METHODS

On-Line Hydrogenation in GC-MS Analysis of Cyclic Fatty Acid Monomers Isolated from Heated Linseed Oil[1]

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A simple on-line hydrogenation method in GC-MS analysis of unsaturated fatty acid esters is described. Using hydrogen as carrier gas, hydrogenation takes place in a capillary reactor connected to the outlet of the analytical column in the oven of the gas chromatograph. The reactor is a fused silica tube (60 cm × 0.32 mm i.d.) coated with palladium acetylacetonate. Selective hydrogenation of olefinic bonds is achieved after a normal chromatographic run. Structural information (carbon-skeleton, double bond equivalents) can thus be deduced, and structural correlations between the saturated and unsaturated components can be obtained. Structures of cyclic fatty acid esters isolated from heated linseed oil were elucidated using this simple method which was found very useful for structural investigations on unsaturated compounds by GC-MS.

Lipids 24, 347-350 (1989).

Separation and spectral characterization of the methyl esters of unsaturated cyclic fatty acid monomers (CFAM) isolated from heated vegetable oils were described recently (1,2). However, many structural problems remained unsolved because the mass spectra of the unsaturated compounds of the mixtures were ambiguous. Generally, the mass spectra of saturated species are interpreted more readily than those of related unsaturated compounds. For example, distinction of the carbon-skeleton types is much easier (ring size, nature of the substituents).

The easiest and most commonly used method to obtain saturated components is by catalytic hydrogenation of the unsaturated mixture before analysis. This was done very recently on CFAM mixtures isolated from sunflower and linseed oils. The carbon-skeletons of the major constituents were elucidated, and the double bond equivalents were determined (1,3).

However, this kind of data is useful only if the correlations between the saturated and their corresponding unsaturated analogues can be carried out easily. Correlations become difficult if various unsaturated species are converted into the same saturated products. Positional and geometrical isomers, for example, apparently rather abundant in the CFAM mixtures (2), will give the same hydrogenated product; therefore, only one peak will be detected by gas chromatography. Consequently, it was impossible to determine correlations between the unsaturated and saturated CFAM mixture obtained after total hydrogenation.

Another method of hydrogenation would be to obtain selective hydrogenation of all the unsaturated species after chromatographic separation. An on-line technique (4,5), just before mass spectrometry, may be used. We now describe an on-line hydrogenation method for use in GC-MS analyses of methyl esters of CFAM isolated from a heated linseed oil. This method proved easy and useful in structural studies.

EXPERIMENTAL

Cyclic fatty acid monomers were isolated from linseed oil heated at 275°C under nitrogen and esterified according to published procedure (1).

Gas chromatography-mass spectrometry (GC-MS). All GC-MS analyses were performed with a Nermag R 10-10 C mass spectrometer directly coupled to a Girdel 31 gas chromatograph. The analytical column used throughout the study was a fused silica capillary column (30 m × 0.25 mm i.d.) coated with DB Wax (J and W Scientific, 0.5 μm film thickness).

Splitless injections were used and oven temperatures were programmed from 60 to 180°C/min at 10°C/min, then to 220°C at 3°C/min and held at 220°C until completion of the analyses. Hydrogen was used as the carrier gas with a linear velocity of 53 cm/s at room temperature. Electron impact mass spectra were generated at 70 eV with a source temperature of 150°C, the instrument scanning from 42 to 310 amu on an 0.8 s cycle.

Hydrogenation capillary reactor. A deactivated fused silica capillary column (5 m × 0.32 mm i.d.) was statically coated with a 0.5% methylene chloride solution of palladium acetylacetonate (Fluka). After drying under a stream of nitrogen, 50 cm of both ends of the column were discarded. A 60-cm piece of the capillary was inserted with zero-dead volume butt connectors (Supelco Inc., Bellefonte, PA) between the analytical column and a deactivated fused silica capillary column (3 m × 0.25 mm i.d.) directly connected, through the heated interface, to the ion source of the mass spectrometer.

Palladium metal was precipitated in a stream of hydrogen carrier gas by heating to the normally-applied column temperature (220°C).

RESULTS AND DISCUSSION

A recent structural study on unsaturated CFAM methyl esters isolated from heated linseed oil revealed that the CFAM fraction was a mixture of cyclohexenyl and cyclopentyl and/or cyclopentenyl derivatives (2). After total hydrogenation of the CFAM fraction, another study (3) partially confirmed an earlier one (6) while extending our knowledge of the MS fragmentation of alkylcyclopentyl esters. The structures of some of them were definitely established by organic synthesis (7).

[1]Presented in part at the AOCS meeting in May 1988 in Phoenix, AZ.

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Abbreviations: CFAM, cyclic fatty acid monomers; GC, gas chromatography; MS, mass spectroscopy; TIC, total ion currents.

However, in the GC-MS analyses of the hydrogenated CFAM mixture isolated from linseed oil, the chromatograms obtained before and after total hydrogenation were, as expected, completely different (2,3). It was then rather difficult to assign structures to positional isomers of unsaturated CFAM which gave only one product after hydrogenation.

In order to obtain better GC correlations between saturated and unsaturated species, we investigated an on-line hydrogenation method first described by Schomburg et al. (4,5). Schomburg and his coworkers determined important parameters (activity of catalysts, hydrogen flow in the capillary reactor and temperature of hydrogenation) using a double oven chromatograph equipped with a switching device (5). However, a simpler way to operate, compared to the method described by Schomburg et al., would be to connect a capillary catalytic reactor either before or after the analytical column, directly in the oven of the gas chromatograph. Hydrogen would be provided as the carrier gas and reaction temperature would be the oven temperature. The availability of deactivated fused silica capillaries and of various zero-dead volume connectors permitted us to investigate this direct simple arrangement.

For the selective hydrogenation of olefinic double bond, a palladium catalyst was chosen, because platinum catalysts may also hydrogenate other functions, such as ester groups (5).

The capillary reactor was placed in the oven of the GC behind the analytical column and ahead of the mass spectrometer. Connecting the reactor to the inlet of the analytical column would not afford an advantage as compared to classical hydrogenation before GC-MS analysis.

The activity of the catalyst, its selectivity for carbon-carbon double bonds, and adsorption on its surface were tested with mixtures of unsaturated esters. For instance, a mixture of methyl oleate and methyl linoleate (Fig. 1A) gave two peaks of methyl stearate when injected with the Pd reactor connected to the outlet of the column (Fig. 1B). Only slight variations of a few seconds were noted between independent runs. Peak tailing, due to adsorption of the eluates on the surface of the catalyst, was considered acceptable. Complete hydrogenation was obtained when the sample load did not exceed 1 μg/peak.

The unsaturated CFAM mixture described above was therefore submitted to post-column hydrogenation. The total ion currents obtained without and with the Pd

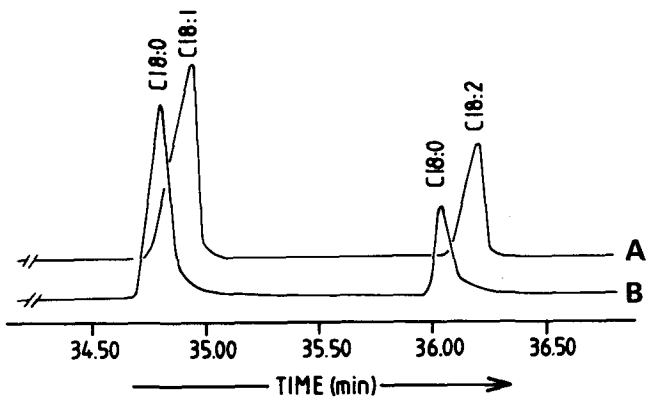

FIG. 1. Total ion currents (TIC) of a mixture of methyl oleate (C18:1) and methyl linoleate (C18:2). (A) Without Pd reactor. (B) With Pd reactor. Column: DB Wax 30 m × 0.25 mm i.d. programmed from 60 to 220°C at 5°C/min. Pd capillary reactor: 60 cm × 0.32 mm i.d. Carrier gas: H_2

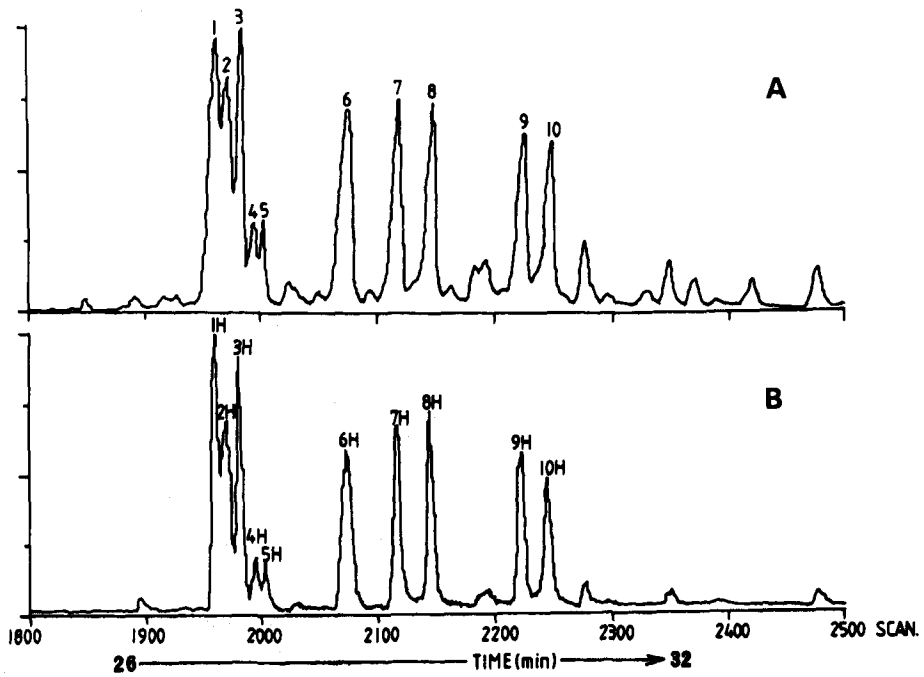

FIG. 2. TIC of CFAM esters isolated from heated linseed oil. (A) Without Pd reactor. (B) With Pd reactor. Column: DB Wax 30 m × 0.25 mm i.d. programmed from 60 to 180°C at 10°C/min, then to 220°C at 3°C/min. Pd capillary reactor: 60 cm × 0.32 mm i.d. Carrier gas: H_2

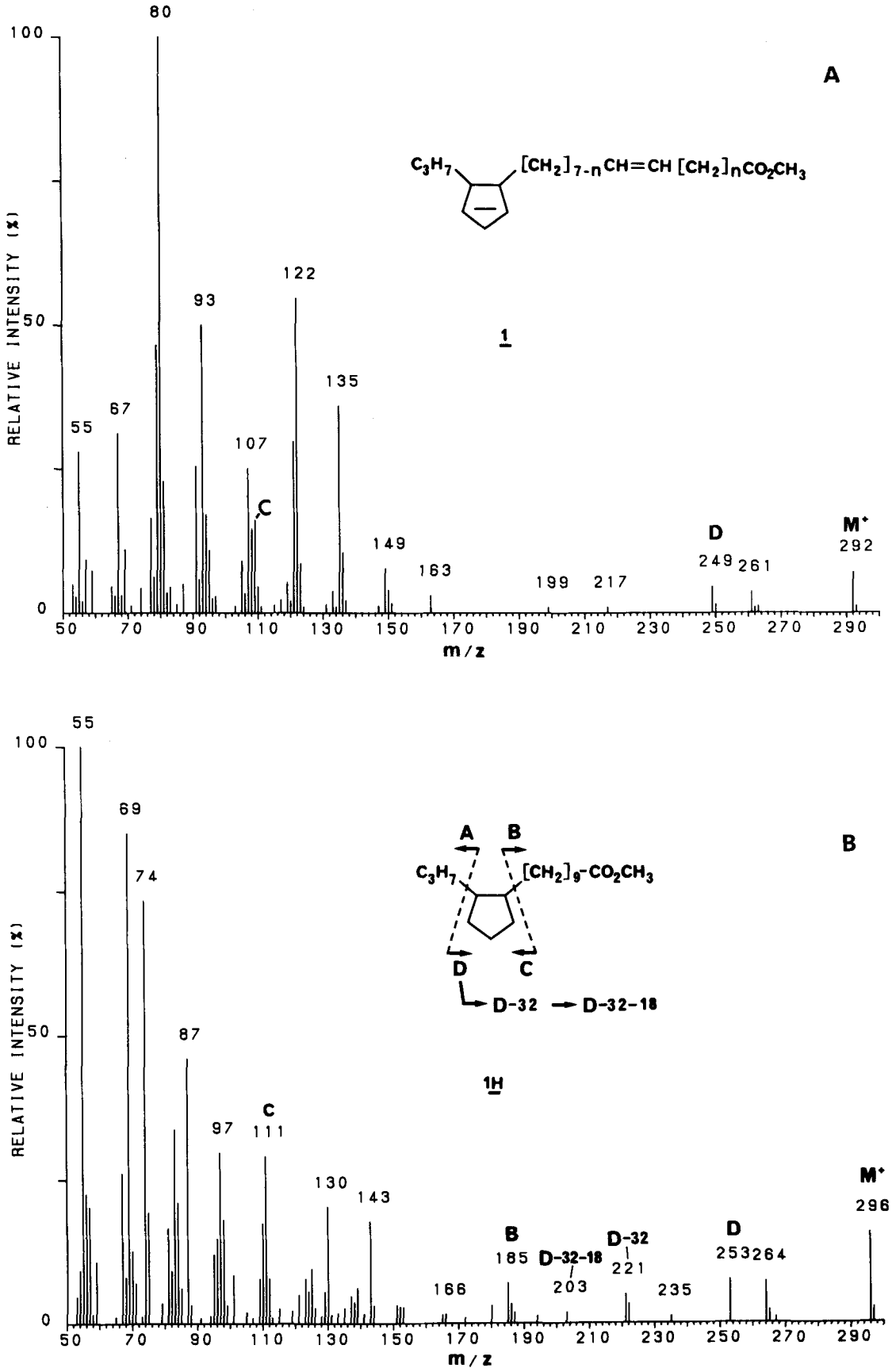

FIG. 3. Mass spectra (70 eV) of compound 1 (n = 0–7, A) and its hydrogenated counterpart 1H (B), with their identification and characteristic fragmentations. Peak numbers are those of Figure 2.

reactor could be superimposed (Fig. 2). Under the same chromatographic conditions, retention times were not altered by inserting the capillary reactor into the system. Only slight tailing of peaks was observed due to adsorption. Mass spectra of the pure hydrogenated species were obtained for each of the major 10 peaks. The carbon skeleton of each of the compounds (peaks 1 to 10) could clearly be established by the information gained through the mass spectra of their hydrogenated counterparts 1H to 10H (Fig. 2). For instance, peak number 1 was identified as a propylcyclopentenyl decenoate, because the mass spectrum of its hydrogenated product 1H (Fig. 3B) was identified as that of methyl (2-propylcyclopentyl) decanoate. The molecular ion was increased by four mass units from 292 to 296 (Fig. 3). Extensive changes in the spectrum occurred in the mass range of 50–150 as a result of hydrogenation. Diagnostic ions (Fig. 3B) B (*m/z*: 185), C (*m/z*: 111), D (*m/z*: 253), D-32 (*m/z*: 221) and D-32-18 (*m/z*: 203) were unambiguously assigned. We also noted ions of low intensity (*m/z*: 267 and 235) and attributed them to fragment ions D_β and D_β-32 resulting from a second cleavage of the alkyl moiety in the β-position. This hypothesis was confirmed by mass spectrometry of some synthesized alkyl-cyclopentyl derivatives (7), and the β-fragmentation of the alkyl chain seems to be characteristic of these alkyl-cyclopentyl esters.

Peak 2 was identified as the *cis*-isomer of 1, peaks 3 to 6 were identified as butylcyclopentenyl nonenoates, differing only by the position of the double bond in the side-chain, and peaks 7 to 10 were confirmed (2) to be isomers of propylcyclohexenyl nonenoates.

Activity and selectivity of the catalyst were tested periodically. Good results were obtained through several days of operation using one 60-cm piece of capillary reactor. The technique was found useful for structural investigations on mono- and polyunsaturated compounds of various origins as they occur in complex mixtures.

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[Received July 22, 1988; Revision accepted December 12, 1988]