
Identification of Aromatic Fatty Acid Ethyl Esters

The only use of gas chromatography is not sufficient to determine which compounds are eluting from the catalytic bed. At the beginning of this work, the comparison of retention times apparently provided the information. However, as more active catalysts were tested, the number of peaks increased considerably, so that other supporting techniques had to be used. The most suitable, and also available, technique was gas chromatography coupled to mass spectrometry. However, a mass spectrum cannot be associated to a particular chemical structure without additional information. Therefore, it resulted of extremely great importance the incorporation of additional identifying parameters, related to UV, IR, NMR spectra, solubility, etc., to build the interpretation of the spectrum [1]. Particularly aromatic Fatty Acid Ethyl Esters (FAEE) were structurally determined by the combined use of GC-MS, UV and ¹H-NMR results. Additionally aromatization had been reported to occur when heating linoleic acid in a batch reactor at 260 °C using Pd/AC [2]. It is worth pointing out the importance of their identification, which represented a key step in the development of this study. The fact of obtaining such compounds was the first evidence that a dehydrogenation reaction was occurring in the reactor.

The final part of this chapter makes reference to those compounds, which were detected in most of the cases and, whose ion molecular presented a value of 302.

3.2.1 Interpretation of spectra

Mass spectra do not show emission or uptake of energy by the compound, unlike spectra obtained by other spectroscopic techniques, such as UV, IR, NMR, or fluorescence, but they reflect the qualitative and quantitative analysis of the processes accompanying ionization (fragment formation, rearrangements, chemical reaction) [1].

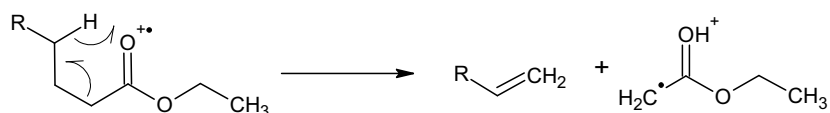
The current methodology used by analysts to determine fatty acids structures involves GC-MS, GC linked to Fourier-transform infrared spectroscopy (FTIR), and silver ion and reversed-phase high-performance liquid chromatography (HPLC), among others. However, their mass spectra rarely contain ions indicative of structural features. Consequently, it is not possible to know the position of double bonds in the aliphatic chain. Instead, the carboxyl group of the ester must be

derivatized with a reagent containing a nitrogen atom giving place to nitrogen-containing derivatives, such as pyrrolidides [3], picolinyl (3-hydroxymethylpyridinyl) ester and 4, 4-dimethyloxazoline (DMOX) derivatives [4]. Although such derivatizations are the optimal method to determine fatty acids esters structures, we did not proceed in such a way to structurally determine the *unknown* compounds. On one hand, the complexity of the mixture obtained at the outlet of the reactor made difficult the separation and derivatization, and on the other hand, the combination of $^1\text{H-NMR}$, UV and GC-MS resulted sufficient to determine the general structure of the compounds.

Concerning aromatic FAEE, their reiterative appearance as catalytic reaction products forced an exhaustive structural determination. The absence of the mass spectra of such compounds in the spectra-library let us know on the scarcity of available published studies.

A usually recorded spectrum of these compounds is shown in Fig. 3.2.1 (see Annex I). All spectra presented a molecular ion at $m/z = 304$. Ethyl linoleate has a molecular ion at $m/z = 308$, so the difference between them is a loss of 4 a.m.u. This difference may suggest a possible loss of 2 molecules of H_2 , confirming that a catalytic dehydrogenation reaction was taking place.

The following discussion may be applicable to spectra MS-01, MS-02, MS-05, MS-06, MS-07, MS-09 and MS-10, since they all show a similar profile. The spectra are certainly of ethyl esters in that there is a gap of 46 a.m.u. from the molecular ion at $m/z = 304$ to 258 as a result of the breaking corresponding to the loss of ethanol. The fragment at $m/z = 88$ is the ion resulting of the Mc Lafferty rearrangement. This reaction consists in the transfer of a γ -hydrogen to carbonyl groups [5]:



Ions at $m/z = 91$ (tropylium ion) and 105 are typical of highly unsaturated structures such as parinarate (conjugated tetra-unsaturated fatty acid and derivatives) or benzene rings. The ions at $m/z = 173$ and 145 (in Fig. 3.2.1, breakings I-3 and I-2, respectively) may be the key to the structure though there would have to be a loss of protons to give the correct numbers. Ions at $m/z = 131$ and 117 could be formed by sequential loss of methylene groups from these ions.

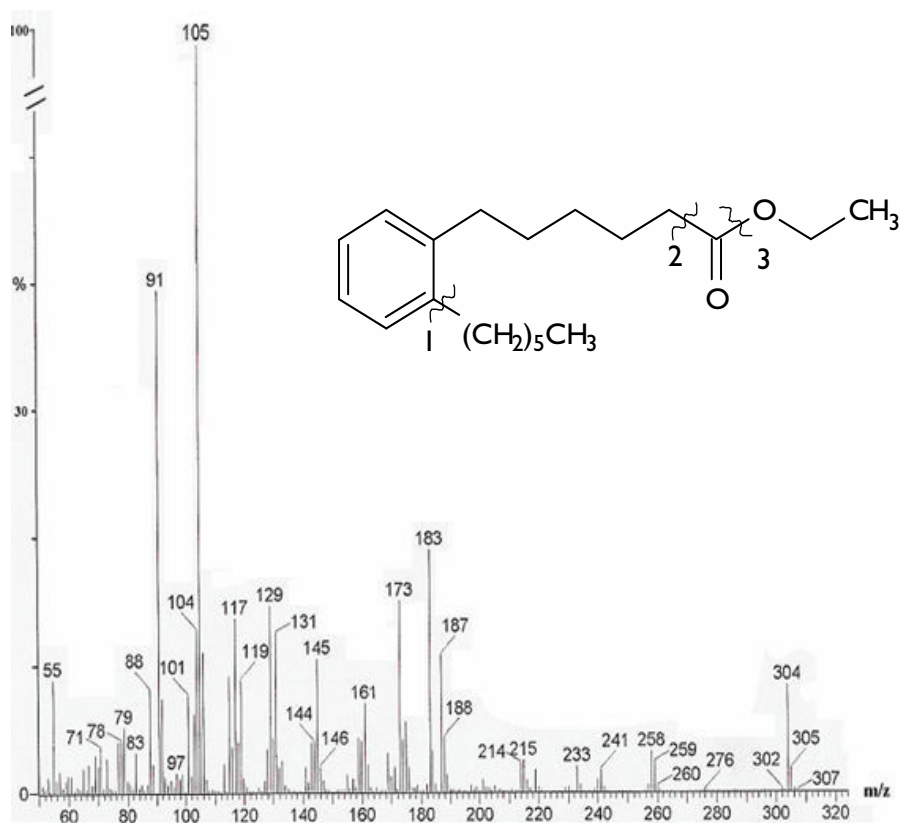


Figure 3.2.1 A typical mass spectrum of the *unknown* compounds.

Tetra-unsaturated fatty acid ester derivatives have been identified and structurally characterized [6-8]. On one hand, when the double bonds of these compounds are conjugated, their mass spectra have a 100 % intensity peak at 91 a.m.u. On the other hand, if they are methylene-interrupted, that is containing a methylene group between the double bonds, the spectra have the base peak (relative abundance of 100%) at 79 a.m.u. However, concerning fatty acids with four or more double bonds, even when non-conjugated, the tropylium ion ($m/z = 91$) becomes the most important component of the spectrum. In our study, all spectra, excepting the mass MS-03 and MS-08, present the most abundant ion at $m/z = 105$.

A great similitude is seen when comparing the spectra of our *unknown* compounds to the spectrum of diethylbenzene (Fig. 3.2.2) [9]. Diethylbenzene presents a base peak at $m/z = 105$. Other abundant ions are those of $m/z = 117, 133$

and 91. This fact suggests one more that the *unknown* compounds have presumably an aromatic structure.

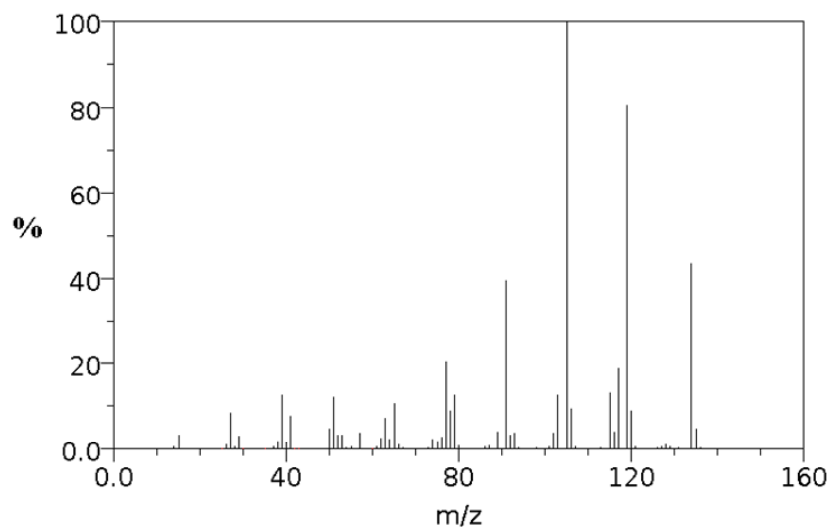
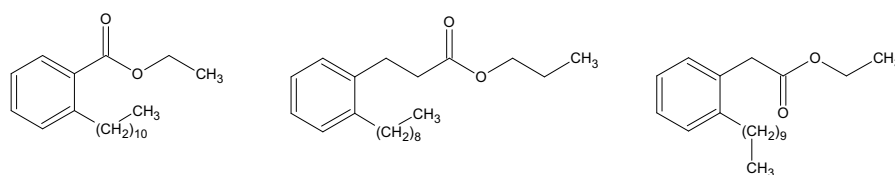


Figure 3.2.2 Mass spectrum of diethyl-benzene.

Concerning MS-08, the absence of ion at $m/z=88$ indicates that the ester group has not hydrogen in γ -position. Regarding this point, three isomers are possible:



Regarding MS-03, the only difference is the base peak, which has a m/z value of 119 a.m.u. In the case of MS-04, most of the peaks have the same values as in other spectra, but it differs from the rest in the relative abundance of the peaks, which is very high in most of the peaks.

Regarding UV spectroscopy results (Fig. 3.2.3), the *unknown* compounds exhibit absorption between 270 nm and 350 nm. Two clear overlapped bands are detected one at 295 nm and another one at 316 nm. The band with the highest absorbance value presents a small valley on the top suggesting the possibility of being the product of two overlapped bands. Then, three bands at 293, 299 and 316 nm are exhibited in Fig. 3.2.3.

Unexpectedly, our sample does not present any band in that region. The effect of the alkyl substitution intensifies and shifts the benzene spectrum to longer wavelengths [10]. An example of this effect is the spectrum of 1-ethyl-2-methylbenzene, which shows three bands at 256, 263 and 271 nm [9].

Surprisingly, the spectrum in Fig. 3.2.3 shows a high coincidence with those found in the bibliography [6] for conjugated 9, 11, 13, 15-octadecatetraenoic fatty acids esters (parinarate). Kuklev et al. [6] characterized the following isomers of parinaric acid methyl esters: β -parinaric acid methyl ester (methyl 9E, 11E, 13E, 15E-octadecatetraenoate), α -parinaric acid methyl ester (methyl 9Z, 11E, 13E, 15Z-octadecatetraenoate), methyl 9Z, 11E, 13E, 15E-octadecatetraenoate and methyl 9E, 11E, 13E, 15Z-octadecatetraenoate. They show three absorption bands between 286 and 319 nm.

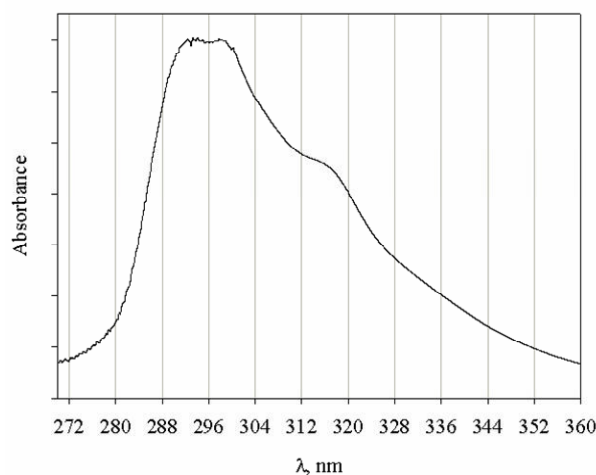


Figure 3.2.3 UV spectrum of the *unknown* compounds.

At this point of the structural determination, no clear results were still available. On one hand, GC-MS measurements suggested a benzene ring in the molecule rather than a linear structure. On the other hand, UV spectrum was similar to that exhibited by a highly linear unsaturated compound, such as parinarate.

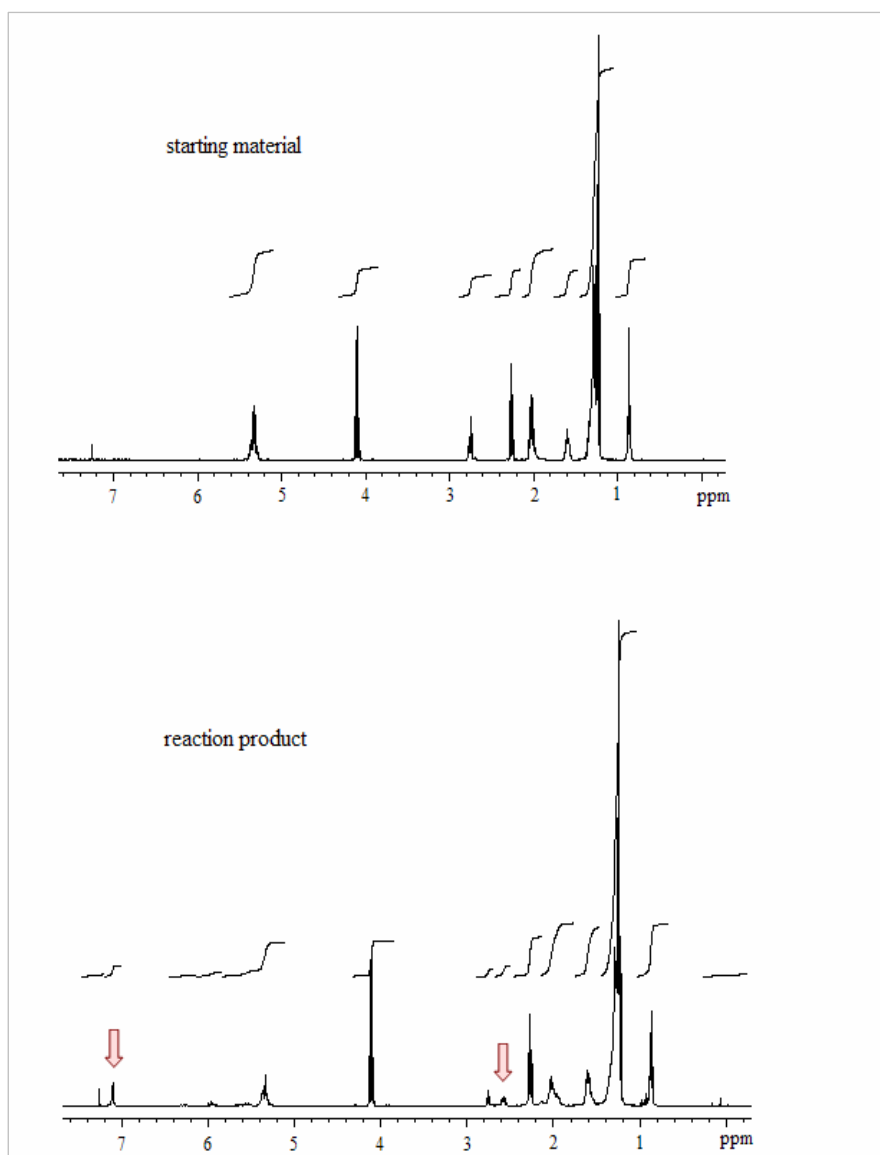


Figure 3.2.4 $^1\text{H-NMR}$ spectra of starting material and reaction eluted product. The red arrows indicate the position of the new multiplets.

Therefore, the results of $^1\text{H-NMR}$ were determinant to decide the structure of the *unknown* compounds. The measurements were performed with the sample eluted

from the reactor, so the spectrum seemed to be very complicated. A comparison between the spectra of starting material and that of the eluted mixture was done (Fig. 3.2.4).

In this case, the main products of the reaction were the *unknown* compounds, so no other products could give confusing data. The spectrum of the reaction product shows basically two new multiplets comparing it to the one corresponding to the starting material: one around 2.5 ppm and another one at 7.1 ppm.

The multiplet of benzenic protons is centred at 7.26 ppm, when no substituents are present. Nevertheless, alkyl chains in ortho position decrease the chemical shift of benzenic protons around 0.1 ppm. The signal at around 2.5 ppm may be originated by the methylenic/methyl groups in ortho position in the aromatic compounds, whose chemical shift has a value between 1.9 and 3.1 ppm [11]. The results obtained in these analyses were in good agreement with the hypothesis of the obtaining of aromatic compounds which was strongly suggested by the mass spectrometry results, too.

3.2.2 Compounds with $m/z = 302$

When GC-MS analyses of the samples were performed, some peaks at higher retention times different of aromatic FAEE ($m/z = 304$) were detected in most of the cases. Their analysis results revealed that their ion molecular was equal to 302 (see Annex II). Once more, a gap of 2 a.m.u. was suggestive of a possible dehydrogenation reaction, because it may be related to a loss of a hydrogen molecule in aromatic FAEE. Although it might have been speculative, the structural determination based on the analysis of GC-MS results of these compounds indicated that m/z 302 compounds possibly came from a dehydrogenation reaction. By observing a representative mass spectrum of one of these compounds (Fig. 3.2.5), one may detect a relevant similarity to those spectra of aromatic FAEE. The main peaks of most of aromatic FAEE showed values at m/z equal to 91, 105, 117, 129, 131, 145 and 161. Analogously, compounds with $m/z = 302$ have the most important peaks at the same values (91, 105, 117, 129, 131, 145, 159). The difference lied in the intensity of those peaks, since the most abundant peaks came out to be those corresponding to 117 and 131. The added unsaturation may presumably favour the conjugation of the molecule due to stability reasons. Furthermore, the likeness of these spectra with those corresponding to molecules with a conjugated system

including a benzene ring (Fig. 3.2.6) may suggest the possibility that the unsaturation is really situated in a conjugated position to the benzene [9].

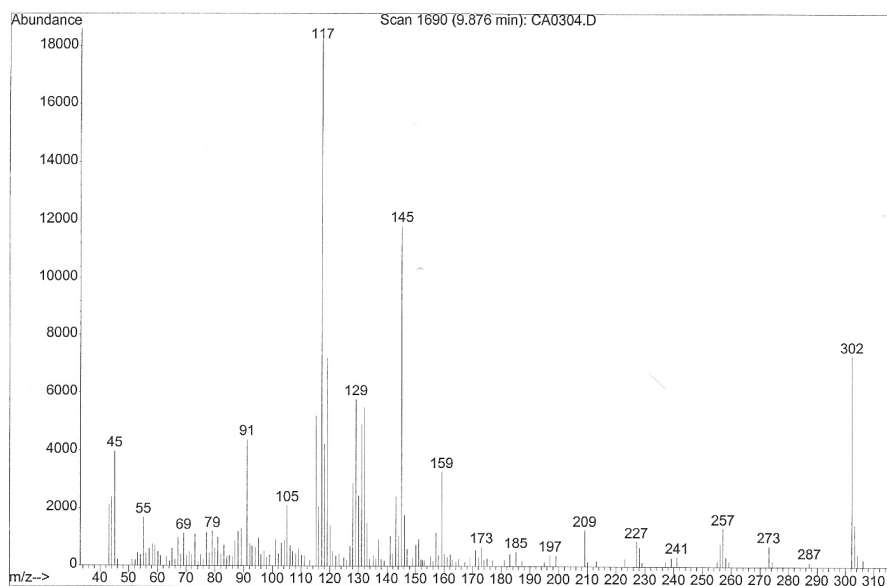


Figure 3.2.5 Representative mass spectrum of compounds with $m/z = 302$.

Due to its very low presence in the mixture of the reaction products, it was not possible to obtain more useful information concerning the structural analysis, when performing UV and $^1\text{H-NMR}$ measurements.

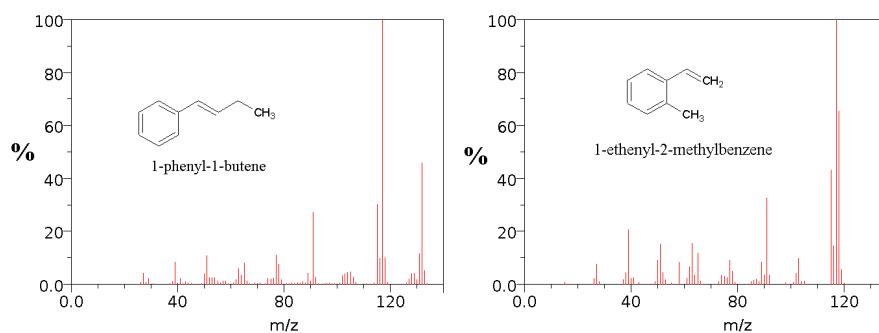
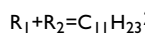
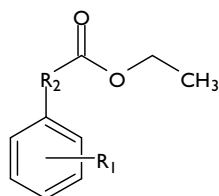


Figure 3.2.6 Mass spectra of molecules with an unsaturation conjugated to the benzenic ring.

3.2.3 Conclusions

The combining use of different techniques (GC-MS, UV, $^1\text{H-NMR}$) gave sufficient information to make a suitable structural analysis of some reaction products, which initially appeared to be unknown. The unknown reaction products have a structure such as the one shown below:



It is worth pointing out that the knowledge of the starting material structure and the study of the reaction and its mechanism complemented the information provided by the structural analysis methodology.

The reaction gave also place to compounds, whose m/z of GC-MS analyse was 302, suggesting the formation of a higher unsaturated structure.

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