theory. Further, trypsin and other proteolytic enzymes, by destroying the protein part of thromboplastin, would increase its dissociation and speed the transfer of cephalin to prothrombin, thus providing a mechanism for the action of trypsin on the compound, the practical significance of which Ferguson has stressed. In the case of those clotting factors which are enzymes, the theory becomes the well-known theory of Michaelis and Menten. From the foregoing it is evident (1) that this reaction of heparin should be considered in experiments requiring its use, and (2) that in studies on blood clotting, heparin activity may be affected by the presence of factors not usually considered.

SUMMARY

Heparin forms stable salts with many proteins. The resulting salt is markedly different in its properties

(isoelectric point, solubility, etc.) from the original protein. In solution, these salts show partial dissociation according to the mass law. The dissociation constant of the compound varies with the protein, and the dissociation is markedly affected by pH and the presence of other anions and cations. The formation of compounds with a sufficiently low dissociation constant is followed by the loss of biological activity on the part of the anticoagulant. The application of these results to quantitative studies on blood clotting is indicated.

The substance of this paper was presented before the meeting of the Division of Biological Chemistry of the American Chemical Society at Buffalo, N.Y., 9 September 1942. The author is greatly indebted to Prof. C. H. Best for his kindly interest in the problem. This study has been supported by the John and Mary R. Markle Foundation.

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The Isolation of Behenic Acid from Refined Mona Wax

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(Received 3 December 1942)

Montan wax has been refined by treatment with chromic acid in glacial acetic acid solution [Marcusson & Lederer, 1931]. Mona wax isolated from Irish peat [Reilly & Wilson, 1940] was not changed by this mixture. A sulphuric acid-chromic acid mixture gave 72.5% of a pale yellow acidic wax. Its properties are compared with those of Mona wax in the following table:

	Mona wax	Refined Mona wax
Acid value	46	149
Saponification value	98	173
Ester value	52	24
Iodine value	30	5.5
M.P.	70°	73.5°

The acids were isolated by the usual procedure [Chibnall, 1934; Reilly & Wilson, 1940] and converted to their methyl esters. By a series of frac-

tional distillations *in vacuo*, the latter were separated into a number of fractions. From one of these, an acid was isolated whose equivalent weight and elementary analysis suggested that it might be behenic acid $C_{22}H_{44}O_2$.

Stohmann & Langbein [1890] found the m.p. of behenic acid, obtained by the reduction of ethyl erucate, to be 77–78°. The value obtained in the present investigation was 77.5°. A more recent determination on the synthetic acid by Francis, Piper & Malkin [1930] gave a value of 80.0°. To determine its identity, the acid was submitted for X-ray crystallographic examination. C. H. Carlisle and D. M. Crowfoot (see below) showed, by measurements of the B and C crystal spacings, that the acid was contaminated with 2.5–10% of $C_{20}H_{40}O_2$, and probably with traces of a higher acid, $C_{24}H_{48}O_2$.

Holde [1924], from a consideration of the theory of fat formation proposed by Buchner & Meisenheimer [1908], concluded that only normal fatty acids with an even number of carbon atoms can occur in nature. Francis *et al.* [1930] fully supported his conclusion by their work on a number of the higher naturally occurring acids. From an examination of the X-ray photographs they also concluded that a separation into single acids was almost impossible (except when one acid occurred in the mixture in preponderating amounts). Stadler [1933] on the other hand, claims that, after a series of carefully executed fractional distillations *in vacuo*, he isolated, from Bohemia Montan wax, two pure acids containing an uneven number of carbon atoms ($C_{27}H_{54}O_2$ and $C_{29}H_{58}O_2$).

The present investigation was an attempt to verify the last author's results. By using similar distilling apparatus, and adhering to his conditions, the present authors isolated behenic acid ($C_{22}H_{44}O_2$), in an impure condition. These results do not, therefore, support his claim, but are further evidence in favour of the conclusions reached by Francis *et al.* [1930].

EXPERIMENTAL

A total of 190 g. of pure refined mona wax was used.

Saponification and isolation of calcium salts. 50 g. wax were dissolved in 500 ml. benzene and 200 ml. ethanol. 25 g. KOH, in 250 ml. ethanol, were added to the hot solution and the mixture boiled for 3 hr. under reflux. The content of the flask was divided in two and a hot solution of anhyd. $CaCl_2$ (200 g.) was added slowly to each portion while nearly boiling with vigorous shaking. The solution was boiled for 1 hr. to complete the reaction. The Ca salts were filtered off, dried, ground and extracted exhaustively with benzene in a Soxhlet apparatus.

Formation of acids and esterification. The powdered calcium soaps were added in small quantities to warm dilute HCl (1 part acid : 2 parts water) and the mixture boiled till the fatty acids floated in a brown layer on top. When cold, the acids were removed and washed free from mineral acid. Acid value, 185.

The acids were esterified in 20 g. lots. 20 g. acid were refluxed with 1200 ml. methanol and 40 ml. conc. H_2SO_4 for 8 hr. The brown solution of the ester was then decanted from the brown-black residue. After distilling off most of the alcohol the remainder was poured into a large volume of water, when the esters were precipitated. The water was then heated to boiling, when a brown layer of esters collected on top. These were separated and washed free from H_2SO_4 . Acid value, 22; saponification value, 178; iodine value, 2.8; m.p. 73° .

An attempt to reduce the acid value by re-esterification failed. The method of Buxton & Kapp [1940], which depends on removing free acid as the K salt, was used, but it reduced the acid value only to 11. Total weight of methyl esters, 115.8 g.

Fractionation. All-glass, electrically heated, fractionation columns, similar to those described by Stadler [1933] and Longenecker [1937], were used. Each consisted of an inner tube packed with Lessing rings. This was surrounded by

another tube on which was wound nichrome resistance wire. An outer insulating tube enclosed both heating tube and column. The flask containing the esters was fused to the bottom of the column for each distillation. The heating of the column was controlled by external variable resistances and the temperature measured by an iron-nickel couple inserted between the column and heating jacket. Jantzen & Tiedecke [quoted by Stadler, 1933], in a study of fractionation, found that the boiling-point was not determinate of the course of fractionation. These workers fractionated according to the melting-points of the distillate. This method was used by Stadler [1933] and was adopted in the present work.

Preliminary distillation was undertaken in order to free the esters from any impurities, and to divide them into a higher and a lower fraction. The ester was divided into three portions (two of 40 g., one of 35.8 g.). Each was distilled (2–5 mm.) from a 250 ml. Claisen flask, and the distillate collected by means of a Perkin triangle.

Lower fraction	56.6 g.
Higher fraction	28.9 g.
Total	85.5 g.

Fractional distillation. The lower and higher fractions were placed separately with some glass wool and powdered pumice stone in two 250 ml. Pyrex flasks which were fused to the bottom of a column 47 cm. long and 3 cm. internal diameter. Test tubes fused on to the side arm were used as receivers. Table 1 gives the details of fractionation of the higher and lower fractions. IF is the lower, and IIF the higher fraction.

Table 1. *Fractional distillation of higher and lower ester fractions*

Fraction	Weight g.	M.P. °C.	Dist. temp. °C.	Pressure mm.
IF. Total 56.6 g.		Distillate 49.41 g.		
(i)	4.10	Liquid	90–125	
(ii)	2.68	Semi-liquid	136	0.10
1	3.68	31.5	126	
2	1.96	34.0	136	
3	5.70	37.5	138	
4	2.33	38.5	146–150	A.V. 7.4
5	1.59	40.5	157	0.15
6	1.82	41.0	160	
7	3.84	42.3	166	
8	4.25	42.5	168	
9	2.02	45.0	155	
10	2.67	46.5	160–166	
11	2.98	47.0	163	0.15
12	3.17	48.5	154	A.V. 7.7
13	4.04	51.5	178	
14	2.58	54.0	186	0.30
IIF. Total 28.9 g.		Distillate 26.62 g.		
(i)	2.97	Liquid	156	0.20
1	4.08	39.5	170	
2	5.09	45.0	172	
3	2.88	49.0	182	
4	4.78	53.0	174	A.V. 1.7
5	3.49	53.5	205	
6	3.33	57.0	210	

The low acid values show that very little decomposition had taken place during the distillations.

Table 2. *Grouping of ester fractions for further distillation*

Group	Fractions combined	M.P. range ° C.
I R.	I 9-11, II 2	45.0-47.0
II R.	I 14, II 4, 5	53.0-53.5
III R.	I 12-13	48.5-51.5
F.F.	(II R 7-10 III R 2-4	48.8-52.5

Table 3. *Data for distillation of the three main fractions*

Fraction no.	Wt. g.	M.P. ° C.	Dist. temp. ° C.	Pressure mm.
(a) Fraction I R. Total 12.7 g. Distillate 12.3 g.				
(i)	0.50	Liquid	92	0.05
1	0.83	41.0	101	
2	0.58	40.5	112.5	
3	1.59	43.0	106	0.01
4	0.60	43.5	109	
5	0.93	45.0	114	
6	1.2	46.0	111	
7	0.87	48.0	112	
8	0.46	47.6	106	
9	0.38	48.5	106-111	
9 A	1.00	49.0	—	
10	1.66	49.5	122.4	
11	0.97	50.5	115	0.01
12	0.73	54.0	135-172	
(b) Fraction II R. Total 10.17 g. Distillate 9.76 g.				
(i)	0.46	Liquid	92	0.05
1	0.84	48.0	100	
2	0.87	51.5	126	
3	1.69	52.5	129	0.01
4	1.87	54.0-54.6	118	
5	0.40	55.0	113	
6	1.11	56.5-57.2	138	
7	1.04	59.0-59.2	130	
8	1.48	59.0	132	
(c) Fraction III R. Total 5.30 g. Distillate 4.81 g.				
1	0.35	48.0	98	0.03
2	0.38	49.7	115	
3	0.59	50.2	120	
4	0.74	52.5	117	
5	1.40	53.5	130	
6	0.23	55.0	111	
7	0.65	56.0	104	
8	0.47	56.4	120	

The fractions were divided into groups according to their melting-points and the groups IR to IIIR were then distilled separately through a smaller column of the same type (26 × 2 cm.). The scheme is clear from Table 2 and the data are given in Table 3.

For the final fractionation, some fractions of the highest weight and shortest temperature range were chosen (Table 4). Fractions IR 7-10 and IIIR 2-4 were combined and distilled through a third fractionating column. Instead of a flask, a small elongated bulb containing the ester was fused on to the end of the column. The bulb was wound with resistance wire (nichrome no. 15) for heating purposes, and the temperature was measured by means of an iron-nickel couple. This heating arrangement was found to be more satisfactory than the metal bath previously used, and allowed of more careful temperature control.

Table 4. *Data from final fractionation*

Total 5.0 g.		Distillate 4.08 g.		
Fraction no.	Wt. g.	M.P. ° C.	Dist. temp. ° C.	Pressure mm.
1	0.27	47.8	93	0.01
2	0.42	48.5	94	
3	0.32	49.0	94	
4	0.36	49.1	94-100	0.04
5	0.33	49.2	100	
6	0.30	49.7	80	
7	0.29	49.7	88	
8	0.25	50.0	90	
9	0.41	50.3	95	
10	0.27	50.2	97	
11	0.25	50.4	100	
12	0.28	50.5	102	
13	0.33	50.8	105	

Fractions nos. 10, 11 and 12 were worked up to obtain the acid. The results are given in Table 5.

Table 5. *Data on isolated behenic acid*

Origin of acid	Quantity mg.	KOH ml.	Normality of alkali	Equiv. wt.	Acid value	M.P. ° C.
A	101.4	11.25	0.0261	344.2	162.7	77.5
	100.0	11.00	0.0261	340.0	164.9	
	100.6	11.2	0.0266	336.0	166.8	
	103.2	—	0.0266	342.6	163.8	

A, therefore, consists mainly of behenic acid ($C_{22}H_{44}O_2$) of acid value 165, and equivalent weight 340.35. (Analysis: found 77.23% C, 12.5% H; 77.23% C, 12.7% H. Calc. for 77.6% C, 12.9% H. $C_{22}H_{44}O_2$.)

X-ray Crystallographic Examination of Fatty Acids Obtained from Mona Wax

By C. H. CARLISLE AND D. CROWFOOT

In order to obtain further evidence on the composition of fraction A, it was submitted to X-ray crystallographic examination. For comparison, one of the higher melting fractions was studied in addition.

The results of the X-ray examination are shown in Table 6, together with the most recent data obtained on pure fatty acids by Francis & Piper [1939]. This table

confirms the general conclusions already reached as to the main constituents of these fractions, and also shows clearly that neither fraction is pure. Unfortunately no exact X-ray measurements have been made on synthetic binary mixtures of the acids most likely to be present, i.e. $C_{20}H_{40}O_2$, and $C_{22}H_{44}O_2$, or some ternary mixtures of these acids with $C_{24}H_{48}O_2$, and the variations in X-ray spacing of

other mixtures, e.g. of $C_{22}H_{44}O_2$ and $C_{24}H_{48}O_2$, which have been examined [Chibnall, Piper & Williams, 1936], show too many irregularities to be used for direct comparison. Some estimate of the nature of the impurities present may, however, be made by correlating the X-ray measurements with the general observations made by Chibnall *et al.* [1936] on the behaviour of fatty acid mixtures. For this purpose measurements had to be made of both the B and C polymorphic modifications, the X-ray photographs usually being taken of samples pressed lightly on to glass slides.

A. The original preparation, when recrystallized from acetone, showed X-ray lines corresponding to the C modification alone. On recrystallization from benzene and from acetic acid, preparations were obtained, both of which showed lines due to B and C modifications on the same photograph. Such behaviour was noted by Piper and co-workers [Chibnall *et al.* 1936] in the binary mixture containing 2.5–10% and 80–90% of the lower constituent. Here the C spacing is slightly lower, the B spacing slightly higher than the best values for pure $C_{22}H_{44}O_2$. Both these types of spacing change may be due to mixture of a small quantity of $C_{26}H_{46}O_2$, say 2.5–10%, with $C_{22}H_{44}O_2$, but it seems very probable that the fraction still contains traces of other acids, particularly $C_{24}H_{48}O_2$.

IIIR 5. This fraction, when recrystallized from methanol-ethyl acetate, showed the B spacing alone. While the spacings, both of this, and of the methyl ester, are intermediate

between those of $C_{22}H_{44}O_2$ and $C_{24}H_{48}O_2$, the data do not fit very well with the observations that have been made on mixtures of synthetic $C_{22}H_{44}O_2$ and $C_{24}H_{48}O_2$, so that the mixture is presumably more complex.

Table 6. X-ray measurements

Acid fraction	M.P. ° C.	B spacing	C spacing	Methyl ester spacing
A	77.5	53.6	47.7	—
III R 5	81.9	56.5	—	61.0
Compare				
$C_{26}H_{46}O_2$	73.5	48.45	44.15	52.3
$C_{22}H_{44}O_2$	79.95	52.95	48.3	57.02

SUMMARY

1. The isolation of behenic acid from refined mona wax is described.

2. The results of X-ray crystallographic examination of the product, by C. H. Carlisle & D. Crowfoot, are reported. They confirm the conclusion that the isolated product consists largely of behenic acid.

The authors wish to thank Mr J. P. Wilson for his assistance during some of the distillations and Mr J. McKenna for constructing the distillation apparatus.

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A Study of the Excretion of Organic Antimonials Using a Polarographic Procedure

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(Received 4 December 1942)

Several attempts have been made to determine the rate of excretion of antimony compounds during the treatment of tropical diseases, and so to control dosage and avoid toxic symptoms. The early qualitative tests for the presence of the metal in urine [Di Cristina & Caronia, 1916; Mallardi, 1921] were replaced by quantitative methods based upon the colorimetric determination of antimony as sulphide

[Brahmachari, Das & Sen, 1923; Khalil, 1931] or by a modification of the Gutzeit test [Boyd & Roy, 1929]. A summary of this work is given in Table 1. All the quantitative procedures were tedious; the degree of accuracy was low, and the chemical treatment made it impossible to distinguish between trivalent and quinquevalent antimony in the original samples. The subjects were various human patients