STUDIES ON TROMBIDIUM TINCTORUM LINN.

Part .I—Chemical Constitution of the Fat of Trombidium Tinctorum Linn.

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Various physico-chemical characteristics of the fat of *Trombidium tinctorum* Linn. have been studied. Relative proportions of solid, liquid and steam-volatile acids have been determined. The water-soluble and the water-insoluble steam-volatile acids were studied as such. The various solid and liquid fatty acids, separated by Twitchell's lead salt alcohol method, were determined after fractionation of their methyl esters under reduced presssure.

Introduction

The species *Trombidium tinctorum* Linn. (local name, bir bohiti) belongs to the members of the family *Trombidiidae* and is known as scarlet mites to the horticulturists. Its phylum is *Arthropoda*, order *Acarida* and class *Arachnida*.The members of this class are in fact spiders. It has been wrongly classified as cochineal insects by several authors.[1,2] The mites were identified as *Trombidium tinctorum* Linn. by Akhtar[3] in Pakistan and Evans[4] in England.

Interest in the investigations on *Trombidium tinctorum* Linn. has been evoked by the fact that the mites in the dried form are administered in the indigenous system for respiratory and reproductive ailments. The mites are reputed to possess nerve stimulating properties in Auyervedic and Unani schools of medicine. Their colouring matter is reported to possess sedative and anti-spasmodic properties. It is also useful in whooping cough, neuralgia, etc.

These mites become plentifully available during the rainy season, when they were hand-picked and preserved in rectified spirit for investigation.

Experimental

Extraction of the Fat.—The mites were extracted exhaustively with petroleum ether (b.p. 50-70°C.) in a Soxhlet apparatus. When most of the colouring matter had been removed, the mites were taken out from the Soxhlet apparatus, crushed in a mortar, and extracted further with petroleum ether (50-70°C.). The solvent was distilled off on a water bath. The residual fat thus obtained was intensely red and had a characteristic odour. It was dried first over anhydrous sodium sulphate and then at a temperature of 75-85°C.

Physical Characteristics.—The determination of various physical constants was carried out by following the standard procedures.[5] These values were: refractive index, 1.4575 (at 32°C.); specific gravity, 0.8923; and tint, equivalent to 9 units of yellow, 11 units of blue and 7 units of red coloured discs of the Lovibond tintometer.

Chemical Values.—The followng chemical values for the oil were determined by the usual methods[6]:—

Saponification value	=	202.50
Iodine value	=	80.10
Thiocyanogen value	=	58.27
Hehner value	=	94.40
Acid value	=	1.59
R.M. value	=	2.585
Polenske value	=	0.65
Kirschner value	=	2.87
Saturated acids (Bertram)	=	42%
Hexabromide value	=	0
Non-saponifiable matter	=	2.6%

Resolution of the Fat into Various Acid Fractions.—Eighty six grams of the fat was saponified with approximately 0.5 N alcoholic caustic potash solution. The alcohol was distilled and the soap dissolved in water. The non-saponifiable matter was extracted with diethyl ether in a continous liquid-liquid extractor. Afterwards the dried soap was thoroughly mixed with acid- washed white sand of 20 mesh and extracted with diethyl ether. This treatment removed almost all the colouring matter in the fat. The soap solution was then decomposed into fatty acids with dilute sulphuric acid. The liberated acids were steam-distilled to recover the steam volatile acids. The steam distillate was treated with ether and the ether-soluble fatty acids were recovered after distilling away the ether. The total amount of steam-volatile acids was determined from the titration of (1) the ether extraction residue, and (2) the ether treated aqueous distillate. The steam non-volatile acids were extracted with ether and dried in 'vacuum' at 100°C. They were separated into 'solid' and 'liquid' acids by Twitchell's lead salt alcohol method as adapted

by Hilditch.[7] Amounts and values of solid, liquid and steam-volatile acids are recorded in Table 1.

TABLE 1.—AMOUNTS AND VALUES OF STEAM NON-VOLATILE AND VOLATILE FATTY ACIDS

	Oil	Total acids	Liquid acids	Solid acids	Volatile acids
Amount	—	100	54.18	42.00	3.82
Saponification value	202.50	200.50	198.50	205.00	592.27
Iodine value	80.10	96.32	108.35	0.60	—

The composition of steam volatile acids was as follows:—

Acids extracted with ether from the steam distillate = 2.48 g.
Saponification equivalent of the acids = 94.72
Calculated amount of butyric acid (C_4) = 1.29 g.
Calculated amount of iso-valeric acid (C_5) = 1.18 g.

Liquid Acids.—The liquid acids were converted into their methyl esters which were fractionally distilled under reduced pressure and at elevated temperature, as recorded in Table 2.

The amount of individual esters and acids in the liquid acid fractions, as indicated in Table 3, was as follows:—

C_{14}	methyl ester	= 3.80 g.
C_{16}	methyl ester	= 20.48 g.
C_{18}	methyl ester	= 8.15 g.
C_{20-22}	methyl ester	= 0.18 g.
	Total	= 32.61 g.
or C_{14}	acid	= 3.67 g.
C_{16}	acid	= 19.40 g.
C_{18}	acid	= 7.76 g.
C_{20-22}	acid	= 0.17 g.
	Total	= 31.00 g.

Solid Acids.—Esterification of the solid acids to methyl esters was accomplished as recommended by Hilditch.[8] Eight successive fractions were collected by distilling the esters under diminished pressure and at elevated temperature, recorded in Table 4.

TABLE 2.—FRACTIONATION OF METHYL ESTERS OF LIQUID ACIDS. WEIGHT OF THE ESTERS DISTILLED = 32.63 g.

Fraction	Pressure	Temp.	Amount
L_1	1mm.	45- 65°C.	11.45 g.
L_2	,, ,,	65- 80°C.	8.12 g.
L_3	,, ,,	80-105°C.	7.00 g.
L_4	,, ,,	105-120°C.	0.82 g.
L_5 (residue)	,, ,,	120°C. falling	3.44 g.
		Total =	30.83 g.
		Loss =	1.80 g.

The loss in distillation was proportionately added to each fraction.

TABLE 4.—FRACTIONATION OF METHYL ESTERS OF SOLID ACIDS. WEIGHT OF ESTERS DISTILLED = 29.92 g.

Fraction	Pressure	Temp.	Amount
S_1	1 mm.	47- 58°C.	2.11 g.
S_2	,, ,,	58- 78°C.	1.04 g.
S_3	,, ,,	78- 88°C.	1.48 g.
S_4	,, ,,	88- 98°C.	4.10 g.
S_5	,, ,,	98-112°C.	4.78 g.
S_6	,, ,,	112-122°C.	6.56 g.
S_7	,, ,,	122-135°C.	4.67 g.
S_8 (residue)	,, ,,	135°C.-falling	4.95 g.
		Total =	29.69 g.
		Loss =	0.23 g.

TABLE 3.—VALUES AND COMPOSITION OF INDIVIDUAL ESTER FRACTIONS OF THE LIQUID ACIDS.

Fraction	Corrected wt. in g.	S.E.	I.V.	Composition			
				C_{14}	C_{16}	C_{18}	C_{20-22}
L_1	12.11	259.22	48.44	3.80	8.31	—	—
L_2	8.59	271.57	54.31	—	7.49	1.09	—
L_3	7.40	279.42	79.73	—	4.38	3.02	—
L_4	0.87	286.16	86.92	—	0.30	0.57	—
L_5 (residue)	3.65	297.38	91.87	—	—	3.47	0.18

The loss in distillation was added proportionately to each fraction. Saponification equivalents and iodine values for each fraction were determined from which mean molecular weight for each, fraction was calculated, as recorded in Table 5.

The amount of individual esters and acids in the solid acids fractions was as follows:—

Methyl caproate	=	0.51 g.
Methyl laurate	=	2.979 g.
Methyl myristate	=	2.816 g.
Methyl palmitate	=	17.249 g.
Methyl stearate	=	6.370 g.
Total	=	29.924 g.
or Caproic acid	=	0.46 g.
Lauric acid	=	2.78 g.
Myristic acid	=	2.65 g.
Palmitic acid	=	16.35 g.
Stearic acid	=	6.07 g.
Total	=	28.31 g.

From the data recorded for liquid and solid acids, the composition of the fat can be represented as below:—

Glycerides of		
Butyric acid	=	2.48 %
Iso-valeric acid	=	1.34 %
Caproic acid	=	0.68 %
Lauric acid	=	4.12 %
Myristic acid	=	3.93 %
Palmitic acid	=	24.25 %
Stearic acid	=	9.05 %
C_{14} unsaturated acids	=	6.09 %
C_{16} unsaturated acids	=	32.33 %
C_{18} unsaturated acids	=	13.07 %
C_{20-22} unsaturated acids	=	2.80 %
Total	=	100.00

The component solid acids were calculated from an equation given by Hilditch.[9] The saponification equivalents of all the fractions were determined by the method of Marcali and Reimann III on the decigram scale.[10]

The determination of the individual unsaturated acids, constituting acids of C_{14}, C_{16}, C_{18}, C_{20} and C_{22} carbon contents, is difficult and misleading when based on iodine and thiocyanogen values or on bromo derivatives. This is so because while determining these values of one fraction it is not certain as to whether lower or higher unsaturated acids are present alongwith.

The derivation of the amounts of unsaturated acids is therefore entirely based upon the saponification equivalents of the ester fractions of the liquid acids.

In working out the composition of the fat, the non-saponifiable matter was not taken into consideration as the studies on this part are still in progress and will be followed up in Part II.

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TABLE 5.—VALUES AND COMPOSITION OF INDIVIDUAL ESTER FRACTIONS OF SOLID ACIDS.

Fraction	Corrected wt. in g.	S.E.	I.V.	Composition				
				C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
S_1	2.12	208.00	0.00	0.46	1.66	—	—	—
S_2	1.05	212.60	0.00	0.05	1.00	—	—	—
S_3	1.49	235.66	0.00	—	0.319	1.171	—	—
S_4	4.13	259.22	0.00	—	—	1.590	2.540	—
S_5	4.82	269.321	0.10	—	—	0.055	4.765	—
S_6	6.61	277.74	0.20	—	—	—	4.780	1.83
S_7	4.70	280.54	0.20	—	—	—	2.930	1.770
S_8 (residue)	5.00	285.50	0.50	—	—	—	2.230	2.770
Total:	29.92			0.51	2.979	2.816	17.245	6.37

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