

268. *The Unsaponifiable Matter of Butter Fat. Part I.*

By ISOBEL M. MORICE.

Chromatography has shown the unsaponifiable matter of butter fat to be extremely complex. From it have been isolated the triterpene lanosterol, previously obtained only from wool fat and yeast, and small amounts of four other colourless crystalline compounds not hitherto known to occur in butter. Compound A, detected by Morton and Heilbron (*Biochem. J.*, 1930, **24**, 870) and reported as an unidentified substance with an absorption maximum at 272 m μ ., has m. p. 108° and probable formula C₁₉H₁₆O; compounds B (m. p. 80°), C (m. p. 65°), and D (m. p. 38°) have probable formulæ C₂₇H₅₆O, C₁₄H₂₈O₂, and C₁₄H₂₈O respectively.

THE unsaponifiable matter of butter is known to contain cholesterol (Kreis and Wolf, *Chem.-Ztg.*, 1898, **2**, 805), β -carotene (Gillam and El Ridi, *Biochem. J.*, 1937, **31**, 251), and squalene (Fitelson, *J. Assoc. Off. Agric. Chem.*, 1943, **26**, 506), which have been isolated from it, vitamin A (Morton and Heilbron, *Biochem. J.*, 1930, **24**, 870), kryptoxanthin, and lycopene (Gillam and Heilbron, *ibid.*, 1935, **29**, 834), which have been detected by their absorption spectra, and vitamin D (Kon and Booth, *ibid.*, 1934, **28**, 111) which biological tests have shown to be present.

The purpose of this investigation was to determine if the unsaponifiable matter of butter fat contained any other substances. Accordingly it was separated into three fractions by its solubility in methanol at 50° and -26°, and each fraction further divided by chromatography. Adsorption of the fraction soluble in methyl alcohol at -26° on alumina gave a number of

sub-fractions. Re-adsorption of these, followed by crystallisation, showed that the material adsorbed immediately below cholesterol was a colourless substance crystallising from methanol in soft needles, m. p. 138—139°, but forming a jelly with light petroleum. This compound formed an acetate and a benzoate, and the melting points of these, together with its slow precipitation by digitonin and the yellow colour with a green fluorescence given by the Liebermann–Burchard reagent (Burchard, *Chem. Zentr.*, 1890, I, 25) led to the supposition that it was lanosterol. Wieland, Pasedach, and Ballauf (*Annalen*, 1937, 529, 68) report that cryptosterol, which has since been proved by Ruzicka, Denss, and Jeger (*Helv. Chim. Acta*, 1945, 28, 759) to be identical with lanosterol, gives a yellow colour with the Liebermann–Burchard reagent.

The melting points and specific rotations of our alcohol, and that from wool fat, and of their acetates and benzoates, are similar (see Table I which gives also data recorded for

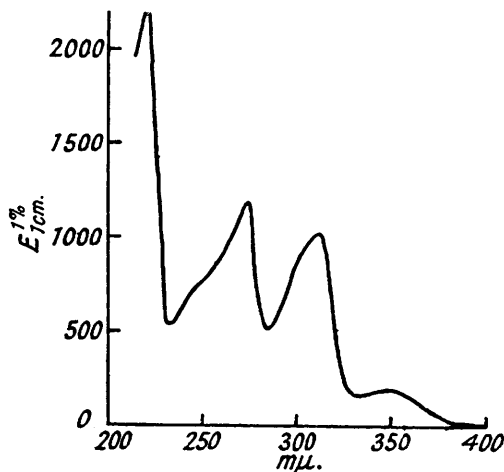
TABLE I.

Source.	Alcohol.		Acetate.		Benzoate.		Reference.
	M. p.	$[\alpha]_D$.	M. p.	$[\alpha]_D$.	M. p.	$[\alpha]_D$.	
Butter fat ...	138—139°	+59.1°	127—128°	+59.0°	191—192°	+76.3°	This paper
Wool fat ...	138—139	61.6	125—127	61.2	191—192	76.9	"
Yeast	138—139.5	60.7	130—131	65.3	190—191	70.5	a, b
Wool fat ...	138—139	62.3	128—129	64.5	191.5	74.5	b, c

(a) Wieland, Pasedach, and Ballauf, *loc. cit.* (b) Ruzicka, Denss, and Jeger, *loc. cit.* (c) Windaus and Tschesche, *Z. physiol. Chem.*, 1930, 190, 51.

cryptosterol and lanosterol); mixed melting points gave no depression. Thus it appears certain that this alcohol occurring in butter fat is lanosterol.

From the absorption spectrum of the unsaponifiable matter of butter Morton and Heilbron (*loc. cit.*) reported the presence of an unidentified substance giving a maximum at 272 m μ . This has now been isolated and provisionally named compound A. It was found on the alumina column below lanosterol, has m. p. 108°, and in ethanol absorbs strongly at 272.5 m μ . with other maxima at 220, 310, and 348 m μ . (see figure). It is highly unsaturated to tetranitromethane, and appears to have the formula C₁₉H₁₆O.



Ultra-violet absorption spectrum of compound A in 95% ethanol.

Three other crystalline substances, provisionally named B, C, and D, not previously known in butter have been isolated. They appear all to be saturated. The probable formulæ are C₂₇H₅₆O, C₁₄H₂₈O₂, and C₁₄H₂₈O, respectively. The four compounds, A, B, C, and D, occur in very small quantities and are not yet fully characterised. Further work on them is in progress.

EXPERIMENTAL.

(M. p.s are uncorrected. Specific rotations are in chloroform. The light petroleum boiled at 50—60° The analyses are by Mr. B. R. Thomas, Dominion Laboratory, and Dr. T. S. Ma, University of Otago.)

The Unsaponifiable Matter of Butter Fat.—Butter fat (4 kg.) was saponified with 0.5N-alcoholic potassium hydroxide (40 l.), and the unsaponifiable matter extracted with ether according to the method

recorded in *The Analyst*, 1933, 58, 203. The unsaponifiable matter (21 g.) was divided into fractions: I, insoluble in 1050 ml. of methanol at 50° (6.2%); II, soluble in methanol at 50°, insoluble at -26° (64.5%); and III, soluble at -26° (29.3%).

Fraction I (1.3 g.), dissolved in light petroleum (125 ml.), was adsorbed on a column of alumina (55 g.; 23 × 1.8 cm.). The column was eluted successively with light petroleum (500 ml.), benzene (450 ml.), ether (300 ml.), and ethanol (350 ml.) in 50 ml. portions. Four fractions, 0.06, 0.50, 0.09, and 0.65 g., in decreasing order of adsorbability, were obtained. The second fraction, in light petroleum, deposited crystals of cholesterol (147 mg.), m. p. 148—149°, which was identified by mixed melting point with authentic cholesterol. Some colourless waxy crystals (50 mg.), m. p. 63—65°, were obtained from the fourth fraction but did not appear to be pure.

Fraction II (13.5 g.), dissolved in benzene (280 ml.), was adsorbed on alumina (140 g.; 28 × 2.5 cm.). The column was eluted successively with benzene (4350 ml.), ether (2250 ml.), and benzene-ethanol (2250 ml.) in 150 ml. portions. The five fractions obtained, in decreasing order of adsorbability, are shown in Table II. Fraction 2 in acetone gave cholesterol (7.87 g.), m. p. 149—150°. Fraction 3 in acetone gave *compound B* (32 mg.) as colourless crystals, 79—80° (Found: C, 81.7; H, 14.3. $C_{27}H_{46}O$ requires C, 81.7; H, 14.2%), giving no colour with tetranitromethane. Fraction 4 in benzene-methanol gave 6 mg. of somewhat impure β -carotene which when pure melted at 182—183°, did not depress the m. p. of authentic β -carotene, and had an absorption spectrum identical with that of authentic β -carotene. From fraction 5 in acetone were obtained colourless crystals (40 mg.), m. p. 60—61° (Found: C, 85.3; H, 14.8. $C_{32}H_{54}$ requires C, 85.2; H, 14.8%), giving no colour with tetranitromethane. Although the m. p. is sharp it seems doubtful whether this is one hydrocarbon only.

TABLE II.

Fraction.	Wt. (g.)	Compound present.	Fraction.	Wt. (g.)	Compound present.
1	0.30	—	4	0.14	β -Carotene
2	10.64	Cholesterol	5	1.98	Hydrocarbon mixture, m. p. 61°
3	0.44	Compound B			

Fraction III (6.2 g.), dissolved in light petroleum (130 ml.), was adsorbed on alumina (130 g.; 26 × 2.5 cm.). The column was divided into nine portions, of which the three upper were eluted with 95% ethanol and the six lower with 95% ethanol and light petroleum.

Fractions 4—8 were dissolved in light petroleum and separately re-adsorbed on alumina. Fraction 3 in light petroleum was re-adsorbed on calcium oxide. Fractions 1 and 2 were dissolved in chloroform and separately re-adsorbed on alumina. In several cases the highest sub-fraction was added to the fraction above before re-adsorption, and the lowest sub-fraction added to the highest remaining sub-fraction of the fraction below. The fractions and sub-fractions obtained are shown in Table III in decreasing order of adsorbability. Fraction 1(3) in acetone gave *compound C* (44 mg.) as colourless crystals, m. p. 64—65° (Found: C, 73.1; H, 12.8. $C_{14}H_{26}O_2$ requires C, 73.6; H, 12.4%), giving no colour with tetranitromethane. From fractions 1(4), 2(1), 2(2), 2(3), and 3(2) light petroleum or methanol gave cholesterol (340 mg.), m. p. 146—147°. Fractions 1(4) and 2(1) had absorption spectra with maxima at 424, 445, and 472 μ . in ethanol, indicating the presence of small amounts of a xanthophyll. Vitamin A was shown to be present in fractions 2(3), 3(2), and 4(1) which gave typical vitamin-A absorption curves in ethanol with a maximum at 324 μ . Lanosterol and *compound A* are further described below. Fraction 8(1) in methanol gave *compound D* (40 mg.) as colourless crystals, m. p. 37—38° (Found: C, 79.2, 79.3; H, 13.1, 13.2. $C_{14}H_{26}O$ requires C, 79.2; H, 13.3%), showing no colour with tetranitromethane. Fraction 8(2) in benzene-methanol gave approx. 3 mg. of β -carotene, m. p. 182—183°.

TABLE III.

Fraction.	Sub-fraction.		Compounds present.	Fraction.	Sub-fraction.		Compounds present.		
	G.	G.			G.	G.			
1	0.92	(1)	0.06	4	0.62	(1)	0.59	Lanosterol, vitamin A	
		(2)	0.10			(2)	0.15		Lanosterol
		(3)	0.50			(3)	0.14		Lanosterol
		(4)	0.12			(1)	1.06		Lanosterol
		(5)	0.02			(6)	0.42		(1)
2	1.37	(1)	0.54	(7)	0.20	(1)	0.12	Compound A	
		(2)	0.43	(2)	0.02	(2)	0.02	—	
		(3)	0.39	(8)	0.28	(1)	0.20	Compound D	
		(4)	0.03	(2)	0.09	(2)	0.09	β -Carotene	
3	0.56	(1)	0.11	9	1.06	—	—		
		(2)	0.27			Cholesterol, lanosterol, vitamin A			

Lanosterol.—From fractions 3(2), 4(1, 2, 3), and 5(1) of fraction III was obtained a colourless substance (183 mg.) crystallising in rosettes of soft needles (from methanol), m. p. 138—139°, $[\alpha]_D^{25} +59.1^\circ$ (*l*, 0.5; *c*, 1.29) (Found: C, 84.1, 83.6; H, 11.7, 11.9. Calc. for $C_{28}H_{48}O$: C, 84.5; H, 11.8%). The Liebermann-Burchard reagent gave a yellow colour with a green fluorescence. Digitonin gave a precipitate after approx. one hour.

The substance was acetylated by warm pyridine-acetic anhydride, and the product was precipitated and after two crystallisations (methanol) yielded shining prisms, m. p. 127—128°, $[\alpha]_D^{18} +59.0^\circ$ (*l*, 0.5;

c, 1.36) (Found : C, 82.1; H, 11.6. Calc. for $C_{27}H_{44}O_2$: C, 82.0; H, 11.2%). Hydrolysis of the acetate with 0.3*N*-alcoholic potassium hydroxide gave the original alcohol, m. p. 138—139°.

The benzoate, formed by pyridine-benzoyl chloride, crystallised from acetone as rosettes of soft shining needles, m. p. 191—192°, $[\alpha]_D^{14} +76.3^\circ$ (*l*, 0.5; *c*, 2.33) (Found : C, 83.4; H, 10.2. Calc. for $C_{27}H_{44}O_2$: C, 83.7; H, 10.3%). Hydrolysis with 0.5*N*-alcoholic potassium hydroxide gave the original alcohol, m. p. 138—139°.

Lanosterol from Wool Fat.—Two adsorptions of the unsaponifiable matter of wool fat on alumina yielded lanosterol, m. p. 138—139°, $[\alpha]_D^{16} +61.6^\circ$ (*l*, 0.5, *c*, 1.30). Lanosteryl acetate formed prisms (from methanol), m. p. 126—128°, $[\alpha]_D^{16} 61.2^\circ$ (*l*, 0.5, *c*, 1.50). Lanosteryl benzoate crystallised from acetone-methanol in rosettes of soft needles, m. p. 191—192°, $[\alpha]_D^{14} +76.9^\circ$ (*l*, 0.5, *c*, 2.08).

Compound A.—Fractions 6(1) and 7(1) of fraction III, dissolved in light petroleum, gave colourless prismatic needles (40 mg.), m. p. 107.5—108° (Found : C, 87.6, 87.6; H, 6.2, 6.2; *M* (Rast), 232. $C_{19}H_{16}O$ requires C, 87.7; H, 6.2%; *M*, 260). The substance gives a blue fluorescence when dissolved in methanol or ethanol but none in light petroleum. With tetranitromethane a brown colour is obtained. The ultra-violet absorption curve in 95% ethanol determined with a Beckman spectrophotometer has maxima at 220, 272.5, and 310 μ . and a smaller one at 348 μ . ($E_{1\%}^{1\text{cm}}$ = 2225, 1180, 1021, and 190 respectively). The compound was not acetylated by ordinary means, neither did it form an oxime.

FATS RESEARCH LABORATORY,
DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH,
WELLINGTON, NEW ZEALAND.

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