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COMPOSITION AND STRUCTURE OF SECONDARY FATTY ALCOHOLS AND KETONES, ISOLATED FROM *PYRUS MALUS* SKIN WAX

MARIA S. DODOVA-ANGHELOVA and CHAVDAR P. IVANOV
Department of Organic Chemistry, Institute of Chemical Technology, Sofia 56, Bulgaria

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Key Word Index—*Pyrus malus*; Rosaceae; varieties, Bouhavitsa, Tetovka; apple skin wax; C_{21-29} secondary alcohols; *n*-nonacosan-10-ol; C_{21-29} ketones; *n*-nonacosan-10-one.

Abstract—A homologous series of long-chain secondary fatty alcohols from C_{21} to C_{29} (C_{29} being predominant) has been isolated from the skin wax of a Bulgarian apple variety, Bouhavitsa. The alcohols were found only in a free state. Long-chain fatty ketones, with C_{29} again markedly prevalent, have been isolated for the first time from the skin wax of this, and another variety, Tetovka. The C_{29}-ketone from both samples was identified by means of MS as nonacosan-10-one. A complete similarity has been found in the relative amounts of C_{29} in the mixtures of secondary alcohols, ketones and paraffins.

INTRODUCTION

AS EARLY as 1931, Chibnall *et al.*[1] isolated a secondary fatty alcohol from the wax of apple skins (different varieties) and showed it to be *n*-nonacosan-10-ol. More recently, Wollrab *et al.*[2] using GLC demonstrated the presence of a homologous series of secondary alcohols, among which C_{29} predominated. By means of MS, Wollrab[3] confirmed that the hydroxy group of the nonacosanol is at C-10. Using column chromatography on silica gel, we obtained six fractions from the unsaponified skin wax of a Bulgarian apple variety, Bouhavitsa[4] and using IR spectra and TLC established that one of them (the second with CCl_4) was a mixture of long-chain secondary fatty alcohols and ketones. We now report here a more detailed study of the composition and structure of these wax components.

RESULTS

The IR spectrum of the alcohol mixture (6% of light petrol. soluble part of the wax) could be assigned to a straight long-chain secondary fatty alcohol (bands at 1100, 3633, 1470, 1375, 2860, 2880, 2940 and 2960 cm^{-1}). The quantitative and qualitative composition of the mixture of secondary alcohols was determined by GLC using suitable standards (Table 1). Under the conditions used, the secondary alcohols yielded symmetric peaks, so that reduction to paraffins was not necessary. It can be seen from the data that a homologous series of secondary alcohols, C_{21}–C_{29}, with C_{29} markedly prevalent, are present in a free

[1] CHIBNALL, A. C., PIPER, S. H., POLLARD, A., SMITH, J. A. B. and WILLIAMS, E. F. (1931) *Biochem. J.* **26**, 2095.
[2] WOLLRAB, V., STREIBL, M. and ŠORM, F. (1968) *Collection* **33**, 2257.
[3] WOLLRAB, V. (1969) *Phytochemistry* **8**, 623.
[4] IVANOV, CH. P. and DODOVA-ANGHELOVA, M. S. (1968) *Year-Book, Higher Inst. Chem. Technol.* **15** (4), 9.

state in the skin wax of the Bouhavitsa apples. Since, the differences between our results and those of Wollrab *et al.*[2] are mainly quantitative, no structural studies were carried out by us. We could not establish the presence of secondary alcohols in the mixture of ester-bound alcohols,[5] in agreement with data on secondary alcohols in other plant waxes.[6]

TABLE 1. PERCENTAGE COMPOSITION OF SECONDARY ALCOHOLS AND KETONES, ISOLATED FROM SKIN WAXES OF BULGARIAN APPLE VARIETIES

No. of C-atoms	Ketones		Secondary alcohols	
	Wax from Tetovka	Wax from Bouhavitsa	Wax from Bouhavitsa	Lit. data[2]
C_{21}	0·1	1·3	Traces	—
C_{22}	0·1	0·1	0·1	1·0
C_{23}	0·2	0·2	0·2	2·5
C_{24}	0·1	0·5	0·1	1·1
C_{25}	0·1	0·4	0·1	0·7
C_{26}	0·1	0·1	0·1	1·7
C_{27}	1·2	8·7	5·0	1·8
C_{28}	0·7	0·4	0·6	2·8
C_{29}	97·4	88·5	93·3	86·1
C_{30}	—	—	—	3·0

The IR spectrum of the ketone mixture (1 % of light petrol. soluble wax) showed clear evidence for the presence of fatty carbonyl compounds with a long straight chain (peaks at 1720, 1410, 1375, 1470, 2860 and 2940 cm^{-1}). The spectrum is similar to the spectra of pure stearone and palmitone, so that it could be inferred that the carbonyl group in these apple ketones is not close to the end of the chain. The individual composition of the ketone mixture was established by GLC under the same conditions as for the secondary alcohols (Table 1). Using the same procedure, we also examined the ketone mixture from the wax of another Bulgarian apple variety, Tetovka. As may be seen from Table 1, both wax samples contain a homologous series of ketones. As with the secondary alcohols from the apple wax, the odd homologues of the ketones are prevalent, and again the C_{29} compound is the major substance present.

By applying combined GC–MS, we succeeded in establishing in both samples the structure of the major nonacosanone. The main peaks were those derived from the α-splitting of the ketone, namely at *m/e* 155 ($C_9H_{19}CO^+$) and at *m/e* 295 ($C_{19}H_{39}CO^+$), which possessed the highest intensity. It is noteworthy that in the case of the Bouhavitsa wax, the cleavage occurred mainly with the formation of the low-molecular fragment, while in the case of the Tetovka wax, the reverse was observed. Peaks at *m/e* 127 ($C_9H_{19}^+$) and at *m/e* 267 ($C_{19}H_{39}^+$) were also present, which are also due to the α-splitting. On the basis of these peaks, the ketone is nonacosan-10-one. This structure is confirmed by peaks, corresponding to rearrangement fragments, arising from β-splitting at *m/e* 170 ($C_9H_{19}C(OH) = CH_2^+$) and *m/e* 310 ($C_{19}H_{39}C(OH) = CH_2^+$). The intensive peak at *m/e* 58 ($CH_3C(OH) = CH_2^+$), derived from the secondary rearrangement of the fragments of the β-splitting shows that there is no methyl group at the α-C-atom. The relatively intensive molecular peak M^+

[5] IVANOV, CH. P. and DODOVA-ANGHELOVA, M. S. (1969) *Compt. Rend. Acad. Bulg. Sci.* **22**, 427.
[6] WOLLRAB, V. (1969) *Collection* **34**, 867.

(422), as well as the absence of the peaks $M^+ - 15(407)$ and $M^+ - 29(393)$ show that the ketone chain is normal. The MS do not offer data on the presence of admixtures from the nonacosan-15-one, found in other plant waxes.

DISCUSSION

The unsymmetrical ketone, nonacosan-10-one, has been so far isolated also from the wax of the stem of *Laserpitium latifolium*,[7] from the surface lipids from leaves of *Brassica oleracea*,[8] as well as from the leaves of different trees (quoted after Wollrab[3]). In all these cases the corresponding secondary alcohol has also been isolated. A similar correspondence is present also between the hentriacontane-9-one, found in rose flower wax,[9] and the secondary alcohol, isolated from the same wax[3]. The results from our investigations on the composition of long-chain secondary alcohols and ketones, isolated from the skin wax of the Bouhavitsa apples also show that these two groups of compounds contain almost the same members and that with both groups the maximum is at C_{29} (Fig. 1).

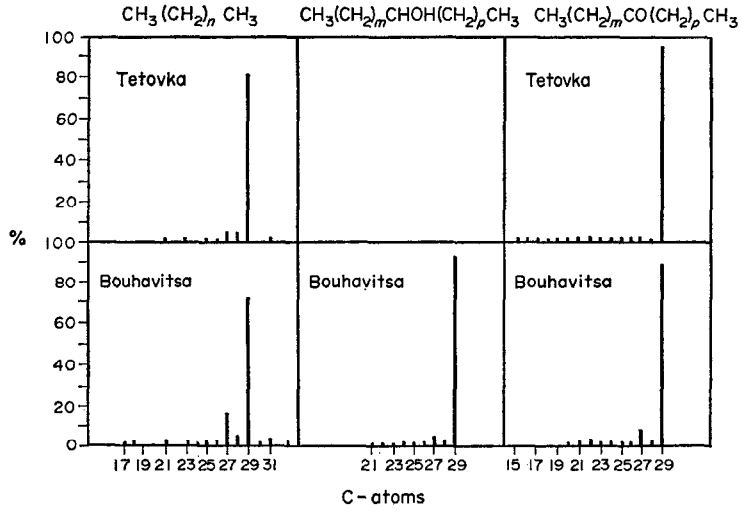

FIG. 1. HISTOGRAMMATIC PRESENTATION OF THE PERCENTAGE COMPOSITION OF LONG-CHAIN HYDROCARBONS, SECONDARY ALCOHOLS AND KETONES, ISOLATED FROM SKIN WAX OF TWO BULGARIAN APPLE VARIETIES, BOUHAVITSA AND TETOVKA.

Comparing the individual composition of the long-chain secondary alcohols and ketones with those of the paraffin hydrocarbons from the same wax, studied previously,[10] it can be seen that there exists an analogy between them, too. In the case of the paraffins, the odd-numbered members are again prevalent, the C_{29} compound being present in the largest amount. All these facts confirm the suggestion put forward by other authors, as well as the

[7] HUNECK, S. (1960) *Naturwissenschaften* **47**, 160.
[8] PURDY, J. and TRUTER, E. V. (1963) *Proc. Roy. Soc.* **158B**, 533.
[9] STOIANOVA-IVANOVA, B., MLADENOVA, K. and POPOV, S. (1971) *Phytochemistry* **10**, 1391.
[10] IVANOV, CH. P. and DODOVA-ANGHELOVA, M. S. (1969) *Compt. Rend. Acad. Bulg. Sci.* **22**, 751.

experimental evidence[11,12] made about other waxes, that there exists a biogenetic relationship between hydrocarbons, secondary alcohols and ketones and that the compounds of these three groups undergo reciprocal transformations.

EXPERIMENTAL

Isolation of the wax. By a 48-hr extraction of dry apple skins with C_6H_6 in a Soxhlet. Along with the typical wax components, triterpenic acids are also extracted. In order to eliminate these acids, the total wax is boiled with light petrol. several times. The average amount of the fraction, soluble in light petrol., is 46% of the total wax.

Column chromatography. About 500 mg of wax, soluble in light petrol., or only its neutral components, are separated on a column, packed with 12–15 g silica gel (Reanal). The gel was ground to 0·125–0·250 mm, washed well with Et_2O and EtOH, and activated according to Spengler.[13] Six fractions were isolated during the elution of the column, consecutively by light petrol., CCl_4 (2), $CHCl_3$ (2) and EtOH.

TLC. Silica gel for TLC (Fluka), mixed with 15% gypsum for medical purposes, was used as adsorbent. In the solvent system C_6H_6: R_f of the secondary alcohols 0·40, R_f of the ketones 0·67.

GLC. Perkin-Elmer analyser, Model 10, with an FID; 1 m columns with 4·5 mm i.d. Phase 2·5% SE-52, on Chromosorb W. Carrier gas N_2 50 ml/min. T. 130–270° (5°/min).

IR. Solution in CCl_4.

MS. They were taken on a combined GC–MS, comprising a Varian 1200 attached to an MAT CH-7 mass spectrometer, operating at 70 eV and using a 3·05 m × 1·6 mm (i.d.) column, packed with 2% OV17.

[11] KOLATTUKUDY, P. E. and LIN, TSUI-YUN J. (1970) *Biochem. Biophys. Res. Commun.* **41,** 1369.
[12] KOLATTUKUDY, P. E. (1970) *Arch. Biochem. Biophys.* **141,** 381.
[13] SPENGLER, G. and HAUT, G. (1955) *Fette, Seifen, Anstrichmittel* **57,** 475.