexane (15%, v/v). After most of the saturated esters had been eluted (at about 800 ml), the eluant was changed to benzene in hexane (22%, v/v) and to 30%, v/v at about 1800 ml. The composition of the benzene–hexane solutions sometimes had to be varied because the binding capacity of the "Absorbosil-CABN" often varied from batch to batch. As far as possible, "cuts" of eluates were made so that each fraction contained only two monoenes, e.g. C22:1 and C20:1.

Later in the experiments, increasing concentrations of diethyl ether in hexane, according to the technique of Willmer,[14] were used in place of the toxic benzene-hexane eluting solutions, and were found to give satisfactory separations.

Pure monoenes, of which at least 15 mg each were needed for further chemical work, were prepared by either of two methods from the fractions containing two monoenes.

A. Preparative GLC, using a Varian Aerograph 204 with a 10 ft × 0·25 in. i.d. glass column packed with 5% of the silicone SE-30 on Chromosorb G, with a long, heated-tube trap described by Wood and Reiser.[15] Normal operating temperature was in the range 190°–220°. A sample of 12 mg could be resolved with a recovery of about 65–70%.

B. Reversed-phase column chromatography using a column 100 cm × 2·5 cm i.d., with heptane and methanol in acetonitrile (15%, v/v) as the stationary and mobile phases respectively, and silanized "Celite" as the solid support. Up to 400 mg of mixed monoenes could be cleanly separated in one run. The monoenes prepared by the two methods were usually >99 per cent pure.

Determination of Positional Isomers

Two methods of determining positional isomers were used for each monoene, (a) $KMnO_4$ oxidation method of Tinoco and Miljanich[16] where the resulting methyl esters of the dibasic acids were characterized and their proportions measured by GLC at 175°; (b) Micro-ozonolysis using the equipment described by Beroza and Bierl,[17] where both the resulting aldehydes and the aldehyde-esters were characterized and measured by isothermal GLC at 75° and 185° respectively. Precise measurements of short-chain aldehydes were sometimes difficult, and therefore reliance was mainly placed on the measurement of aldehyde-esters, with the aldehydes providing a qualitative check.

The results obtained by the two methods usually agreed approximately, although ozonolysis was the more sensitive in being able to reveal small amounts (< 2%) of some isomers. For instance, $KMnO_4$ oxidation of C18:1 of *Orites diversifolia* gave 90% Δ^9 and 10% Δ^{13}, while ozonolysis showed 2% Δ^8, 90% Δ^9, 2% Δ^{11} and 6% Δ^{13}. The results from ozonolysis are recorded where the two methods gave different results.

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I.r. spectra of pure monoene esters were prepared on a Perkin-Elmer double-grating spectrophotometer using film thicknesses of 0·008–0·025 mm. The spectra were examined for absorption at 965 cm^{-1} and 1170 cm^{-1}.

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