492. *The Seed Fat of* Macadamia ternifolia.

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The nuts of *Macadamia ternifolia*, a tree found in South Queensland and elsewhere in Australia, contain seeds with a high content of a fatty oil resembling olive oil in general character. Its component acids, however, are made up of oleic (60%) and hexadec-9-enoic * (palmitoleic) acid (20%) with about 8% of palmitic, 3% of stearic, and very small proportions of linoleic, eicosenoic, myristic, and arachidic acids. So large a proportion of hexadecenoic acid in the seed fat of a terrestrial plant has not been observed previously, and further studies of the seed fats of related genera would be of value.

The evergreen tree *Macadamia ternifolia*, belonging to the family Proteaceæ, grows between the latitudes of 25° and 32° S. in Eastern Australia (northern New South Wales and southern Queensland) and attains about 60 feet in height. It is cultivated to a considerable extent in these areas and also in Hawaii and California. It bears hard woody nuts (termed in Australia Queensland nuts or Australian Bush nuts) which contain a single seed. According to Lathrop (*J. Oil Fat Ind.*, 1925, **2**, 44) Hawaii nuts of this species consist of shell 70% and kernel 30%, whilst Morrison (*J. Sydney Tech. Coll. Chem. Soc.*, 1922, **1**, 84) reported that Australian kernels yielded 73% of a liquid fatty oil (iodine value 74·5—75·8, saponification equivalent 287) which consisted mainly of glycerides of oleic acid, accompanied by small proportions of palmitic and stearic acids, and thus closely resembled the best quality of olive oil.

* Geneva numbering.

Some time ago Dr. J. R. Vickery, Chief of the Division of Food Preservation, Australian Commonwealth Scientific and Industrial Research Organisation, sent us a small specimen of Macadamia oil, the detailed composition of which we have determined. The analytical characteristics of this oil were very close to those given by the earlier observers (*loc. cit.*), namely, iodine value 74·8, saponification equivalent 292·0, free fatty acid (as oleic) 0·5%, with 1·0% of unsaponifiable matter. When, however, the mixed fatty acids of the oil were determined in detail by separating them by crystallisation from acetone and then from ether at low temperatures into four groups of differing unsaturation, followed by fractional distillation of the methyl esters of each of the four groups of acids, it became evident that a considerable amount of monoethenoid acid other than oleic accompanied the latter. The acid in question was shown to be hexadec-9-enoic * (palmitoleic) acid, present in traces in many, probably all, seed fats (Hilditch and Thompson, *J. Soc. Chem. Ind.*, 1937, **56**, 434T; Hilditch and Jasperson, *ibid.*, 1938, **57**, 84; 1939, **58**, 187), but not hitherto observed in major proportions in any seed fat.

Our detailed examination of the mixed fatty acids of the *Macadamia ternifolia* oil led to the following composition: myristic 1·6, palmitic 8·0, stearic 3·3, arachidic 2·2, behenic 0·8, hexadecenoic 20·4, oleic 59·3, linoleic 2·2, and eicosenoic 2·2% (wt.). Macadamia oil is thus distinguished by its very low content of diethenoid (linoleic) acid, by the presence of a very small proportion of, apparently, an eicosenoic acid $C_{20}H_{38}O_2$, and, above all, by its content of 20% of the monoethenoid acid of the C_{16} series. The total amount and the relative proportions of saturated acids in the oil (with palmitic acid predominating) are similar to those in many other liquid seed oils of comparable general unsaturation (the minor proportions of myristic, arachidic, and behenic acids, it may be pointed out, are deduced from the ester-fractionation data, but the amounts present were too small to permit of definite identification as individual acids).

The seemingly exceptional occurrence of so great a proportion of hexadecenoic acid in this seed fat merits some consideration. This acid is present in similar proportions, but is accompanied by equally marked proportions of highly-unsaturated acids containing 20 and 22 carbon atoms in the molecule, in the fats of vegetable plankton and other marine flora (Lovern, *Biochem. J.*, 1936, **30**, 387; Hilditch, " Chemical Constitution of Natural Fats," 2nd Edn., 1947, pp. 24—26). It has also been observed in quantity in fats of some lower forms of land flora, such as those of diphtheria bacilli (Chargaff, *Z. physiol. Chem.*, 1933, **218**, 223), yeast (Newman and Anderson, *J. Biol. Chem.*, 1933, **102**, 219), or lycopodium spores (Riebsomer and Johnson, *J. Amer. Chem. Soc.*, 1933, **55**, 3352). The botanical family Proteaceæ, in which *Macadamia* is placed, includes over 900 species: of these about two-thirds are indigenous to Australia, whilst the remainder are natives of Cape Colony and the adjacent parts of South Africa. The members of this family are thought to be survivors from one of the more primitive types characteristic of the original flora of the Australian continent. It is conceivable that plants of such origin should elaborate seed fats differing in some respects from those of other plants. Again, many members of the Proteaceæ are xerophytic in character and habituated to arid conditions of growth (although this probably does not hold entirely in the case of *Macadamia*); seed fats of such species have not, however, yet been studied in detail.

Rather, therefore, than regarding the presence of hexadecenoic acid in quantity in *Macadamia ternifolia* seed fat as a completely abnormal feature in a terrestrial seed fat, we prefer merely to record our findings and to suggest that further study of seed fats, of other species of Proteaceæ, of other species of the elemental Australasian flora, or of other xerophytic species, would be of great interest and might reveal further specific relations between seed-fat composition and the biological development of plant species.

Experimental.

Determination of the Component Acids of Macadamia ternifolia *Seed Fat.*—The mixed acids (93·2 g.) obtained by hydrolysis of 100 g. of the fat were crystallised, first from 10% solution in acetone at −50°. The acids left in solution were then crystallised from acetone at −60°, most of the polyethenoid acid present being left in solution (*D*), and the acids (*C*) deposited at −60° consisting mainly of monoethenoid compounds. The acids deposited from acetone at −50° were further crystallised from 10% solution in ether at −40°, the material deposited consisting almost wholly of saturated acids. The following groups of acids were thus obtained:

	Wt. (g.).	%.	Iodine value.
A, Insoluble in ether at −40°	13·7	14·7	8·0
B, Soluble in ether at −40°	50·8	54·5	87·9
C, Insoluble in acetone at −60°	20·1	21·6	94·5
D, Soluble in acetone at −60°	8·6	9·2	109·3

Each group of acids was separately converted into methyl esters, which were distilled in a vacuum through an electrically-heated and packed column. Table I shows the distilled fractions obtained from each group, with the temperature at the head of the column, and the equivalent and iodine value of each ester fraction.

The values in Table I clearly indicate the presence of hexadecenoic esters (methyl hexadecenoate, equiv. 268, iodine value 94·8) in quantity in the ester fractions *B*2, *B*3, C2, *C*3, *C*4, and *D*2. The residual fractions from esters *B*, *C*, and *D* evidently contained, in addition to unsaturated esters of the C_{18} series, a monoethenoid ester of an acid of higher carbon content; the amounts present were too small to permit its identification, and it has been calculated as methyl eicosenoate (equiv. 324, iodine value 78·4).

TABLE I.

Fractionation of methyl esters of fatty acids of groups A, B, C, *and* D.

Methyl esters from group *A*.

Fraction.	Wt. (g.).	Temp.	Sapon. equiv.	Iodine value.
1	1·08	120—135°	265·2	1·7
2	3·16	135—140	271·3	1·8
3	2·73	140—142	276·5	12·2
4	2·29	142—145	292·1	21·4
5	1·00	145—150	301·5	8·9
6	2·52	Residue	339·6 *	3·3 *
	12·78			

Methyl esters from group *B*.

Fraction.	Wt. (g.).	Temp.	Sapon. equiv.	Iodine value.
1	1·29	60—120°	257·3	55·5
2	1·89	120—135	267·4	79·4
3	2·57	140—145	271·4	79·7
4	2·21	,,	286·2	82·4
5	2·55	,,	291·8	84·5
6	2·58	,,	292·6	85·4
7	3·31	,,	293·8	85·4
8	4·92	,,	294·5	85·5
9	5·01	,,	294·8	85·4
10	3·98	,,	295·5	86·1
11	3·46	,,	295·8	85·5
12	3·45	,,	296·4	85·2
13	3·14	,,	295·7	85·9
14	3·64	,,	295·8	84·8
15	1·83	Residue	313·7 *	73·2
	45·83			

Methyl esters from group *C*.

Fraction.	Wt. (g.).	Temp.	Sapon. equiv.	Iodine value.
1	1·18	50—120°	257·2	61·5
2	1·54	120—135	268·1	91·9
3	3·07	135—140	268·7	93·8
4	2·55	,,	268·9	93·4
5	2·57	140	283·7	92·7
6	3·54	140—142	295·8	92·4
7	2·55	142—145	296·9	90·7
8	1·86	Residue	319·9 *	80·7 *
	18·86			

Methyl esters from group *D*.

1	1·11	135—137°	260·1	83·2
2	1·89	137—138	267·5	93·3
3	1·98	140—145	279·2	109·5
4	1·66	,,	294·6	114·2
5	0·46	Residue	307·8 *	98·6 *
	7·10			

* Residual esters, freed from unsaponifiable matter:

*A*6 Sap. equiv. 332·4, iodine value 2·6. *C*8 Sap. equiv. 316·5, iodine value 79·3.
*B*15 ,, 307·1, ,, 72·6. *D*5 ,, 291·3, ,, 92·5.

The unsaturated C_{18} acids (which form the greater part of the oil) occurred to varying extents in all the separate groups, but those in groups *A* and *B* were wholly mono-ethenoid (methyl oleate, equiv. 296, iodine value 85·8). The polyethenoid C_{18} acids of the oil were concentrated in groups *C* and *D*, in representative C_{18} ester fractions of which the proportions of linoleic and oleic acids were determined spectrophotometrically after isomerisation with alkali at 180° for 1 hour as described by Hilditch, Morton, and Riley (*Analyst*, 1945, **70**, 68). The acids of ester fraction *D*4 were also isomerised with alkali at 170° for 15 minutes, but then gave negligible absorption at 268 mμ., indicating the absence of linolenic acid. The spectrophotometric data for the acids isomerised at 180° for 1 hour were:

	Mixed acids,		Composition of mixed acids (% wt.).	
Ester fraction.	Iodine value.	$E^{1\%}_{1\,cm.}$ at 234 mμ.	Linoleic.	Oleic.
*C*6	97·0	66	7·3	92·7
*D*4	119·9	289	31·9	68·1

The components of each ester fraction were then calculated by procedures which have been described elsewhere in detail (Hilditch, *op. cit.*, pp. 505—509). The percentages of the component acids in each of the four groups, and therefrom those for the total fatty acids of the Macadamia oil, then follow (Table II).

TABLE II.

Component fatty acids of Macadamia oil.

Acid.	Acid groups. *A*. % (wt.).	*B*. % (wt.).	*C*. % (wt.).	*D*. % (wt.).	Total fatty acids (excluding unsaponifiable). % (wt.).	% (mol.).
Myristic	1·4	1·3	2·2	2·4	1·6	1·9
Palmitic	46·5	1·9	0·7	—	8·0	8·6
Stearic	21·9	0·2	—	—	3·3	3·2
Arachidic	14·9	—	—	—	2·2	1·9
as Behenic	5·5	—	—	—	0·8	0·6
Hexadecenoic	2·6	10·5	46·0	46·5	20·4	22·0
Oleic	6·8	85·4	39·8	33·3	59·3	57·7
Linoleic	—	—	3·3	16·3	2·2	2·0
as Eicosenoic	—	0·6	7·9	1·4	2·2	2·0
Unsaponifiable	0·4	0·1	0·1	0·1	—	—

Identification of Unsaturated Acids Present in Macadamia Oil.—Hexadecenoic, oleic and linoleic acids were identified in one or other of the ester fractions as follows :

Hexadecenoic acid. The acids (4·3 g.) from the mixed ester fractions *C*1, *C*2, *D*1, and *D*2 (equivalents 268 or below) were mixed and oxidised in acetone solution with powdered potassium permanganate (Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 43T, 180T). After separation of the mono- and di-carboxylic acids produced, azelaic acid was isolated (m. p. 103—104°, unchanged on admixture with the pure acid; Found : equiv., 92·5. Calc. : equiv., 94·0). The monobasic acids (separated by distillation in steam) were recovered and fractionally distilled through a small column, giving fractions of acids with equivalents from 126 to 132 (heptoic acid, equiv. 130). The acid is thus hexadec-9-enoic acid.

Another portion of acids (3·0 g., from ester fraction *C*3) was oxidised in dilute aqueous alkaline solution at 0° with potassium permanganate (Lapworth and Mottram, *J.*, 1925, **127**, 1628), a dihydroxy-acid being formed which melted at 127—128° (unchanged on admixture with 9 : 10-dihydroxypalmitic acid).

Oleic acid. Acids (7·9 g.) from the mixed ester fractions *B*10—13 (equivalents 295·5—296·4) were oxidised as above in acetone solution with potassium permanganate. The dibasic acid product melted at 104—105° (unchanged on admixture with azelaic acid), and had an equivalent of 94·4. The monobasic acids produced were converted into ethyl esters and the latter fractionally distilled, fractions with equivalents from 178 to 185 being produced (ethyl nonoate, equiv. 186).

Another portion of the same acids (3·2 g.) was converted into the dihydroxy-acid by oxidation with dilute ice-cold alkaline permanganate, giving an acid of m. p. 130° (unchanged when mixed with 9 : 10-dihydroxystearic acid).

The octadecenoic acid present was thus ordinary oleic acid.

Linoleic acid. Acids (1·6 g.) from ester fraction *D*4 were dissolved in light petroleum (b. p. 40—60°), and bromine was added to the solution at 0°. A small deposit of crystalline bromo-addition product was produced, which after recrystallisation from the same solvent melted at 112° (unchanged when mixed with tetrabromostearic acid, m. p. 114°, from authentic linoleic acid).

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