5. In a period of 15 min., adenosine 5'-di- and -tri-phosphate, but not adenosine 5'-monophosphate, suppress the incorporation of [32 P] orthophosphate into only the adenosine 5'-monophosphate residues of ribonucleic acid.

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Further Observations on the Paraffins and Primary Alcohols of Plant Waxes

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In a series of papers dealing with researches carried out between 1930 and 1938 on the long-chain constituents of plant and insect waxes it was inferred, 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, contained an even number of carbon atoms, whereas the paraffins, ketones and secondary alcohols, which are probably formed from their respective acids by de-

carboxylation, contained an odd number of carbon atoms (Chibnall, Piper, Pollard, Williams & Sahai, 1934). Such findings, in so far as they related to the saturated acids and primary alcohols, seemed to bring the wax constituents into line with the views then current about the constituents of shorter chain-length present in glycerides and similar natural products. There was much less evidence at the time with respect to the *n*-paraffins. The above-mentioned workers examined the meagre

data then available and concluded that in all cases the products hitherto isolated consisted of one or more of the odd-number members from C_{25} to C_{37} . A more comprehensive investigation was carried out in 1937–38 on the paraffins present in rose-petal wax (Chibnall, Mangouri & Piper, 1954). A preliminary separation was made by vacuum-distillation and it was possible finally to obtain a series of fractions of recognizable purity showing the presence of all the odd-number members from C_{21} to C_{37} . Such of the intermediary fractions as had melting points corresponding to the even-number members of the series, however, were all shown to be complex mixtures and in no instance was definite evidence for the presence of any such member obtained.

In more recent years many new techniques have been used on the problem and the evidence so adduced casts doubt on the general validity of the odd-even relationships mentioned above. It is now well established, for instance, that small, generally very small, amounts of the odd-number fatty acids are present in certain glycerides. By column chromatography Ivanoff, Stoianova-Ivanova & Ivanoff (1955) claim to have isolated both odd- and even-number paraffins from Bulgarian rose-petal wax, and Wanless, King & Ritter (1955), who submitted their column fractions to mass spectrography, were able to demonstrate the presence of a similar mixture in pyrethrum wax. These newer findings call for a re-examination of the materials used by Chibnall and his colleagues, whose conclusions had been based in large part on data derived from a series of synthetic paraffins of appropriate chain-length. If the glyceride fatty acids from which these had been prepared had unwittingly contained small amounts of neighbouring odd-number acids the final products could have been contaminated with nearby odd- or even-number homologues as the case may be. It has therefore been considered advisable to submit such of the earlier synthetic paraffins as are still available, as well as some of the paraffins isolated from waxes or derived from wax primary alcohols, to analysis by mass spectroscopy.

MATERIALS

Synthetic paraffins. (Main reference: Piper *et al.* 1931.) *n*-Tricosane had been prepared from a sample of Kahlbaum's purest lauric acid that had been recrystallized four times successively from large volumes of acetone. *n*-Heptacosane had been prepared from Kahlbaum's purest myristic acid treated in a similar way. A sample of myristic acid that had been fractionated as the ethyl ester to constant setting point had been used to prepare *n*-hexacosane (Garner, van Bibber & King, 1931) and (via *n*-tetradecanol) *n*-octacosane. *n*-Nonacosane had been prepared from a sample of *n*-pentadecanoic acid (Eastman

Kodak Co.) that had been fractionally distilled four times successively and then repeatedly recrystallized from light petroleum (b.p. below 40°).

Paraffins isolated from plant waxes. The products from runner-bean leaves and cocksfoot have not hitherto been described. The following comments refer to Chibnall *et al.* (1934) unless otherwise stated. Candelilla wax, series no. B372; apple cuticle, series no. C136 (Chibnall, Piper, Pollard, Smith & Williams, 1931); swede turnip, series no. B379; Brussels sprout, unfractionated sample (Sahai & Chibnall, 1932); white mustard; rye grass, unfractionated sample (Pollard, Chibnall & Piper, 1931); green tobacco leaf, unfractionated sample; cactus, unfractionated sample.

Paraffins of rose-petal wax. The fractions selected for further analysis had melting points close to those of even-number paraffins. The previous work had suggested that these were all complex mixtures (Chibnall *et al.* 1954).

Paraffins derived from primary alcohols isolated from various waxes. The samples from barley and runner-bean leaves have not hitherto been described. Cocksfoot, one of several samples that collectively had been used to demonstrate that the primary alcohol was C_{28} , with small amounts of C_{24} and C_{22} (Pollard *et al.* 1931); wheat, series no. B261 (Pollard, Chibnall & Piper, 1933); apple cuticle, series no. C72 (Chibnall *et al.* 1931); lac wax (bulk samples 5, 6, 7, 11, 13 and 14), wild white clover, cactus and white mustard (Chibnall *et al.* 1934); lucerne, series no. B226 (Chibnall, Williams, Latner & Piper, 1933).

METHODS

The method of chemical analysis by mass spectrometry has been described by several authors, e.g. Washburn (1950) and Waldron (1956). Only a brief account of the experimental technique will be given here.

Fig. 1. Schematic diagram of heated inlet system.

The method of analysis of *n*-paraffin hydrocarbons employed in the present work closely followed that originally developed by O'Neal & Weir (1951), except that the mass spectrometer used was the Associated Electrical Industries type MS2-H, and that a different type of sample-introduction system was employed. A detailed description of the latter has been given by Craig & Errock (1959) and a sketch is shown in Fig. 1. The main feature of this introduction system is that it enables a weighed quantity of a solid sample to be introduced from atmospheric pressure through metal valves into a heated glass reservoir. The glass sample tube (Fig. 1) can be detached from the inlet system and a melting-point tube containing the sample placed in the tube. The glass sample tube is permanently attached to a metal insert through a glass-metal seal and the whole assembly is fitted to the inlet system by means of a screw connexion and gold gasket. After pumping away the air above the tube, the sample can be evaporated directly into the reservoir. About 1 mg. of material was usually employed and the partial pressure of paraffin in the reservoir was approx. 0.05 mm. Hg. In the present work the reservoir and valves were operated at a temperature of approx. 320°, which was sufficiently high to maintain the paraffin hydrocarbon in the vapour phase but yet was sufficiently low to avoid thermal-decomposition effects. Any products of pyrolysis would have been readily detected since the mass spectrum would have been observed to change with time.

From the reservoir the sample flowed at a steady rate through a porous disk, which reduced the pressure to about 5×10^{-6} mm. Hg. and then through a glass tube heated to 150° and into the ionization chamber of the mass spectrometer. The rate of loss of sample from the reservoir was small, approx. 10%/hr. for *n*-hexadecane, which was negligible in the 5–10 min. required to scan the mass spectrum. In the ionization chamber, the molecules of hydrocarbon vapour were bombarded with electrons of 70 v energy and a record of the mass spectrum of the positive ions produced was obtained in the usual way by magnetic scanning.

Two typical examples of the mass spectra obtained are reproduced in Figs. 2 and 3. The mass spectrum is essentially a plot of the relative ion intensity against mass. The height of the peak at any mass number is directly pro-

portional to the number of positive ions of that particular mass formed in the ionization chamber, and this, in turn, is directly proportional to the partial pressure of the compound from which the ions originate. If the ions originate from more than one compound then the peak height is proportional to the sum of the partial pressures of the compounds concerned. It should be noted that the mass scale is known exactly and that, consequently, there can be no doubt about the mass assignments in any spectrum.

Interpretation of the mass spectra and method of calculation

The method is best described in terms of an example, and Fig. 2 shows the upper portion of the mass spectrum of the paraffin derived from the primary alcohol of wheat, which is almost pure *n*-octacosane.

Two types of positive ions are produced on electron bombardment, namely, parent or molecular ions and fragment ions. The molecular ions are simply those which are formed by removing one electron from the molecule; they occur at the mass corresponding to the molecular weight of the compound and the intensity of the ion current at that mass is directly proportional to the partial pressure of the compound in the reservoir. Molecular-ion peaks are designated 'm' in Figs. 2 and 3. Thus the peak at mass 394 in Fig. 2 is due to the ions $C_{28}H_{58}^+$ produced from *n*-octacosane.

Fragment ions are produced by ionization and dissociation of the neutral molecule and the most prominent in the spectrum of a *n*-paraffin are the alkyl ions, $C_nH_{2n+1}^+$, designated 'a' in Figs. 2 and 3. These are unimportant from the point of view of quantitative analysis, but, qualitatively, they serve to distinguish a *n*-paraffin from other compounds of the same molecular weight. The regular pattern of alkyl-ion peaks in Fig. 2, increasing in intensity towards lower mass, is quite characteristic of *n*-paraffins; ketones or isoparaffins do not exhibit such a regular pattern. For example, with an isoparaffin the regular pattern is interrupted by a peak of very large intensity at the mass corresponding to the formation of the alkyl ion by rupture of the side chain.

In addition to the major peaks found at the masses calculated from the molecular formulae, both parent and

Fig. 2. Partial mass spectrum of the normal paraffin derived from wheat. For further explanation see text.

fragment ions give small isotope peaks (designated 'i' in Figs. 2 and 3). These are produced by ions in which one or more carbon atoms is substituted by the heavy isotope, ^{13}C , or in which a hydrogen atom is replaced by deuterium. For example, the peak at mass 395 in Fig. 2 is due to the molecular ions $^{12}\text{C}_{27}^{13}\text{CH}_{57}^+$ and $^{12}\text{C}_{28}\text{H}_{56}\text{D}^+$ and the peak at mass 338 is not due to the molecular ion $\text{C}_{24}\text{H}_{50}^+$ but to the alkyl ions $^{12}\text{C}_{23}^{13}\text{CH}_{49}^+$ and $^{12}\text{C}_{24}\text{H}_{48}\text{D}^+$. The intensity of the isotope peaks can be calculated exactly from the intensity of the unsubstituted ion peak and the known isotopic abundance of ^{13}C and deuterium. The method of calculation is discussed by Washburn (1950).

Apart from isotopic ions, for which correction can be made, a *n*-paraffin does not produce on electron bombardment any ions at mass numbers corresponding to the molecular weights of other *n*-paraffins. This forms the basis of the mass-spectrometric method of analysis. Thus in Fig. 2 it can be seen that the paraffin derived from wheat is mainly *n*- C_{28} with a trace of *n*- C_{30} , and, after correction for the isotopic contributions, traces of *n*- C_{27} and *n*- C_{26} . Similarly, in Fig. 3 the mass spectrum of the paraffin derived from runner bean indicates that this is mainly *n*- C_{28} , but that substantial amounts of other *n*-paraffins, in particular *n*- C_{26} , are also present.

In detail, the method of analysis consists of five steps: (1) At least five repeat scans of the mass spectrum are recorded. (2) The average intensity of the ion current at the molecular-ion peaks, $\text{C}_n\text{H}_{2n+2}^+$, is tabulated at each carbon atom number. (3) The contribution of the isotope of the alkyl ion, $\text{C}_n\text{H}_{2n+1}^+$, at the $\text{C}_n\text{H}_{2n+2}^+$ peak is calculated and subtracted from the measured value. (4) The value remaining is then due entirely to the molecular ion and this is divided by the appropriate relative sensitivity. (5) The quotients are summed, normalized to 100% and the individual values reported as mole/100 moles concentration.

The relative sensitivity in step (4) is simply the intensity of the molecular-ion peak/unit pressure; the value varies with the molecular weight of the paraffin and is established by calibration. In the present work the instrument was calibrated with synthetic mixtures made with the paraffins listed in Table 2 and some *n*-hexatriacontane obtained from the American Petroleum Institute. In Table 1 the values for paraffins C_{23} , C_{26} , C_{27} , C_{28} , C_{29} and C_{30} were determined experimentally. A smooth curve of relative sensitivity against carbon atom number was then plotted, and from this values for the relative sensitivities of the other paraffins were obtained by interpolation.

Accuracy and limits of detection

The lack of pure normal paraffins of each carbon atom number for calibration means that the relative sensitivities used may be in error, possibly by 2–3%. The method of analysis is such that an error in relative sensitivity is reflected directly as an error in concentration, and the absolute values given in Tables 2–5 therefore may be in error by 2–3% of the reported concentration. Thus, for example, in Table 4 the values for C_{22} and C_{23} paraffins in fraction 12 could be in error by as much as 1.0 mole/100 moles, but the smaller concentrations such as those for C_{21} and C_{24} will be relatively unaffected. In the last-named the error is likely to be no more than 0.1 mole/100 moles. From the point of view of the present work the accuracy of the calibration data is relatively unimportant since the main interest is in the presence or absence of small concen-

Fig. 3. Partial mass spectrum of the normal paraffin derived from runner bean. For further explanation see text.

Table 1. *Relative sensitivity of normal paraffin hydrocarbons*

C no.	21	22	23	24	25	26	27	28	29
Rel. sensitivity	4.3	3.5	2.7	2.1	1.55	1.3	1.15	1.00	0.88
C no.	30	31	32	33	34	35	36	37	38
Rel. sensitivity	0.79	0.73	0.67	0.64	0.60	0.58	0.56	0.54	0.52

trations of odd homologues in mixtures containing the even-number members of the series and vice versa. It is, however, important to consider the factors which affect the limit of detection. These are: (1) The minimum ion current that can be detected. (2) The accuracy of measurement of small peaks. (3) The accuracy with which the isotopic correction can be determined (step 3 in the above-described calculation).

Compared with the first two, factor (3) can be ignored since isotope ratios are known to be better than 0.5%. The limit of detection of higher paraffins in mixtures of lower paraffins is determined primarily by the first factor and the limit of detection of lower paraffins in mixtures of higher paraffins is determined primarily by the second. In both cases the predominant instrumental effect is the noise level of the system. With the instrument used the signal-noise ratio of the molecular-ion peak with a pure sample of *n*-octacosane was approx. 300:1. The limit of detection of *n*-octacosane is thus 0.3% and figures for other paraffins can be readily calculated from a knowledge of the appropriate relative sensitivity.

RESULTS AND DISCUSSION

The mass-spectrograph analyses of the synthetic *n*-paraffins (Table 2) show, for example, that the sample of lauric acid used to prepare C_{23} had contained about 2% of myristic acid, and that the samples of myristic acid used for C_{26} , C_{27} and C_{28} had contained about 1% of lauric acid and 2% of palmitic acid together with (in one case) about 1% of *n*-tridecanoic acid. These preparations had been made at a time when the criteria necessary to define the purity of a *n*-fatty acid were only slowly emerging, from the pioneer work of Francis, Piper & Malkin (1930) and of Malkin (1930), and when the application of X-ray analysis to long-chain compounds had not yet been fully exploited. Under the circumstances it is gratifying to find that these early synthetic paraffin preparations were as pure as the new analyses indicate.

The paraffins isolated from plant sources (Table 3) fall into two groups. In the first of these the products had been submitted to a laborious fractional crystallization with the object of identifying if possible the minor as well as the major components present. Apple-cuticle wax was an example. This contained 85–90% of C_{29} paraffin, and an examination of the residual fractions for shorter-chain material by comparing the melting-point and long-crystal-spacing data with those of appropriate synthetic mixtures of C_{25} – C_{28} and C_{29} – C_{27} paraffin led to the conclusion that the

minor component was C_{27} and not C_{28} . The issue, unfortunately, could have been fogged at the time by the presence of about 0.5% of C_{28} in the synthetic C_{29} standard (Table 2), an amount as large as that now suggested as a minor component in the purified apple product (C134). For a similar reason the small percentage of even-number paraffins in Brussels-sprout wax would have been missed. The other group of plant paraffins had been given a more cursory examination. A fairly simple fractionation had shown that in each case the product contained more than one component, and by comparison with appropriate synthetic mixtures a satisfactory resolution in terms of the odd-number members seemed the most probable. It is clear, however, from the results given in Table 3, that although the odd-number members do indeed largely predominate, in no case was the interpretation on such an exclusive basis valid. All the even-number members between C_{28} and C_{34} are present as minor components in one or other of these waxes. The new finding receives confirmation from the revision of the rose-petal data (Table 4). The fractions that have been re-examined represent but a small proportion of the original paraffin mixture and each one was known to be of complex composition. In agreement with Ivanoff *et al.* (1955) the results show that all the even-number members from C_{22} to C_{30} are represented in one or other of these particular fractions. Unfortunately a sample of the original paraffin mixture was no longer available for overall quantitative analysis, and in the present research no more can be claimed than that the even-number homologues were minor components. The mass spectra of fractions 58 and 77 indicate that small quantities of *isoparaffins* were present. Apart from green tobacco leaf, which is discussed below, these were the only cases in which any *isoparaffin* was detected.

Carruthers & Johnstone (1959) have recently prepared hydrocarbon fractions from both green and fermented tobacco leaves and have had these submitted to mass-spectrographic analysis at the Thornton Research Centre, Shell Research Ltd. In each case the main component *n*-paraffin present was C_{31} , but appreciable quantities of the neighbouring odd-number homologues and small amounts of the even-number homologues were also present. The observed peak intensities, moreover, suggested that about half of each fraction consisted of *iso*-paraffins of chain-length similar to those just

Table 2. *Analysis of synthetic paraffins*

Paraffin	Series no.	Composition (moles/100 moles)													
		No. of C atoms													
	21	22	23	24	25	26	27	28	29	30	31	32	33		
C ₂₁ H ₄₄	B 491	—	98.4	—	1.6	—	—	—	—	—	—	—	—	—	
C ₂₅ H ₅₄	C 67	—	—	0.4	1.0	97.5	—	1.1	—	—	—	—	—	—	
C ₂₇ H ₅₈	B 13	—	—	—	0.8	—	97.3	—	1.9	—	—	—	—	—	
C ₂₉ H ₆₂	B 10	—	—	—	—	0.6	—	98.8	—	0.6	—	—	—	—	
C ₃₁ H ₆₆	C 141	—	—	—	—	—	1.4	0.5	97.3	—	0.8	—	—	—	
Limit of detection	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.5	0.5	0.5	0.5	

Table 3. *Analysis of paraffins isolated from plant sources*

Source	Analysis by mass spectrometry (M) or as mentioned in earlier work (E)	Composition (moles/100 moles)													
		No. of C atoms													
		26	27	28	29	30	31	32	33	34	35	36	37	38	
Apple peel (C134)	M	—	2.2	0.3	97.5	—	—	—	—	—	—	—	—	—	
	E	—	—	—	100	—	—	—	—	—	—	—	—	—	
Apple peel (C136)	M	—	12	1.8	86.2	—	—	—	—	—	—	—	—	—	
	E	—	17	—	83	—	—	—	—	—	—	—	—	—	
Swede-turnip leaf	M	—	1.3	1.3	95.0	—	2.3	—	—	—	—	—	—	—	
	E	—	—	—	95	—	5	—	—	—	—	—	—	—	
Brussels sprout	M	—	0.4	0.5	87.6	1.7	9.8	—	—	—	—	—	—	—	
	E	—	—	—	(approx.)	—	2.5-10	—	—	—	—	—	—	—	
Cocksfoot grass	M	—	1.3	1.5	35.2	3.5	48.8	—	9.7	—	—	—	—	—	
Runner-bean leaf	M	—	3.3	3	44	3.6	48	2.7	5.4	—	—	—	—	—	
White-mustard leaf	M	—	0.7	0.8	35.7	3.7	52.4	3.2	3.5	—	—	—	—	—	
	E	—	—	—	35	—	65	—	—	—	—	—	—	—	
Rye grass	M	—	6.5	1.2	40.2	4.6	39.2	—	8.3	—	—	—	—	—	
	E	—	—	—	Too complex to suggest	—	—	—	—	—	—	—	—	—	
Green tobacco leaf*	M	—	—	—	~5	~5	~60	~10	~20	—	—	—	—	—	
	E	—	—	—	†	—	†	—	†	—	—	—	—	—	
Cactus leaf	M	—	—	—	—	—	2.6	1.5	14.7	5.8	64.6	—	10.8	—	
	E	—	—	—	—	—	—	—	†	—	†	—	†	—	
Limit of detection	0.3	0.3	0.3	0.3	0.3	0.4	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.6	

* Contains substantial quantities of isoparaffin; values must therefore be regarded as approximate.

† Detected.

Table 4. *Analysis of rose-petal paraffin fractions*

Fraction no.	Composition (moles/100 moles)											
	No. of C atoms											
	21	22	23	24	25	26	27	28	29	30	31	
12	4.1	42.6	48.7	4.6	—	—	—	—	—	—	—	
27	—	1.6	29.7	33.7	26.1	6.2	2.7	—	—	—	—	
58*	—	—	—	—	—	1	2	21	47	3	2	
77*	—	—	—	—	—	1	1	2	40	24	32	
Limit of detection	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.5	

* Mass spectra suggest that these samples may also contain small quantities of isoparaffin.

Table 5. *Analysis of paraffins derived from various primary alcohols*

Source	Analysis by mass spectrometry (M) or as mentioned in earlier work (E)	Composition (moles/100 moles)											
		No. of C atoms											
		24	25	26	27	28	29	30	31	32	33	34	
Barley leaf	M	1.1	—	94.4	—	4.5	—	—	—	—	—	—	
Cocksfoot grass	M	—	—	94.6	—	5.4	—	—	—	—	—	—	
Wheat blade	M	—	—	0.5	0.3	98.5	—	0.7	—	—	—	—	
	E	—	—	—	—	100	—	—	—	—	—	—	
Apple peel	M	—	—	5.8	1.7	66.8	4.8	20.9	—	—	—	—	
	E	—	—	*	—	*	—	*	—	—	—	—	
Wild-white-clover leaf	M	—	—	10.8	1.8	26.1	2.0	59.3	—	—	—	—	
Runner-bean leaf	M	—	—	16.1	0.3	50.8	0.8	24.1	0.6	7.3	—	—	
Cactus leaf	M	—	—	1.1	1.2	52.2	6.8	31.5	2.3	4.9	—	—	
	E	—	—	—	—	50	—	50	—	—	—	—	
Lucerne leaf	M	—	—	—	—	2.2	2.2	95.1	—	0.5	—	—	
	E	—	—	—	—	—	—	99	—	—	—	—	
White-mustard leaf	M	—	—	—	—	15.2	2.7	35.2	4.2	29.2	—	13.6	
	E	—	—	—	—	20	—	40	—	40	—	—	
Laos wax	M	—	—	—	—	27	0.6	20.7	0.5	33	—	18.3	
	E	—	—	*	—	*	—	*	—	*	—	*	
Limit of detection		0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.5	0.5	0.5	0.5	

* Detected.

mentioned. The corresponding fractions prepared by Chibnall *et al.* (1934), which were thought at the time to consist largely of C_{31} with subordinate amounts of C_{29} and C_{33} paraffins have now been passed through the mass spectrometer. In agreement with Carruthers & Johnstone (1959) the new analyses confirmed the presence of small amounts of the even-number homologues and also of *iso*-paraffins (Table 3). It is clear from all these new findings that the older methods of fractionation employed by Chibnall and his colleagues were not sufficiently selective to resolve binary and ternary mixtures containing about equal amounts of neighbouring homologues and that the composition of such mixtures could not be predicted with certainty from melting-point and long-crystal-spacing data.

A similar story emerges from the re-examination of the paraffins obtained by reduction via the iodide from the wax primary alcohols (Table 5). The new analysis confirms the earlier deduction that the paraffin from the wheat primary alcohol was as pure a sample of C_{28} as the synthetic standard used for comparison (Table 2). The lac-wax primary alcohols had been extensively fractionated and in this case the new analysis of the bulked material confirms the presence of the even-number members from C_{28} to C_{34} , the odd-number members being virtually absent. In all the other cases, however, one or more of the odd-number members are definitely present as minor components. From the known relationship between the *n*-fatty acids and primary-alcohol constituents of waxes it may be inferred that the odd-number acids may also be minor components in some cases.

SUMMARY

1. A number of the *n*-paraffin and primary-alcohol fractions of plant waxes characterized in former years by Chibnall and his colleagues have been re-examined by mass spectroscopy.

2. Contrary to the earlier suggestion that the *n*-paraffin fractions comprised exclusively the odd-number members of the series, it is now shown that

in some cases the even-number members may be present as minor components.

3. Likewise the primary-alcohol fractions are not composed exclusively of the even-number members of the series; in some cases the odd-number members may be present as minor components.

4. *iso*Paraffins are present in rose-petal wax and tobacco-leaf wax but in no other wax examined in the present research.

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