

## UREA ADDUCTS OF FATTY ACIDS. PART V. COMPONENT FATTY ACIDS OF MUSTARD OIL

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Fractionation of mustard oil fatty acids has been carried out by (1) stepwise addition of urea and (2) solvent crystallisation of urea adducts. Erucic acid of about 93% purity and 43% yield has been obtained. From the neutralisation equivalent and iodine values of different fractions obtained by crystallising the urea adducts, the component fatty acids of mustard oil have been calculated and compared.

Bengen<sup>1</sup> has first shown that urea can be used to separate straight-chain compounds from branched chain or cyclic compounds. Since then numerous investigators have used urea to separate straight-chain compounds and to obtain high purity fatty acids. Oleic acid of high grade purity has been obtained by Schlenk and Holman<sup>2</sup>, and linoleic and linolenic acid concentrates by Swern and Parker<sup>3</sup>, and highly unsaturated polyethenoid fatty acids with four or more double bonds by Abu-Nasr, Potts and Holman<sup>4</sup>. Schlenk and Holman<sup>5</sup> separated the mixture of lauric and stearic acids of 240 A. V. into two fractions of 207 and 266 by the use of urea. The urea method is being developed by Mehta *et al.*<sup>6</sup> for determining the composition of fatty acids of sesame oil, linseed oil and castor oil. The mixed fatty acids have been separated into fractions by varying quantities of urea or by stepwise addition of urea, to calculate the composition of fatty acids. The main interest was focussed in the previous work on the fractionation according to unsaturation, and it was therefore thought desirable to study the urea-adduct formation of the fatty acids in the light of both chain length and unsaturation. Mustard oil, which contains erucic acid, oleic and linoleic acids and C<sub>16</sub>-C<sub>24</sub> saturated acids, has therefore been chosen. While this work was in progress, Skellon and Taylor<sup>7</sup> claimed to have obtained pure erucic acid by the addition of urea and subsequent lead-salt separation. Knafo<sup>8</sup> prepared erucic acid from mixed fatty acids of rape seed oil by adding saturated solution of urea.

The present work is being carried out to evolve a method of fractionation of fatty acids by urea to determine the composition of the fat. In stepwise addition, as the small portions of urea are added and the fatty acids are in excess, the composition of urea adduct varies giving a low mol. ratio of urea to fatty acids. The formation of urea adducts with insufficient amounts of

urea is competitive for the various components of a mixture, and the compositions of the solids largely follow the individual equilibrium constants. Due to the "trailing in" effect a clear cut separation of a single straight-chain compound is not possible.

Urea binds fatty acids and their derivatives in a ratio of approximately 3 : 1 by weight. In preparing adduct it is necessary to add excess of urea in order to obtain a satisfactory yield, as seen from the equilibrium,



To obtain proper fractionation of fatty acids the addition of an excess of urea has been found to furnish stable urea adducts. For these reasons urea, about three times the amount of fatty acids, is dissolved in alcohol and the adducts are obtained by slow crystallisation, removing the alcohol by distillation in stages.

#### EXPERIMENTAL

*Addition of varying amounts of urea.*—To 25 g. of mustard oil fatty acids (I. V. 93.7; N.V. 195.3), prepared from alkali-refined mustard oil by the usual method, varying amounts of urea (1 part) in C.P. methanol (2 parts) were added and the procedure was followed as shown in Part I<sup>6</sup>. The results are recorded in Table I.

TABLE I\*  
*Mustard oil fatty acids.*  
(I.V. 98.7 ; N.V. 195.3)

Fatty acids.	Methanol.	Urea.	Fatty acids in adduct.	Fatty acids in raffinate.	Fatty acids in adduct.	Fatty acids <sup>2</sup> in raffinate.	Loss.	Fatty acids in adduct.	Fatty acids in adduct.	Fatty acids in raffinate.
								I.V.	N.V.	I.V.
1. 25.23 g.	25 ml.	12.5 g.	5.32 g.	16.71 g.	21.1%	66.2%	12.7%	66.1	165.4	96.4
2. 25.20	50	25.0	12.99	11.62	47.6	46.1	6.3	75.3	175.5	105.0
3. 25.20	75	37.5	14.94	9.89	55.3	39.2	5.5	78.4	195.4	110.5
4. 25.08	100	50.0	16.41	6.19	65.4	24.7	9.9	81.6	196.4	115.3

*Fractionation by stepwise addition of urea.*—(a). To 50 g. of the mustard oil fatty acids 10 g. of urea in 75 ml. C.P. methyl alcohol was dissolved and kept overnight. As no adduct was obtained, another 10 g. of urea was added. The adduct formed was filtered through a sintered glass funnel and decomposed by hot distilled water. The fatty acids were extracted with ethyl ether and dried over anhydrous sodium sulphate. In the second step 10 g. of urea was added to the filtrate, warmed to dissolve and kept overnight. The

adduct was removed and to the filtrate urea was again added. The results are shown in Table II.

TABLE II\*

50 g. Mustard oil fatty acids + 75 ml. methanol.

Urea.	Adduct.	Fatty acids in adduct.		Wt. ratio urea Fatty acids	I.V. of fatty acids in adduct.
20 g.	17.5 g.	5.8 g.	11.6%	2.0	68.2
10	7.5	2.99	6.0	1.5	72.5
20	<del>3.45</del>	10.90	21.8	2.2	87.5
10	10.5	3.76	7.5	1.8	91.6
—	—	15.08	30.1	—	114.0
		Total	77.0		
		Loss	23.0		126.5 (calc.)

\* The experiments were carried out by R.M. Warad in March, 1952.

(b). Ethyl alcohol (95%) was used as the solvent for urea in the following experiments because of its cheapness and ready availability. To 25 g. of mustard oil fatty acids in 75 ml. ethyl alcohol 12.5 g. of urea were added, warmed to dissolve and kept overnight. No crystalline adduct was obtained. Further 12.5 g. of urea was added and kept overnight. Fatty acids liberated from the adduct in the usual manner were extracted with ethyl ether. The filtrate was treated with further quantity (12.5 g.) of urea and the same process was repeated. The last filtrate comprised the final fraction. The results are shown in Table III.

### TABLE III

### Stepwise fractionation of mustard oil fatty acids.

**Urea added stepwise—125 g. in every step.**

**25 g. Fatty acids (I.V. 104, N.V. 177-4) + 75 ml. ethyl alcohol.**

No. of step.	Adduct.	Fatty acids in adduct.		Wt. ratio urea fatty acids	I.V. of fatty acids in adduct
1	Nil	—	—	—	—
2	20 g.	6.26 g. (solid)	25.05%	2.19	65.5
3	17	5.58	22.32	2.04	75.4
4	20	5.83	23.32	2.13	100.0
5	15	2.95	11.80	4.07	146.5
6	—	2.47	9.88	—	184.7
	Loss	1.91	7.64		189.1 (calc.)
	Total	25.00	100		

(c). To mustard oil fatty acids (50 g.) urea (12.5 g.) in 200 ml. ethyl alcohol was added and kept overnight. No crystalline adduct was obtained. Further 10 g. of urea was added and kept overnight. The adduct was separated and fatty acids were recovered in the usual manner. To the filtrate further 12.5 g. of urea was added and thus the process was repeated. The results are recorded in Table IV.

TABLE IV

50 g. Fatty acids + 200 ml. ethyl alcohol.					
No. of step.	Adduct.	Fatty acids in adduct.	Wt. ratio urea fatty acids		I.V. of fatty acids in adduct.
1	Nil	—	—	—	—
2	18 g.	5.23 g. (solid)	10.46	2.44	68.0
3	20	6.24	12.48	2.20	75.0
4	20	4.47	8.94	3.47	85.3
5	20	4.46	8.92	3.49	85.2
6	15	4.56	9.12	2.07	99.4
7	18	4.18	8.36	3.31	108.5
8	17	4.22	8.44	3.03	108.6
—	—	12.85	25.70	—	172.0
		Loss	7.58	—	I.V. 183.1 (calc.)

*Fractionation by decreasing solvent crystallisation method.*—Mustard oil fatty acids (25 g.) were taken in a 750 ml. conical flask and ethyl alcohol (650 ml.) was added and kept overnight. No solid acids crystallised out. To this 75 g. of urea was added and kept overnight. The adduct was removed and fatty acids were recovered in the usual manner. From the raffinate ethyl alcohol was distilled off successively and the urea adducts were fractionally crystallised out. The results are shown in Table V and those obtained by repeating the experiment in Table VI.

TABLE V

Mustard oil fatty acids (I.V. 104; N.V. 177.4) = 25 g.

Urea = 75 g. Ethyl alcohol = 650 ml.

	Adduct.	Fatty acids in adduct.		Wt. ratio urea fatty acids	I.V. of fatty acids in adduct.	N.V. of fatty
Kept overnight	45 g.	11.5 g.	46.0%	2.91	73.3	168.3
250 ml. alcohol distilled off and kept overnight.	10	2.5	10.0	2.95	85.5	168.6
Do	22	4.6	18.4	3.77	106.2	199.0
100 ml. alcohol distilled off and kept overnight.	15	2.4	9.6	5.25	160.4	199.3
Raffinate	—	1.85	7.4	—	191.0	200.5
Loss			8.6		158.9 (calc.)	

## DISCUSSION

The yield of urea adduct increases with the amount of urea added keeping the ratio of urea to methanol constant. As the quantity of urea is increased the unsaturated acids also form the adducts (vide Table I.)

The segregation of fatty acids of mustard oil by adding urea successively to the filtrate has been shown in Table II. The last raffinate of I.V. 114 has been obtained from the fatty acids of I. V. 93.07. It is clear that the yield of adducts depends on the quantity of urea that

has been added. For example, in the first step by the addition of 20 g. urea a yield of 17.5 g. of an adduct has been obtained, while in the second step by adding 10 g. of urea a yield of 7.5 g. has been obtained. The I.V. of adducts are gradually increasing as seen from Table II. Thus it is necessary to add equal quantities of urea in successive steps. The results of such fractionation have been shown in Table III. In these experiments ethyl alcohol has been used as a solvent. The yields of adducts are decreasing in this case, as the unsaturation is increasing. In the first fraction almost all saturated acids have formed adducts.

FIG. 1

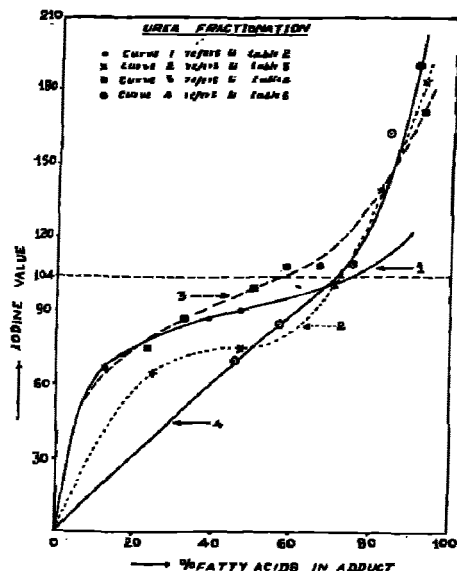
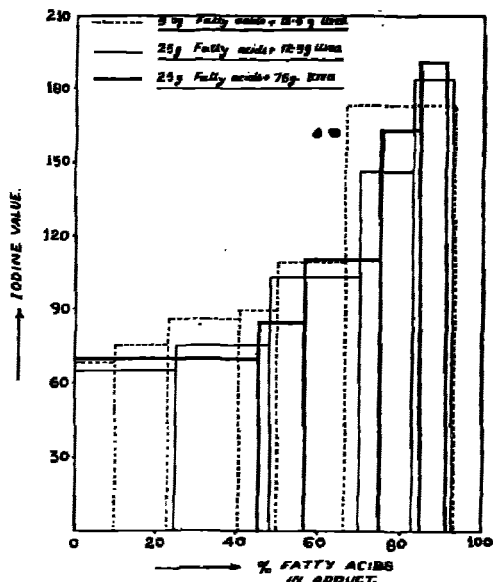


FIG. 2



In data, reported in Tables III and IV, the original proportion of urea is changed. The first lot of urea is very insufficient to precipitate out any fatty acids from the mixed fatty acids, but further additions of urea furnish adducts. In this fractionation also first fraction is of saturated acids and the second fraction is of erucic acid and then oleic and linoleic and linolenic acids follow. Better fractionation is obtained when the solvent is used in large amount. This is found by comparing the data in Tables III and IV; the losses are also much less. Redlich *et al.*<sup>9</sup> have found that the complex from the mother-liquor, washing, and analysis give erratic results. Narayan and Kulkarni<sup>10</sup> also noted that the urea-fatty acids adduct formation was a highly complex reaction involving two or possibly more phases and extremely sensitive to changes in operating conditions. The varying mole ratio of urea to fatty acids indicates that the urea molecules

TABLE VI

25g. Mustard oil fatty acids (I.V. 104, N.V. 177.4) + 75 g. urea + 650 ml. ethyl alcohol.

	Adduct	Fatty acids in adduct	Urea. F.A.	I.V. of F.A. ( $I_w$ )	S.E. of F.A. ( $I_w$ )	Satura- ted acids.	Approximate composition calculated from iodine value.			
							Unsaturated acids.	Erucic acid.	Oleic acid.	Linolenic acid.
							$U = \left[ W \times \frac{I_w}{I_u} \right]$			
Kept overnight	49 g.	11.42g. (solid)	45.68%	3.23	70.2	331	0.73 g.	10.69 g.	10.69 g.	...
250 ml. alcohol was distilled										
off and kept overnight.	10	2.80	11.20	2.56	85.3	297.7	...	0.88	1.92 g.	...
"	25	4.47	17.88	4.54	109.6	281.9	...	4.47	3.51	0.96 g.
100 ml. alcohol was distilled										
off and kept overnight.	10	1.82	7.28	4.91	173.8	281.2	...	1.82	0.14	1.68
Alcohol was removed	...	2.40	9.60	...	190.6	280.4	...	2.40	...	2.15
completely	...	...	...	...	...	...	...	...	...	0.25
2										
						Total	0.73	22.18	11.57	4.79
						Total %	2.92	88.72	22.28	13.16
						Loss %	8.36	I.V. (calc.)	156.4	1.0

may wind around the fatty acid chain covering partially or wholly, giving rise to the adducts of varying composition and stability, and thus the reaction of adduct formation is erratic and irreproducible. However, if the concentrations of urea and fatty acids and the solvent are maintained and if the temperature is controlled, the results may be duplicated as shown in Tables V and VI.

In the fractionation by the method of distilling off the solvent, it is found that erucic acid comes along with saturated acids indicated by S.E. (331) and I.V. (70.2) and that the segregation of acids on the basis of unsaturation is more selective as seen from Table VI. Abu-Nasr, Potts and Holman<sup>4</sup> have also found the selectivity more when a large quantity of solvent is used with gradual addition of urea. Sakurai<sup>11</sup> also reported less selectivity when less quantity of solvent was used.

The composition of mixed fatty acids of mustard oil has been calculated from the analytical data of each fraction as given by Hilditch<sup>12</sup> on the assumption that there are only two acids in each fraction, as done by Hilditch for the calculation of fatty acid composition by ester fractionation method. Further, from the previous work on urea complex of fatty acids it has been found that higher saturated fatty acids form urea complex more readily than lower and unsaturated fatty acids. In the calculations therefore it is assumed that saturated and erucic acids first form adducts and later erucic and oleic acids, oleic and linoleic acids, and the iodine values indicate the presence of unsaturated acids and the neutralisation equivalents indicate the chain length. The neutralisation equivalents of the first and second fractions (331 and 297.7 respectively) show that they contain erucic acid (N.E. 340.5). The first fraction has been calculated to contain saturated acids and erucic acid, and the proportion of each has been found out from the iodine values. In further fractions it is assumed that each fraction contains a binary mixture of acids. Thus the second fraction contains oleic and erucic acids, and the proportion of each has been calculated on I.V. basis. The last three fractions consist wholly of  $C_{18}$  unsaturated acids as seen from their determined equivalents (280 to 282) and linolenic acid is present in a very minor quantity as indicated by the iodine values. Hence, the proportion of each acid in fractions have been calculated directly from iodine values. From Table VI the fatty acid composition of mustard oil is as follows: saturated acids 2.92%, erucic acid 46.28%, oleic acid 22.28%, linoleic acid 19.16% and linolenic acid 1.00%. This composition compares well with the common varieties of mustard oil. Erucic acid (free from polyethenoid acids) of about 93% purity and in 43% yield has been obtained by crystallisation of urea adducts by the decreasing solvent crystallisation method. It is difficult to obtain erucic acid of higher purity due to the presence of mixed saturated acids of  $C_{14}$ - $C_{22}$  series which also have the tendency of urea-adduct formation along with erucic acid ( $C_{22}$  acid).

## R E F E R E N C E S

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