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Short Communications

Mass Spectrometry of Terpenes: Comments and Correction

ERIK von SYDOW

Swedish Institute for Food Preservation Research (SIK), Göteborg, Sweden

Mass spectra of monoterpene hydrocarbons, alcohols, aldehydes and ketones have been published [1-3] prior to similar data [4,5] on hydrocarbons and alcohols. Different techniques were used in the two cases.[6,7] Our main purpose was to introduce a technique enabling rapid and simultaneous identification by mass spectrometry of microquantities of fractions eluted from a gas chromatographic column fed with complex mixtures. The Swiss work (statement in Ref.[5]) obviously aims at clarifying the fragmentation patterns in detail by using deuterium labeling. These results will be very important in the advancement of knowledge in this field. It is also likely that by using deuterium labeling less speculative suggestions regarding the formulas of different fragments formed can be achieved, than has been given by Thomas and Willhalm [4] in their work on monoterpene hydrocarbons.

It seems that the experimental conditions used in the mass spectrometers in both investigations have their advantages and disadvantages. Owing to the direct coupling to a gas chromatograph we have had only limited opportunities to vary some of these conditions. The apparatus has also been continuously improved during the course of our investigation. It is obvious that differences in the mass spectra obtained in the two investigations are due to instrumental differences other than the use of different energies of the bombarding electrons. This is evident from comparison of the low m/e-regions of the spectra of linalool in Fig. 4 in Ref.[2] and Fig 1 in Ref.[5] Willhalm *et al.*[5] claim that this difference is due solely to the presence of linalool oxide. In an attempt to prove this it seems rather unsuitable to use a spectrum of the latter compound recorded under different experimental conditions, *i.e.* at a much higher energy of the bombarding electrons. The author feels that the differences between the spectra are largely due to instrumental differences and possibly to a minor extent to the presence of an impurity, possibly linalool oxide, as indicated by $m/e = 155$. It is well known in several cases that by using higher pressures in the source $m/e = M + 1$ is formed, although this is not suggested in this case.

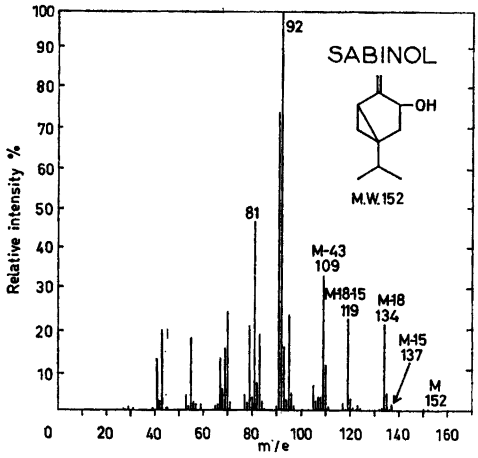

Fig. 1. Mass spectrum of sabinol recorded in a combined gas chromatograph-mass spectrometer.
Gas transfer system: 100°C. Column temperature: 110°C. Column material: 10 % Dow Corning Silicone Oil 200 on Embacel Kieselguhr (60–100 mesh, acid washed). See also Ref.[1]

Thermal decomposition of monoterpene hydrocarbons has been discussed exten-

sively by Ryhage and von Sydow.[1] This phenomenon was overlooked by Thomas and Willhalm [4] in their study of γ-terpinene, where the high intensity of $m/e = 119$ indicates the occurrence of a thermal breakdown in the mass spectrometer.

Similar decompositions of monoterpene alcohols were discussed by von Sydow,[2] who is very well aware of the instability of sabinol, although this was inadvertently overlooked when Ref.[2] was prepared. Fig. 14 in that paper shows the mass spectrum of sabinol when the combining system was kept at 200°C. It contains a large amount of thujone, as pointed out by Willhalm *et al.*[5] When the combining system was kept at 100°C the spectrum shown in Fig. 1 of this paper was obtained (March 25, 1963). This should substitute Fig. 14 in Ref.[2] and agrees nicely with the one published by Willhalm *et al.*[5] In the case of thujone there is also good agreement.[3,5]

As to fenchol,[2] it seems likely that the specimen contained 10 % isofenchol.[5] A better gas chromatographic separation has recently been obtained.

The data presented in Refs.[1-3], although in need of future improvements — like all experimental data — have been applied successfully in two investigations on black currant material.[8,9]

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2. von Sydow, E. *Acta Chem Scand.* **17** (1963) 2504.
3. von Sydow, E. *Acta Chem. Scand.* **18** (1964) 1099.
4. Thomas, A. F. and Willhalm, B. *Helv. Chim. Acta* **47** (1964) 475.
5. Willhalm, B., Thomas, A. F. and Stoll, M. *Acta Chem. Scand.* **18** (1964) 1573.
6. Ryhage, R. *Anal. Chem.* **36** (1964) 759.
7. Willhalm, B., Thomas, A. F., Stoll, M. and Hawkins, E. G. E. *Helv. Chim. Acta* **46** (1963) 2098.
8. Andersson, J., Bosvik, R. and von Sydow, E. *J. Sci. Food. Agr.* **14** (1963) 834.
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Proteolytic Activity in Extracts from Human Thyroid Tissue

GUNNAR LUNDBLAD and JANIS IMMERS

National Bacteriological Laboratory, and Wenner-Gren Institute for Experimental Biology, University of Stocholm, Stockholm, Sweden

The thyroid gland is an aggregate of follicle cells, acini, which are filled with a gelatinous substance called colloid. It is known that the thyroid hormones thyroxine and triiodothyronine are stored within the colloid as thyroglobulin. Thyroglobulin, however, has a molecular weight of about 650 000 to 670 000 and cannot pass the follicle membranes. Gersh and Caspersson [1] postulated that in the normal animal the production of colloid is a continuous process and they suggested that proteases in the gland bring about hydrolysis of thyroglobulin into polypeptides and peptones which can pass the cell membranes. In extending these observations De Robertis [2] was able to show that thyroid follicle extract possessed proteolytic activity and Dziemian [3] stated that the activity of the protease depended upon the state of the thyroid. The hydrolysis of thyroglobulin and the proteases and peptidases in thyroid extracts, in most cases of animal origin, have been studied by several authors.[4-12] The proteolytic activity is considered to be of catheptic nature but has not yet been identified. Attempts to purify the proteases in thyroid extracts from different animals have been carried out by some of the above authors, but only in extracts of sheep thyroids have two proteases with pH optima at 3.8 and 5.7 been isolated.[13] In human thyroid only one protease has been found. In the present communication the proteolytic activity of thyroid extracts on gelatin and haemoglobin, and the nature of a thyroid cathepsin are examined and the separation of two of these enzymes by means of gel filtration is reported.

Material and methods. Thyroid tissue from non-toxic goitre patients was frozen at −20°C, freed from fat and connective tissue, cut in pieces and extracted (toluene added) for 20 h at + 4°C in a volume of 3.0 ml per g tissue of a mixture of 4 parts 0.9 % NaCl and 1 part 0.067 M Na-phosphate buffer, pH 7.4.