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SECONDARY ALCOHOLS AND PARAFFINS IN THE PLANT WAXES OF THE FAMILY ROSACEAE

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Abstract—Secondary alcohols detected in waxes of some plants of the family Rosaceae occur in homologous series. Hentriacontan-9-ol, nonacosan-7-ol and nonacosan-10-ol in the wax of rose flowers and nonacosan-10-ol in the cuticle wax of three apple varieties and in the wax of the fruit and leaves of hawthorn have been identified. Secondary alcohols and paraffins from one source occur in similar homologous series, the same homologue being dominant, thus suggesting a biogenetic relationship of these two classes of compound. Secondary alcohols are present only as free alcohols in contrast to the situation found among the primary alcohols. It may be postulated that these two classes of compound are formed by different biosynthetic routes. It is suggested that classes of compounds with odd carbon numbered homologues prevailing are synthesized by a different pathway from those with a predominance of even numbered homologues.

INTRODUCTION

SECONDARY alcohols are well established constituents of plant waxes. In brussels sprouts,[1] nonacosan-15-ol (I) has been found, while apple cuticle wax[2] and the wax from the leaves of

$$CH_3(CH_2)_{13}\underset{\displaystyle OH}{\underset{|}{CH}}(CH_2)_{13}CH_3 \qquad \text{(I)}$$

various trees[3–7] contain nonacosan-10-ol (II). Except for the apple cuticle wax, the corresponding ketones (III and IV) are also present.[1–7] Nonacosane (V) has been reported in all

$$CH_3(CH_2)_{18}\underset{\displaystyle OH}{\underset{|}{CH}}(CH_2)_8CH_3 \qquad \text{(II)}$$

$$CH_3(CH_2)_{13}CO(CH_2)_{13}CH_3 \qquad \text{(III)}$$

$$CH_3(CH_2)_{18}CO(CH_2)_8CH_3 \qquad \text{(IV)}$$

$$CH_3(CH_2)_{27}CH_3 \qquad \text{(V)}$$

cases. In *Brassica oleracea*, Purdy and Truter[8] identified compounds I and II as well as other C_{29} compounds for which they suggested a biogenetic relationship. The present paper deals with secondary alcohols and paraffins found in plants of the family Rosaceae.

[1] P. N. SAHAI and A. C. CHIBNALL, *Biochem. J.* **26**, 403 (1932).
[2] A. C. CHIBNALL, S. H. PIPER, A. POLLARD, J. H. B. SMITH and E. F. WILLIAMS, *Biochem. J.* **25**, 2095 (1931).
[3] T. KARIYONE and H. AGETA, *Yakugaku Zasshi* **79**, 47 (1959).
[4] T. KARIYONE, H. AGETA and A. TANOKA, *Yakugaku Zasshi* **79**, 51 (1959).
[5] T. KARIYONE, H. AGETA and K. ISOI, *Yakugaku Zasshi* **79**, 54 (1959).
[6] H. AGETA, *Yakugaku Zasshi* **79**, 58 (1959).
[7] T. KARIYONE, Proceedings of Symposium on Phytochemistry, Kuala Lumpur, 1957, p. 160 (UNESCU Science Cooperation Office for SE Asia).
[8] S. J. PURDY and E. V. TRUTER, *Proc. R. Soc. B*, **158**, 553 (1963).

RESULTS AND DISCUSSION

Flowers of *Rosa damascena* Mill. var. trigintipetala, leaves of *Rubus idaeus* L., *R. fruticosa* L., spec. coll., and *Crataegus oxyacantha* L., fruits of *C. oxyacantha* L., stems of *Agrimonia eupatoria* L., and *Potentilla anserina* L., and the fruits and leaves of three varieties of apple (Wagener's apple, Raspberry apple, and Hajek's muskatreinette) and two varieties of pear (Conference pear and Comtesse de Paris) have been investigated. Paraffins are present in all of these plants[9, 10] but secondary alcohols have been detected only in waxes of the rose flowers (8·6 per cent of waxes), fruits (0·3 per cent) and leaves (2·8 per cent) of hawthorn and in the fruit cuticle waxes of the apple varieties[11] (20 per cent). The corresponding ketones, however, have not been found. The occurrence of the secondary alcohols is unexpectedly specific.

The isolation procedure used involves a group separation by column chromatography and TLC on silica gel with subsequent hydrolysis of the ester fractions. The i.r.-absorption 1027 cm^{-1}, 1043 cm^{-1}, 1067 cm^{-1} and 3628 cm^{-1} showed the presence of a secondary alcohol in some chromatographic fractions. The oxidation to ketones also showed, that the alcohols are secondary. They occur in the waxes only as free alcohols and not as esters.

For identification by GLC, aliquots of the secondary alcohol fractions have been converted[11] to their corresponding paraffins, the retention times of the latter were then compared with those for standard substances. The resulting GLC data showed that the secondary alcohols constitute a straight chain homologous series (see Table 1). The dominant secondary

TABLE 1. COMPOSITION* OF SECONDARY ALCOHOLS AND PARAFFINS (%)

No. of carbon atoms	Apple cuticle wax (Raspberry apple)		Fruits of hawthorn		Leaves of hawthorn		Rose flowers	
	Paraffin	alcohol	Paraffin	alcohol	Paraffin	alcohol	Paraffin	alcohol
18	0·1	—	—	0·1	—	—	0·1	—
19	0·1	—	—	0·1	2·7	—	2·6	—
20	0·1	—	—	0·4	0·3	0·2	0·4	0·3
21	0·6	—	—	1·1	2·8	0·8	3·6	0·3
22	0·1	1·0	0·1	2·3	0·4	2·4	0·2	0·8
23	0·2	2·5	0·6	2·5	3·5	3·0	2·9	0·8
24	0·1	1·1	0·2	3·0	0·5	3·0	0·3	1·3
25	0·5	0·7	0·5	2·7	2·5	2·5	2·6	0·7
26	0·4	1·7	0·2	3·0	0·4	2·6	0·6	0·6
27	3·0	1·8	1·4	4·0	2·6	3·0	17·7	0·7
28	1·4	2·1	0·4	2·6	0·8	1·3	2·3	0·3
29†	—	—	—	1·5	—	1·3	—	—
29	*92·0*	*86·0*	*94·5*	*73·0*	*73·8*	*77·0*	21·0	2·1
30	0·4	3·0	0·6	1·6	1·0	1·1	1·3	0·6
31	0·6	0·1	1·5	2·1	7·5	1·8	*32·8*	*88·4*
32	0·2	—	—	—	—	—	0·8	1·3
33	—	—	—	—	—	—	10·6	1·8

* The above quantitative determinations were calculated by comparison of GLC peak areas.

† Secondary alcohol with branched chain which can be reduced to a paraffin of carbon number 28·75 (consistent with a 2- or 3-methylparaffin).

[9] V. WOLLRAB, *Coll. Czech. Chem. Commun.* **32**, 1304 (1967).

[10] V. WOLLRAB, *Coll. Czech. Chem. Commun.*, in press.

[11] V. WOLLRAB, M. STREIBL and F. ŠORM, *Coll. Czech. Chem. Commun.*, in press.

alcohol has the same chain length as the dominant paraffin isolated from the same part of the plant. The overall distribution patterns are also similar except that in the range where the minor homologues of the secondary alcohols occur the carbon preference index* approaches 1.

Mass spectrometry of ketones gives a characteristic fragmentation pattern which allows the determination of the position of the ketone group. To establish the position of the hydroxyl groups in the secondary alcohols these were oxidized to the corresponding ketones which were examined by mass spectrometry. Here an interesting fact was observed: in the ketone fragments (Fig. 1) derived from the longer chain end, $(RCOCH_3)^+$ is stronger than

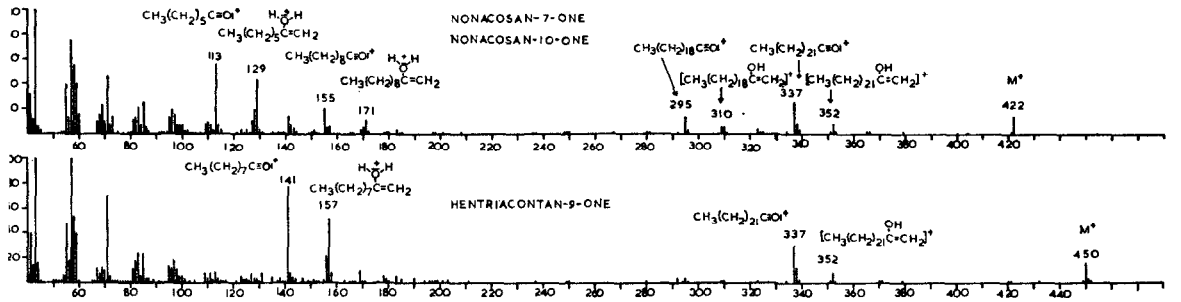

FIG. 1. MASS SPECTRA OF THE KETONE HOMOLOGUES DERIVED FROM SECONDARY ALCOHOLS OCCURRING IN ROSE FLOWER WAX.

$(R\overset{+}{C}OH\,CH_3)$, whereas in the fragments from the shorter chain the opposite is true. The results show (Table 2) that the dominant secondary alcohol in apple cuticle wax as well as in fruit and

TABLE 2. MASS SPECTRA OF KETONES DERIVED FROM SECONDARY ALCOHOLS

Plant part	Structure of secondary alcohol found from ketone fragmentation pattern	Fragmentation pattern
Apple cuticle wax (Raspberry apple)	Nonacosan-10-ol	*m/e* 155, 170, 171, 183, 110, 295, 310, 311, 250
Fruit of hawthorn	Nonacosan-10-ol	M^+ 422
Leaves of hawthorn	Nonacosan-10-ol	M^+ 422
Rose flowers	Hentriacontan-9-ol	*m/e* 141, 156, 157, 337, 352 M^+ 450
	Nonacosan-7-ol	*m/e* 103, 118, 119, 337, 342 M^+ 420
	Nonacosan-10-ol	*m/e* 155, 170, 171, 295, 310 M^+ 420

leaves of hawthorn is in each case nonacosan-10-ol (II). In rose flowers, where the C_{29} and C_{31} components were separated by preparative GLC, the dominant homologue is hentriacontan-9-ol (VI). The C_{29} component (see Fig. 1) consists of two isomers, the more abundant

* The carbon preference index (CPI) value is defined as the mean of two ratios which are determined by dividing the sum of concentrations of odd-carbon-numbered homologues by the sum of even-carbon-numbered homologues within a given range of homologues.[12]

[12] J. E. COOPER and E. E. BRAY, *Geochim. Cosmochim. Acta* **27**, 113 (1963).

$$CH_3(CH_2)_{21}\underset{\displaystyle OH}{\underset{|}{CH}}(CH_2)_7CH_3 \qquad \text{(VI)}$$

$$CH_3(CH_2)_{21}\underset{\displaystyle OH}{\underset{|}{CH}}(CH_2)_5CH_3 \qquad \text{(VII)}$$

one is nonacosan-7-ol (VII), the other is nonacosan-10-ol (II). Because of the small quantities of material available the position of the hydroxyl groups in the other homologues could not be established. Hentriacontan-9-ol (VI) from rose flowers was esterified to form the hydrogen phthalate which has $[\alpha]_D^{25} = 1°$ (0·2 per cent, $CHCl_3$). The nonacosan-10-ol hydrogen phthalate (alcohol from apple wax) has[2] $[\alpha]_{5461}^{17·5} = 0·6°$ (20 per cent, $CHCl_3$). The same sign of rotation suggests the absolute configuration of both secondary alcohols to be the same.

Hentriacontan-9-ol (VI) and nonacosan-7-ol (VII), both occurring in rose flower wax, differ only by a two carbon unit in the short chain. The same wax[10] contains *cis*-hentriacont-7-ene (VIII) and *cis*-nonacos-5-ene (IX). It seems possible in this case that the alcohols are formed from the olefins by a mechanism which introduces the hydroxyl group in the allylic position.

$$CH_3(CH_2)_{22}CH{=}CH(CH_2)_5CH_3 \qquad \text{(VIII)}$$

$$CH_3(CH_2)_{22}CH{=}CH(CH_2)_3CH_3 \qquad \text{(IX)}$$

The secondary alcohols from the rose flower wax are the first known case with the dominant C_{31} homologue, thus showing that the long chain secondary alcohols are not only confined to those having the C_{29} homologue dominant.

The secondary alcohols and paraffins occur in similar homologues series, the same homologue being dominant (Table 1), thereby suggesting a biogenetic relationship between secondary alcohols and paraffins. Secondary alcohols are not present as esters in contrast to the situation found among the primary alcohols and which suggests that the two classes of compound are formed by different biosynthetic routes.

Two types of compounds are present in waxes, one with predominance of odd numbered homologues, to which belong the secondary alcohols and paraffins and the other type with even numbered homologues prevailing, such as primary alcohols and wax acids. The present results and those of previous papers[9–11] show that the two types of compound are biosynthesized by two distinct pathways—not necessarily independent. This is supported by the following observations: (*a*) secondary alcohols occur only as free alcohols, (*b*) the striking differences between the distribution patterns of compounds with odd and even homologues prevailing, (*c*) the distribution patterns of both types do not show mutual regularities, and (*d*) the coincidence of the most abundant homologues in both the secondary alcohols and paraffins which bear no relationship to those of the primary alcohols and acids. In this connexion it should be noticed that Kolattukudy[13] on the basis of experiments with trichloroacetic acid postulates different sites for the synthesis of different classes of compounds which originate from the same precursor, namely acetate. The present paper does not postulate the particular biosynthetic pathways followed by the two types of compounds, thus leaving this question still open.

[13] P. E. KOLATTUKUDY, *Biochemistry* **4**, 1844 (1965).

EXPERIMENTAL

Isolation of Waxes and Column Chromatography

The dried plant material was extracted with light petroleum. The residue obtained by evaporation of the extract was chromatographed on the 20–50 times its weight of silica gel CH X (Chemapol, Prague) particle size 10–30 μ, deactivated with 15 per cent water. The solvents were cyclohexane, benzene, $CHCl_3$ and ether used successively in a gradient elution procedure. The secondary alcohols were eluted with benzene.

Thin-layer Chromatography

The fractions obtained by column chromatography were examined by TLC on glass plates (2·5 × 7 cm) with silica gel G (Merck). Light petroleum–benzene (65:35) was used as solvent for development (R_f values: paraffins 0·95, waxes 0·8, secondary alcohols 0·6 and primary alcohols 0·3). The components were detected by spraying with a 0·06% solution of Rhodamine 6 G or with H_2SO_4 followed by heating with a resistance wire (charred spots appear).

Oxidation of Secondary Alcohols to Ketones

A solution of the secondary alcohols (300 mg) in HOAc (15 ml) at 60° was treated with CrO_3 (100 mg) in HOAc (10 ml). The mixture was kept at 60° for 10 min, diluted with water (100 ml), extracted with light petroleum (3 × 50 ml) and the combined extracts were evaporated to give the ketones as residue.

Conversion of Secondary Alcohols to Paraffins

The secondary alcohols (40 mg) were heated with I_2 (28 mg) and red P (8 mg) in a sealed tube at 100° for 3 hr, the reaction product was dissolved in light petroleum (1 ml) and filtered through a small column of silica gel (0·5 g). The column was washed with more light petroleum (5 ml), the combined eluates were evaporated, 0·5 N methanolic HCl (5 ml) and Zn granules (0·5 g) were added and the mixture was refluxed for 2 hr with occasional addition of more Zn and of a few drops of conc. HCl. The solution was extracted with light petroleum (10 ml) and the extract was filtered through a small column of alumina (activity I).

Gas–Liquid Chromatography

Analytical procedure. The GLC was performed on a Pye argon chromatograph, column 120 × 0·8 cm o.d., 60 ml/min Ar with liquid phase 7% QF-1 9n kieselguhr Merck, temperature 230° for alcohols and ketones and 1% Apiezon L on ground tile, temperature 235° and 250° for paraffins. The retention times are quoted in an earlier paper.[11]

Preparative procedure. The GLC of the ketones on preparative scale was carried out with an Aerograph A 90 P 3, column 30 × 0·3 cm o.d., 7% QF-1 on Kieselguhr for chromatography (Merck), at 270°, 40 ml/min N_2.

Mass Spectrometry

Mass spectra were measured on an MS 9 or M CH 1303 mass spectrometer, both with a direct inlet system, ionizing current voltage 75 eV and at a temperature of the ion source 160°.

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