Mass spectrometric determination of methyl group positions in long chain methyl ethers and alcohols *via* nitriles

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Mass spectra of long chain branched nitriles are used to determine the position of the methyl groups along the chain in naturally occurring methyl ethers, alcohols and fatty acids.

Recently we showed that lipids occur on the silk of spiders.[1,2] Extracts of this silk contain long chain, methyl branched 1-methoxy alkanes as their major component[2] which are not known from any other natural source. The silk of the golden orb weaver *Nephila clavipes* (Araneidae) is currently being intensively studied because of its high tensile strength and elasticity.[1,3] Investigations of web extracts by GC–MS showed the presence of alkanes, alcohols and 1-methoxy alkanes with multiple methyl branches. The 1-methoxy alkanes exhibit a more complex branching pattern than those previously known from the Linyphiidae.[2] For the determination of the position of the methyl groups in the chain we developed a new method applicable to microgram amounts of complex mixtures.

Methyl branches in long chain alkanes can be determined by their mass spectra and gas chromatographic retention indices.[4] This becomes more difficult with alcohols, aldehydes and 1-methoxy alkanes because they easily loose water or methanol under the mass spectrometric conditions. The localization of the methyl groups is thus only possible in special cases.[5] Methyl ester branches between C-2 and C-4 can be easily determined because of the high ion-density caused by carbonyl induced fragmentations, but methyl groups at other positions are much more difficult to identify due to low ion intensities.[6,7]

Derivatives of such compounds can be used in some cases to determine the branching positions. Fatty acids can be transformed into alkanes *via* reduction, chloride formation and reductive dehalogenation.[7] Free acids can also be converted *via* their acid chlorides into 3-pyridylmethyl esters or pyrrolidides, the mass spectra of which clearly show the branching positions.[8] Long chain branched alcohols have been converted into alkyl chlorides, deuterated alkanes and nicotinic acid derivatives.[8,9] All methods have drawbacks when used on the microgram scale, *i.e.* the low intensity of diagnostic ions and by-products introduced into the sample during derivatization or interference with other compounds present in the extract.

Investigations of branched alkyl nitriles showed that their mass spectra exhibit intense molecular ions and an ion series C_nH_{2n-2} dominating in the region above 100 amu. Branching positions can be easily determined by the ions **A** and **A** + 1 which arise by cleavage besides the methyl group (see Fig. 1). In the higher mass region the ions **B** and **B** + 1 also become prominent. The respective ions **A** − 14 are of low abundance. Nitrogen containing ions dominate the spectrum above 100 amu. The cyano group obviously stabilizes the cations obtained by cleavage of σ-bonds, so that the information on the branching positions is preserved in the spectra (see Fig. 2). Below 100 amu this fragmentation pattern is overlayed with ions arising from other processes,[10] thus making the detection of the methyl groups at C-2, C-3 and C-4 difficult. While a methyl group at C-6 is clearly visible [see Fig. 2(*a*)], a methyl group near the cyano group can only be detected by the ion *m/z* 111, which intensity becomes comparable to the one of the ion *m/z* 97 [see Fig. 2(*b*)]. On the other hand, methyl ethers can be converted in methyl esters on a microscale using RuO_4,[2] thus enabling the location of the methyl groups between C-2 and C-4.

Methyl ethers or alcohols can be cleanly transformed in high yield into nitriles by a microprocedure.† They are first converted into the respective iodides using Me_3SiI,[2] followed by reaction with Et_4NCN in dichloromethane. After completion, the solvent is substituted with pentane, leaving a clean derivatized extract, almost free of reagents and impurities. Extracts of silk from *Nephila clavipes* containing complex mixtures of alkanes, alcohols and methyl ethers in microgram amounts were successfully characterized by this method.[11] As an example, 1-methoxy-16,20,24,28-tetramethylhentriacontane

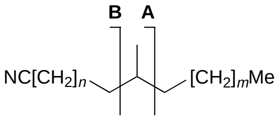

Fig. 1 Mass spectrometric fragmentation pattern of long chain branched 1-methoxy alkanes

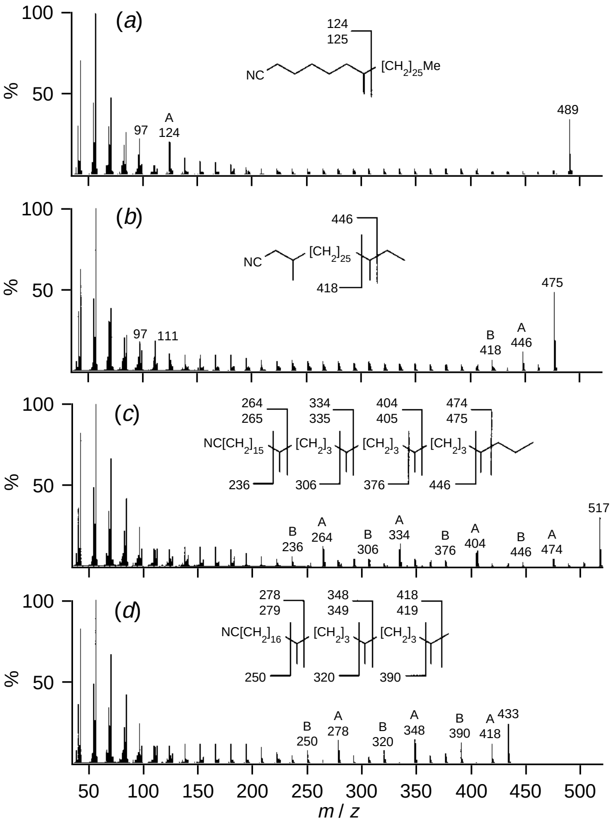

Fig. 2 Mass spectra (70 eV, EI) of (*a*) 6-methyldotriacontyl cyanide (synthetic); (*b*) 2,28-dimethyltriacontyl cyanide (from *N. clavipes*); (*c*) 16,20,24,28-tetramethylhentriacontyl cyanide (from *N. clavipes*); (*d*) 17,21,25-trimethylhexacosyl cyanide (synthetic)

and 1-methoxy-2,28-dimethyltriacontane could be identified (the mass spectra of their nitriles are shown in Fig. 2). Advantages of the analysis of the methyl branching positions *via* nitriles are the very characteristic mass spectra carrying the information of the functional group position, mild reaction conditions and clean products. In addition, all compounds easily convertible by microprocedures into alcohols, like fatty acids or their esters, can also be analysed.[12]

Footnotes

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† *Experimental procedure for the microderivatization of methyl ethers or alcohols.* The reactions were performed in 1 ml mini-vials (Chromatography Research Supplies). To 50 µl of a CH_2Cl_2 solution of the educts 20 µl Me_3SiI was added under nitrogen. After 2 h at 60 °C in a dark place (oven) and cooling, 50 µl sat. aq. $NaHCO_3$ and 200 µl CH_2Cl_2 were added and the mixture thoroughly shaken. The aqueous phase was removed and NaCl added for drying. The organic phase was transferred into another vial and the NaCl residue was again washed with 200 µl CH_2Cl_2. After addition of Et_4NCN (5 mg, washed with pentane prior to use) to the combined organic phases the mixture was stirred overnight, 50 µl H_2O was added and the aqueous phase removed after vigorous stirring. The solvent was evaporated at 45 °C bath temperature, the residue twice taken up in 100 µl pentane, and the combined pentane extracts concentrated to a volume suitable for analysis.

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