COMPOSITION OF THE WAX OF RYE STRAW*

M.Streibl, K.Konečný, A.Trka, K.Ubik and M.Pazlar**

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Combination of chromatographic and spectrometric methods, especially gas–liquid chromatography and mass spectrometry, were employed for analysis of the epicuticular wax of rye straw. The wax contains hydrocarbons (6·5%), wax esters (5·0%), free alcohols (11·0%), acids (3·5%), sterols (4·5%), β-diketone (32·0%), hydroxy β-diketone (17·0%) and an unidentified residue (20·5%).

The composition of surface waxes of cultured crops, particularly of wheat, has been reported several times before[1–4]. Since the cereal straw might serve even in this country as a source of interesting compounds, we studied the waxes of rye straw in the context of a broader study of natural waxes. According to Tulloch and coworkers[3,4] who published recently the results of a detailed analysis of wax of several cultivars of green wheat, the usual components are accompanied in these waxes by substantial amounts of an aliphatic β-diketone and a mixture of isomeric hydroxy β-diketones. From this point of view we analyzed the wax of rye straw, *i.e.* of a ripe desiccating plant, the results being presented in this communication.

EXPERIMENTAL

Extract. 2·5 kg of the overground part of ripe rye (cv. Petkus normalstroh) from the surroundings of Prague was extracted at room temperature twice with 20 l distilled light petroleum. The authors are indebted to Dr J. Kudela, Research Institute of Plant Production, Prague for a kind gift of the rye sample. A total of 13 g extract was obtained (0·5%).

Chromatography. Colum chromatography[5] and thin-layer chromatography[6] were done on silica gel. Double elution of the plate with tetrachloromethane with a trace of ethyl acetate gave the following R_F values: hydrocarbons 1·0; esters 0·8; β-diketone 0·75; free alcohols 0·6; hydroxy β-diketone 0·5; free acids 0·4; sterols 0·3; resins 0·24; residue 0·0. Gas chromatography was done on a PYE series 104 Chromatograph Model 124 with flame-ionization detectors and a dual system of glass columns (0·4 . 150 cm). The following packings were used: 3% OV-17 on Chromosorb G (phase A); 3% SE-30 on Gas Chrom Z (B); 3% SE-30 GC grade (General Electric, USA)

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** Present address: Czech Academy of Agricultural Sciences, Research Institute of Food Chemistry, 150 00 Prague 5.

on Gas Chrom P (C); 2·5% QF-1 on Gas Chrom Q (D); 10% 1,4-butanediol succinate on HMDS-Chromosorb W (E). All the supports were 100—200 mesh. As required, either a constant temperature or linearly programmed temperature (2—3°C/min) was used. The samples were injected with a syringe as 1% solutions in CCl_4. For identification of homologues in homologous series the usual graphical method was employed[7]. For a quantitative evaluation the product of the retention time and the peak height, or the triangulation method were used.

Spectroscopy. IR spectra were recorded in a UR-10 (Zeiss Jena) spectrometer in 0·1 cm cuvettes. The sample concentration varied at about 5% in CCl_4. The mass spectra were measured in a AEI 902 spectrometer either by direct inlet or in connection with the PYE series 104 Chromatograph Model 64. As the separating phase in the chromatographic column served packing C, temperature of the ion source of the spectrometer being 240°C, the energy value being 65 eV. The NMR spectra were obtained in a Varian HA-100 in deuteriochloroform using tetramethylsilane as internal standard. The chemical shifts are expressed in the δ-scale.

Separation and identification of components. Separation of normal paraffins from other hydrocarbons was done by sorption in a 5Å molecular sieve (Linde Air Products Co., USA)[8]. The presence of unsaturated hydrocarbons was detected by thin-layer chromatography[9] on silica gel impregnated with 20% silver nitrate. The esters were gas-chromatographed for analytical purposes as well as for mass spectral analysis on packing at a temperature program of 220—320°C (3°C/min). Reesterification was done with methanol and gaseous hydrogen chloride[5], gas chromatography of the alcohols formed (free and in acetate form) was done on columns A and B. The methyl esters after reesterification were analyzed on columns B and D at constant and programmed temperatures. The IR spectrum of the original ester fraction showed absorption bands at 1 172 and 1 735 cm^{-1}. 14,16-Hentriacontanedione was isolated *via* cupric salts in the usual way[2,10]. Its IR spectrum showed absorption at 1 608, 1 702 and 1 728 cm^{-1}. The mass spectrum was recorded under direct inlet conditions. In GLC (packing B, 250°C) a decomposition of the sample was occasionally observed but with careful application with a long syringe needle directly into the column packing no decomposition was observed. M.p. 57—58·5°C, without depression with an authentic sample. 25-Hydroxy-14,16-hentriacontanedione melted at 75—76·5°C (ethyl acetate). Its IR spectrum contains bands at 1 206, 1 607, 1 702, 1 726 and 3 620 cm^{-1}. Acetylation was done with acetic anhydride in pyridine at room temperature and the two acetoxyenolacetates formed showed IR absorption bands at 1 205, 1 245, 1 628, 1 675, 1 699, 1 735 and 1 770 cm^{-1}; 1 220, 1 245, 1 623, 1 699, 1 735, 1 765 respectively. In gas chromatography using column A (280°C) the second derivative had a substantially longer (1·37) retention time than the first. The NMR spectra contained the following common signals: 0·88 triplet, 6H ($2 \times CH_3—CH_2—$); 2·02 singlet, 3H ($—OCOCH_3$); 4·88 triplet, 1H ($—CH—OCOCH_3$). Proton signals of the $C{=}C—OCOCH_3$ differed somewhat (2·17 and 2·24), just as the signals of the $C{\equiv}CH—CO—$ grouping (5.86 and 6·04). For $C_{35}H_{64}O_5$ (564·9) calculated: 74·40% C, 11·42% H; found: 74·82% C, 11·42% H. Primary free alcohols were gas-chromatographed on column B at 260°C free and at 240°C in acetate form, prepared by the action of acetic anhydride in pyridine. The IR spectrum of free alcohols showed absorption maxima at 1 055 and 3 622 cm^{-1}, of the acetates at 1 040, 1 240 and 1 738 cm^{-1}. Free acids were analyzed by GLC as methyl esters (CH_2N_2) on packings B and E at different temperatures (140—180°C). The preparative separation of saturated acids from unsaturated ones for IR spectra measurements was done on impregnated silica gel[11]. Double bonds exhibited absorption bands at 1 655 and 3 005 cm^{-1}. The retention times of the methyl ester of unsaturated acid C_{18} were in all GLC analyses identical with that of standard oleic acid. Sterols could be resolved by gas chromatography in the free form both on columns A (270°C) and B (250°C). Suitable separation of their acetates was accomplished even on packing B (250°C), the relative retention times of the acetyl derivatives being as follows: cholesterol acetate 0·63;

campesterol acetate 0·81; stigmasterol acetate 0·91, β-sitosterol acetate 1·0. The mass spectra of the individual chromatographic peaks were recorded during GLC on column B at a temperature program between 220 and 250°C (2°C/min).

RESULTS AND DISCUSSION

Group separation. Light petroleum was used to obtain a 0·5% yield of an extract. Crude separation of the extract was done on a column of silica gel and the components were eluted first with light petroleum (hydrocarbons), then with light petroleum

TABLE I
Composition of the Individual Groups[a]

Number of carbon atoms	n-Alkanes[b]	Acids in esters	Alcohols in esters	Free acids[c]	Free alcohols
11		—	—	—	—
12		—	—	2·5	—
13		—	—	0·5	—
14		—	0·5	12·5	—
15	2·0	tr	tr	4·6	—
16		15·1	0·5	36·2	—
17		0·7	tr	7.0	—
18		11·0[d]	1·5	15·6	—
19		tr	tr	—	—
20		28·0	4·0	1·2	—
21	0·7	2·6	1·5	—	—
22	0·5	18·0	11·0	—	1·8
23	1·3	1·2	2·5	—	0·5
24	0·6	14·2	38·0	—	17·3
25	3·0	0·5	2·0	—	0·5
26	1·1	5·1	33·0	—	74·0
27	13·1	tr	0·5	—	0·6
28	2·6	1·1	3·5	—	4·6
29	39·2	—	tr	—	—
30	1·6	—	—	—	tr
31	28·0	—	—	—	—
32	tr	—	—	—	—
33	2·0[e]	—	—	—	—
Total	95·7	97·5	98·5	80·1	99·3

[a] According to GLC analysis. [b] 4·1% forms branched hydrocarbons and chromatographic background. [c] Traces of the C_{35} homologue are present. [d] 1·5% of $C_{18:1}$ is also present. [e] Unidentified peaks 7%; unsaturated acids 11·8% (C_{15} traces, C_{16} 1·8%, C_{17} traces; C_{18} 10·0%).

plus 5% ethyl acetate (a mixture of esters and diketone), light petroleum plus 15% ethyl acetate (hydroxy diketone, alcohols, acids, sterols), and finally with ethyl acetate alone (resinous fraction). The column then still contained some 10% of the original extract. The incompletely separated fractions were then separated on silica gel, if necessary.

Hydrocarbons and esters. The hydrocarbons (6.5%) represent according to GLC analysis an extensive series of normal alkanes $C_{11}-C_{33}$ with a maximum of occurrence of the odd-numbered members C_{29}, C_{31} and C_{27} (Table I). Using a molecular sieve[5] (5 Å) the hydrocarbon fraction yielded 4% branched or cyclic hydrocarbons, whose GLC record displays the so-called "chromatographic background"[12] formed by a broad flat elution peak of undefined hydrocarbons, and two shorter homologous series of branched alkanes. Unsaturated hydrocarbons have not been detected[9]. The fraction of simple wax esters (5%) contains according to GLC analysis a homologous series of predominantly even-numbered homologues in the range C_{32}—C_{52} with striking peaks at C_{40} (16%), C_{42} (26%), C_{44} (20%) and C_{46} (10%). The mass spectra of the C_{40} and C_{42} peaks recorded during GLC indicate that the individual chromatographic peaks do not correspond to individual esters but that they contain a mixture of isosteric esters[5]. Reesterification of the ester fraction, separation of the reaction components on silica gel and subsequent GLC revealed that the original esters are formed by a mixture of normal acids C_{14}—C_{26} with the highest participation of C_{20}, esterified by a series of primary alcohols C_{18}—C_{28} with predominant tetra- and hexacosanol. The presence of a small amount of unsaturated acids with predominanting oleic acid (C_{18}) has been detected.

Diketones. Of further chromatographic fractions a substantial amount (32%) of individual 14,16-hentriacontanedione has been isolated *via* cupric salts. It was identified on the basis of retention data during GLC,* with the aid of IR spectra and especially mass spectra[13]. Cupric salts of other fractions were used for isolating a similar group of compounds which were found to be hydroxy β-diketones according to IR spectra. During gas chromatography, however, the sample gave no response in the detector. After acetylation the reaction mixture could be separated into two fractions differing in GLC as well as TLC. The IR spectra of both acetylated compounds contained the expected bands for the enol-acetate grouping of the β-diketone and for another alcohol acetate. Similarly, the NMR spectra of both acetylated compounds contained the expected signals of the isolated acetoxy group as well as of the acetylated β-diketone enolate, the isolated acetoxy group not occurring at the terminal or penultimate carbons of the aliphatic chain. Both compounds probably differ only in the isomerism of the enolacetate grouping of the β-diketone. This is supported by a mass spectrum analysis of the free hydroxy β-diketone[13] which made it possible

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to localize the hydroxy group and to identify thus the compound as 25-hydroxy-14,16--hentriacontanedione.

Alcohols. The mother liquors after insoluble cupric complexes of the diketone were subjected to column chromatography and a homologous series of free primary alcohols (11%) C_{20}—C_{30} has been isolated. The even-numbered homologues predominate over the odd-numbered ones, most of all hexacosanol. They were identified with the aid of retention data from GLC (free and in the form of acetates) and with the aid of the mass spectrum of the C_{26} chromatographic peak. Using GLC and MS it was possible to identify in the late fractions a group of phytosterols (on the basis of Knight's study)[14]: β-sitosterol (63%), campesterol (29%) and stigmasterol (7%). They are accompanied by a small amount of cholesterol (1%).

Acids. A minor fraction of free fatty acids (3·5%) was analyzed in the form of methyl esters by GLC. It contains a homologous series C_{12}—C_{20} and, in addition to saturated acids, homologous unsaturated acids were present, especially *cis*-9-octadecenic acid (oleic acid) (Table I). It was identified on the basis of IR spectrum and of retention times at GLC.

The results indicate that the composition of rye straw wax resembles that of the waxes of green Canadian wheat[3,4], including the high diketone content. 14,16-Hentriacontanedione has been described in all the waxes of the wheat cultivars studied[3,4]. 25-Hydroxy-14,16-hentriacontanedione as a chemical individual has been described so far in a single cultivar[4] while in others the hydroxy-β-diketones contain OH-groups in position 8 or 9. Higher aliphatic aldehydes, formerly described in wheat waxes[15], have not been found in rye.

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