387. *The Seed Wax of* Simmondsia Californica.

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GREENE and FOSTER (*Bot. Gazette*, 1933, **94**, 826) found that the seeds of *Simmondsia californica* Nutt., an evergreen shrub indigenous to southern California and southern Arizona, contained about 46% of a liquid oil which resembled sperm or Arctic sperm oil closely in its analytical characteristics, and which they therefore concluded to be a liquid wax; qualitative tests suggested that the wax might consist principally of fatty acid esters of decyl alcohol. The genus *Simmondsia* was formerly placed by botanists in the Box section of the *Euphorbiaceæ*, but modern authors place it in a distinct family, *Buxaceæ*. The seeds of members of the latter family, like those of the *Euphorbiaceæ*, usually contain endosperm, but in this respect *Simmondsia* is an exception, its seeds (about 12 mm. × 8 mm.) consisting wholly of embryo and cotyledons enclosed in a thin, hard testa.

The nature of the fatty content of the seeds, as given by Greene and Foster, is also entirely exceptional, for the occurrence of wax-esters instead of glycerides in the proportions observed in *Simmondsia* seeds has not, so far as we are aware, ever been observed in any other seed. We have therefore been interested to undertake a further examination of the fatty material present in about 400 g. of mature seeds of *Simmondsia californica* which were kindly placed at our disposal by Prof. R. A. Greene and Dr. E. Lewkowitsch.

The decorticated ground kernels yielded, on extraction with light petroleum (b. p. 40—60°), 48% of a golden-yellow oil which, after complete hydrolysis, gave approximately equal weights of fatty acids and fatty alcohols. We examined the products of hydrolysis for the presence of glycerol, with negative results, confirming in this respect the previous observation of Greene and Foster. Sufficient of the crude acids and alcohols from the wax was collected for a more detailed examination, which revealed that their constitution was still more unusual than the preliminary study by the former workers had suggested.

The Acidic Constituents of Simmondsia *Seed-wax.*—The mixed acids were subjected to a lead salt separation from alcohol; the acids recovered from the insoluble and the soluble lead salts were separately converted into methyl esters and fractionally distilled in a vacuum. From the details of the fractionation data (Tables I—III) it was evident (*a*) that the acids yielding insoluble lead salts were not saturated fatty acids, but differed little from the main bulk of those acids whose lead salts had remained in solution in the alcohol; and (*b*) that the acids from the soluble lead salts were for the most part similar to the former, except for the presence of quite minor amounts (not more than 6—7% of the total acids) of acids, the mean equivalent (292·0) and iodine value (71·3) of the methyl esters of which indicated the probable presence of oleic and palmitic acids. The greater part of the methyl ester fractions from both groups of acids had equivalents of 325—328 and iodine values of 77·5—79, but a small portion (about 10% of the total acids) possessed a higher equivalent and lower iodine value, indicating the presence of small proportions of an unsaturated acid of higher molecular weight than the main component. The equivalent of the methyl esters was unchanged after acetylation, from which the absence of hydroxy-acids was concluded. One of the middle ester-fractions (L4, Table II, equiv., 326·0; iod. val., 79·1) was found to contain C, 77·8; H, 11·9% (methyl eicosenoate, $C_{21}H_{40}O_2$, requires C, 77·8; H, 12·3%; equiv., 324; iod. val., 78·4). The acids from another ester-fraction (L5, Table II, equiv., 327·0; iod. val., 78·6) gave, after oxidation with ice-cold alkaline permanganate, a *dihydroxy-saturated acid*, m. p. 130·5° (Found: C, 69·2; H, 11·5. $C_{20}H_{40}O_4$ requires C, 69·8; H, 11·6%). The main component acid was therefore an eicosenoic acid, $C_{19}H_{37}\cdot CO_2H$, the structure of which was determined by the following further experiments.

The ester-fractions L15, L4—7, and S4—6 (Tables I—III) were united. A portion of these was hydrogenated as far as possible, and the product oxidised with permanganate in acetone in order to remove all traces of unsaturated ester. The purified saturated ester, after crystallisation from absolute alcohol, melted at 44° (equiv., 325·6) and the corresponding acid, after crystallisation from ethyl acetate, at 74·5° (unchanged when mixed with *n*-eicosanoic acid). Dr. Malkin, of the University of Bristol, kindly undertook the *X*-ray spectrographic examination of both the ester and the acid and reported that the respective spacings (52·7 A. and 44·5 A.) were those of methyl *n*-eicosanoate and *n*-eicosanoic acid.

The remainder of the combined methyl esters was oxidised with permanganate in acetone, and the acidic products of oxidation examined. Nonoic acid was identified, together with nonane-1 : 9-dicarboxylic acid, proving that the chief acid present in the seed-wax is $\Delta^{11:12}$-eicosenoic acid.

The Alcoholic Constituents of Simmondsia *Seed-wax.*—The alcohols were fractionally distilled in a vacuum and separated into three main fractions of about equal weight (Table IV), none of which appeared to be composed wholly of an individual compound, although the iodine values (84·7 and 80·4) of the alcohols in fractions A2 and A3, and the equivalents of their acetates (350·5, 350·1), were not far removed from those for a heneicosenol, $C_{21}H_{42}O$ (iod. val., 82; equiv. of acetate, 352).

A portion of fraction A2 was hydrogenated and some of the saturated alcohols formed were recrystallised several times from ethyl acetate; they then melted at 67—68°. *X*-Ray spectrographic examination of this specimen by Dr. Malkin gave the spacing 59·7 A. and showed that it was not an individual alcohol, but by further crystallisation from alcohol he obtained a specimen, m. p. 71°, which gave the spacing 60·2 A., agreeing closely with that of the vertical form of *n*-docosanol, $C_{22}H_{46}O$ (m. p. 72°).

Another portion of the fully-hydrogenated alcohols was oxidised with chromic acid in acetic acid solution to the corresponding carboxylic acid or acids. The latter, after purification from unchanged alcohols and crystallisation from ethyl acetate, melted at 74—75° (depressed on admixture with *n*-eicosanoic acid); this product gave results on analysis corresponding fairly closely with heneicosanoic acid. After several further crystallisations from alcohol a specimen of acid was obtained which melted at 78—79° and showed no depression in melting point when mixed with *n*-docosanoic acid, while the melting point fell to 71° on admixture with *n*-eicosanoic acid. Dr. Malkin informed us that the *X*-ray spacings (B form 53·5 A., C form 48·3 A.) given by this acid showed definitely that it was *n*-docosanoic (behenic) acid.

A portion of the succeeding fraction 3 of the original unsaturated alcohols was oxidised by means of permanganate in acetone, and the products of oxidation examined (p. 1755). Nonoic acid was substantially the only monocarboxylic acid present. With the dicarboxylic acids (as with the monobasic acids from the chromic acid oxidation of the hydrogenated alcohols), much difficulty was experienced in obtaining a pure specimen by means of crystallisation, but ultimately an acid was obtained (equiv., 122·8) which melted at 108—110° and showed no depression when mixed with undecane-1 : 11-dicarboxylic (brassylic) acid (equiv., 122).

We have therefore definite evidence of the presence of $\Delta^{13:14}$-docosenol as one of the constituents of the alcohols present. From the repeated observation of analytical values for the alcohols and their derived acids corresponding with a mean carbon content of C_{21}, together with the non-appearance of any decane-1 : 10-dicarboxylic acid (derived from a heneicosenol) in the ultimate oxidation products, we consider that an eicosenol is probably the other main component of the alcohols. Since no monocarboxylic acid except nonoic was identified among the oxidation products of the unsaturated alcohols, it seems probable that this is $\Delta^{11:12}$-eicosenol.

The seed-wax of *Simmondsia californica* is thus a marked exception to the usual fatty material of seed endosperm or embryo, not only in being composed of wax-esters instead of triglycerides, but also in the nature of its component alcohols and acids. Oleic and palmitic acids are almost, if not wholly, absent from the wax, the main components of which are esters of $\Delta^{11:12}$-eicosenoic acid (with possibly a little docosenoic acid) in combination with $\Delta^{13:14}$-docosenyl and (?) $\Delta^{11:12}$-eicosenyl alcohols, probably in something approaching equal proportions. So far as we are aware, no instance of this kind had been observed until Greene and Foster's observation on this species. It is not without interest, however, that seed-fats of the *Euphorbiaceæ* (with which family *Simmondsia* evidently has a near botanical relationship) include some examples such as those of *Aleurites montana* and *Ricinus communis*, in which the acyl part of the triglycerides is composed very largely of unusual unsaturated acids (elæostearic, ricinoleic) instead of the more common linoleic and oleic acids.

The observation that the unsaturated alcohols and acids of *Simmondsia* seed-wax are of the general formulæ

$$CH_3{\cdot}[CH_2]_7{\cdot}CH{:}CH{\cdot}[CH_2]_m{\cdot}CH_2{\cdot}OH \text{ and } CH_3{\cdot}[CH_2]_7{\cdot}CH{:}CH{\cdot}[CH_2]_n{\cdot}CO_2H$$

is also of interest in the light of Chibnall and Piper's hypothesis (*Biochem. J.*, 1934, **28**, 2217) that a number of cuticle and leaf wax components may be derived from unsaturated compounds of this general form.

An attempt was made to ascertain whether the *Simmondsia californica* seeds presented any unusual feature in the development of the seed wax by comparing the analytical characteristics of the material from immature seeds of the 1936 crop with that from mature seeds of the 1935 and 1936 crops, specimens of all of which were very kindly obtained for us by Professor Greene. The amount of wax from the immature seeds was so small that an accurate measure of the equivalent could not be determined, but it was evident that there was no great variation in any of the specimens :—

		Mature seeds 1935.	Half-mature seeds 1936.	Mature seeds 1936.
Soluble in light petroleum		33·7%	6%	31·9%
Wax	iodine value	83·4	82·3	83·1
	saponification equivalent	594·3	*ca.* 500	590·0
Acid constituents	iodine value	79·8	—	80·7
	mean molecular weight	303·4	—	311·0
Alcohol constituents	iodine value	80·8	—	81·5

EXPERIMENTAL.

The kernels of the seeds were removed from the testa, ground, and extracted exhaustively with light petroleum (b. p. 40—60°). 400 G. of the ground kernels yielded 195 g. of a golden-yellow neutral oil with saponification equivalent 604·1 and iodine value 86·3. The oil (184 g.) was hydrolysed with excess of boiling alcoholic potassium hydroxide, and the alcohols produced were separated from the solution of potassium soaps; from the latter the acids were recovered and re-submitted to the saponification process, in order to ensure complete hydrolysis of the wax. There were thus obtained 95·6 g. of acids (M.M.W. 309·6, iod. val. 82·1) and 91·9 g. of alcohols (M.M.W. 307·7, iod. val. 84·3).

Examination of the Mixed Acids of the Seed-wax.—The mixed acids (95·6 g.) were dissolved in boiling 95% alcohol (500 c.c.), mixed with a boiling solution of lead acetate (65 g.) in 95% alcohol (500 c.c.) containing a few drops of acetic acid, and set aside: a quantity of low-melting syrupy insoluble lead salts separated, and was redissolved in 1000 c.c. of hot 95% alcohol containing 5 c.c. of glacial acetic acid and again allowed to separate. The acids from the insoluble and from the soluble lead salts were recovered separately and converted into their methyl esters for fractional distillation. The yield of acids from the insoluble lead salts was 31·8 g., and that from the soluble lead salts 63·7 g.

Fractional Distillation in a Vacuum of the Methyl Esters.—The weights and characteristics of the fractions obtained from distillation of the methyl esters of the acids from the insoluble lead salts are given in Table I, those from the primary fractionation of the methyl esters of the acids from the soluble lead salts in Table II, while redistillation of the primary fraction L1 (Table II) gave the results shown in Table III.

TABLE I.

Methyl esters of acids from insoluble lead salts.

Fraction.	G.	B. p./0·2 mm.	Sap. equiv.	Iod. val.
S1	3·47	121—144°	316·6	75·3
S2	3·37	144—146	321·8	77·7
S3	3·71	146	323·8	79·3
S4	3·83	146—147	325·3	79·0
S5	3·61	147	325·6	78·9
S6	3·77	147—155	326·3	77·7
S7	4·31	155	328·5	77·4
S8	5·48	Residue	335·8	74·2
	31·55			

TABLE II.

Methyl esters of acids from soluble lead salts.

Fraction.	G.	B. p./0·2 mm.	Sap. equiv.	Iod. val.
L1	21·06	118—143°	309·6	79·2
L2	5·84	143—146	321·8	80·6
L3	6·01	146—147	324·7	80·0
L4	5·68	147—152	326·0	79·1
L5	6·04	152—155	327·0	78·6
L6	6·01	155	327·9	77·6
L7	6·21	155—157	334·8	76·3
L8	4·15	Residue	350·6*	73·2*
	61·00			

* The esters in fraction L8, after allowance for unsaponifiable matter present, had sap. equiv. 329·7, iod. val. 71·4.

TABLE III.

Refractionation of fraction L1 (*Table II*).

Fraction.	G.	B. p./0·2 mm.	Sap. equiv.	Iod. val.
L11	3·47	110—134°	292·0	71·3
L12	3·20	134—135	304·0	81·8
L13	3·42	135	312·0	83·8
L14	3·43	135—145	318·9	81·9
L15	3·52	Residue	325·9	79·3
	17·04			

It is clear from the data in Table III that the esters of the usual component acids of seed fats—for example, methyl oleate (equiv., 296; iod. val., 85·8) or linoleate, or methyl palmitate (equiv., 270)—can only be present in very small quantities in the *Simmondsia* seed-wax; fraction L11 might have been composed wholly, and fractions L12 and L13 partly, of such esters. The chief component of the esters listed in both Tables I and II is evidently a compound with equivalent about 325—328 and iodine value about 78—79, and this is accompanied by smaller proportions of one or more esters of somewhat higher molecular weight and lower iodine value.

Hydrogenation of the Methyl Esters.—Fractions L15, L4—7 and S4—6 (Tables I—III) were united for the purpose of further experiments to determine the structure of the main component acid. A portion of the esters (15 g.) was hydrogenated (in solution in purified kerosene) in presence of nickel at 180°; subsequently the solvent was removed by distillation in a vacuum and the hydrogenated esters were fractionated. The main fraction (12·1 g.) was oxidised, and the purified neutral esters examined, as described on p. 1751.

Oxidation of the Methyl Esters.—Another portion of the esters (9 g.) was dissolved in acetone (90 c.c.) and oxidised with potassium permanganate (36 g.). After decolorisation and removal of unchanged ester or neutral products (0·4 g.), the acidic products were separated by light petroleum into a soluble (3·4 g.) and an insoluble (4·6 g.) portion.

The acids soluble in light petroleum were recovered and distilled at atmospheric pressure, a main fraction being collected (Found : equiv., 161. Calc. for $C_9H_{18}O_2$: equiv., 158). Those insoluble in light petroleum were solid and were recrystallised several times, first from methyl alcohol, then from water and finally from chloroform. The acid finally melted at 109° [mixed with synthetic nonane-1 : 9-dicarboxylic acid (m. p. 111°), at 110—111°] (Found : C, 61·0; H, 9·0; equiv., 108·6. Calc. for $C_{11}H_{20}O_4$: C, 61·1; H, 9·3%; equiv., 108).

The original acid was therefore $\Delta^{11:12}$-eicosenoic acid.

The potassium salts from the hydrolysis of fraction L5 (Table II) were oxidised in dilute ice-cold aqueous solution with alkaline permanganate according to the procedure of Lapworth and Mottram (J., 1925, **127**, 1628); 11 : *12-dihydroxyeicosanoic acid* was produced, m. p. 130·5° after crystallisation from ethyl acetate (Found : C, 69·2; H, 11·5. $C_{20}H_{40}O_4$ requires C, 69·8; H, 11·6%).

Lapworth and Mottram (*ibid.*, p. 1987) showed that 9 : 10-dihydroxystearic acid (from oleic acid), on subjection to further oxidation with cold aqueous alkaline permanganate, was converted into *n*-octoic, oxalic and suberic acids (instead of *n*-nonoic and azelaic acids). We similarly oxidised 11 : 12-dihydroxyeicosanoic acid (1·7 g.), dissolved in water (680 c.c.) and sodium hydroxide (3·4 g.), with potassium permanganate (6·8 g.) in water (510 c.c.) for 2½ hours at room temperature, after which only 0·4 g. of the acid had been oxidised. The oxidation of the remaining acid was still incomplete after 48 hours at room temperature (0·3 g. remaining unattacked); it is clearly much more resistant to the action of alkaline permanganate solution than the dihydroxystearic acid. The dibasic acid product of oxidation was recovered from the united products and, after crystallisation from chloroform, melted at 130—132° (mixed with authentic sebacic acid, m. p. 131—132°) (Found : C, 59·2; H, 9·0; equiv., 103·4. Calc. for $C_{10}H_{18}O_4$: C, 59·4; H, 8·9%; equiv., 101). The alkaline permanganate oxidation of $\Delta^{11:12}$-eicosenoic acid thus follows the same course as that of oleic acid, but is much slower in its second stage.

Examination of the Mixed Alcohols of the Seed-wax.—The mixed alcohols (87 g.) were distilled in a vacuum, with the results shown in Table IV.

TABLE IV.

Fractional distillation of the mixed alcohols.

Fraction.	G.	B. p./0·2 mm.	Iod. val. (alcohols).	Equiv. (acetates).
A1	28·9	140—182°	86·6	342·8
A2	31·9	182—185	84·7	350·5
A3	20·9	185	80·4	350·1
A4	5·1	Residue	88·1	385·4
	86·8			

The figures in Table IV suggest the presence of monoethenoid alcohols with a carbon content of not less than C_{20}, but also indicate a mixture of homologous alcohols which, as would be expected, has not been resolved into any individual compound. The analytical characteristics of fractions A2 and A3 correspond fairly well with those for a heneicosenol ($C_{21}H_{42}O$ requires iod. val. 82, equiv. of acetate 352).

Hydrogenation of the alcohols. Some of fraction A2 (15 g.) was hydrogenated in solution in purified kerosene, the latter being subsequently removed by distillation and the hydrogenated alcohols crystallised repeatedly from ether and ethyl acetate until their melting point was 67—68°. The results of the *X*-ray spectrographic examination are given on p. 1751.

A portion of the fully-hydrogenated alcohols (m. p. 63°, 5 g.), dissolved in glacial acetic acid (100 c.c.), was mixed with chromium trioxide (2·2 g.) at room temperature, and oxidation allowed to proceed for 20 minutes. The acetic acid was removed and the acid products (2·9 g.) from the oxidation were recovered and crystallised several times from ethyl acetate; m. p.

74—75° (depressed to 70—71° on admixture with *n*-eicosanoic acid) (Found : C, 77·3; H, 13·0; equiv., 326·8. Calc., respectively, for $C_{20}H_{40}O_2$, $C_{21}H_{42}O_2$, $C_{22}H_{44}O_2$: C, 76·9, 77·3, 77·6; H, 12·8, 12·9, 12·9% ; equiv., 312, 326, 340). Further repeated crystallisation from alcohol finally yielded a small specimen of acid (m. p. 78—79°; unaltered when mixed with *n*-docosanoic acid, depressed to 71° on mixing with *n*-eicosanoic acid) (cf. also *X*-ray spectrographic examination, p. 1752).

Oxidation of the unsaturated alcohols. A portion (15 g.) of fraction A3 was dissolved in acetone (150 c.c.) and oxidised in the usual manner with powdered potassium permanganate (60 g.), and the acidic products of oxidation recovered and distilled in steam until no further steam-volatile acids passed over. The acids in the aqueous condensate were extracted with ether, thoroughly dried, and distilled at atmospheric pressure; there were then obtained three fractions (1·3, 0·7, 0·7 g.; equiv. found, respectively, 155·5, 158·1, 156·6) and a residue (1·5 g., equiv., found, 158·5). The barium salt was prepared from the distilled acids (Found : Ba, 30·8. Calc. for $C_{18}H_{34}O_4Ba$: Ba, 30·5%). The only monocarboxylic acid formed by oxidation of the unsaturated alcohols is therefore *n*-nonoic acid, and all the alcohols have the general structure $CH_3\cdot[CH_2]_7\cdot CH{:}CH\cdot[CH_2]_x\cdot CH_2\cdot OH$.

The acids not volatile in steam (5 g., primarily ω-hydroxycarboxylic acids) were oxidised further by chromium trioxide (3·8 g.) in glacial acetic acid (100 c.c.), as described above. The dicarboxylic acids produced were crystallised from chloroform to remove any unchanged hydroxy-acid, and then melted indefinitely at 93—101°. They were obviously a mixture of acids and required much further crystallisation from chloroform before a small specimen was obtained, m. p. 107—109° (equiv., 122·8). The m. p. was raised to 108—110° after still further crystallisations and remained unaltered when the specimen was mixed with undecane-1 : 11-dicarboxylic (brassylic) acid (Found : C, 64·1; H, 9·9. Calc. for $C_{13}H_{24}O_4$: C, 63·9; H, 9·8% ; equiv., 122).

Oxidation of the unsaturated alcohols to trihydric alcohols. Fraction A2 (5 g.) was dissolved in glacial acetic acid (50 c.c.) and oxidised at 80° with perhydrol (10 c.c.) for 6 hours (cf. Collin and Hilditch, J., 1933, 246). A mixture of solid trihydric alcohols was obtained which, after crystallisation from ethyl acetate, melted at 77—78° (Found : C, 73·0; H, 13·1. $C_{21}H_{44}O_3$ requires C, 73·3; H, 12·8%). The product, in the light of the previous results, was probably a mixture of docosane-1 : 13 : 14-triol and eicosane-1 : 11 : 12-triol.

SUMMARY.

1. The fatty matter present in the seeds of *Simmondsia californica* Nutt., as previously observed by Greene and Foster (*loc. cit.*), is a mixture of wax-esters, and not glycerides.

2. Detailed examination of the acid and alcohol components of the seed-wax has shown that these are of an unusual nature. The chief acid is $\Delta^{11:12}$-eicosenoic, probably accompanied by small quantities of a higher (possibly docosenoic) acid and of oleic and palmitic acids; the amount of the latter two acids is, however, quite unusually small. A mixture of C_{20} and C_{22} unsaturated alcohols forms the alkyl portion of the wax esters. $\Delta^{13:14}$-Docosenol has been definitely identified and the other alcohol component (present in equal or slightly larger proportion) is probably $\Delta^{11:12}$-eicosenol.

3. *Simmondsia* presents certain morphological anomalies in its seed; the chemical nature of the fatty matter in the latter is also abnormal both in its general character of a wax-ester and in the constitution of the component acids and alcohols of the seed-wax.

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