The C₂₀ Unsaturated Acids of Pig Back Fat

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Analyses by the ester-fractionation process described by Hilditch (1940) of pig depot fats have shown the presence of small amounts (1–3%) of unsaturated C₂₀–C₂₂ acids, which have generally been regarded as highly unsaturated (Hilditch, Lea & Pedelty, 1939) and probably similar to the corresponding liver glyceride fatty acids. Experimental difficulties in purifying the residual ester fractions (in which the C₂₀–C₂₂ acids accumulate, along with polymerized or partially oxidized acids) have prevented full identification. The amount of C₂₀–C₂₂ acids determined from the polybromide number has, however, been much less than the amount of these acids calculated from the saponification equivalent of the residual ester fractions, a fact which led Hilditch & Pedelty (1939) to suggest an empirical method for the rapid determination of unsaturated C₂₀–C₂₂ acids in pig depot fats.

Elsewhere the writers (de la Mare & Shorland, 1945) have presented preliminary evidence that the bulk of the C₂₀–C₂₂ unsaturated acids of the back fat from buttermilk-fed pigs consists of acids with fewer than three double bonds. This conclusion is confirmed by the present work, and the monoethenoid C₂₀ acid has been identified as Δ^{11-12} eicosenoic acid.

EXPERIMENTAL

(a) *Preparation and purification of C₂₀ unsaturated acids.* Bulk of inner and outer back fat (4019 g.) from pigs fed buttermilk during the fattening period* and killed at approximately 200 lb. live weight, was extracted as previously described (Shorland, Hansen & Hogan, 1944). After hydrolysis and removal of unsaponifiable matter as described by Hilditch (1940), the recovered fatty acids were converted into ethyl esters (3908 g.). These were fractionated at c. 1 mm. pressure from a 5 l. flask, using an electrically heated column 24 in. high and 22 mm. in diameter, packed with sections of glass tube 10 mm. long and 7 mm. in diameter. After removal of 3518 g., boiling-point below 155°, the residue was hydrolyzed. The recovered acids were crystallized twice from 4 l. of acetone at –10°. The soluble acids (251 g.) gave on methylation 257 g. of methyl esters (i.v. 92.4, saponification equivalent (sap. equiv.) 295.6). Fractionation of these with a column of the type described by Longenecker (1937) gave three fractions, L₁ (174 g., mainly C₁₈ esters), L₂ (48 g., mainly C₂₀ esters) and L₃ (35 g., undistilled residue).

* With access to pasture, which Shorland *et al.* (1944) have shown to have little effect on the iodine value of pig back fat.

Table 1 gives details of the fractionation of L₂ (43.5 g.).

Table 1. *Fractionation of L₂ (mainly C₂₀ methyl esters)*

Fraction	Weight (g.)	b.p. (°)*	Sap. equiv.	i.v.
L ₂₁	2.74	120–122	293.6	89.7
L ₂₂	2.02	122	297.4	92.7
L ₂₃	1.29	122–124	296.8	91.2
L ₂₄	2.30	124–130	299.0	96.7
L ₂₅	2.01	130–134	303.3	104.5
L ₂₆	2.51	134–138	312.6	109.7
L ₂₇	2.95	138	317.1	110.1
L ₂₈	4.41	138	318.4	106.7
L ₂₉	2.83	138	319.8	105.9
L ₂₁₀	2.76	138	318.6	103.1
L ₂₁₁	3.13	138	320.8	103.5
L ₂₁₂	2.90	138	320.5	100.0
L ₂₁₃	3.14	138	320.5	93.9
L ₂₁₄	3.80	138	323.1	104.9
L ₂₁₅	1.63	138–145	326.8	165.5
L ₂₁₆	2.95	Residue	328.3	123.4

* In this and subsequent tables, boiling-points are at c. 0.1 mm.

The acids recovered from fractions L₂₇–L₂₁₅ were crystallized from acetone as shown in Table 2.

Table 2. *Crystallization of C₂₀ unsaturated acids*

L ₂₇ –L ₂₁₅	
24.7 g., sap. equiv. 305.9, i.v. 109.0, crystallized twice from 250 ml. acetone at –30°	
B ₁ insoluble 9.5 g., sap. equiv. 306.5, i.v. 84.1	Soluble (15.2 g.) crystallized twice from 100 ml. acetone at –50°
B ₂ insoluble 3.2 g., sap. equiv. 314.2, i.v. 76.6	Soluble (12.0 g.) crystallized twice from 100 ml. acetone at approx. –80°
B ₃ insoluble 2.7 g., sap. equiv. 305.4, i.v. 96.9	B ₄ soluble 9.3 g., sap. equiv. 293.3, i.v. 159.4

(b) *The C₂₀ monoethenoid fraction.* Methyl esters prepared from fractions B₁ and B₂ (which from their iodine values were substantially free from polyethenoid acids) were fractionated as shown in Table 3.

Table 3. Fractionation of C₂₀ 'monoethenoid' methyl esters

Fraction ...	B ₁₂₁	B ₁₂₂	B ₁₂₃	B ₁₂₄	B ₁₂₅	B ₁₂₆	B _{12R}
Weight (g.)	0.97	1.77	1.38	1.70	1.60	2.34	0.80
b.p. (°)	130-140	140-146	146	146	146	146	(Residue)
Sap. equiv.	302.3	320.0	321.2	321.2	321.2	322.5	322.5
i.v.	75.2	74.9	75.0	76.3	74.4	86.8	

The acids recovered from fractions B₁₂₂-B_{12R} were converted into ethyl esters and fractionated, giving the results of Table 4.

Table 4. Fractionation of C₂₀ 'monoethenoid' ethyl esters

Fraction ...	C ₁	C ₂	C ₃	C ₄
Weight (g.)	1.67	1.52	5.77	1.36
b.p. (°)	140-146	146	146	(Residue)
Sap. equiv.	331.1	335.6	336.2	342.1
i.v.	—	—	65.5	—

A portion of fraction C₃ (1.43 g.) was oxidized with 15 g. KMnO₄ in 20 ml. acetone by the method of Armstrong & Hilditch (1925). The unoxidized material (0.32 g.) had i.v. 14. The acids recovered from the acidic products of oxidation were extracted several times with hot light petroleum (b.p. 40-70°). The material insoluble in petroleum ether was crystallized from water, giving 0.19 g. of dibasic acid m.p. 105-107°, which on recrystallization from water and from chloroform melted at 109-109.5°. Analysis: C=61.2, 60.9, 60.6; H=9.2, 9.2, 9.5%; neutralization equivalent (neut. equiv.) 107.4. Nonane 1:9 dicarboxylic acid requires C=61.1, H=9.3%, neut. equiv. 108. The acid, mixed with genuine nonane 1:9 dicarboxylic acid (prepared by the method of Walker & Lumsden (1901), m.p. 111-112°, neut. equiv. 106.8) melted at 110-111°. A further crop (0.134 g.) of dibasic acid was obtained by concentration of the first mother liquors; this after recrystallization from water had equivalent 108.1 and melted at 101-103°.

The amount of material insoluble in petroleum ether was too small for accurate characterization. A *p*-toluidide was prepared, however, which after recrystallization melted at 78-79°, and showed no depression of m.p. on admixture with genuine nonoic *p*-toluidide, m.p. 82°.

The potassium salts from the hydrolysis of fraction C₃ (1.70 g. acids) were oxidized with alkaline KMnO₄ as described by Lapworth & Mottram (1925). The crude oxidation products were extracted with petroleum (b.p. 40-70°) giving 0.67 g. of soluble material i.v. 22. The residue (1.00 g.) was crystallized twice from ethyl acetate, giving 0.63 g. of a hydroxy-acid, m.p. 129-129.5°. Analysis: C=69.5, 69.6; H=11.9, 11.6%. Dihydroxy-arachidic acid prepared from Δ¹¹⁻¹² eicosenoic acid has m.p. 130.5

(Green, Hilditch & Stainsby, 1936), and requires C=69.8, H=11.6%.

0.80 g. of the ethyl esters (C₃) was hydrogenated at 180-200°, using reduced nickel as catalyst. The product (i.v. 2.5) was hydrolyzed and the acid after crystallization from acetone melted at 72.5-73.5°. Recrystallization twice from acetone and once from petroleum raised the m.p. to 74.5-75°, and no depression of m.p. was detected on admixture with arachidic acid prepared from hydrogenated shark oil. The purified arachidic acid was methylated; the methyl ester after recrystallization from methanol had m.p. 46.0-46.5°. Shinowara & Brown (1940) record the m.p.'s of arachidic acid and methyl arachidate respectively as 76.3-77° and 46-47°.

The monoethenoid C₂₀ acid of this pig depot fat is therefore Δ¹¹⁻¹² eicosenoic acid, previously reported only in the vegetable kingdom as a major component acid of the seed wax of *Simmondsia californica* (Green *et al.* 1936). From the iodine values of the C₂₀ fractions of Tables 1 and 2, and allowing for the presence in the material insoluble in acetone (results to be published elsewhere) of approximately 0.1%, it may be estimated that the original fat contained approximately 0.5% of this acid.

(c) *The C₂₀ polyethenoid fraction.* Methyl esters from fraction B₄ (9.6 g., sap. equiv. 313.3, i.v. 159.4) were fractionated, giving the results of Table 5.

Table 5. Fractionation of C₂₀ 'polyethenoid' methyl esters

Fraction ...	B ₄₁	B ₄₂	B ₄₃	B ₄₄	B ₄₅
Weight (g.)	1.06	1.53	1.52	3.61	1.88
b.p. (°)	137	137	137	137-140	(Residue)
Sap. equiv.	298.0	314.7	316.6	320.4	312.5
i.v.	142.6	163.1	162.8	165.3	134.1

Bromination of the acids from fraction B₄₃ (1.22 g.) in dry ether at 0° gave 0.18 g. of bromides, m.p. 248° (decomp.), Br=66.6% (octabrom-arachidic acid requires Br=67.8%).

The acids recovered from fraction B₄₂ (1.0 g.) were oxidized by the method of Lapworth & Mottram (1925). Only a trace of water-insoluble acids was formed, and no hydroxy-acid was isolated from the water-soluble products of oxidation. A similarly inconclusive result was obtained when the recovered acids from fraction B₄₄ (0.95 g.) were

oxidized by alkaline KMnO_4 under the conditions described by Riemenschneider, Wheeler & Sando (1939).

The absorption spectrum of a portion of fraction B_{44} (sap. equiv. 320.4, i.v. 165.3) was examined in absolute ethanol. A further portion was heated with a solution of KOH in ethylene glycol as described by Mitchell, Kraybill & Zscheile (1943), and the soaps were examined spectroscopically in absolute ethanol. Table 6 summarizes the specific extinction coefficients.

Table 6. *Extinction coefficients ($E_{1\text{ cm.}}^{1\%}$) of fraction B_{44} (C_{20} polyethenoid esters) before and after isomerization*

Wave-length (A) ...	$E_{1\text{ cm.}}^{1\%}$			
	2340	2680	3010	3160
Fraction B_{44} , unisomerized	143	16	8	6
Fraction B_{44} , isomerized	539	205	64	52

From the above data, using the results for arachidonic acid given by Beadle & Kraybill (1944), the amount of arachidonic acid in fraction B_{44} can be calculated to be 24 %, as compared with 17 % from the weight of ether-insoluble polybromides.* On the basis of the whole fat, these values represent 0.07 and 0.05 % respectively, as compared with the value of 0.4 % recorded by Brown & Deck (1930) for commercial lard.

The specific extinction coefficients for isomerized eicosadienoic and eicosatrienoic acids are not known. Assuming, however, that the known values for octadecadienoic acid and for octadecatrienoic acid can be applied without modification to their C_{20} analogues,† and correcting for the presence of conjugated material in the unisomerized esters,‡ the acids from fraction B_{44} can be calculated to have the following composition: eicosatetraenoic acid 24 % (24 %); eicosatrienoic acid 15 % (0 %); eicosadienoic acid 20 % non-conjugated, 14 % conjugated (35 %); eicosenoic acid 27 % (39 %). The figures in brackets are obtained by assuming that the value for arachidonic acid is correct, and that all the remaining iodine value of the fraction is due to eicosadienoic acid and eicosenoic acid, saturated acids being absent.

The authors interpret the above results as indicating the presence in pig depot fat of one or more previously unknown acids which on isomerization

by alkali give acids with diene and triene conjugation. The iodine value of the polyethenoid concentrate is regarded as consistent with the presence of either an eicosadienoic acid, or an eicosatrienoic acid, or both. The amount of either of these acids in the original fat is probably less than 0.1 %.

DISCUSSION

In contrast to the ease generally experienced in purifying esters of fatty acids of the C_{18} series, considerable difficulty was found in obtaining a C_{20} fraction of theoretical saponification equivalent (cf. Tables 1, 3, 4). A similar difficulty in obtaining pure methyl arachidonate by fractional crystallization and distillation was attributed by Mowry, Brode & Brown (1942) to the formation of a constant boiling-point mixture with C_{18} methyl esters.

During fractionation of the combined C_{20} methyl esters, some separation of monoethenoid from polyethenoid esters occurred, as shown by the fall in iodine value from fractions L_{27} to L_{213} (Table 1). The rise in iodine values of the final fractions L_{214} and L_{215} might be explained by the presence of a trace of C_{22} acids; no conclusive evidence for the presence of such acids was, however, obtained. Further distillation of the undistilled residues L_3 in a molecular still gave only fractions of equivalents below 310, and probably either decomposition products or dimers. If C_{22} acids were present in the fat, then either they were selectively destroyed during manipulation, or they were present in amount smaller than the detected amount of arachidonic acid (0.07 %).

The monoethenoid acid. The major component acid of many natural fats is accompanied by small amounts of homologues both of higher and of lower molecular weight. It is not unexpected, therefore, that in pig depot fat, containing 40–50 % of oleic acid, both hexadecenoic acid and an eicosenoic acid should occur. It is known, however, that the decenoic, tetradecenoic and hexadecenoic acids of cow milk fat (Hilditch & Longenecker, 1938), the tetra- and hexadecenoic acids of ox depot fat (Hilditch & Longenecker, 1937), and the hexadecenoic acid of pig depot fat (Hilditch *et al.* 1939) have the double bond in the 9–10 position. It is somewhat surprising, therefore, that the eicosenoic acid of pig depot fat should have the Δ^{11-12} structure.

Other Δ^{11-12} unsaturated acids are, however, known to occur naturally. Thus cetoleic acid (Δ^{11-12} docosenoic acid) has been found in various marine animal and fish oils (Toyama, 1927). More relevant to a discussion of land animal fats is the report by Bertram (1928) of the presence in various animal fats of vaccenic acid, shown by oxidation to be Δ^{11-12} octadecenoic acid. Other workers (Grossfeld & Simmer, 1930; Viollier & Iselin, 1941) have determined the amount of this acid present in animal fats

* Ault & Brown (1934) give the polybromide number of arachidonic acid as 90.

† Probably correct if each double bond is separated from a neighbouring double bond by one $-\text{CH}_2-$ group only, but not otherwise; see discussion below.

‡ Using the molecular extinction coefficient of $\Delta^{9-10, 11-12}$ eicosadienoic acid given by Bradley & Richardson (1942), and assuming that the unconjugated material was unchanged by the isomerization.

by a method essentially depending on a determination of the iodine values of lead salts soluble in aqueous ethanol containing a small amount of acetic acid. Swine fat, beef fat, mutton fat and butter fat are recorded as containing 0.2, 0.8–1.6, 1.0–1.8 and 1.1–4.7% respectively of vaccenic acid. It seems quite possible that at least a part of the vaccenic acid detected by the above workers may have been Δ^{11-12} eicosenoic acid; preliminary work in this laboratory has indicated, however, that sheep fat as compared with pig back fat contains less Δ^{11-12} eicosenoic acid.

No conclusion can be reached in respect of the origin of Δ^{11-12} eicosenoic acid in pig depot fat, as the corresponding dietary fats have not been examined in sufficient detail. Sow's milk fat has been shown by the writers (de la Mare & Shorland, 1944) to contain approximately 3.9% of C₂₀–C₂₂ acids, of which most, however, seemed to be highly unsaturated. In regard to cow's milk fat, Hilditch & Longenecker (1938) do not mention the possible presence of eicosenoic and eicosadienoic acids suggested by Bosworth & Brown (1933). It may be noted, however, that Baldwin & Longenecker (1944*a*) record the possible presence of approximately 0.6% of eicosadienoic acid in cow's colostrum; their analyses are not inconsistent, as will be shown below, with the presence of an eicosenoic acid in this fat. It is possible, therefore, that the trace of milk fat present in the diet of pigs used in the present work is the source of Δ^{11-12} eicosenoic acid.

Di- and triethenoid acids. Although spectroscopic examination determines with fair certainty the types of conjugation present in a sample, the appearance of diene conjugation after isomerization by alkali is not conclusive evidence that a diene was present in the unisomerized material, nor does the failure to obtain conjugated diene material in this way prove the absence of dienes. It is known, for instance, that in linoleic acid the double bonds are separated by one CH₂ group only; and the mechanism of isomerization by alkali is regarded by Kass & Skell (quoted by Wood, Jackson, Baldwin & Longenecker, 1944) as involving removal of a

proton from the active methylene group of the 1–4 diene system. Hence it would be expected that, as compared with linoleic acid, an isomeric octadecadienoic acid, in which the double bonds were separated by two or more methylene groups, would not rearrange to a diene system as readily.* Similarly, a tetraene acid

might be expected to rearrange on heating with alkali to yield a compound showing only diene conjugation.

In this connexion it is interesting that conjugation develops during the reactions of oxygen with methyl linolenate and with methyl docosahexaenoate, both of which contain the methylene-interrupted system

velops, however, during the similar reaction between oxygen and squalene, which contains the grouping

(1943) regard this reaction as having a free radical mechanism involving attack on the CH₂ group adjacent to a double bond, the rearrangement to form a conjugated system being made possible by resonance in the intermediate free radical. Similar considerations may apply to the rearrangement of 1–4-diene acids under the influence of alkali.

Baldwin & Longenecker (1944*a, b*) have concluded from evidence similar to that presented by the present writers that human milk fat and cow colostrum fat contain eicosadienoic acids as minor component acids. Their methods differed from ours in that saturated acids were not removed from their

* It should be noted, however, that migration of a double bond in oleic acid from the 9–10 position to the 2–3 position is regarded by Hilditch (1940, p. 315) as explaining the isolation of palmitic acid after fusion of oleic acid with KOH.

Table 7. Comparison of different methods of calculation of the composition of fractions containing di- and polyethenoid C₂₀ acids

Fraction	Fraction 17 (Table 3) of Baldwin & Longenecker (1944 <i>a</i>). Sap. equiv. 316.0; i.v. 130.2		Fraction 26 (Table 2) of Baldwin & Longenecker (1944 <i>b</i>). Sap. equiv. 315.9; i.v. 120.8	
	Our assumptions	B. and L.'s assumptions	Our assumptions	B. and L.'s assumptions
Eicosatetraenoic acid (%)	15.5	15.3	20.8	20.8
Eicosatrienoic acid (%)	6.7	—	1.6	—
Eicosadienoic acid (%)	13.3	51.6	10.1	34.5
Eicosenoic acid (%)	55.0	—	44.2	—
Eicosanoic acid (%)	9.5	33.1	23.3	44.7
Total	100.0	100.0	100.0	100.0

fractions by crystallization, and in their calculations all the iodine value not accounted for as arachidonic acid was attributed to eicosadienoic acid. Alternatively the assumptions of our work (i.e. that the specific extinction coefficients for isomerized linoleic and linolenic acids can be applied to the corresponding C_{20} analogues) could be applied to their results. Comparisons of the two methods of calculation are given in Table 7 for two of their fractions. It can be seen that further investigation and more accurate characterization of the C_{20} di- and polyethenoid acids is necessary before reaching a final judgement.

Polymerization. Bradley & Johnston (1940) have discussed the products from, and mechanism of, thermal polymerization of methyl esters of unsaturated fatty acids. The predominant reaction was shown to be polymerization by bimolecular addition at the double bonds, often preceded by conjugation and accompanied by cyclization. Small amounts of new low boiling-point materials of low molecular weight were also formed, probably by thermal decomposition of unsaturated esters. In the case of the esters from soybean oil and from olive oil, new high boiling-point methyl esters were isolated. Thus from olive oil methyl esters (b.p. (1 mm.), 140–160°; sap. equiv. 295; i.v. 83.4), a fraction was isolated with the following properties: b.p. (1 mm.), 180–200°; sap. equiv. 404; i.v. 108.6.

Although the possibility cannot be entirely eliminated that our polyethenoid C_{20} acids were contaminated with materials similar to those described by Bradley & Johnston (1940), it is believed that substances of the latter type were not major constituents of our fractions for the following reasons. First, refractionation of our C_{20} fractions did not in any case reveal the presence of methyl esters of equivalent greater than 327, whereas in the work of the former authors, fractions of equivalent greater than 350 were consistently obtained. Secondly, the products of low equivalent should have been detected, contaminating the C_{18} fraction. Thirdly, the conditions of distillation would be expected to favour rather the continuous removal of any intermediates of low molecular weight than the further condensation of such intermediates with the C_{18} methyl esters. Fourthly, the iodine values of our C_{20} polyethenoid fractions are considerably higher (even after allowing for the presence of 23% of arachidonic acid) than the iodine values of the fractions obtained by the former authors. Fifthly, the iodine values and structures suggested by the former authors for the relevant fractions appear to be inconsistent with the iodine values and results of isomerization by alkali of the polyethenoid fractions obtained in the present work.

Conjugation. In view of the extensive treatment necessary during the preparation of the C_{20} poly-

ethenoid fraction, no certainty can be felt concerning the origin of the conjugation present in this material to the extent of about 13% (calculated as $\Delta^{9-10, 11-12}$ octadecadienoic acid). Purification by distillation of linoleic acid (Wood *et al.* 1944) and of methyl arachidonate (Mowry *et al.* 1942) is known to result in development of only traces of diene conjugation. The products of thermal polymerization of the methyl esters of unsaturated, non-conjugated fatty acids have been shown (Bradley & Richardson, 1940) not to be conjugated, though conjugation is regarded as generally preceding polymerization. Tentatively, therefore, it is suggested that the conjugation found in the C_{20} polyethenoid concentrate is likely either to have been derived from C_{20} di- or triethenoid acids by alteration during manipulation, or to have been present in the original fat. It may be noted that Miller (1940) records for pig leaf fat an $E_{1\text{cm}}^{1\%}$ of 2.0 at 2340 Å, corresponding (as $\Delta^{9-10, 11-12}$ octadecadienoic acid) with approximately 0.2% of diethenoid material.

General considerations. Evidence is gradually accumulating that the C_{20} acids of animal depot and milk fats are probably more complex mixtures than had until recently been realized. The work of Baldwin & Longenecker (1944 *a, b*) has been discussed somewhat critically above; but the writers wish to emphasize their belief that the conclusions of the former authors are qualitatively correct. Cramer & Brown (1943), from crystallization data, reached similar conclusions in respect of human depot fat.

The possible function of C_{20} di- and triethenoid acids as essential factors in nutrition makes further investigation of the structure of these acids an interesting problem which we hope to investigate further.

SUMMARY

1. The C_{20} unsaturated acids of the back fat of bacon pigs fed only buttermilk during the fattening period have been isolated by crystallization of the fatty acids and fractional distillation of their methyl and ethyl esters.

2. Δ^{11-12} eicosenoic acid has been shown to occur probably to the extent of 0.5%; milk fat is a possible source of this acid.

3. Isomerization by alkali at 180° has been used to characterize the C_{20} polyethenoid acids.

4. Subject to the limitations of the method discussed in the text, evidence is presented for the presence of eicosadienoic acid and/or eicosatrienoic acid.

5. Arachidonic acid was identified by bromination as a minor constituent of the C_{20} unsaturated acids.

6. No conclusive evidence for the presence of unsaturated C_{22} acids was obtained.

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The Non-identity of Thudichum's 'Glycoleucine' and *nor*Leucine

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The natural occurrence of *nor*leucine is generally considered in the literature as having been first reported by Thudichum (1901), who obtained an isomer of leucine, originally called 'glycoleucine', from the proteins of ox brain by baryta hydrolysis. When Abderhalden & Weil (1912) first applied Fischer's 'ester' method to the proteins of nervous tissue they were familiar with the passage in Thudichum's (1901) book on the chemistry of brain, in which occurs (pp. 257, 301) the description of this amino-acid C₆H₁₃O₂N. The acid, differing charac-

teristically from ordinary *L*-leucine by the insolubility of its Cu salt and of the free acid, had a sweet taste, and was therefore called 'glycoleucine' in distinction from the tasteless ordinary leucine. Although Thudichum considered 'glycoleucine' as identical with the aminocaproic acid which he had obtained from caproic acid by the well-known bromine process, he cautiously refrained from drawing a conclusion as to its constitution on account of the insufficiently characterized commercial caproic acid used for the synthesis.