GAS-CHROMATOGRAPHIC CONTRIBUTION TO THE CHEMISTRY OF BEET SAPONINS, I

By

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In the past decade the problem of "floc" which had arisen in connection with the widespread production of aerated refreshments aroused the interest of workers dealing with the production of beet sugar, so they began to investigate the problem in more detail. It was soon proved that the deposit formed during the storing of aerated refreshments came from the sugar, and consisted mostly of saponin [1], (Fig. 1).

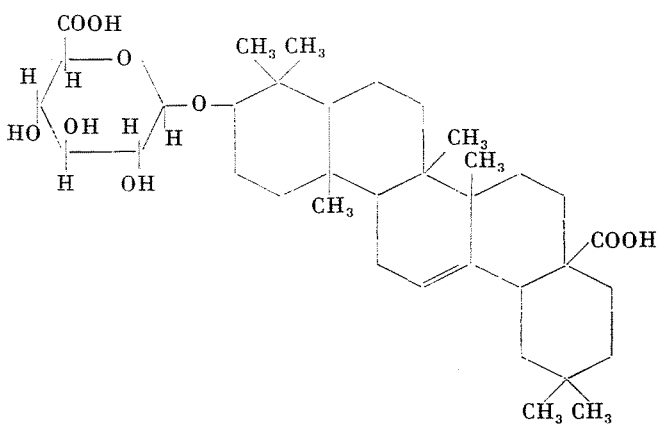

Fig. 1. The stucture formula of beet saponin according to HARDEGGER et al. [Helv. Chim. Acta **35**, 824 (1952)]

Most plants contain various saponins, though their physiological role is rather unknown.

During the production of beet sugar about 39% of the saponin content of sugar-beet go into the raw liquid [2] and have a remarkable effect on the subsequent manufacturing process. Saponin causes strong bubbling, and being a surface-active substance it affects the removal of other colloidal non-sugar compounds during the raw liquid purification. 96—97% of the saponin content of the raw liquid can be removed by caustic sludge [3]. Most of the sapo-

nins left back in the liquid goes through the technological process and gets into the molasses. As a surface-active material, it delays the crystallization of sugar and contaminates the product because of adsorption to the surface of sugar crystals.

According to HARDEGGER et al. [41] beet saponins are the D-glucuronides of oleanic acid. This statement was affirmed by EIS et al. [1].

As shown by paper- and thin-layer chromatographic tests [5, 6], beet saponins are no homogeneous materials. According to our present knowledge they cannot be crystallized.

According to recent investigations of Japanese workers [7] the glucon component of beet saponins may be not only glucuronic acid but also glucose and arabinose. It is probable that also other saccharides may be present in saponins as glucon components.

To our present knowledge, upon heating, beet saponins can be precipitated in acid alcoholic medium to their glucon and aglucon components, to field beet sapogenins, glucuronic acid, glucose and arabinose. Beet sapogenin six times recrystallized and purified could be obtained in chromatographically pure form (giving a single spot).

This pure beet sapogenin was used as standard in the determination of beet saponins. Beet sapogenins selected for this purpose were tested by thermal and gas-chromatographic methods leading to unexpected results.

Gas-chromatographic methods

There are some papers [8, 9] in which attempts are described to use gas-chromatography to solve problems in connection with sapogenins. Since these papers appeared, gas chromatography has much developed and the application of pyrolysis gas chromatography and programmed gas chromatography to analyse beet sapogenins seemed to be promising. The selection of the adequate conditions of gas-chromatographic measurements is highly facilitated if the thermal properties of the material are known. The thermoanalytical curves of crystallized beet sapogenin were prepared by a derivatograph [10], in argon atmosphere. The derivatograms are given in Fig. 2.

The substance is of constant weight up to 240 °C, and decomposition starts only above this temperature. Other data read off the thermal curves are of no importance for gas-chromatographic measurements, so they are not treated here.

Programmed gas chromatography has been preferred to pyrolysis gas chromatography the latter giving only indirect data. Programmed gas chromatography gives reproducible results, it is highly sensitive, and the produced data can be readily used for qualitative and quantitative evaluation.

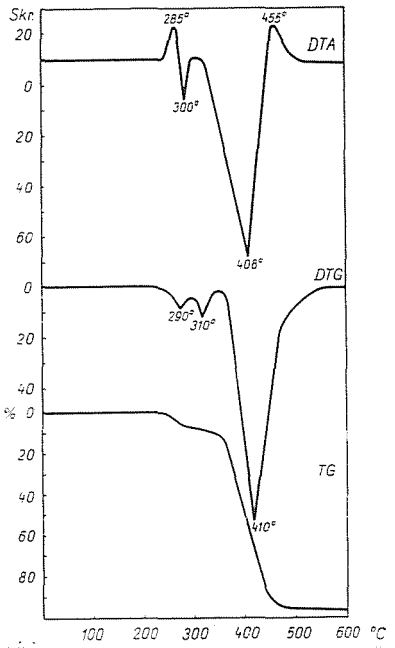

Fig. 2

Testing beet sapogenin by programmed temperature gas chromatography

The optimal conditions for the programmed temperature gas-chromatographic method were chosen on the basis of the derivatogram of beet sapogenin. An adequate solvent had to be selected first to meet the following requirements:

1. It had to be of gas-chromatographic purity.
2. Its peak had to be at the beginning of the chromatogram.
3. It had to be stable at the temperature of the vaporizer and column.
4. It had to be a fairly good solvent for beet sapogenin.

Dioxane was found to be best from among several solvents studied. A combined temperature program was used in the analyses, the essence of which was as follows: The sample was introduced into the column kept at constant temperature of 64 $\pm$ 0.1 °C through a vaporizer of a temperature of 295 $\pm$ 1.0 °C. A 44 °C/min temperature program was started in the 30th minute after the sample introduction, and the program was automatically switched off at 178 °C and the analysis continued at 176 °C, under isothermal conditions, in a spiral column 2 m in length and 4 mm inner diameter, filled with Chromosorb W 60/80 carrier wetted with 20% Carbowax 1550. Since the temperature was relatively low during the measurements, no decomposition occurred and only the solvent and beet sapogenin and their contaminants

were indicated in the chromatograms, as shown in Fig. 3. Fig. 4 shows the chromatogram taken of the solvent itself under the same experimental conditions.

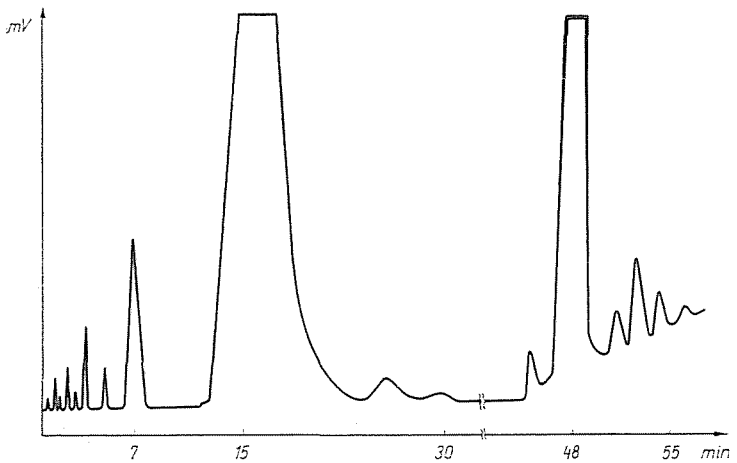

Fig. 3

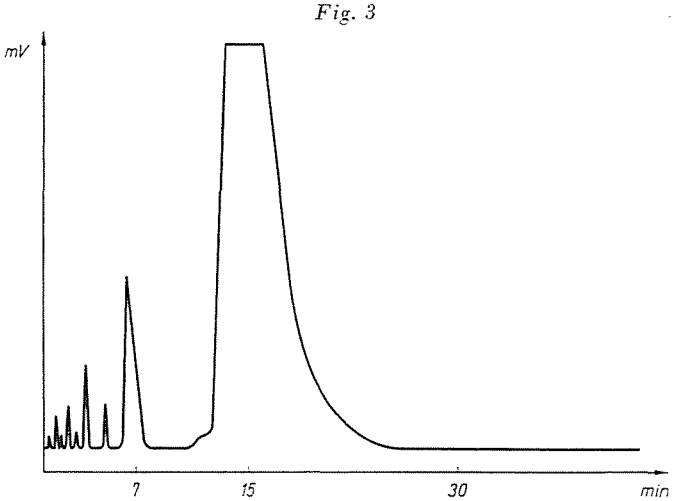

Fig. 4

The second series of experiments covered the thermal decomposition of beet sapogenin tested by gas chromatography. In these experiments new parameters and another column had to be used.

A column of 2 m length and 4 mm inner diameter filled with Chromosorb W, wetted with 10% SE-30 silicon polymer was used. The sample was introduced into the column maintained at 154 $\pm$ 0.1 °C, through a vaporizer of a temperature of 450 $\pm$ 1.0 °C. An 8 °C/min temperature program was started in the 12th minute after the sample introduction, and switched off automatically

at 320 °C. The analysis was then continued at 318 °C, under isothermal condif tions. Since the evaporation of the sample was effected at its temperature of decomposition, breaking of bonds of the molecule was expected and also the appearance of the peaks of new components on the chromatogram. These really appear, as indicated in Fig. 5.

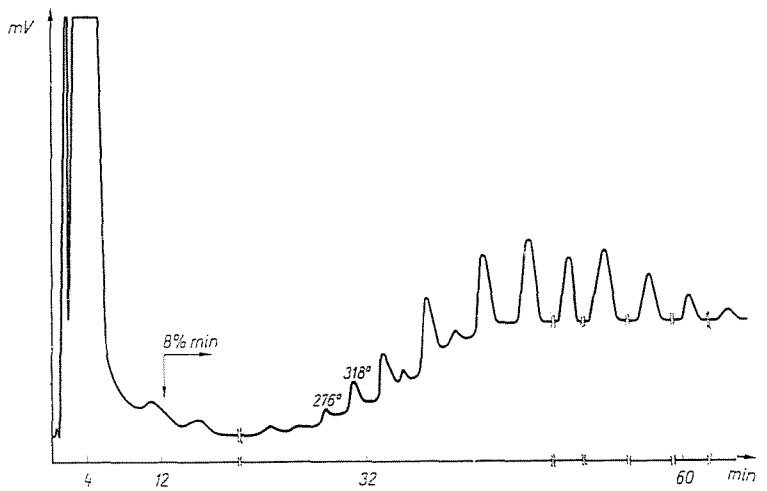

Fig. 5

Unexpected results were obtained during the evaluation of the chromatograms. The logarithms of retention times corresponding to the peaks of the decomposition products were found to be the linear function of the number of carbon atoms (Fig. 6) making probable a homologous series. However, at present we do not have the pure decomposition products which would be necessary for proving this interesting finding. We wish to study the problem further and give the results later.

The experiments were carried out in a gas chromatograph Carlo Erba Fractovap Model D with flame-ionization detection. Nitrogen was used as carrier gas, its pressure at the inlet being 1.90 kp/cm^2 for the column with Carbowax 1550 and 0.75 kp/cm^2 for the column with WSE-30-10.

The pressure of auxiliary gases at the inlet was the same in each case:

0.25 kp/cm^2 for hydrogen

0.75 kp/cm^2 for oxygen.

A Hamilton syringe was used for sample introduction. The volume of sample was 1—1 in each case, at a sensitivity of 16×10.

A Speedomax G recorder with 2.5 mV final amplitude, 1 sec time constant, and 12.7 mm/min chart speed has been applied.

Although we wish to continue the analysis of beet saponins, it has to be stated that programmed temperature gas chromatography could give new

data to the chemistry of beet saponins, and is a suitable means of studying these compounds. The rather interesting finding of the gas-chromatographic analysis that part of the decomposition products are members of a homologous series, is considered by us as a supposition until it is proved by means of standard substances.

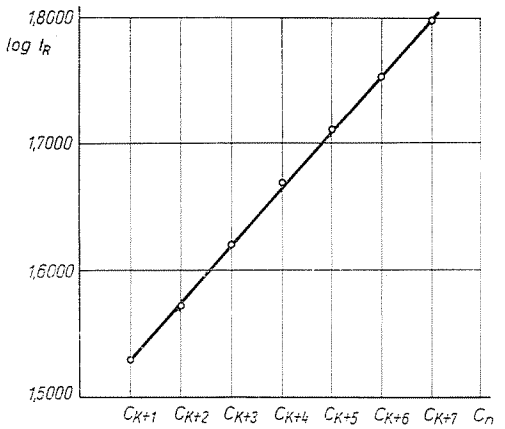

Fig. 6

We wish to express our thanks to Professor L. ERDEY for his valuable help and advice and of senior lecturer S. GÁL for his assistance in thermoanalytical measurements.

Summary

In the past decade the problem of "floc" arisen along with the widespread production of aerated refreshments raised the interest of workers dealing with the production of beet sugar. It was soon proved that the deposit formed during the storage of aerated refreshments came from the sugar, and consisted mostly of saponin. Our purpose was to analyse beet sapogenins by gas-chromatographic methods. The outcome of the gas-chromatographic analysis of the decomposition products, that part of them are members of a homologous series, we now consider as a supposition until it is proved by means of standard substances.

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