123. *Vegetable Oils. Part VI.* The Component Acids of Ergot Oil.*

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Despite earlier contradictory reports ergot oil contains an unsaturated hydroxy-acid (34%) along with palmitic (24%), oleic (21%), and linoleic (12%) acids; hexadecenoic, stearic, myristic, and arachidic acids are minor components. The acids are not present entirely as triglycerides but there is some esterification of the hydroxyl group of the hydroxy-acid. The hydroxy-acid is shown to be identical with ricinoleic acid.

In continuation of our study of fats believed to contain hydroxy-acids we have examined ergot oil. Ergot contains about 30% of a fatty oil. In the earliest reference [1] the oil is stated to have a high acetyl value (63) indicating the presence of a hydroxy-acid which, however, was not isolated. Subsequently, several authors [2-8] have referred to an acetyl value or to the presence of a hydroxy-acid but Dieterle, Diester, and Thimann [9] and Baughman and Jamieson [10] state that they could find no evidence of a hydroxy-acid. Matthes and Schütz [3] and Marqués and Rodríguez [8b] have also drawn attention to the optical activity of the oil and of the mixed acids derived from it but only Matthes and Kürscher [4] reported any evidence for the structure of the hydroxy-acid which they believe to be ricinoleic [D-(+)-12-hydroxyoctadec-*cis*-9-enoic †] acid; this conclusion is based on the isolation of azelaic acid and (—)-β-hydroxypelargonic acid on ozonolysis and subsequent oxidation. We have now confirmed this result by alternative procedures.

Table 1. *Characteristics of ergot oil.*

	n^t/t	Sapon. equiv.	Iodine value	Acetyl value
Mjöen [a]	—	314·5	71·1	62·9
Rathje [b]	1·4685	312·9	74·0	27·4
Gander and Zellner [c]	—	286·0	—	—
Matthes and Schütz [d]	1·4694/20°	287·2	70·1	—
Dieterle *et al.* [e]	1·5420/40°	290·2	66·5	—
Baughmann and Jamieson [f]	1·4691/25°	284·9	73·8	7·3
Novák [g]	—	302·4	71·6	—
Caines [h]	—	301·8	—	—
Present investigation :				
Oil (i)	1·4748/20°	282·4	75·7	—
Oil (ii)	1·4730/20°	280·0	69·6	—
Oil (iii)	1·4730/20°	322·8 *	71·3	—

(* This oil contains 13·4% of unsaponifiable material.)

[a] Ref. 1. [b] Ref. 2. [c] Ref. 2. [d] Ref. 3. [e] Ref. 9. [f] Ref. 10. [g] Novák, *Magyar Gyógyszerészt. Társaság Értesítõge*, 1942, **18**, 342. [h] Caines, *Mfg.-Chemist*, 1948, **19**, 447.

Three samples of ergot oil were used and after removal of a little insoluble impurity and of some volatile solvent they had the characteristics shown in Table 1. These suggest that, apart from variable amounts of unsaponifiable material, the oils do not differ greatly from samples which have been investigated previously.

* Part V, *J. Sci. Food Agric.*, 1956, 7, 606.

† Geneva numbering, CO_2H = 1.

[1] Mjöen, *Arch. Pharm.*, 1896, **234**, 278.
[2] Rathje, *ibid.*, 1908, **246**, 692; Gander and Zellner, *Seife*, 1921, **6**, 411.
[3] Matthes and Schütz, *Arch. Pharm.*, 1927, **265**, 541.
[4] Matthes and Kürscher, *ibid.*, 1931, **269**, 88.
[5] Bodendorf and Reichner, *ibid.*, 1932, **270**, 291.
[6] Fiero, *J. Amer. Pharm. Assoc.*, 1933, **22**, 608.
[7] Vandermeulen, *J. Pharm. Belg.*, 1939, **21**, 195, 213, 237.
[8] Marqués and Rodríguez, *Anal. Fis. Quím.*, (*a*) 1948, **44**, *B*, 467; (*b*) 1949, **45**, *B*, 433; (*c*) 1949, **45**, *B*, 89.
[9] Dieterle, Diester, and Thimann, *Arch. Pharm.*, 1927, **265**, 171.
[10] Baughman and Jamieson, *Oil and Fat Ind.*, 1928, **5**, 85.

A detailed study of oil (i) and a less detailed examination of oil (ii) have been made.

The largest sample was examined by a procedure previously described by us.[11] Briefly, the mixed acids freed from unsaponifiable matter were partitioned between light petroleum and aqueous methanol and were divided thereby into two fractions in which the non-hydroxy- and the hydroxy-acids (fraction *C*) were separately concentrated, the former was further divided into concentrates of saturated (fraction *A*) and unsaturated acids (fraction *B*) by low-temperature crystallisation. Fractions *A* and *B* were subsequently esterified, distilled, and analysed by the usual procedures whilst the composition of fraction *C* followed from the equivalent before and after acetylation. The results are given in Table 2 along with values given by previous investigators and with values obtained for oil (ii) from certain composite values measured on the mixed acids and esters. Our results clearly indicate that an unsaturated hydroxy-acid is present to the extent of 34%, a value which agrees well with those of Matthes and Schütz,[3] Fiero,[6] and Vandermeulen.[7] The oil also contains about 30% of saturated acids (almost entirely palmitic), oleic acid, and linoleic acid. Our investigation reveals, for the first time, the presence of hexadecenoic acid as a minor component. The identity of the acids has been confirmed in the usual way and the unsaturated hydroxy-acid has been shown to be ricinoleic acid, identical with that present in castor oil.

TABLE 2. *The component acids of ergot oil.*

	Saturated				Unsaturated			
	C_{14}	C_{16}	C_{18}	C_{20}	C_{16} (−2·0 H)	C_{18} (−2·0 H)	C_{18} (−4·0 H)	Ricinoleic
Oil (i)	1·0	24·0	3·0	1·0	4·0	21·0	12·0	34·0
Oil (ii)	——	27 *	——		——	24 ——	10	39
Rathje [a]	—	5	—	—	—	72	—	23
Matthes & Schütz [b]	——	29	——		—	32	4	35
Dieterle *et al.* [c]	——	21	——		—	74	5	—
Baughman & Jamieson [d]	Tr	22	5	1	—	63	9	—
Bodendorf & Reichner [e]	—	—	—	—	—	—	—	47
Fiero [f]	3	25	2	—	—	21	13	36
Vandermeulen [g]	—	30	12	—	—	23	Tr	35

[All values are given to the nearest unit-% except for oil (i) where the values are given to the nearest 0·5%.]

* Including any unsaponifiable material.

[a] Ref. 2. [b] Ref. 3. [c] Ref. 9. [d] Ref. 10. [e] Ref. 5. [f] Ref. 6. [g] Ref. 7.

Marqués and Rodríguez have concluded from the variation in reported acetyl values,[8a] by comparison of the optical activity of the oil with that of the derived acids,[8b] and by comparison of its behaviour on vacuum distillation with that of castor oil,[8c] that some part of the ricinoleic acid is acylated with another fatty acid molecule. We have confirmed this for our main sample. In a preliminary examination the mixed acids free from unsaponifiable matter had a saponification equivalent of 280·1 and this fell to 223·4 after acetylation (this fall of 56·7 units indicates the presence of 33% of hydroxy-acid); with the oil, however, the equivalent fell by only 17·2 units. This shows that the oil contains fewer free hydroxyl groups than the mixed acids. A similar phenomenon has been reported for kamala oil containing 18-hydroxyelæostearic acid.[12] (It has been stated [13] that acetylation of hydroxy-acids leads to mixed anhydrides which are incompletely hydrolysed with water; though normally we prefer to use esters we have not encountered this difficulty with acids and have frequently obtained similar results with acids and with esters after making suitable allowance for the presence of the ester group.)

The unsaturated hydroxy-acid was next examined by using a portion of fraction *C* which contains 98% of the hydroxy-acid. The position of the hydroxyl group was

[11] Bharucha and Gunstone, *J. Sci. Food Agric.*, 1955, **6**, 373.

[12] von Mikusch, *Deutsch. Farb.-Z.*, 1954, **5**, 166; O'Neill, Dennison, and Ahlers, *Chem. and Ind.*, 1954, 756.

[13] Meara, "Modern Methods of Plant Analysis," Springer-Verlag, Berlin, 1955, Vol. II, p. 341.

determined by Baruch's method.[14,15] The unsaturated acid was first hydrogenated and then oxidised to an oxostearic acid, the *syn*- and *anti*-oximes of which, when submitted to Beckmann rearrangement, afforded two amides hydrolysed to (i) a monobasic acid and an ω-amino-acid and (ii) an aliphatic amine and a dibasic acid. Three of these—heptanoic acid, hexylamine, and dodecanedioic acid—were identified, indicating that the unsaturated acid has been converted into 12-oxostearic acid and was therefore a derivative of 12-hydroxystearic acid. The iodine value of the hydroxy-acid and the hydrogen uptake on reduction indicate the presence of one double bond and since oxidation affords azelaic and heptanoic acids this must be in the 9 : 10-position (the hydroxyl group having already been shown to be present on $C_{(12)}$). The hydroxy-acid is thus 12-hydroxyoleic acid and its identity with ricinoleic [D-(+)-12-hydroxyoctadec-*cis*-9-enoic] acid is derived from the following observations : (i) the optical rotation of the crude acid is very close to that recorded for ricinoleic acid—the important point is that it has the same sign of rotation and is therefore the same optical isomer, (ii) oxidation by dilute alkaline potassium permanganate gives two 9 : 10 : 12-trihydroxystearic acids identical in melting point and optical rotation with similar compounds obtained from the mixed acids of castor oil.[16]

Experimental

Ergot Oils.—Gifts of ergot oil were received from Burroughs Wellcome and Co. [oil (i)], Carnegies [oil (ii)], and Roussel Laboratories Ltd. [oil (iii)]. All were dark greenish-brown, had a strong disagreeable odour, and contained insoluble impurity and volatile solvent which were removed before investigation. The characteristics of the oils are reported in Table 1 but the following were also determined : free acid (as % of oleic acid) 8·6 (i); unsaponifiable matter 2·4% (i), 13·4% (iii); absorption ($E^{1\%}_{1\,cm.}$) at 234 mμ after alkali isomerisation at 180° for 60 min. 115·5 (i), 87·0 (ii), 97·2 (iii). [The last determination was made on mixed acids, freed from unsaponifiable material from oils (i) and (iii).]

Component Acids of Ergot Oil.—To 1 l. of light petroleum (b. p. 40—60° *) in each of three separating funnels were added about 40 g. (total 117 g.) of mixed ergot acids [from oil (i)]; 400 ml. of the same solvent were placed in two other funnels. 80% Methanol * (400 ml.) was added to the first funnel and, after equilibrium, was passed to each of the other four funnels in turn. Eight portions of methanol extracted 38·3 g. (32·5%) of acids (6·4 + 9·2 + 6·9 + 5·5 + 4·0 + 2·2 + 2·3 + 1·8 g.; Fraction *C*). The acids remaining in the light petroleum were crystallised from methanol (10 ml. per g. of acid) at −20° whereupon 43·3 g. (36·9%; Fraction *B*) remained in solution and 35·9 g. (30·6%; Fraction *A*) crystallised. Fractions *A*, *B*, and *C* had iodine values of 9·5, 119·0, and 85·5, respectively.

Table 3. *The component acids of ergot oil (i).*

	Fraction				Exclg. unsapon.	
Acid	*A*	*B*	*C*	Total	% (wt.)	% (mol.)
Myristic	0·39	0·46	—	0·85	0·9	1·1
Palmitic	22·95	0·76	—	23·71	23·9	26·1
Stearic	3·15	—	—	3·15	3·2	3·1
Arachidic	0·91	—	—	0·91	0·9	0·8
Hexadecenoic	0·24	3·50	—	3·74	3·8	4·1
Oleic	2·89	17·83	—	20·72	20·9	20·6
Linoleic	—	12·18	—	12·18	12·3	12·2
Ricinoleic	—	1·97	31·95	33·92	34·1	32·0
Unsaponifiable	0·07	0·20	0·55	0·82	—	—

Fraction *A* was methylated with boiling methanol containing a little concentrated sulphuric acid, and the esters were fractionally distilled; from the iodine value and saponification

* These solvents were previously equilibrated by being shaken together.

[14] Baruch, *Ber.*, 1894, **27**, 172.
[15] Cf. Gunstone, *J.*, 1952, 1274, and references cited there.
[16] Kass and Radlove, *J. Amer. Chem. Soc.*, 1942, **64**, 2253.

equivalent of each ester fraction the composition of Fraction *A* was determined in the usual way. Fractionation data are not reproduced but the final results are given in Table 3.

After methylation with methanolic hydrogen chloride at room temperature and acetylation with boiling acetic anhydride * the acetylated esters of Fraction *B* were distilled and the composition of each ester fraction determined from the iodine value, saponification equivalent, and from ultraviolet absorption after alkali isomerisation of selected fractions. The details are not reproduced but the final results are given in Table 3.

From the saponification equivalent of Fraction *C* acids before (297·6) and after (171·8) acetylation it follows that this fraction contains 97·2% of hydroxyoctadecenoic acid; the values for the corresponding methyl esters (310·3 and 177·0) indicate 99·4% of hydroxy-acid. The mean value (98·3%) was taken, and since Fraction *C* also contains 1·7% of unsaponifiable material no other acids are present. (All the equivalents quoted here are the mean of four determinations as recommended by Riley.[17]) These results are included in Table 3 where the values are summed to give the composition of this sample of ergot oil.

Palmitic (m. p. 63—63·5°) and stearic (m. p. 69—70°) acid were isolated from appropriate ester fractions and identified by melting point and mixed melting point. A concentrate of hexadecenoic acid was oxidised by dilute alkaline potassium permanganate [18] to *erythro*-9 : 10-dihydroxypalmitic acid, m. p. 126—127°, identical with an authentic specimen. A concentrate of oleic acid, obtained as a complex with urea from a fraction rich in unsaturated C_{18} acids, was similarly oxidised to *erythro*-9 : 10-dihydroxystearic acid, m. p. 128·5—130° (a mixture with an authentic specimen had m. p. 130—130·5°). Bromination of an unsaturated C_{18} acid mixture gave 9 : 10 : 12 : 13-tetrabromostearic acid the m. p. (113·5—114°) of which was identical with that of a mixture with a pure sample.

The composition of oil (ii), reported in Table 2, is derived from the following values: iodine value of the oil (69·6), absorption ($E^{1\%}_{1cm.}$ = 87·0) at 234 mμ after alkali isomerisation (180°/60 min.) of the mixed acids, and equivalent of the mixed esters before (295·1) and after (226·4) acetylation.

The Structure of the Unsaturated Hydroxy-acid.—(a) Determination of the position of the hydroxy-group. A portion (4 g.) of fraction *C* acids was hydrogenated in the presence of 5% palladium–charcoal, the hydrogen uptake indicating the presence of 1·0 double bond. The m. p. of the resulting hydroxystearic acid (m. p. 78—80° after crystallisation from ether) was identical with that of a mixture with 12-hydroxystearic acid derived from castor oil but depressed to 71—74° when mixed with 9-hydroxystearic acid (m. p. 81—82·5°).[15]

The hydroxystearic acid (4·36 g.) in acetic acid (44 ml.) was oxidised with a 10% solution (11 ml.) of chromium trioxide in acetic acid at room temperature for 30 min. The product (4·13 g.) which separated after dilution of the sulphur dioxide-decolorised solution melted, after crystallisation from ether–light petroleum (b. p. 40—60°), at 82·5—83°. This value was unchanged when mixed with 12-oxostearic acid but depressed (73—77°) on admixture with 9-oxostearic acid (m. p. 79·5—81°).

The mixed oximes (4·7 g.) resulting from boiling an alcohol solution of the oxo-acid (4·6 g. in 72 ml.) with an aqueous (18 ml.) solution of hydroxylamine hydrochloride (4 g.) and sodium acetate (6 g.) for 2 hr., were rearranged (Beckmann) during 1 hr. at 100° with concentrated sulphuric acid (30 ml.) and the resulting amides hydrolysed by refluxing for 3 hr. after addition of water (37 ml.—thereby altering the solvent to 60% sulphuric acid). After further dilution (200 ml.) the solution was steam-distilled and the distillate (750 ml.) extracted with ether (5 × 200 ml.) giving crude monobasic acid (0·44 g.); the distillation residue similarly extracted gave crude dibasic acid (2·64 g.). The aqueous residue from this extraction was made alkaline (50% potassium hydroxide solution) and steam-distilled, the distillate (1 l.) after extraction (5 × 200 ml. of ether) afforded a volatile amine (0·28 g.). Attempts to isolate the amino-acid present in the distillation residue were unsuccessful.

The monobasic acid was largely heptanoic acid since the melting point of its *p*-bromophenacyl ester (66—68°) was raised (67—70·5°) when mixed with an authentic sample but depressed (62—63°) when mixed with the ester of hexanoic acid.

The crude dibasic acid afforded a purer sample (0·51 g.; m. p. 127—129°) after extraction with boiling water (15 × 100 ml.), concentration to 30 ml., and cooling to 0°. This did not depress the m. p. of dodecanedioic acid.

* Full experimental details are given in Ref. 11.

[17] Riley, *Analyst*, 1951, **76**, 40.
[18] Lapworth and Mottram, *J.*, 1925, **127**, 1628.

The volatile amine was *n*-hexylamine since it formed a derivative with phenyl *iso*thiocyanate (m. p. 75·5—77°) identical with a similar derivative (m. p. 76·5—77·5°) prepared from the commercial amine.

(*b*) *Determination of the position of the double bond.* The hydroxy-acid (4 g.; fraction *C*) dissolved in acetic acid was oxidised by the gradual addition of powdered potassium permanganate (16 g.) at such a rate that the temperature rose to but did not exceed 50°. After 3 hr. at this temperature the solvent was removed under reduced pressure and the residue diluted with dilute sulphuric acid (300 ml.), decolorised with sulphur dioxide, and then steam-distilled. Both the residue and the distillate (1 l.) were extracted with ether (5 × 200 ml.) to give crude dibasic acid (2·98 g.) and crude monobasic acid (0·89 g.), respectively. The former was extracted with boiling water (8 × 100 ml.) and after concentration of the solution to 25 ml. and cooling to 0° gave azelaic acid (0·98 g.), m. p. 106—107·5° (from ethyl acetate) undepressed with an authentic specimen. The volatile acid when distilled (11 mm.) gave two fractions (0·20 g. and 0·25 g.) both of which readily gave *p*-bromophenacyl heptanoate, m. p. 69—70° (raised to 69·5—71° with the ester of heptanoic acid, depressed to 63—64° with the derivative of hexanoic acid).

(*c*) *Identity with ricinoleic acid.* Fraction *C* acids, freed from unsaponifiable material, had $[\alpha]_D^{18\cdot5}$ +9·3° (2 dm.; 5·03% solution in acetic acid). [This value compares with those of +7·86° (21°; no solvent),[19] +7·15° (26°; acetone),[20] and +7·79° (25°; no solvent),[21] the difference probably being due largely to the different solvents used.]

The unsaturated hydroxy-acid (1·57 g.) when oxidised with cold dilute alkaline potassium permanganate[16,18] gave mixed trihydroxystearic acids (1·58 g.). The fraction (1·26 g.) insoluble in light petroleum (b. p. 80—100°) was extracted with chloroform (3 × 25 ml.) and left some insoluble acids (0·76 g.) which after two crystallisations from ethanol had m. p. 136·5—137·5°, $[\alpha]_D^{18\cdot5°}$ −10·7° (2 dm.; 1·174% solution in acetic acid) [lit.[21] 138°, −11·6° (23°; acetic acid)]. The chloroform-soluble acid (0·23 g.) after crystallisation from aqueous ethanol had m. p. 110—111·5°, $[\alpha]_D^{18\cdot5}$ −6·7° (2 dm.; 0·601% solution in acetic acid) [lit.[21] 112°, −6·6° (23°; acetic acid)]. Both samples of 9 : 10 : 12-trihydroxystearic acid were identical in m. p. and mixed m. p. with specimens prepared from castor oil.

The authors thank Burroughs Wellcome & Co., Carnegies, and Roussel Laboratories Ltd. for gifts of ergot oil, and one of them (K. E. B.) is indebted to the University of St. Andrews and the J. N. Tata Endowment for the Higher Education of Indians (Bombay) for financial assistance.

CHEMISTRY DEPARTMENT, UNIVERSITY OF ST. ANDREWS. [*Received, September 12th*, 1956.]

[19] Straus, Heinze, and Salzmann, *Ber.*, 1933, **66**, 631.
[20] Brown and Green, *J. Amer. Chem. Soc.*, 1940, **62**, 738.
[21] Hawke, *J. S. African Chem. Inst.*, 1949, **2**, 125.