700. *The Constitution and Properties of a Conjugated Diene Acid present in Stillingia Oil.*

By A. Crossley and T. P. Hilditch.

The unusual short-chain component of the mixed fatty acids of stillingia oil (from seeds of *Sapium* species) has been identified as deca-2 : 4-dienoic acid,* a homologue of sorbic acid. Its ultra-violet absorption spectrum resembles that of sorbic acid, a single absorption band with head at 260 mμ. (acid) or 264 mμ. (ester) and ε 24,000. Complete hydrogenation of the methyl ester yields methyl *n*-decoate, and semi-hydrogenation gives a mixture of esters in which methyl dec-3-enoate predominates (*ca.* 40%), accompanied by about 30% of the Δ^2- and Δ^4-monoethylenic isomers and about 15% each of unchanged diene ester and of saturated methyl *n*-decoate.

It has recently been shown (Hilditch, *J. Oil Col. Chem. Ass.*, 1949, **32**, 18) that stillingia oil, from the seeds of *Sapium sebiferum* Roxb., is unique amongst unsaturated fatty oils in the presence, along with the usual seed-oil unsaturated C_{18} acids (linoleic, linolenic, oleic), of a shorter-chain acid, *n*-deca-2 : 4-dienoic acid. The component acids of stillingia oil had been studied earlier by Jamieson and McKinney (*Oil and Soap*, 1938, **15**, 295) by separation into saturated and unsaturated portions by means of their lead salts, and by fractional distillation of the methyl esters of the saturated (but not the unsaturated) acids; consequently, although these authors remarked upon the somewhat unusually low saponification equivalent of stillingia oils compared with that of other vegetable seed oils derived from similar higher unsaturated acids, the presence of the short-chain acid was overlooked. Our observation of an absorption band in the ultra-violet absorption spectrum at about 260 mμ. revealed the presence of an unusual acid, which was then found to be due to an unsaturated component of relatively low molecular weight (*ca.* 170), which was readily separable by fractional distillation of the methyl esters of the stillingia oil mixed fatty acids and was later found by us to be deca-2 : 4-dienoic acid (Hilditch, *loc. cit.*). In this communication we record the properties of the acid and the evidence on which its constitution is based, and also describe the products of partial hydrogenation of its methyl ester.

The short-chain unsaturated acid was readily obtained from the mixed fatty acids of stillingia oil (prepared by cautious hydrolysis with only a small excess of alkali) by converting them into methyl esters and separating the lowest-boiling constituents of the latter by distillation through an efficient fractionation column at 0·2 mm. pressure. Refractionation of the low-boiling esters led ultimately to the production of an apparently homogeneous methyl ester, a colourless liquid with a characteristic odour and the following analytical characteristics : Found : equiv., 183; C, 71·5; H, 10·0. $C_{11}H_{18}O_2$ requires equiv., 182; C, 72·5; H, 10·0%. On hydrolysis with alkali the ester developed an intense yellow colour (*cf.* Hilditch, *loc. cit.*), but this feature apparently was caused by traces of autoxidation products. The ester, and still more so the acid, is extremely susceptible to atmospheric oxygen, and there is reason to believe that the low carbon content recorded is caused by slight attack by oxygen before the analysis could be undertaken.

The purified ester, on hydrogenation with Raney nickel at 110°, smoothly and rapidly absorbed 2 moles of hydrogen. The hydrogenated product was distilled and hydrolysed to an acid with an equivalent of 171·8 (Calc. for $C_{10}H_{20}O_2$: equiv., 172). The acid melted sharply at 31—31·5° (unchanged when mixed with synthetic *n*-decoic acid) (Found : C, 69·8; H, 11·5. Calc. for $C_{10}H_{20}O_2$: C, 69·7; H, 11·7%). The identity of the acid was further checked by the melting points of its *p*-toluidide (76·5°) and of its *p*-bromophenacyl ester (67·0—67·5°), which were unaltered by admixture with the respective synthetic compounds.

Oxidation of the purified diene ester in acetone with potassium permanganate yielded a steam-volatile monobasic acid which was proved to be *n*-hexoic acid by the melting-points of its *p*-toluidide and its *p*-bromophenacyl ester, both of which were unchanged when mixed with the respective derivatives prepared from authentic *n*-hexoic acid. The natural acid is therefore *n*-deca-2 : 4-dienoic acid, $CH_3\cdot[CH_2]_4\cdot[CH{:}CH]_2\cdot CO_2H$. The presence of a C_{10} acid with the more usual linoleic, linolenic, and oleic acids in an unsaturated vegetable seed fat is most unusual. The occurrence in a fatty oil of a homologue of sorbic acid is moreover unique.

Absorption Spectrum of Deca-2 : 4-dienoic Acid.—In our preliminary work (Hilditch, *loc. cit.*) on the mixed fatty acids of stillingia oil a band with a head at 257 mμ. was observed. In

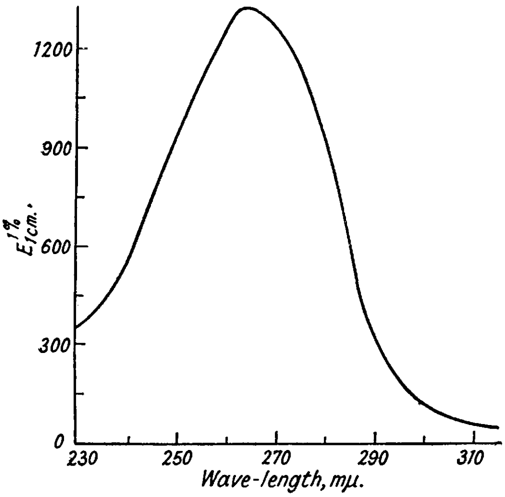
---

* In this paper the acids are numbered on the Geneva system, the number 1 being assigned to the carboxyl carbon atom.

the present series of experiments it was found that the band head (cf. fig.), for the purest specimen of ester obtained, was at 264 mμ. (in alcohol), whilst that for the corresponding acid was at 260 mμ. The mean value (six determinations) of $E^{1\%}_{1\,\text{cm.}}$ at 264 mμ. for the purest specimens of freshly-distilled methyl ester in alcohol was 1317, corresponding with a molecular extinction coefficient ε 24,000. Hausser, Kuhn, Smakula, and Hoffer (*Z. physikal. Chem.*, 1935, **29**, *B*, 371) found that, in alcohol at 254 mμ., sorbic acid has ε 24,800, a value with which our determination is in satisfactory general agreement.

Deca-2 : 4-dienoic acid, peculiar so far as is known to the glycerides of stillingia (*Sapium*) seed oils, appears to form about 5% (by weight) or about 8% (by mol.) of the total fatty acids present. An account of the proportions of the component fatty acids present in seed oils from *Sapium* species of known origin will be published elsewhere, with, it is hoped, some data as to the mode of combination of the conjugated diene acid with the higher fatty acids in the glycerides of the oils.

Partial Hydrogenation of Methyl Deca-2 : 4-dienoate.—The hydrogenation of conjugated ethenoid systems in an aliphatic chain has been relatively little investigated, except in the case of esters of elæostearic acid (Boeseken *et al.*, *Rec. Trav. chim.*, 1927, **46**, 629; 1930, **49**, 247; Steger, van Loon, and van Vlimmeren, *Fette u. Seifen*, 1944, **51**, 49; Hilditch and Pathak, *Proc. Roy. Soc.*, 1949, *A*, **198**, 323). It was therefore of interest to examine the products of semi-hydrogenation of the conjugated decadienoic ester. A portion of the purified ester was allowed to combine with half the volume of hydrogen necessary for complete saturation in presence of Raney nickel at 110°, its extinction coefficient $E^{1\%}_{1\,\text{cm.}}$ at 264 mμ. having then fallen from the original value of 1317 to 216. The product was fractionally distilled (for details see Experimental) into five fractions, the middle one representing about 64% of the total product. Unchanged diene ester was determined in each fraction from the observed values of $E^{1\%}_{1\,\text{cm.}}$ at 264 mμ. Completely hydrogenated (*n*-decoic) ester was determined by quantitative oxidation of the fractions with potassium permanganate in acetone, and concurrently the amounts of monobasic acids produced by the oxidation of the mono- and di-ethylenic esters present were determined from the equivalents of the fractions obtained when they had been fractionally distilled. From the proportions of hexoic, heptoic, and octoic acids so determined, an approximate measure of the amounts of the three possible decenoic esters present in the semi-hydrogenated ester was obtained. It was found that, at the half-way stage, about 15% each of unchanged diene ester and of completely hydrogenated ester (decoate) was present, about 40% of the product was methyl dec-3-enoate, and the remaining 30% consisted of the isomeric Δ^2- and Δ^4-monoethylenic esters. The hydrogenation is largely selective in character in that mono-ethylenic esters form about 70% of the half-hydrogenated ester, and that, of these, over half consists of the dec-3-enoate, *i.e.*, over half reacts by 1 : 4-addition of hydrogen to the conjugated diene system; but in the remaining molecules hydrogen appears to have united with one or other of the double bonds of the original diene system.



Farmer and Hughes (*J.*, 1934, 304, 1929) found that, whilst fixation of 1 mole of hydrogen by sorbic acid in presence of newly-prepared platinum catalyst led mainly to the production of hexoic and unchanged sorbic acid with relatively little formation of hexenoic acids, the employment of aged platinum catalysts caused the production of up to 44% of hexenoic acids (36% of hex-2-enoic acid) in some instances; the use of Raney nickel, and still more so of palladium, as catalysts also resulted in the predominant formation of the dihydro-derivatives (apparently mainly hex-2-enoic acid).

EXPERIMENTAL.

Isolation of Decadienoic Acid from Stillingia Oil.—A good commercial specimen of stillingia oil (1200 g.) was cautiously hydrolysed with only a slight excess of alcoholic potassium hydroxide, and the mixed acids were liberated from the soaps and converted into methyl esters by heating them under reflux for a short time in methyl alcohol in presence of a trace of sulphuric acid. The neutral methyl esters (1143·5 g.) were distilled through an electrically-heated and packed column under 0·2 mm. pressure, the following lowest-boiling fractions being obtained: (*a*) 42·2 g., b. p. 84—96°, $E^{1\%}_{1\,\mathrm{cm.}}$ 1118 at 264 mμ.; (*b*) 8·1 g., b. p. 96—110°, $E^{1\%}_{1\,\mathrm{cm.}}$ 676 at 264 mμ.; (*c*) 4·3 g., b. p. 110—125°, $E^{1\%}_{1\,\mathrm{cm.}}$ 417 at 264 mμ.; and (*d*) 11·8 g. (left in column), $E^{1\%}_{1\,\mathrm{cm.}}$ 102 at 264 mμ.

Fractions (*b*), (*c*), and (*d*) were re-combined and re-fractionated, giving 10·0 g., b. p. 90—94°, $E^{1\%}_{1\,\mathrm{cm.}}$ 1276 at 264 mμ.; this fraction was united with fraction (*a*), and the mixture re-distilled at 0·2 mm. pressure through the column, whereupon the following fractions were obtained :

G.	B. p./0·2 mm.	$E^{1\%}_{1\,\mathrm{cm.}}$ at 264 mμ.	G.		$E^{1\%}_{1\,\mathrm{cm.}}$ at 264 mμ.
2·7	86°	1154	3·0	Left in column	1279
37·7	86	1317	4·7	Residue	167
1·0	86—90	1359			

Other specimens of the ester were similarly obtained by working up further batches of stillingia oil. The purified ester was a colourless mobile liquid with a characteristic, slightly pungent odour, quite different in character from that of the corresponding saturated ester. It was unstable when kept in presence of air and light; when it was heated with alcoholic potassium hydroxide a strong yellow colour of varying intensity always developed, but it is considered that this is a secondary effect caused by the presence of traces of autoxidised compounds since it appeared to be minimal when the ester was freshly distilled. The corresponding acid is still less stable and rapidly commences to autoxidise and polymerise Several analyses led to low values for carbon (cf. p. 3353). Tests for the presence of aldehyde or ketone groups gave negative results, and the ester possessed no acetyl value, indicating the absence of alcoholic hydroxyl groups.

The methyl ester and the free acid exhibit a single broad absorption band in the ultra-violet spectrum (figure) with a well-defined head at 264 mμ. (ester) or 260 mμ. (acid). The extinction coefficient (mean of six determinations) of the purest specimens of the methyl ester (in alcohol) at 264 mμ. was $E^{1\%}_{1\,\mathrm{cm.}}$ 1317 (molar extinction coefficient ε 24,000). When kept at 0° under nitrogen for a few days the ester developed a slight yellow colour, and the value of $E^{1\%}_{1\,\mathrm{cm.}}$ fell by about 30 units; it remains unchanged in these respects, however, indefinitely when kept at −70°. When the ester is heated with an excess of alkali in ethylene glycol at 170° or 180° (as in the spectroscopic determination of linolenic and linoleic acids), the band at 260 mμ. gradually disappears and a new band appears at 238 mμ. ($E^{1\%}_{1\,\mathrm{cm.}}$ 1056 after heating at 180° for 1 hour with potassium hydroxide in ethylene glycol); the cause of this has not been traced, although it is consistent with decarboxylation of a diene acid and production of a conjugated diene hydrocarbon. The absorption band of the original ester is similar in position and molar extinction coefficient to that of sorbic acid.

Iodine-absorption methods are of little value in characterising deca-2 : 4-dienoic acid or esters, as would be expected in view of the presence, not only of a conjugated diethenoid system, but also of the contiguity of a carboxyl group. The methyl ester gave somewhat variable results both with Wijs reagent (iodine value *ca.* 130, ½ hour's contact) and by the Toms bromine-vapour absorption method [Found: *ca.* 240. $C_{11}H_{18}O_2$ (2 double bonds) requires 279]. Similarly, in the semi-hydrogenated ester, distillate fractions 3 and 4 (p. 3356), in which methyl dec-2-enoate as well as the deca-2 : 4-dienoate are present, possess iodine values (Wijs) which fail to correspond with the unsaturation shown to be present in the respective fractions.

Constitution of the Unsaturated C_{10} *Acid.—Hydrogenation of the methyl ester.* The purified methyl ester (20·3 g.; $E^{1\%}_{1\,\mathrm{cm.}}$ 1317 at 264 mμ.), hydrogenated in presence of Raney nickel at 110°, absorbed 4300 ml. of hydrogen in 40 minutes and continued to absorb hydrogen less rapidly during the next 60 minutes, the total absorption being 5100 ml. (measured at 15°). This corresponds with the fixation of 1·95 moles of hydrogen per mole of ester. The product (18·6 g.) yielded on distillation 17·6 g. of a liquid ester which on hydrolysis gave an acid which, after crystallisation from ether at −45°, melted sharply at 31—31·5° (unchanged when mixed with authentic *n*-decoic acid) (cf. p. 3353).

Oxidation of the methyl diene ester. The ester (18 g.) was dissolved in acetone (180 ml.) and oxidised with powdered potassium permanganate (180 g.), the solution being gently refluxed for 2 hours after all the permanganate had been added. After removal of the acetone and decolorisation of the aqueous solution of the acidified products of oxidation, the lower fatty acids were removed by distillation with steam, recovered from the condensate, and, after being dried in ethereal solution, were distilled through a semimicro-fractionating column with the following results :

Fraction.	G.	B. p./760 mm.	Equiv.	Fraction.	G.	B. p./760 mm.	Equiv.
1	0·13	70—110°	600	6	1·13	198°	113·9
2	0·68	110—140	99·7	7	0·46	200	116·4
3	0·72	160—184	110·3	8	0·17	200—190	121·6
4	2·26	184—188	113·1	9	0·45	Residue	217·9
5	0·99	194	114·3				

Total wt. : 6·99 g.

The equivalents show that, apart from traces of ether, water, and small proportions of acids of lower molecular weight than hexoic, the main component approximated exclusively to a molecular weight of 114—116 (hexoic acid, 116). Fractions 5 and 6 gave a *p*-toluidide, m. p. 74·5—75·5° which, when mixed with an authentic specimen (m. p. 76°) prepared from synthetic *n*-hexoic acid, melted at 74·5—76°, and a *p*-bromophenacyl ester of m. p. 70·5—71·5° which remained unchanged when mixed with an authentic specimen (m. p. 70·5—72°).

There is little difference in the melting points of the *p*-toluidides and *p*-bromophenacyl esters of *n*-hexoic and *n*-decoic acid (above); but mixed melting points of, *e.g.*, the *p*-toluidides of *n*-hexoic and *n*-decoic acid gave depressions of about 30°. The identity in these cases is established almost entirely by the behaviour on admixture with known derivatives rather than by the specific melting point of any single derivative.

*Semi-hydrogenation of Methyl Deca-*2 : 4-*dienoate.*—The ester (24·4 g.) was hydrogenated at 110° in presence of Raney nickel until approx. 1 mole (3050 ml.) of hydrogen per mole of ester had been absorbed. The filtered product (which had $E^{1\%}_{1\,\text{cm.}}$ 216 at 264 mμ.) was then fractionally distilled under reduced pressure giving :

Fraction.	G.	$E^{1\%}_{1\,\text{cm.}}$ at 264 mμ.	Iodine value (Wijs, ½ hr.).	Saturated ester (Me decoate), %.
1	0·69	5	87·5	34·0
2	3·20	18	76·0	
3	14·68	171	56·1	11·5
4	3·61	551	63·7	6·0
5	0·84	672	93·1	

Fractions 1 and 2, and 4 and 5, were combined and these two groups, and also fraction 3, were quantitatively oxidised in acetone solution with excess of powdered permanganate; any completely hydrogenated methyl decoate remained unchanged and was recovered and weighed, whilst the monobasic acids produced by oxidative scission of the decenoic and decadienoic esters present were also recovered and submitted to micro-fractionation with the following results :

Fraction 1 + 2 : 3·18 g. yielded 1·08 g. of saturated ester (34·0%).
,, 3 : 13·11 g. ,, 1·51 g. ,, ,, (11·5%).
,, 4 + 5 : 3·20 g. ,, 0·19 g. ,, ,, (6·0%).

Monobasic acid (C_6, C_7, C_8) *fractions obtained from oxidation of fractions* 1—2, 3, *and* 4—5. The acid oxidation products yielded the following fractions :

Fractions 1–2.			Fraction 3.			Fraction 4–5.		
G.	B. p.	Equiv.	G.	B. p.	Equiv.	G.	B. p.	Equiv.
0·09	160°	113·7	0·31	204°	127·5	0·17	190°	119·1
0·28	160—180	112·4	0·56	204—206	120·3	0·29	190—207	118·4
0·36	180—205	122·9	0·56	206	125·7	0·37	207—210	127·2
0·13	205—210	129·1	0·59	206—210	128·9	0·37	210—215	134·6
			0·41	210—212	133·7	0·15	213	139·8
			0·47	212	136·5			
			0·48	214	139·5			
			0·41	212	142·0			

The lowest-boiling fractions of group 1—2 contained traces of acids lower than hexoic. The rest of the acids conformed with mixtures of hexoic, heptoic, and octoic, corresponding respectively with the scission-products of dec-2, -3-, and -4-enoic acids. The presence of *n*-heptoic acid was not conclusively established, but the steady increase in molecular weight (*cf.*, especially, the third—sixth fractions of group 3) differs quite definitely from the more abrupt change between about 125 and 135 which is characteristic in similar fractionations of hexoic and octoic acids alone (*e.g.*, in the lower fatty acids of milk fats). The *p*-bromophenacyl ester of the fourth fraction in group 3 (equiv., 128·9; corresponding with a mixture of about 90% of heptoic and 10% of hexoic acid) was prepared, but its melting point could not be raised above 62° (m. p. of *p*-bromophenacyl ester of synthetic *n*-heptoic acid, 71°), probably owing to the impossibility of separating the admixed ester of hexoic (and possibly also traces of octoic) acid present; the melting point (62°) of the ester was raised to 65—66° on admixture with synthetic *p*-bromophenacyl *n*-heptoate (m. p. 71°) and depressed to 48—53° by admixture with synthetic *p*-bromophenacyl *n*-hexoate (m. p. 70·5—71·5°).

An approximate estimate of the proportions of hexoic, heptoic, and octoic acids can, however, be obtained on the assumption that each of the separated fractions is a binary mixture of the three homologues. The proportions of these acids, and those of the corresponding decenoic acids from which they were produced, can thus be roughly given as follows :

				Corresponding decenoic acids,		
Semi-hydrogenated ester.	Hexoic, %.	Heptoic, %.	Octoic, %.	-4-. %.	-3-. %.	-2-. %.
Fractions 1–2	62	38	—	65	35	—
Fraction 3	21	51·5	27·5	23·5	51·5	25
Fractions 4–5	33	49·5	17·5	36	48	16

The approximate composition of the esters in the original fractions 1—5 of semi-hydrogenated methyl deca-2 : 4-dienoate can then be deduced as follows :

fully hydrogenated methyl decoate from the oxidation data above;
unchanged methyl deca-2 : 4-dienoate from the spectroscopic data;
methyl dec-4-enoate, from the observed proportions of hexoic acid after deducting the amount of hexoic acid produced from the unchanged diene ester;
methyl dec-2- and -3-enoates, from the proportions respectively of octoic and heptoic acids.

The approximate data thus obtained are :

Semi-hydrogenated ester.		Decoate.	Decenoates,			Deca-2 : 4-dienoate.
			-4-.	-3-.	-2-.	
	g.	g.	g.	g.	g.	g
Fractions 1–2	3·89	1·33	1·62	0·90	—	0·04
Fraction 3	14·68	1·69	1·16	6·68	3·24	1·91
Fractions 4–5	4·45	0·26	—	2·03	0·65	1·51
Total weights	23·02	3·28	2·78	9·61	3·89	3·46
	100·0%	14%	12%	42%	17%	15%

We wish to mention that Dr. H. C. Dunn was responsible for earlier preliminary work on stillingia oil, which led up to the results now recorded, and to express our thanks to him. We thank the Colonial Products Research Council for a grant to one of us (A. C.), and for authority to publish this paper.

THE UNIVERSITY, LIVERPOOL.

[*Received, August* 27*th*, 1949.]