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SHORT COMMUNICATION

BIOGENESIS OF ALKANES IN THE FLOWERS OF THE ESSENTIAL OIL ROSE (*ROSA DAMASCENA* MILL.)

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Abstract—In the flowers of *Rosa damascena* Mill. appreciable amounts of alkanes occur which are essentia components of rose oil. The possibility of their formation via acetate was studied. It was found that acetate-2-^{14}C was incorporated into the hydrocarbons. It was also shown that formate-^{14}C was likewise incorporated into these compounds suggesting that branch-chain or odd-chained hydrocarbons were synthesized.

INTRODUCTION

THE hydrocarbons in rose flowers have been studied earlier;[1,2] Prophète reported the isolation of the normal hydrocarbons C_{30}, C_{27}, C_{26}, C_{22}, C_{21}, C_{20} and C_{16},[2] which, according to Naves and Mazuyer[3] represent a mixture of hydrocarbons.

Studies on the composition of stearoptene in Bulgarian rose oil[4–6] have established that it contains the alkanes C_{17}, C_{19}, C_{20}, C_{21} and the unsaturated hydrocarbon—10-eicozene. According to Schuette and Hanif[7] the normal even-chained hydrocarbons C_{24} to C_{36} occur in waxes of Ouricuri.

The presence of normal even-chain hydrocarbons along with odd-chain ones is of considerable interest. Consequently, Ivanoff and co-workers[8] examined the unsaturated hydrocarbon pattern in waxes (light petroleum extracts) from *R. damascena* Mill., grown in Bulgaria, and the presence of even- and odd-chained hydrocarbons was confirmed.

According to Chibnall *et al.*[9] rose wax contains only paraffins with odd number of carbon atoms from C_{21} to C_{35}. The authors found no evidence for the presence of even-chained paraffins, and on these grounds suggested their scheme for the formation of odd-chained hydrocarbons by the combination of two molecules of even chain acids (occurring in natural products) to give an odd-chain ketone. This, via the secondary alcohol, unsaturated hydrocarbon, gives the odd saturated hydrocarbon.[10] However, the presence of both normal

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even- and odd-chained hydrocarbons in rose wax[11] and other plant waxes[12] may be considered as proven. Moreover, there is evidence for the occurrence of small amounts of terpenic hydrocarbons[13] and branch-chained paraffins[11] in rose flowers.

The biogenesis of hydrocarbons in rose wax has been studied through a more detailed examination of the wax composition. The pattern of the unsaturated hydrocarbons,[14] of the acids[15] and of the alcohols[16] was examined and parallel investigations were conducted into the composition of waxes in small and large flower buds from rose shrubs of the same origin.[17]

Until recently there was little information available concerning the biogenesis of alkanes. A review on the work in this area has been made by Douglas and Eglinton.[18]

During the past few years investigations in this respect have been carried out more extensively using labelled compounds. However, the evidence presented by different workers contain a considerable degree of uncertainty. Thus Sandermann and Schweers[19] have shown that acetate is involved in the formation of *n*-heptane in *Pinus jeffrei*, presumably via octynic acid. Recently Mazliak[20] made a comprehensive examination of the wax composition in apple peels including the aliphatic hydrocarbons, and for the first time subjected Chibnall's hypothesis for the biogenesis of aliphatic hydrocarbons via higher aliphatic acids to experimental check. He fed apple-fruit rinds labelled acetate and found that it was quickly incorporated into the fatty acids, followed by incorporation into the corresponding alcohols, but at the end of his experiment (48 hr), the aliphatic hydrocarbons remained inactive. As far as paraffins were concerned he suggested, contrary to Chibnall's hypothesis, that these were not formed from fatty acids but from another unknown precursor and via unknown route.

We consider that Mazliak's observations offer no substantial evidence for rejecting Chibnall's hypothesis. The formation of hydrocarbons requires a longer duration since, according to Chibnall, they are the final product in the biogenetic pathway: acetate–higher fatty acids–paraffins. It is possible that Mazliak obtained non-radioactive hydrocarbons because of his relatively short exposure times.

The aim of the present study was to establish whether the acetate was incorporated into the molecule of the higher paraffins in the flowers of *R. damascena* and to thus attempt to elucidate the biogenesis of alkanes.

Unlike Mazliak, our feeding experiments using acetate-2-^{14}C were conducted for 1, 2 and 5 days and in another series from 5 to 11 days. Moreover, taking into account that normal even- and odd-chained hydrocarbons as well as branch-chained ones occur in rose flowers, feeding experiments were undertaken using sodium formate-^{14}C in order to determine if C_1-units were incorporated into some of these latter hydrocarbons. The lipids were extracted from the rose flowers with light petroleum, and after purification from oxygen-containing compounds, the hydrocarbons isolated by chromatography. The unsaturated hydrocarbons (terpenes and olefins) were separated by means of thin-layer chromatography

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(silica gel and silver nitrate) and the paraffins were purified by recrystallization from alcohol to constant relative activity. The results from the acetate and formate feeding experiments are given in Tables 1 and 2.

TABLE 1. INCORPORATION OF ACETATE-2-^{14}C INTO THE PARAFFIN WAXES IN *R. damascena* Mill.

Exposure (days)	Rose flowers (g)	Light Petroleum extract (g)	Wax (g)	Neutral substances (g)	Paraffins (g)	Relative activity of paraffins (cpm/mg)
1	38	0·1105	0·0532	0·0388	0·0063	374
2	19	0·00774	0·0386	0·0282	0·0081	340
5	55	0·0936	0·0274	0·0253	0·0037	197
10	64	0·1416	0·0382	0·0141	0·0060	158

TABLE 2. INCORPORATION OF FORMATE-^{14}C INTO THE PARAFFIN WAXES IN *R. damascena* Mill.

Exposure (days)	Rose flowers (g)	Light Petroleum extract (g)	Wax (g)	Neutral substances (g)	Paraffins (g)	Relative activity of paraffins (cpm/mg)
5–6	24	0·0434	0·0143	0·0093	0·0053	125
8–9	22	0·1007	0·0872	0·0208	0·0026	77
10–11	55	0·1581	0·0434	0·0215	0·0070	63

DISCUSSION

The data in Table 1 show that saturated hydrocarbons of undoubted activity were obtained in all experiments using sodium acetate-2-^{14}C. It was noteworthy that the hydrocarbons assume radioactivity as early as the first day after feeding. Thus, on the basis of this evidence it may be assumed that acetate is the precursor of paraffins in the rose flower and that the biogenetic pathway involving the sequence: acetate–fatty acids–paraffins, is most probable.

The disparity of our findings with the observation of Mazliak concerning the biogenesis of saturated normal hydrocarbons may be attributed first of all to the faster changes in the developmental stage of the rose flowers and to the intensive metabolism during blossom-time[21] than those occurring in apple peel. Our results confirm the evidence obtained by Sandermann and Schweers.[19] In the course of preparation of the present paper we came across the work of Kolattukudy[22,23] who, in a study on the formation of leaf waxes in *Brassica oleracea*, found that acetate and a number of other aliphatic acids were incorporated into the molecules of the higher alkanes. Our data are in accordance with those of Kolattukudy.

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Of considerable interest were the results of the formate experiments, shown in Table 2, which indicate that the formate, i.e. C_1-units, was incorporated into the paraffin molecules. These data reasonably suggest a possible pathway in the building-up of branched hydrocarbons in the rose flower. The possibility that C_1-units may be incorporated into odd-chained paraffins is not excluded.

Experiments to determine the intermediate steps that give rise to paraffins from acetate and the mode of formate incorporation are in progress.

EXPERIMENTAL

The experiments were carried out in the open in May–June 1965 and 1966 in the Research Institute for Roses, Aromatic and Medicinal Plants, Kazanlik.

1. *Feeding Experiments up to 5 Days*

Short excised shoots (up to 10 cm), bearing several flower buds, selected in such a stage of development that all should open by the end of the experiment and in nearly equal numbers within each of the 5 days, were used. The shoots were immersed into small vessels containing about 100 ml aqueous solution of 0·5 mc sodium acetate-2-^{14}C (17 mg). In the course of the experiment, flowers were taken after 1, 2 and 5 days. The experiment could not be extended beyond the fifth day due to the appearance of wilting symptoms.

2. *Long-term Feeding Experiments*

Intact field-grown plants were used in this experiment in which aqueous solutions of 0·5 mc sodium acetate-2-^{14}C (19 mg) and 0·5 mc sodium formate-^{14}C (17 mg) resp. were allowed to pass into different plants by means of cotton wicks drawn through the stem.

3. *Isolation of the Hydrocarbons*

Flower samples, harvested at the indicated intervals, were extracted separately trice with light petroleum (b.p. 50°). After the solvent was distilled off the resulting samples were worked-up each with 1 ml ethanol at 0°. The undissolved residue (waxes) was saponified under reflux with 2 N alcoholic KOH for 3 hr, and additionally by boiling in water for 45 min. The neutral substances (alcohols and hydrocarbons) were extracted with light petroleum ether on a column of aluminium oxide (activity I-II according to Brockmann). Thus, hydrocarbons free from oxygen-containing compounds were obtained.

4. *Separation of the Saturated from the Unsaturated Hydrocarbons*

The separation was effected by thin-layer chromatography on silica gel G impregnated with silver nitrate solution, using as solvent light petroleum b.p. (50°). The plates were developed by spraying with conc. sulphuric acid followed by heating. By chromatography, the saturated hydrocarbons were proved to be free from unsaturated ones.