A C T A A L I M E N T A R I A P O L O N I C A

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THE USE OF COMBINED GAS CHROMATOGRAPHY — MASS SPECTROMETRY FOR THE IDENTIFICATION OF ALIPHATIC CARBOXYLIC ACID IN SMOKE PRODUCED FROM TWO SPECIES OF WOOD

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Key words: carboxylic acids, smoke, mass-spectra identification.

The GLC-MS technique was used for the identification of aliphatic carboxylic acids in smokes obtained from alder wood (*Alnus incana* L.) and fir wood (*Abies alba*). The identification procedure was based on comparing the analysed mass spectra in a data processing system with spectra obtained for carboxylic acid standards. Where standards were not available general fragmentation rules for carboxylic acids were applied, and the results were checked by means of interpretation maps and comparison with registry of mass spectral data.

Smoke from alder wood appeared to have 11 aliphatic carboxylic acids, whereas smoke from fir wood contained only 8 such acids — those with chains longer than 5 carbon atoms were absent.

Several analytical methods were used by various authors for the identification of carboxylic acids in wood smoke. Initially it was a simple paper chromatography [7, 8] which soon was replaced by gas chromatography (GLC) [5].

More refined techniques made use of infrared spectra (IR) or nuclear magnetic resonance (NMR) after separating the analysed mixture by means of preparative scale GLC [3, 4, 9, 11].

The most modern and simultaneously most reliable method of identification carboxylic acids in wood smoke makes use of the combined gas chromatography — mass spectrometry technique (GLC-MS) [4, 9, 11].

This method was used in the experiments described in this paper, which aimed in the identification of carboxylic acids in smoke obtained from alder wood and from fir wood.

EXPERIMENTAL

1. MATERIALS

The wooden material used for developing smoke were two kinds of sawdust: Silver Fir (*Abies alba*) as a representative of coniferous tress, and Grey Alder (*Alnus incana L.*) — representing deciduous tress. The sawdust originated from a timber works, and is used as rawmaterial for producing smoke condensates used in the production of a smoke flavouring. The percentage share of the main components in these two species of wood is presented in Table 1.

Table 1. Proximate composition of analysed wood species (in percents)

Component / Species	Cellulose*) %	Lignin**) %	Hemicelluloses %
Grey Alder	48.6	26.0	18.8
Silver Fir	52.2	34.0	13.6

*) Kürschner method
**) Hägglund method

2. DEVELOPMENT AND ABSORPTION OF SMOKE

The smoke was developed in a single-step laboratory generator by combusting the sawdust under controlled conditions of grain size, moisture contents, air flow rate and temperature. The construction of this generator is published elsewhere [2].

Smoke was produced in three parallel repetitions for each of the two kinds of wood. Each time about 100 g air-dry sawdust was burned. The average size of wood particles was 0.48 mm (alder) and 0.58 mm (fir). The temperature in the burning zone was 1200°C on the average and the linear air flow rate — 1592 cm/min. The smoke was absorbed in 3 gas washing bottles fitted with sintered discs and linked in series, each of which was filled with 300 cm^3 of chloroform.

3. ISOLATION OF CARBOXYLIC ACIDS

Isolation of acids from the absorbed wood smoke was performed according to the method presented in an earlier paper [2].

4. SEPARATION AND IDENTIFICATION OF CARBOXYLIC ACIDS

Separation and identification of the acids was carried out by means of a gas chromatograph coupled with a mass spectrometer. The operational parameters were as follows:

1. Gas chromatograph Varian Model 2700 with an all-glass stream splitter. Splitting ratio 1:1.

— flame ionization detector (FID), termostated at 250°C,
— chromatographic column (glass), 2.75 m long, 4 mm I.D.,
— stationary phase: 10% SP-1000 on Chromosorb WAW 80/100 mesh,
— programmed column temperature: 140°C-250°C; temperature increment — 4°C/min.,
— carrier gas: helium; flow rate — 30 cm^3/min,
— auxiliary gases: hydrogen — 30 cm^3/min; air — 300 cm^3/min,
— attenuation: 256×10^{-11} A/fsd.

The chromatograph was coupled with the mass spectrometer by a glass pipe and a Ryhage all-glass separator kept at 250°C.

2. Mass spectrometer, Du Pont, Model 21-492 B, with a data processing system Model 21-094 B:
— ion source temperature: 250°C,
— ion energy: 70 eV,
— mass scan rate: 2 decades/sec., 10 sec/cycle,
— scanning range: 2 to 1000 amu,
— resolution: 800,
— acquisition range: 27 to 600 amu,
— acquisition rate: 4 kHz.

RESULTS AND DISCUSSION

The use of the stationary phase SP-1000, whose properties are similar to Carbowax 20-M and FFAP usually employed for separation of free fatty acids, but having a better thermostability, enabled the separation of the analysed acids at a higher column temperature with less background in the mass spectra (less bleeding). Besides on the SP-1000 a better separation of propionic and isobutyric acids was achieved. The obtained separation of peaks is illustrated by Fig. 1 and Fig. 2.

To identify the individual acids mass spectra of known carboxylic acids (from acetic to stearic) were taken making possible the determination of the regularities in the spectra of these acids. These spectra also were stored in a data processing system, where they served for the identification of their counterparts present in the spectra of the analysed smokes. It was observed that for aliphatic carboxylic acids the parent peak is usually less intensive (Fig. 3), with the exception of acetic and propionic acids having intensive parent peaks. The latter may be explained by the short chain in these two acides limiting the possibilities of fragmentation (Fig. 4 and Fig. 5). The long-chain acids have greater possibilities of fragmentation which is manifested by a bigger number of fragmentary peaks and the diminishing intensity of the parent peak. Beginning, however, with nonanoic acid up to stearic acid the intensities of the parent peak in comparison to the base peak tend to increase. This

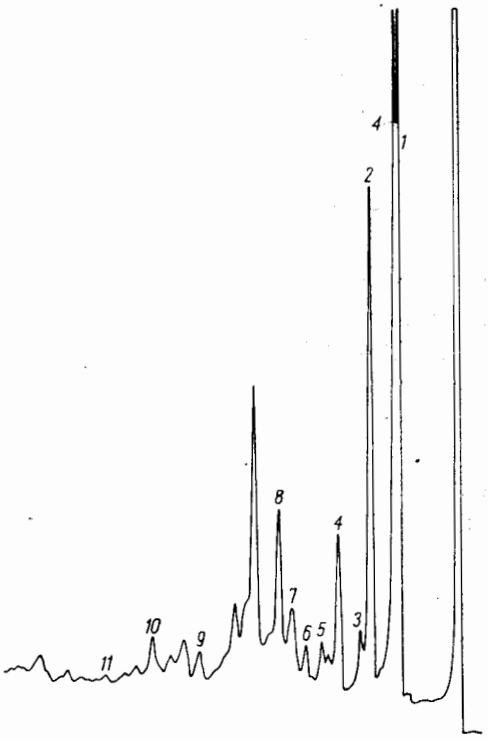

Fig. 1. Gas chromatogram of carboxylic acids in smoke from alder wood

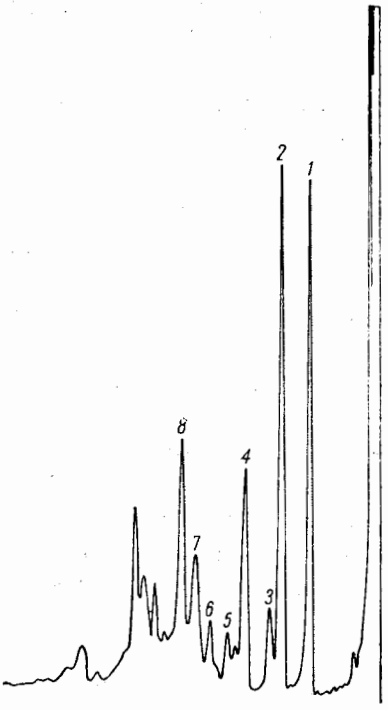

Fig. 2. Gas chromatogram of carboxylic acids in smoke from fir wood

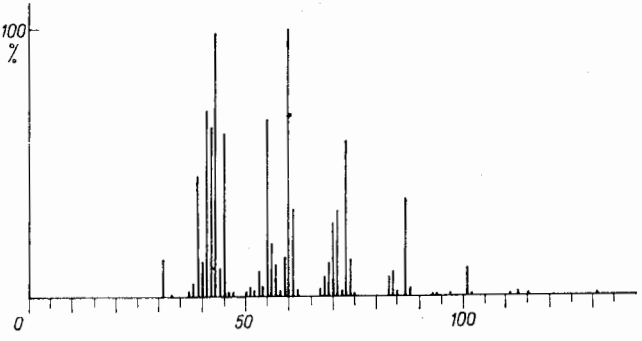

Fig. 3. Mass spectrum of heptanoic acid standard (mol. weight 130)

may be due to decreased possibilities of splitting the bond between the carboxylic group and the alpha-carbon and the formation o-OH and -COOH ions. This observation is only partially in agreement with the data given by Beynon [1], who suggests that the intensities of the parent peak begin to increase as early as with valeric acid.

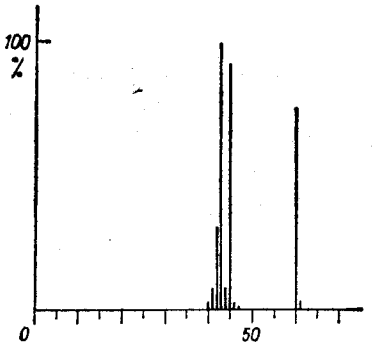

Fig. 4. Mass spectrum of acetic acid standard (mol. weight 60)

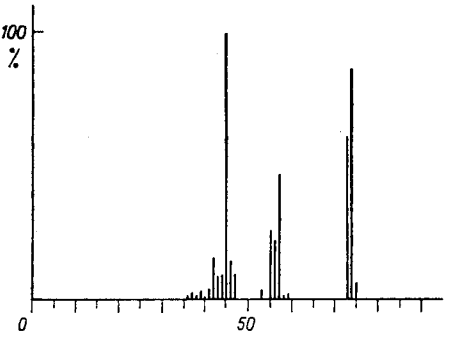

Fig. 5. Mass spectrum of propionic acid standard (mol. weight 74)

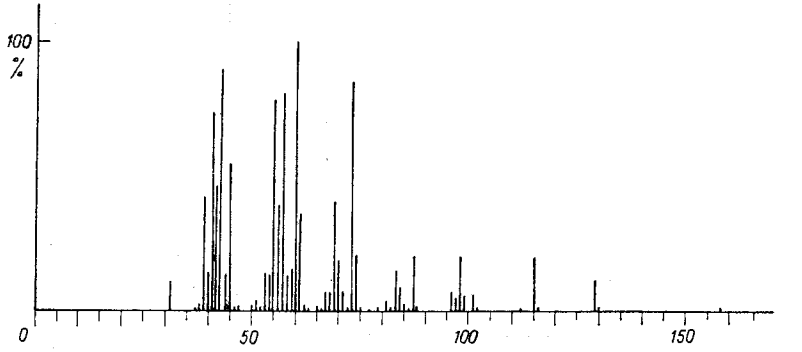

Fig. 6. Mass spectrum of nonanoic acid standard (mol. weight 158)

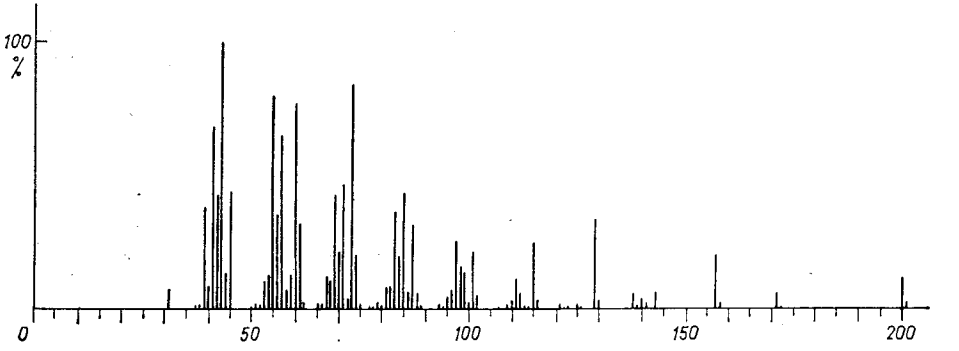

Fig. 7. Mass spectrum of lauric acid standard (mol. weight 200)

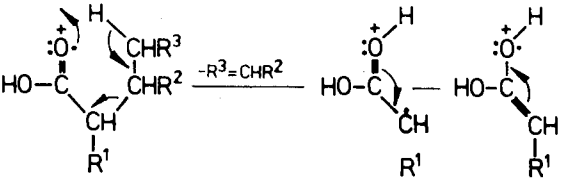

Fig. 8 Formation mechanism of the rearranged ion, after Silverstein

The most typical peak in the mass spectra of saturated aliphatic carboxylic acids is that one at m/e 60. In the spectrum of acetic acid it corresponds to the parent ion (Fig. 4), and in the other spectra (Fig. 6 and Fig. 7) except for that of propionic acid, it corresponds to a rearranged ion whose constritution and origin, as suggested by Silverstein [13] is shown in Fig. 8. Propionic acid does not form a rearranged ion because of its short chain.

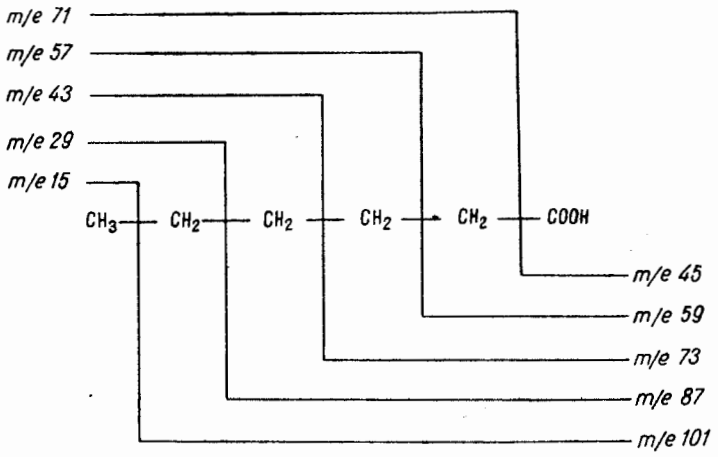

Fig. 9. Fragmentation mechanism of caproic acid

Another typical feature in the mass spectra of acids with longer chains is the presence of two series of ions resulting from splitting of their C-C bonds according to the mechanism shown in Fig. 9 for the example of caproic acid. Examples of such spectra are given in Fig. Fig. 3, 6 and 7. These peaks do not occur singularly but rather in bunches with the period of 14 mass units. This is due to elimination of hydrogen from the fragmentary ions. The presence of these two series in a mass spectrum considerably facilitates the identification of aliphatic fatty acids.

The above detailed analysis of the mass spectra of standard fatty acids was the starting point for the identification of their isomeric forms and unsaturated acids whose standards were not at hand, and the data processing system could not be applied successfully. As the presence of side chains or double bonds may modify to some extent the general course of fragmentation, the analysis of the mass spectra of isomers and unsaturated acids was performed taking also in consideration Hammings's interpretation maps and a registry of mass spectral data [6, 14]. Crotonic (Fig. 10), isobutyric (Fig. 11) and isovaleric acids were indentified in this way.

The crotonic acid spectrum has a very intensive parent peak, which is

at the same time its base peak. This is a result of the presence of a double bond, which stabilises the parent ion. The next characteristic feature is the intensive peak at m/e 41. Its presence suggests that fragmentation of crotonic acid proceeds by dissociating the bond between the carboxyl group and the alpha-carbon atom with a simultaneous rearrangement of the oxygen-free ion, which results in the formation of a resonance stabilized allyl cation. This remains in agreement with the views that in the presence of a double bond the allyl splitting is more privileged [1, 10, 13].

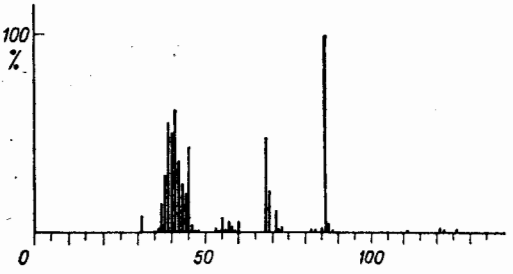

Fig. 10. Mass spectrum of crotonic acid in smoke from alder wood (mol. weight 86)

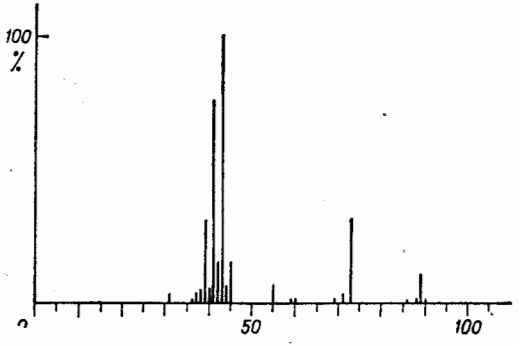

Fig. 11. Mass spectrum of iso butyric acid in smoke from alder wood (mol. weight 88)

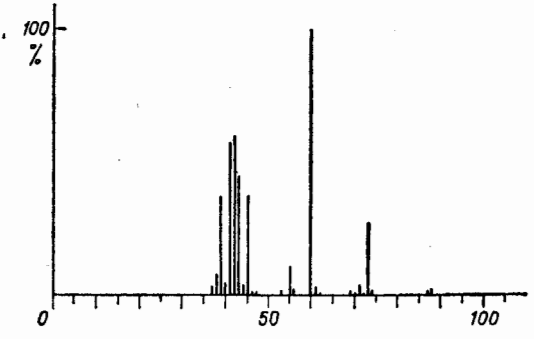

Fig. 12. Mass spectrum of butyric acid in smoke from alder wood (mol. weight 88)

From the comparison of branched isomers with their nonbranched counterparts, e.g. butyric acid (Fig. 12) and isobutyric acid (Fig. 11) follows that isomer spectra include more intensive peaks corresponding to the ions formed by dissociation of bonds at carbon atoms at which branching occurs (peaks at m/ 41 and 73). Easier fragmentation at these sites is ascribed to a greater stability of the tertiary carbonium ion in comparison with the secondary one, which in turn is greater than that of the primary ion [10, 13]. This phenomen enables location of the branching sites.

The identified components of the acid fractions of alder and fir smokes are presented in Tables 2 and 3. The acid fraction of the alder smoke (hard wood) includes altogether 11 acids, among which heptanoic and nonanoic acids were identified for the first time. Hitherto the literature on the subject did not report the presence of these acids in wood smoke.

The acid fraction in smoke produced from silver fir (soft wood) includes fewer acid components. There are only 8 carboxylic acids altogether.

Table 2. Carboxylic acids identified in smoke from alder wood

Peak No	Compound	Molecular weight	M/e values in decreasing order
1.	Acetic acid	60	43, 45, 60, 42
2.	Propionic acid	74	45, 74, 73, 57, 55, 56
3.	Isobutyric acid	88	43, 41, 73, 39, 45
4.	Butyric acid	88	60, 42, 41, 43, 45, 39
5.	Isovaleric acid	102	60, 41, 43, 45, 74, 87
6.	Isocrotonic acid	86	39, 41, 40, 86, 68
7.	Valeric acid	102	60, 73, 43, 45, 41, 55
8.	Crotonic acid	86	86, 41, 39, 68, 45, 69
9.	Heptanoic acid	130	60, 43, 41, 74, 55, 45
10.	Caprylic acid	144	60, 43, 73, 55, 41, 42, 45
11.	Nonanoic acid	158	60, 43, 73, 57, 55, 41, 45

Table 3. Carboxylic acids identified in smoke from fir wood

Peak No	Compound	Molecular weight	M/e values in decreasing order
1.	Acetic acid	60	43, 45, 60, 42
2.	Propionic acid	74	45, 74, 73, 57, 55, 56
3.	Isobutyric acid	88	43, 41, 73, 39, 45
4.	Butyric acid	88	60, 42, 41, 43, 45, 39
5.	Isovaleric acid	102	60, 41, 43, 45, 74, 87
6.	Isocrotonic acid	86	39, 41, 40, 86, 68
7.	Valeric acid	102	60, 73, 43, 45, 41, 55
8.	Crotonic acid	86	86, 41, 39, 68, 45, 69

Acids with chains longer than five carbons are not present. These differences in the composition of the acid fractions no doubt must be related to the differences in the chemical structure of alder wood and fir wood. It is known that in the pyrolysis of wood the thermal destruction of carbohydrates, i. e. cellulose and hemicelluloses is the main source of carboxylic acids in the destruction products. From the comparison of data collected in Table 1 follows that there is less cellulose in alder wood (48.6%) than in fir wood (52.2%), however with regard to the contents od hemicelluloses the opposite is true — its contents in alder is bigger (18.8%) than in fir (13.6%). This leads to the conclusion that the richer composition of the acid fraction in smoke from alder wood may be linked in some way with the bigger amount of hemicelluloses in this wood species. However, it is deemed that the main reason for the above described differences in the composition of the acid fraction are the differences in the chemical structure of hemicelluloses between the analysed wood species. As follows from literature reports [12] alder wood contains 1.75 times more pentosans and 7.72 times less hexosans than fir wood.

Hence, it may be concluded that the richer composition of the acid fraction in smoke from alder wood is in some relation with the pentosan fraction of hemicelluloses. This finding corroborates the earlier suggestion made by Wasserman and coworkers [15] that bigger amounts of carboxylic acids in smoke from hard wood probably are linked with the bigger amount of the pentosans in these species.

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IDENTYFIKACJA ALIFATYCZNYCH KWASÓW KARBOKSYLOWYCH WYSTĘPUJĄCYCH W DYMIE WĘDZARNICZYM

Instytut Przemysłu Mięsnego i Tłuszczowego

Streszczenie

Identyfikację alifatycznych kwasów karboksylowych w dymie wędzarniczym pochodzącym z drewna olchowego (*Alnus incana* L.) oraz z drewna jodłowego (*Abies alba*) przeprowadzono przy zastosowaniu sprzężonej techniki GLC-MS. Podstawę identyfikacji stanowiło porównywanie badanych widm masowych z widmami otrzymanymi dla substancji wzorcowych kwasów karboksylowych przy wykorzystaniu systemu przetwarzania danych.

W tych przypadkach, w których nie dysponowano odpowiednimi substancjami wzorcowymi identyfikację przeprowadzono na podstawie ogólnych zasad fragmentacji kwasów karboksylowych, posługując się również mapami interpretacyjnymi Hamminga oraz katalogiem widm masowych.

Stwierdzono, że dym olchowy zawierał 11 alifatycznych kwasów karboksylowych, podczas gdy w dymie jodłowym występowało ich tylko 8. Brak było kwasów o łańcuchach dłuższych niż 5-węglowe.