siderable purification is required before it can be demonstrated. Brewer's yeast extracts, which require little further treatment, are therefore very convenient for the preparation of AM^{32}P . As dialysis results in inactivation of these extracts of yeast the method used here to decrease phosphate content is to be preferred, although treatment with such high concentrations of magnesium and ammonium chlorides may diminish the activity of the enzyme system.

The concentrations of adenosine ($1.4 \times 10^{-3} \text{ M}$) and ATP ($9.1 \times 10^{-4} \text{ M}$) used in the method described are respectively 7 and 1.8 times higher than those required for optimum activity of the enzyme system (Kornberg & Pricer, 1951), but to use less would introduce difficulties in the isolation of the final AM^{32}P which would contain a smaller proportion of the total radioactivity.

The chemical isolation of AM^{32}P from these extracts resulted in heavy losses even with added carrier AMP, but with the paper-chromatographic technique described here the recovery was quantitative and comparatively simple.

SUMMARY

1. A method is described for the preparation of ^{32}P -labelled adenosine 5'-phosphate in an extract of brewer's yeast. The product is quantitatively separated and is chromatographically homogeneous. It has an activity of approximately 0.2 mc/mg.

2. After deamination of this adenosine 5'-phosphate with nitrous acid, radioactive inosine 5'-phosphate, chromatographically pure, can be separated in a similar manner.

3. Radioactive ribose 5-phosphate is obtained by heating this adenosine 5'-phosphate in N hydrochloric acid and can be separated by paper chromatography.

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The Long-chain Paraffin Constituents of Rose-petal Wax

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In a series of papers dealing with the long-chain constituents of plant and insect waxes it has been shown, from evidence based largely on melting point and long crystal spacing data, that all the saturated, ketonic and hydroxy acids, as also the corresponding primary alcohols, contain an even number of carbon atoms, whereas the paraffins, ketones and secondary alcohols, which are probably formed from these respective acids by decarboxylation, contain an odd number of carbon atoms (Chibnall & Piper, 1934 (for main references); Blount, Chibnall & Mangouri, 1937; Chibnall, Piper, Mangouri, Williams & Iyengar, 1937). Such findings, in so far as they relate to the saturated

acids and primary alcohols, bring the wax constituents into line with those of somewhat shorter chain length present in glycerides and similar natural products. There is, indeed, an overlap between the two sets of components and collectively the complete series of even-numbered members from C_{10} to C_{36} is represented, suggesting that all of them can be regarded as manifestations of the same sequence of metabolic events.

The evidence with respect to the paraffins is much less widespread. Chibnall, Piper, Pollard, Williams & Sahai (1934) analysed the data then available, and concluded that in all cases other than the one mentioned below the products that had

hitherto been isolated consisted of one or more of the odd-numbers from C_{25} to C_{37} . The exception was rose-petal wax, which had been investigated by Prophète (1926), who showed that the main component was a mixture of hydrocarbons with a melting-point of 53–55°, from 7 g. of which he claimed to have isolated by fractional distillation and crystallization the paraffins C_{16} , C_{20} , C_{21} , C_{22} , C_{23} , C_{26} , C_{27} , C_{35} . No analyses but only melting and boiling points were quoted. For reasons given by Chibnall *et al.* (1934) we did not consider that the identification of these paraffins was valid, for the amount of material in the various distillates was small, and on fractional crystallization the separation of several products from each of them was claimed. Prophète's results, however, did suggest that paraffins of chain length shorter than C_{25} were present in this particular wax, which was accordingly a very suitable one for re-investigation by our own procedure. A generous supply of raw material having been placed at our disposal by Schimmel and Company, the scent manufacturers of Leipzig, we have been able to show that the wax contains all the odd-number paraffins between C_{21} and C_{37} . None of the even-number paraffins could be isolated, and it seems certain that if they are present at all they must be very minor constituents. When, for various reasons, the work was interrupted in 1938, a low-melting product obtained by steam distillation of the residual unsaponifiable material was under investigation. A number of fractions has been prepared from it by vacuum distillation, and some of these are liquid at room temperature. Paraffins of chain length shorter than C_{21} are definitely represented, but on account of the existing inadequacy of the relevant melting-point, transition-temperature and crystal-spacing data we have been unable to make satisfactory progress in the identification of the components by our present procedure. These low-melting fractions should be amenable to chromatographic methods of analysis.

EXPERIMENTAL

The melting points recorded in this paper were obtained by the method of Piper *et al.* (1931) and are corrected. The crystal measurements were all made by the method previously described (Piper *et al.* 1931). This method, employing the radiation reflected from a layer of the material, has been retained, since much information on purity can be obtained by recording as many orders of reflexion as possible (see, for example, Piper *et al.* 1931, p. 2081, fig. 1). Transmission powder photographs do not register these reflexions so clearly.

Preliminary fractionation of the crude wax

The crude wax was a brown, opaque sticky substance with a faint odour of rose, indicating the presence of a small amount of essential oil. It had a saponification value of 51.9

and no definite m.p. On heating it started to liquefy at 53°, but the melt did not clear until 70°. The material (500 g.) was extracted under reflux with 3.5 l. of light petroleum (b.p. 40–60°), filtered hot through a jacketed filter kept at 40° and the filtrate allowed to stand at 0° overnight. The pale yellow crystalline material which separated was filtered off (fraction A; m.p. 61.5°). On evaporation the filtrate gave a sticky residue (fraction B). Fraction A was dissolved in 1200 ml. of warm ether, 2400 ml. of acetone were added and the mixture was kept overnight at 0°. The crystalline mass was filtered off, washed with ether, then with acetone, and air-dried (fraction C; wt. 220 g.). The filtrate was taken to dryness *in vacuo* (fraction D; wt. 9 g.). Fraction B was treated in a similar way to give crystalline material (fraction E; wt. 146 g.) and a reddish brown oil (fraction F; wt. 100 g.).

Treatment of the wax fractions. Fractions C, D and E were first saponified by the procedure of Chibnall *et al.* (1934), the acids being separated as the calcium soaps. In each case the unsaponifiable material was then passed through the phthalate treatment of Chibnall, Piper, Pollard, Smith & Williams (1931) to remove the primary alcohols, giving paraffin fractions G, H and K respectively (Table 1).

Fraction F was treated somewhat differently. It was saponified for 12 hr. in 2 l. of 5% (w/v) ethanolic KOH. The major part of the ethanol was next removed by distillation, the residue diluted with water and then exhaustively extracted with ether. The ethereal solution was washed, the solvent removed and the residue taken up in 300 ml. warm acetone. The crystalline material that had separated after standing for some days at 0° was filtered off and then passed through the phthalate treatment to give paraffin fraction L (Table 1). As the product recovered from the acetone filtrate was a dark brown, very viscous liquid it was decided to treat it with phthalic anhydride at room temperature (Weidemann, 1926). The material (59 g.) was dissolved in freshly distilled pyridine (400 ml.) and phthalic anhydride (60 g.) added. The mixture was allowed to stand at room temperature for 3 days and poured into 1 l. of dilute HCl, which was then brought nearly to the boil. After standing for some time at 0° the aqueous solution was siphoned off and the residue treated repeatedly with hot water until the washings were neutral to litmus. It was then taken up in ether at 20° and shaken with aqueous Na_2CO_3 . A small amount of primary alcohol separated in the aqueous phase as its sodium phthalate. The ethereal solution was drawn off, washed with water and the ether removed. The residue was taken up in hot ethanol and allowed to stand at 0°, when a semi-solid dark brown mass of material separated. This was removed and submitted to steam distillation during 10 working days. The product (12.9 g.) that separated from the distillate was a soft paste, and, as mentioned in the Introduction, it contained low-melting paraffins that have not been fully characterized. The residue in the flask, after removal of the water, was transferred to a vacuum still. Only a very small amount of a coloured oil came over at 120°,

Table 1. *Paraffin fractions from rose-petal wax*

Fraction	Weight (g.)	C (%)	H (%)	Melting point (°)
G	140	85.4	14.8	62.0–62.5
H	87	85.4	14.6	51.5–52.0
K	4	—	—	53.5–54.0
L	20.5	—	—	43.5–44.0

and 0.1 mm., showing that low-boiling paraffins, if present in fraction *F*, had been removed by distillation in steam.

The fatty acids (89 g.) were recovered in the usual way from the calcium soaps, and the primary alcohols (50 g.; m.p. 79–82°) from the sodium primary alcohol phthalates. Neither fraction has been characterized further. The 500 g. of crude wax had thus given 402.4 g. of long-chain material, of which 65.5% was paraffin, 12.4% primary alcohol and 22.0% fatty acid. Prophète's wax was a hard, olive-green solid, melting at 61°, which gave 67.2, 13.0 and 19.8% of the above-mentioned products respectively.

Fractionation of the paraffins

For the fractionation of the paraffins a special type of vacuum still was designed, and we are indebted to Mr J. H. Burrow for its construction and for many helpful suggestions. This apparatus permitted the establishment of a definite reflux ratio, and thermometers suitably placed allowed the temperatures of the condenser water and the column to be kept under control by suitable variation of the heating rates. The condenser was conical in shape with the apex below, and the condensed liquid dripped into a small funnel. From here it passed down a delivery tube, maintained at a temperature high enough to keep it liquid, into a collecting vessel. This vessel was provided with six collecting tubes, which could be rotated in succession below the delivery exit and collect the distillate over controlled temperature intervals. A full description of the apparatus is given elsewhere (Mangouri, 1937).

Fractional distillation of the paraffins. For brevity, the upper limit only of the melting points of the various fractions, except those in Table 3, will be quoted. Fraction *G* (Table 1), on distillation, gave twenty-two fractions. The melting points of 1–5 ranged from 47.5 to 58.5°; the remainder from 60.25 to 67.2°. The still residue, on extraction with warm benzene, gave 25 g. melting indistinctly at 67.5°. Fractions *H* and *K* (Table 1), together with the above-mentioned fractions 1–5 (collected wt. 111 g.), on distillation gave sixteen fractions. The melting points of 1–3 ranged from 44.5 to 48.25°, the remainder from 50.4 to 60.25°. The still residue, treated as before, gave 5.14 g. melting at 60.75°. Fraction *L* (Table 1), together with the above-mentioned fractions 1–3 (collected wt. 38.8 g.), gave eight fractions melting between 39.5 and 50°, with a residue melting at 52°.

This preliminary separation gave forty-one fractions with melting points ranging from 39.5 to 67.5°, and could thus be between them contain all the paraffins from C_{21} to C_{31} . When, however, the derived data for an appropriate fraction were compared with those of the pure paraffin of similar melting point, the transition temperatures were always too low and the X-ray photographs much inferior. One fraction was exceptional, and was in excellent agreement with C_{29} . Clearly the treatment so far applied had not been sufficiently exacting.

For the next series of operations the above-mentioned fractions were collected into groups which, on a basis of melting point, represented $C_{21} + C_{22}$ (24.8 g.), $C_{23} + C_{24}$ (35.2 g.), C_{24} (40.6 g.), $C_{25} + C_{26}$ (32.0 g.), $C_{26} + C_{27}$ (20 g.),

Table 2. *Fractions of paraffin tabulated according to their melting point*

Fraction no.	Weight (g.)	Melting point (°)	Fraction no.	Weight (g.)	Melting point (°)	Fraction no.	Weight (g.)	Melting point (°)
1	0.71	38.0	32	5.0	53.2	63	7.21	62.75
2	0.35	39.25	33	1.27	53.25	64	8.21	63.4
3	1.03	40.25	34	2.7	54.25	65	5.22	63.7
4	2.29	41.25	35	3.0	54.5	66	4.54	63.75
5	2.01	41.75	36	2.57	54.5	67	5.22	63.75
6	0.29	41.75	37	3.05	54.75	68	2.66	63.75
7	1.47	42.0	38	6.04	55.0	69	1.44	64.0
8	1.38	42.5	39	5.65	56.0	70	2.15	64.25
9	1.59	42.75	40	4.14	56.25	71	2.41	64.5
10	1.1	43.0	41	2.41	56.25	72	0.62	64.5
11	1.39	43.2	42	2.01	56.6	73	1.21	64.75
12	0.63	44.8	43	0.45	56.75	74	1.04	65.0
13	0.84	45.2	44	0.75	57.0	75	1.80	65.0
14	0.74	45.75	45	1.78	57.5	76	1.66	65.25
15	1.27	46.25	46	1.71	57.5	77	0.11	65.5
16	0.52	47.70	47	2.21	58.0	78	1.53	65.75
17	7.2	47.70	48	2.55	58.0	79	1.44	65.75
18	0.9	48.8	49	1.77	58.25	80	1.88	65.75
19	1.1	48.75	50	2.85	58.25	81	1.15	65.8
20	0.4	49.0	51	1.11	58.25	82	2.89	66.0
21	3.0	49.25	52	0.7	58.5	83	0.56	66.25
22	4.08	49.25	53	7.12	59.5	84	0.69	66.40
23	3.93	49.75	54	10.17	61.0	85	0.20	66.75
24	2.4	50.0	55	3.7	61.25	86	0.87	67.0
25	4.38	50.5	56	1.8	61.25	87	0.87	68.0
26	3.38	50.5	57	3.3	61.5	88	1.11	68.5
27	1.21	50.5	58	2.51	61.80	89	1.2	68.5
28	1.55	51.0	59	2.42	62.5	90	0.98	68.75
29	0.75	51.75	60	1.27	62.5	91	1.3	69.0
30	1.1	52.0	61	0.62	62.5	92	1.1	69.8
31	0.7	52.0	62	4.0	62.5	93	0.24	73.2

C_{28} (37.3 g.) and C_{30} with higher members (51.2 g.). Each of these were then fractionated in the still. The last group gave nineteen fractions, and of these the final six (including the residue) gave indistinct melting points between 67.0 and 68.0°. They were bulked (27.3 g.) treated with H_2SO_4 at 120° for 2 hr., and the clarified product was submitted to fractional crystallization from light petroleum (b.p. 40–60°). Fourteen fractions were separated and after repetition of the treatment with H_2SO_4 where necessary, the melting points of these ranged from 61.25 to 73.2°. The final separation achieved is set out in Table 2.

Only a limited number of these new fractions could be given a full analysis, and the samples chosen were such as had melting points in fair agreement with those of the odd-number and even-number paraffins from C_{31} to C_{34} . The results are set out in Table 3, together with data for paraffins or mixtures of paraffins which have corresponding melting points or spacings.

DISCUSSION

The data collected in Table 3 are the most comprehensive yet made for a series of fractions obtained from a naturally occurring paraffin mixture. The constitution we have attributed to the various fractions has been derived from the following criteria: the melting point, the transition temperatures, the crystal spacing and modification, and the number and sharpness of the orders of reflexion. As has been shown elsewhere (Piper *et al.* 1931), melting and mixed melting points alone are quite inadequate to indicate the purity of a paraffin specimen. It has been possible, however, in most cases to suggest synthetic mixtures which agree quantitatively in behaviour with the fractions listed in Table 3. Where two or more mixtures appear to agree equally well in melting point, transition temperatures, and spacing with the fraction concerned, the number and sharpness of the orders of reflexion have had an important influence in the selection of which of these is the most probable solution.

Fraction 4. The absence of a first transition on heating and of a second on cooling is in keeping with low-melting synthetic paraffins such as C_{19} and C_{20} . The setting point shows that the fraction contains only a small amount of a second paraffin, as also does the X-ray photograph, which shows six clear orders. The composition is definitely 90 % C_{21} + 10 % C_{23} .

Fraction 12. The melting point suggests C_{22} , but for this even-number paraffin the 'B' form with a spacing of 28.1 Å would be the stable one at room temperature. The low transition temperatures are in keeping with the suggested mixtures of 60 % C_{21} + 40 % C_{23} . The X-ray photograph shows seven sharp orders, which actually is better than that given by the corresponding C_{29} – C_{31} synthetic mixture (Piper *et al.* 1931).

Fraction 16. The X-ray photograph shows eight clear orders and the product cannot therefore be

a gross mixture. All the data are consistent with 5 % C_{21} + 95 % C_{23} .

Fraction 27. The melting point would suggest that this is C_{24} , but the low transition temperatures and the X-ray photograph with five poor orders show that it is a mixture. The composition cannot be given with certainty and the presence of even-number components cannot be excluded. 55 % C_{23} + 45 % C_{25} is a possible suggestion from the poor data available.

Fraction 35. The transition temperatures are sharp and the photograph shows six well defined orders, strongly suggesting that one paraffin is present in predominating amount. All the data are in fair agreement with 90 % C_{25} + 10 % C_{27} . The possible 'two-even' mixture would have too high a spacing and indefinite transition temperatures, while the 'odd-even' mixture would have a low spacing and transition temperatures that are too high.

Fraction 42. The photograph shows only four poor orders and the transition temperatures are indistinct. It is definitely not C_{26} , but a crude mixture of three or more paraffins with C_{25} and C_{27} as probably major components.

Fraction 53. The photograph shows six good orders and the transition temperatures are sharp. The data are consistent with 90 % C_{27} + 10 % C_{29} , with probably a small amount of a third component. The possible mixture 40 % C_{26} + 60 % C_{28} has too low a spacing, shows only four to five poor orders and has transition temperatures that are not sharp. The possible odd-even combination has a spacing that is too low.

Fraction 58. The photograph shows six poor orders and the transition temperatures are not sharp, suggesting a mixture. Possible two-even and even-odd combinations give good photographs with spacings that are definitely too low. The data are in fair agreement with 30 % C_{27} + 70 % C_{29} .

Fraction 65. The X-ray photograph shows seven good orders. The somewhat low transition temperatures suggest C_{29} with about 1 % of both C_{27} and C_{29} .

Fraction 77. The melting point might suggest C_{30} . The indefinite transition temperatures and poor X-ray photograph indicate a gross mixture of three or more paraffins. Comments given under fraction 27 apply and the major components are probably C_{29} and C_{31} .

Fraction 87. This is a good example of how the melting points, transition temperatures and the spacings of two-odd and two-even paraffin mixtures can fall into agreement, and the decision must rest on the character of the X-ray photograph. In this case the eight sharp orders definitely exclude the two-even combination and the fraction is undoubtedly 85 % C_{31} + 15 % C_{33} .

Fraction 92. The melting and setting points are sharp, and the transition temperatures clear but cover a wide range. The photograph shows seven poor orders with broad lines. It can be deduced that the fraction contains only two paraffins and that both are present in fair amount. The derived data make it clear that the fraction is probably 40% C_{31} + 60% C_{33} .

Fraction 93. The X-ray photograph with four fairly sharp orders and the low transition temperatures show that the product is a mixture. The data are consistent with 40% C_{33} + 60% C_{35} , with other components possibly present in small amount.

General conclusions

These detailed criticisms of the individual fractions permit one to draw the following general conclusions:

(1) In all cases in which the melting-point of the fraction suggests a composition close to that of an odd-number paraffin (fractions 4, 16, 35, 53, 65 and 87) the agreement between the melting points, transition temperatures, and crystal spacings is excellent. The transition temperatures are fairly sharp, the 'cone and glassy structure' mentioned in an earlier paper (Piper *et al.* 1931) is clearly visible, and the X-ray photographs show good clear lines,

Table 3. *Composition of various paraffin fractions*

Values marked * are extrapolated.

Fraction no. or composition of synthetic mixture	Transition point on heating (°)	Melting point (°)	Setting point (°)	Transition point on cooling (°)	Crystal spacing (Å)	Composition suggested by melting point
4	Glassy at room temperature	41.2-41.4	40.8 Long needles	39.5 Cone appears and material changes to glass	29.45	C_{21}
90% C_{21} + 10% C_{23}	—	41.5*	—	—	29.4*	—
12	33-34	44.8-45.0	44.8 Short needles	Indefinite	30.95	C_{23}
C_{22}	42.8-43.3	44.5-44.7	44.1 Long needles	37 Sharp	A 30.2 B 28.1	—
60% C_{21} + 40% C_{23}	33-34*	45.0*	—	32* Indefinite	31.0*	—
16	35.5-36.5	47.5-47.7	47° 3	34.5	31.45	C_{25}
5% C_{21} + 95% C_{23}	36.5*	47.7*	47.2*	34.6*	31.5*	—
55% C_{23} + 45% C_{24}	39*	47.7*	—	36.37*	32.2*	—
27	40-41 Indistinct	50.3-50.6	50.1	35.5-36.5 Indistinct	33.0	C_{24}
C_{24}	46.5-47.0	50.9-51.1	50.4	41.5	A 32.5 C 28.8	—
55% C_{23} + 45% C_{25}	40.0-41.0*	50.6*	—	36-35*	33.2*	—
5% C_{22} + 95% C_{24}	43.0*	50.6*	—	40.5*	32.5*	—
10% C_{23} + 90% C_{24}	—	50.7*	—	—	32.7*	—
35	43.0-43.5	54.5-54.7	54.2	43.5-43.0	34.5	C_{25}
C_{25}	47.0*	54.0*	54.0*	45.0*	33.8*	—
90% C_{25} + 10% C_{27}	45.0-46.0*	—	54.5*	44.0*	34.5*	—
30% C_{24} + 70% C_{26}	46.0-47.0*	—	54.9*	45.0*	35.2*	—
80% C_{25} + 20% C_{26}	46.0-47.0*	—	54.5*	44.0*	34.1*	—
42	46.0-47.0	56.6-57.3	57.0-56.4	Indefinite	36.2	C_{26}
C_{26}	51.4-52.0	56.4-56.6	56.1	48.3	A 34.95 C 31	—
50% C_{25} + 50% C_{27}	47.0*	56.5*	—	44.0*	36.2*	—
53	48.5-49.0	59.3-59.5	58.6	47.0-46.0	37.25	C_{27}
C_{27}	52.5-53.0	59.0-59.1	58.5	51.0	36.3	—
90% C_{27} + 10% C_{29}	51.0-52.0*	59.3-59.5*	58.6*	50.0*	37.15*	—
40% C_{26} + 60% C_{28}	49.0-50.5	59.6-59.7	59.2	48.8	36.8	—
90% C_{27} + 10% C_{28}	52.5-53.0	59.2-59.5	58.9	Indistinct 50.0	36.55	—
58	52.0-53.0	61.6-61.8	61.3	Indefinite	38.6	C_{28}
95% C_{28} + 5% C_{30}	54.5-55.0	61.5-61.7	61.2	52.5	37.5	—
90% C_{28} + 10% C_{29}	55.2	61.5-61.7	61.3	54.2	37.8	—
30% C_{27} + 70% C_{29}	54.0-55.0*	61.9*	—	52.0*	38.6*	—

Table 3 (cont.)

Fraction no. or composition of synthetic mixture	Transition point on heating (°)	Melting point (°)	Setting point (°)	Transition point on cooling (°)	Crystal spacing (Å)	Composition suggested by melting point
65	55.6-55.8	63.7-63.9	63.6	53.8	38.7	C ₂₉
C ₂₉	57.3-57.5	63.4-63.6	63.2	55.8	38.68	—
95% C ₂₉ + 5% C ₃₁	57.0-57.2	63.6-63.7	63.1	55.3	39.1	—
50% C ₂₉ + 50% C ₃₀	55.0*	63.6*	—	53.0*	39.7*	—
85% C ₂₉ + 15% C ₃₀	56.0-56.5*	63.7-63.9*	—	54.0*	40.0*	—
77	53.5-54.5	65.8-66.1	65.7-65.3	Indefinite	41.2	C ₃₀
	Indefinite					
C ₃₀	61.0	65.6-65.8	65.3	58.8	A 40.0	—
					C 35.5	
85% C ₃₀ + 15% C ₃₁	58.0-58.5*	65.9-66.1*	65.6*	58.5*	40.9*	—
40% C ₃₀ + 60% C ₃₁	57.0-58.0	65.8-66.0	65.5	56.0-55.0	41.2	—
				Indefinite		
70% C ₃₀ + 30% C ₃₂	59.0*	67.2*	—	56.0-55.0*	41.2*	—
90% C ₃₀ + 10% C ₃₂	58.0*	66.2*	—	56.0*	40.6*	—
87	60.0-60.5	68.1-68.3	67.9	60.0-59.0	42.78	C ₃₁
85% C ₃₁ + 15% C ₃₃	60.5-61.0*	68.2-68.4*	67.8*	61.0-60.0*	42.59*	—
30% C ₃₀ + 70% C ₃₂	60.0-61.0*	68.3*	68.0*	60.0*	42.7*	—
				Indefinite		
70% C ₃₁ + 30% C ₃₂	62.0-62.5*	68.2-68.4*	—	61.0-60.0*	43.2*	—
92	63.5-64.5	69.8-70.0	69.6	62.0-61.0	44.15	C ₃₂
C ₃₂	65.2-65.4	69.5-69.7	69.2	63.9	A 42.3	—
					B 37.8	
40% C ₃₁ + 60% C ₃₃	63.0-64.0*	70.0*	—	62.0-61.0*	44.05*	—
90% C ₃₂ + 10% C ₃₄	62.0-61.0*	70.1*	—	62.0-61.0*	43.0*	—
80% C ₃₂ + 20% C ₃₃	63.5-64.0*	70.1*	—	64.0*	43.0*	—
93	65.0-66.0	72.8-73.2	72.4-72.2	65.0-64.0	46.3	C ₃₄
	Indefinite			Indefinite		
C ₃₄	69.2-69.4	72.6-72.8	72.4	68.5	A 45.5	—
					C 39.86	
40% C ₃₃ + 60% C ₃₅	68.0*	73.2*	—	67.0*	46.2*	—

The most probable constitution of each fraction is given in heavy type.

all of which facts are in keeping with simple binary mixtures in which one component predominates. The two even-number paraffin mixtures appropriate either to the melting point or spacing will not fit, nor will those of the odd-even number mixtures. It has been possible to show, therefore, that the odd-number paraffins C₂₁, C₂₃, C₂₅, C₂₇, C₂₉, C₃₁, C₃₃ and C₃₅ are all present in the original mixture.

(2) In all cases in which the melting-point of the fraction suggests a composition close to that of an even-number paraffin (fractions 12, 27, 42, 58, 77 and 92) the data will not permit a simple interpretation in terms of even-number binary mixtures. The transition temperatures in all cases are too indistinct (suggesting mixtures between 30 and 70%) and the spacings are too high for the suggested mixtures. The same criticism applies to simple binary odd-even number mixtures. With odd-number binary mixtures agreement is in all cases fair, but as the suggested composition lies between 30 and 70%, the transition temperatures are too indistinct and the photographs too poor to warrant the definite conclusion that the mixture is binary. Such fractions may indeed be ternary or more complex, and no. 42 definitely is so, but as in-

sufficient standard data for ternary mixtures are available to make direct comparison possible the presence of even-number paraffins therein cannot be excluded. The interpretation of each individual fraction 12, 27, 42, 58, 77 and 92, therefore, on the odd-number mixture basis may not appear to be strictly warranted. Yet one must remember that each of these fractions was obtained after an *ad hoc* fractional distillation of collected samples having apparently the correct melting point. In the case of the fractions mentioned under (1) above, a good separation was undoubtedly obtained and odd-number paraffins of recognizable purity resulted. In the case of the fractions which we are discussing at the moment the separation was equally good, yet we have been unable to prepare an even-number paraffin of recognizable purity or even to prove definitely the presence of such a paraffin in any fraction at all. The conclusion, therefore, seems warranted, that fractions 4, 16, 35, 53, 65 and 87 collectively contain all the odd-number paraffins from C₂₁H₄₄ to C₃₅H₇₂, that fractions 12, 27, 42, 58, 77 and 92 are binary or more complex mixtures of these particular paraffins, and that even-number paraffins are absent or are present in such small

Table 4. *Approximate composition of the higher paraffin fraction from rose-petal wax*

Paraffin	%
C ₂₁ H ₄₄	6.5
C ₂₃ H ₄₈	16.5
C ₂₅ H ₅₂	17.5
C ₂₇ H ₅₆	23.1
C ₂₉ H ₆₀	25.1
C ₃₁ H ₆₄	10.0
C ₃₃ H ₆₈	1.2
C ₃₅ H ₇₂	0.1

100

amount that they remain undetected by our procedure—a finding in keeping with all our earlier work on plant and insect waxes.

The approximate composition of the higher paraffin fraction from the rose-petal wax calculated from our results is given in Table 4.

SUMMARY

1. The mixture of higher paraffins present in rose-petal wax has been separated into a number of fractions by vacuum distillation.

2. A comprehensive analysis of each of these fractions has been made by comparing the melting point, transition temperatures and long crystal spacing with those of synthetic paraffins and appropriate mixtures thereof.

3. It has been shown that all the odd-number paraffins from C₂₁ to C₃₅ are present. No definite evidence for the occurrence of even-number paraffins has been obtained.

4. Paraffins of chain length shorter than C₂₁ are also present in small amount, but these have not yet been characterized.

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