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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF β-DIKETONE-CONTAINING PLANT WAXES

USE OF TRIMETHYLSILYL ETHERS*

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SUMMARY

A number of β-diketones and β-diketone-containing waxes have been trimethylsilylated and analyzed by gas chromatography–mass spectrometry. The trimethylsilyl enol ethers of β-diketones and hydroxy and oxo β-diketones are completely separated by gas chromatography. Mass spectrometry of these derivatives gives simpler fragmentation patterns than those obtained with untreated β-diketones so that structure determination of β-diketones and oxygenated β-diketones, including structural isomers, is considerably facilitated. Trimethylsilyl derivatives of free acids and alcohols formed at the same time are separated from each other and from hydrocarbons by gas chromatography and are also identified by mass spectrometry.

INTRODUCTION

Analysis of unfractionated waxes from many grass species by gas–liquid chromatography (GLC) shows the presence of β-diketones and hydroxy β-diketones[1–3], but when oxo β-diketones are also present[4] they cannot be distinguished from hydroxy β-diketones. Also when the hydroxy β-diketones are mixtures of isomers[5–7] they cannot be identified by GLC alone. Mass spectrometry (MS) of β-diketones[8,9] and gas chromatography (GC)–MS of β-diketone containing whole waxes[10] has shown that these compounds give characteristic spectra and that the positions of the β-diketone grouping and of hydroxyl substituents can be established[1,9,10]. However MS of β-diketones gives a relatively large number of fragments due to the many possible α-cleavages and also to McLafferty rearrangements[9] which is a disadvantage when mixtures of isomers are present.

We have now found that β-diketones and oxygenated β-diketones can be largely converted to trimethylsilyl (TMS) enol ethers and that the derivatives are better resolved and more conveniently identified by GC–MS analysis than in previous analytical procedures.

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EXPERIMENTAL

Materials

Long-chain acids, alcohols and esters were previously[1] prepared synthetic compounds. β-Diketones and hydroxy and oxo β-diketones were previously isolated from the grass waxes analyzed earlier[4,5]. Mixtures containing β-diketones and natural waxes were trimethylsilylated as follows: wax (5 mg) was dissolved in 0.3 ml toluene–pyridine (5:1) and 25 μl hexamethyldisilazane; 12 μl trimethylchlorosilane were added and the mixture was kept at room temperature overnight[11].

GC–MS conditions

A Finnigan Model 3300 GC–MS system with an Incos Model 2000 data system was used. The transfer lines and the jet separator were maintained at 275° and the GC injector was kept at 300° and the ionization voltage was 70 eV. The glass column was 1.7 m × 2 mm I.D. and was packed with 1.5% Dexsil 300 on 80–100 mesh acid washed and silanized Chromosorb W. The initial helium pressure was 25 p.s.i. and rose to 40 p.s.i. during programming; temperature was programmed from 175° to 380° at 4°/min. For the first 60 sec after sample injection column effluent was diverted through the GC vacuum divert valve into a mechanical pump to avoid contamination of the ion source.

RESULTS AND DISCUSSION

The reconstructed gas chromatogram (RGC) obtained when a trimethylsilylated mixture of approximately equal amounts of octacosanol, hentriacontane-14,16-dione, 25-hydroxyhentriacontane-14,16-dione, 25-oxohentriacontane-14,16-dione, and docosyl docosanoate was examined by GC–MS is shown in Fig. 1. Some

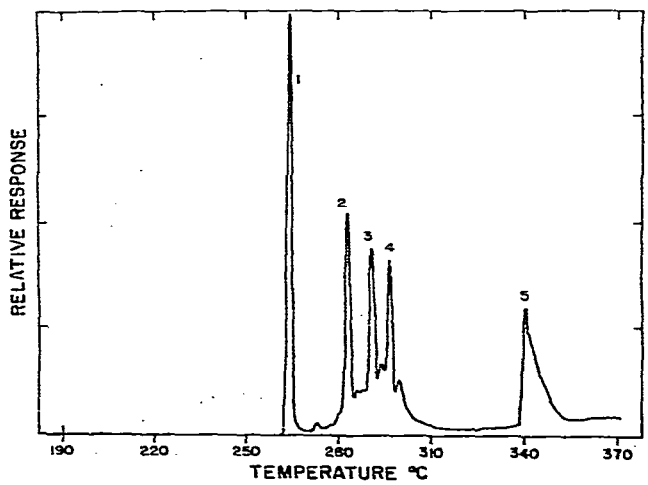

Fig. 1. Reconstructed gas chromatogram from GC–MS analysis of trimethylsilylated mixture of approximately equal amounts of octacosanol (1), hentriacontane-14,16-dione (2), 25-hydroxyhentriacontane-14,16-dione (3), 25-oxohentriacontane-14,16-dione (4) and docosyl docosanoate (5).

decomposition of the β-diketone derivatives occurred during GC but they mainly appeared as TMS ethers of the enol forms. The TMS ether of the hydroxy β-diketone (peak 3) eluted earlier than, and well separated from the oxo β-diketone derivative (peak 4). The intensities of the peaks were not proportional to the amounts of the components and the response of the alcohol TMS ether is particularly large.

Fragment ions produced from the above three β-diketone TMS derivatives and from some other oxygenated β-diketone wax components (mentioned below) were 1. hentriacontane-14,16-dione *m/e* (rel. int.).: 536 M^+ (2), 521 M − 15 (8), 446 (6), 353 (37), 325 (36), 250 (6), 185 (12), 172 (12), 169 (12), 157 (16), 75 (18), 73 (100); 2. 25-hydroxyhentriacontane-14,16-dione: 624 M^+ (1), 609 M − 15 (5), 539 (22), 441 (5), 351 (7), 325 (16), 187 (24), 169 (9), 157 (6), 75 (28), 73 (100); 3. mixture of 8- and 9-hydroxyhentriacontane-14,16-diones: 624 M^+ (0.1), 609 M − 15 (1), 525 (0.2), 511 (0.1), 413 (2), 353 (18), 215 (4), 201 (6), 157 (7), 75 (23), 73 (100); 4. 25-oxohentriacontane-14,16-dione: 550 M^+ (1), 535 (5), 465 (1), 367 (19), 325 (29), 185 (10), 183 (5), 169 (12), 157 (12), 113 (19), 75 (20), 73 (100); 5. 10-oxohentriacontane-14,16-dione: 550 M^+ (1), 535 M − 15 (4), 423 (1), 353 (25), 339 (8), 255 (8), 249 (9), 225 (16), 222 (9), 197 (18), 185 (19), 183 (6), 169 (17), 155 (11), 75 (21), 73 (98), 43 (100).

Fig. 2 shows the probable mass fragmentation patterns of these derivatives, the TMS group is shown as attached to only one of the two equally likely positions.

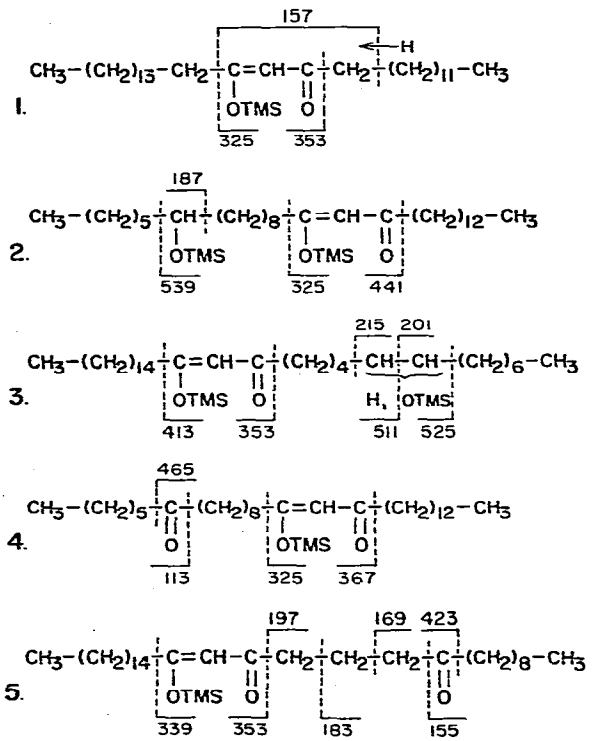

Fig. 2. Mass fragmentation of TMS enol ethers of β-diketones. 1 = Hentriacontane-14,16-dione, 2 = 25-hydroxyhentriacontane-14,16-dione, 3 = mixture of 8- and 9-hydroxyhentriacontane-14,16-diones, 4 = 25-oxohentriacontane-14,16-dione and 5 = 10-oxohentriacontane-14,16-dione.

The unsubstituted β-diketone derivative (Fig. 2.1) undergoes cleavage on both sides of the oxygenated carbons giving only two principal ions *m/e* 325 and 353. The MS of the underivatized β-diketone, on the other hand, shows 6–8 major α-cleavage ions[9]. The fairly prominent ion *m/e* 157 may arise as shown from one McLafferty rearrangement, it is analogous to the ion *m/e* 100 in MS of the β-diketone which arises from two McLafferty rearrangements[9]. This ion also appears in most of the other spectra.

The hydroxy β-diketones form di-TMS derivatives. When the hydroxyl group is at C-25 (Fig. 2.2) the ion *m/e* 353 is replaced by a much less intense ion *m/e* 441 showing that the hydroxyl is attached to the C_{15}-side of the molecule. Location of the hydroxyl group is shown by the expected α-cleavage[12] giving intense ions *m/e* 187 and 539. When hydroxyl groups are at C-8 or C-9 (Fig. 2.3), as they are in the mixed hydroxy β-diketones from spring wheat wax[5], the ion *m/e* 353 shows that the hydroxyls are attached to the C_{13}-side of the molecule. In MS of these isomers, presumably because the hydroxyl is relatively close to the β-diketone group, the α-cleavage fragments *m/e* 525 and 511 are extremely weak and only ions *m/e* 201 and 215 indicate the point of substitution. When the hydroxyl is at C_5, however, α-cleavage fragments are more prominent again (5-hydroxy β-diketone was isolated from wax of another grass species; A. P. Tulloch, unpublished results).

The β-diketone with an oxo group at C-25 also forms a TMS derivative (Fig. 2.4) which gives prominent ions *m/e* 325 and 367 showing that the carbonyl is on the C_{15}-side of the β-diketone. One ion, *m/e* 465, formed by α-cleavage at the carbonyl group is very weak but the other, *m/e* 113, is strong and shows the position of the group in the chain. Considerably more fragments are produced on GC–MS of the isomeric 10-oxo β-diketone derivative (Fig. 2.5) probably because the oxo and β-diketone groups are quite close. There is a strong ion at *m/e* 353 but the corresponding ion, *m/e* 339, is weaker. The position of the oxo group in the C_{13}-chain is indicated by the α-cleavage ion *m/e* 155, however, there is a stronger ion at *m/e* 169 which might be taken to indicate oxygenation at C_{11}. There are also strong ions at *m/e* 185 and 197 and at *m/e* 255, 249, 225 and 222. The latter ions are probably due to decomposition on the column (which also occurs with the underivatized β-diketone[4]) since they are stronger in scans taken before the main peak. GC–MS identification of this oxo β-diketone as the TMS derivative would be unreliable but additional GC–MS of the parent compound would give useful results since the same misleading ions do not occur in both spectra[4].

The results of GC–MS analysis of two trimethylsilylated plant waxes are shown in Fig. 3, the upper RGC was obtained with wax from *Agropyron intermedium*[4] and the lower RGC from wax of spring wheat (Selkirk variety)[5]. In Fig. 3.1 peaks 7, 8 and 10 are due to TMS derivatives of the same β-diketone discussed above, the 25-hydroxy β-diketone and the 25-oxo β-diketone (together with a minor amount of the 10-oxo derivative), respectively; peak 9 is due to decomposition products of oxo β-diketones and peak 11 to unreacted oxo β-diketone. In Fig. 3.2 peak 11 is due to the same β-diketone and peak 12 to a mixture of 8- and 9-hydroxy β-diketones as TMS derivatives. These results agree with those obtained previously[4,5] in which the β-diketones were isolated by silicic acid column chromatography and their structures determined by examination of the products of alkali degradation.

Free acids and free alcohols in the waxes were also converted to TMS derivatives and identified by GC–MS. Thus C_{24} and C_{26} alcohol TMS ethers appear

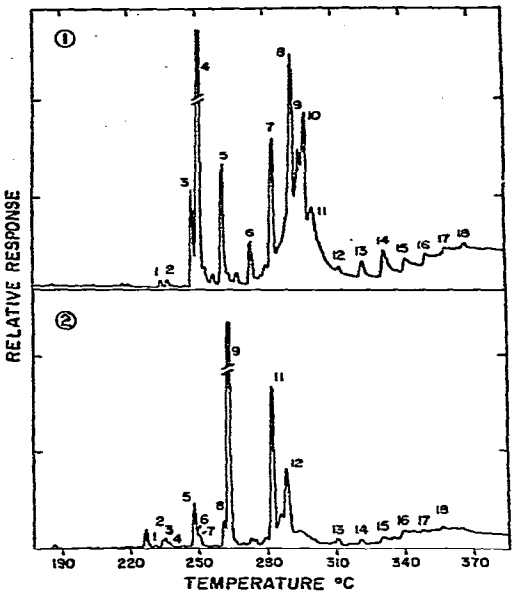

Fig. 3. Reconstructed gas chromatograms from GC–MS analysis of trimethylsilylated waxes. (1) Wax from *Agropyron intermedium*. Peaks 1, 3, 5 and 6 are C_{27}–C_{33} hydrocarbons; peaks 2 and 4 are C_{24} and C_{26} alcohol TMS ethers; peak 7 is hentriacontane-14-16-dione; peak 8 is 25-hydroxyhentriacontane-14,16-dione; peak 10 is a mixture of 25-oxo and 10-oxohentriacontane-14,16-diones all as TMS enol ethers; peak 9 is decomposition productions; peak 11 is unreacted oxo β-diketone; peaks 12–18 are C_{38}–C_{50} esters. (2) Wax from Selkirk variety of spring wheat. Peak 1 is C_{22} acid TMS ester; peaks 3 and 6 are TMS esters of *trans* 2,3 unsaturated C_{22} and C_{24} acids; peaks 2, 5 and 8 are C_{27}–C_{31} hydrocarbons; peaks 4, 7, 9 and 10 are TMS ethers of C_{24}–C_{30} alcohols; peak 11 is hentriacontane-14,16-dione; peak 12 is a mixture of 8- and 9-hydroxyhentriacontane-14,16-diones, all as TMS enol ethers; peaks 13–18 are C_{38}–C_{48} esters.

as peaks 2 and 4 in Fig. 3.1 and C_{24} to C_{30} TMS ethers appear as peaks 4, 7, 9 and 10 in Fig. 3.2. The TMS ethers were identified by the strong M — 15 ion[12]. Free acids were not present in the wax from *A. intermedium*[4] but in Fig. 3.2, peak 1 is the TMS ester of saturated C_{22} acid and peak 3 and 6 are due to TMS esters of *trans* 2-docosenoic and *trans* 2-tetracosenoic acids. The TMS ester of the saturated acid was identified by characteristic ions at *m/e* 117, 129, 132, 145 and 201 (ref. 13) together with the molecular ion and the intense M — 15 ion. The presence of ions *m/e* 129, 132 and 145 and of a parent ion distinguish TMS esters of saturated fatty acids from TMS ethers of 2-alkanols, which also give a strong ion at *m/e* 117[12]. TMS esters of the *trans* 2,3-unsaturated acids were characterized by ions at *m/e* 117, 129, 143 and 155 and by the molecular ion and the intense M — 15 ion. These acids are found in waxes of certain *Triticum* species and have characteristic GLC emergence times[2,5] but the GC–MS results confirm the chain length.

The hydrocarbon compositions of the waxes illustrated in Fig. 3 were known so that peaks 1, 3, 5 and 6 in Fig. 3.1 can be assigned to C_{27} to C_{33} odd-carbon hydrocarbons and peaks 2, 5 and 8 in Fig. 3.2 to C_{27} to C_{31} hydrocarbons. The MS of the hydrocarbons lacked parent ions so that the chain length could not be determined.

Thus the order of emergence on GLC analysis of these other wax components is C_n hydrocarbon, C_{n-3} alcohol TMS ether and C_{n-3} acid TMS ester, all three components being completely resolved. When *trans* 2,3-unsaturated acids are present their TMS esters appear between the peaks due to hydrocarbon and alcohol (with 2 more carbons than the unsaturated acid) TMS ether and partly overlap them. Previously whole waxes were treated with diazomethane to convert free acids to methyl esters and then acetylated to convert alcohols to acetates and analyzed by GLC with Dexsil 300 as liquid phase. The order of elution was C_n hydrocarbon, C_{n-3} acid methyl ester and C_{n-3} alcohol acetate[1,2,14].

Long-chain ester peaks also appeared in the RGC (Figs. 1 and 3) but since the jet separator was not heated above 275° the peaks were broader than they are in normal GLC analysis[1,14]. Esters could, however, be identified by the M^+ ion and some of the parent acids were indicated by $RCO_2H_2^+$ fragments.

Thus most components of plant waxes are resolved by GC analysis of the trimethylsilylated whole wax and almost all are identifiable from MS. The procedure is shorter and more convenient, from a qualitative point of view, than previous GLC procedures[1,2] which involve diazomethane treatment and acetylation. Also β-diketone and substituted β-diketones can be identified without preliminary separation and chemical degradation. At present the procedure is less satisfactory as a quantitative method since acids and alcohols, as TMS derivatives, give much larger responses than the β-diketone derivatives, though use of internal standards could give useful results. Quantitative analysis of waxes containing appreciable amounts of long-chain esters would also be quite difficult by this method.

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