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THERMAL DECOMPOSITION OF FUROSTANOL GLYCOSIDE - TOMATOSIDE

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The thermal degradation of furostanol glycoside, tomatoside has been investigated by thermal analysis (TA), thin-layer chromatography (TLC) and IR-spectroscopy. The thermal curves of tomatoside were obtained under various conditions. The structures of the main intermediates were elucidated. It has been established that on heating above 150° furostanol glycoside is converted into the spirostanol one. Simultaneously the stepped splitting of the carbohydrate fraction at C-3 of the aglycone takes place. The steroid part of the glycoside is destructed at a temperature above 270°. The process is identical both in air and nitrogen atmospheres.

(Furostanol glycoside, tomatoside: 5 α-furostane-3 β, 22, 26-triol-3-[O - β- D- glucopyranosyl (1 → 2) - β - D - glucopyranosyl (1 → 4) - β - D - galactopyranoside] 26 - O - β - D - glucopyranoside, has been studied by thermal analysis (TA).

By comparing the TA curves with the results of the intermediates examined by infrared (IR) and mass spectrometry, thin-layer (TLC), paper (PC) and gas-liquid (GLC) chromatography, it has been shown that in the course of the heating of a furostanol glycoside sample, the water of crystallization is released first, then a number of furo- and spirostanol progenins are formed as a result of the degradation of the carbohydrate fraction at C-3 and C-26 of the aglycone, resulting, at a further rise of temperature, spirostanol sapogenin, the decomposition of which is the last stage of steroid glycoside degradation.

Some 80 steroid glycosides of the furostane series [1] of vegetable origin with a wide range of biological activity [2] are described in the literature.

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As the glycosides extracted from natural sources lack sufficiently broad structural diversity, attempts have been made to chemically and microbiologically modify their structures, which would allow to find new types of activity and practically valuable qualities, as well as to determine the "structure-activity" relationship.

There appears to be no information in the literature on the modification of glycoside molecules by physical factors. In this connection, we have attempted to study how thermal analysis can be used for the investigation of furostanol glycosides and for determining the temperatures where their molecular structures change. With this in view, we investigated tomatoside (Formula 1, Fig. 7), a furostanol glycoside, contained in tomato seeds [3] (molecular mass 1082 daltons).

Experimental

The thermal curves of tomatoside have been obtained using a Derivatograph (Hungarian Optical Works MOM, Budapest) and air and nitrogen atmosphere, in the temperature range 20-500°. The sample size was 100 mg, TG sensitivity 100 mg, DTA sensitivity 1/5, DTG sensitivity 1/10; coverless crucibles of platinum and a multiplate-sampleholder (six plates, 9 mm in diameter) were used. Calcined aluminium oxide was used as reference material. Thermal analysis of sapogenin (neotigogenin) was carried out under the same conditions in air atmosphere.

IR-spectra of tomatoside were taken using a Specord-75-IR spectrophotometer (GDR) within the range of 1300-800 cm^{-1}. Readings were taken from a thin triturated sample paste in petrolatum.

TLC was carried out on 13x18 cm plates, silicagel L 5/40 being a carrier (produced by Chemapol, Czechoslovakia), eluents: chloroform - methanol - water (13:6:1 volume ratio) (1) for glycosides and chloroform - methanol (19:1) (2) for sapogenins.

Tomatoside was isolated from tomato seeds by methanol extraction. The extracted substances were separated by chromatography on a silicagel column, and the tomatoside fraction was recrystallized by methods described earlier [3].

The thermal decomposition products of tomatoside, neotigogenin, monoside, bioside and neotigogenin trioside were obtained by column chromatography from thermally treated tomatoside, with silicagel as a carrier L 40/160 (Chemapol, Czechoslovakia). Elution was performed with system 2 first, up to neotigogenin isolation, and then with system 1. Fractions

were collected, the composition of which was determined by TLC. Chromatographically individual thermolysis products were reprecipitated from ethanol.

Glycoside and sapogenin spot detection in TLC was carried out by spraying the plates with concentrated sulphuric acid, followed by heating for about 3 min at 100-105°, and then with Ehrlich's reagent (E-reag.) specific for furostanol glycosides [4], and with Sanie's reagent [5] for spirostanol ones.

Acid hydrolysis of the thermal splitting products of tomatoside was conducted in 5% sulphuric acid, in sealed glass vials at a temperature of 105-110° for 4 hours.

Descending PC of the acid hydrolysis products was carried out on FN-3 chromatographic paper (GDR) with the following solvent system: n-butanol-benzene-pyridine-H_2O (5:1:3:3, upper layer). Before application on the paper, the hydrolyzate was neutralized on Dowex-8 resin and evaporated to dryness. The residue was dissolved in 0.1-0.2 ml of aqueous methanol and applied onto the paper next to the known monosaccharide samples (glucose, galactose, xylose, rhamnose). The carbohydrate arrangement in PC was detected by spraying the paper with aniline phthalate (2% solution of the phthalic acid in water-saturated butanol x 1% aniline) and heating at 105° for 3-4 minutes.

Before subjecting the monosaccharide mixture to GLC after hydrolysis, monosaccharides were converted into aldonitrile derivative sugar acetates [6]. Chromatography of the derivatives obtained was carried out with a Chrom-5 device fitted with a flame-ionization detector, a glass column 2 m in length and 0.35 cm in diameter, filled with 5% XE - 60 on N-AW-HMOG chromatone (0.125-0.25 mm); carrier gas helium. V_{He}-50 ml/min, column temperature range 180-225°, programmed heating rate 3 deg/min.

Results and discussion

The thermal curves of the investigated tomatoside sample (Fig. 1) made in air atmosphere show the sample to contain 10% crystallization water, corresponding to seven H_2O molecules per one tomatoside molecule, being released with an endoeffect in the temperature range 35-120°. The thin layer chromatogram of the tomatoside sample, heated up to 120°, was identical with that of the native sample. An exoeffect is observed in the temperature range 165-215°, with maximum at 175° (3.5% loss). Weak endo- and exoeffect overlapping with a one-stage mass-loss, equalling 65%, occurs from

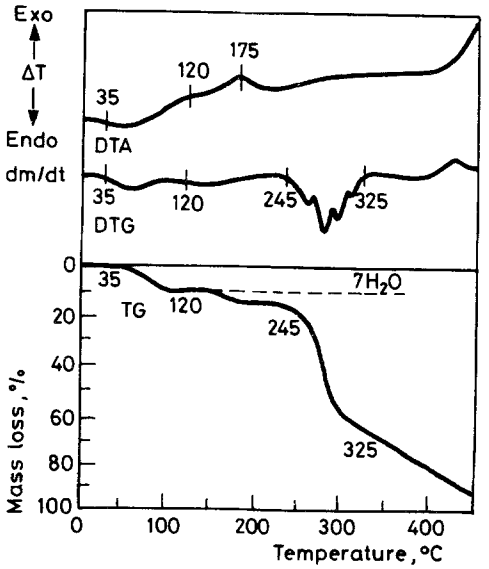

Fig. 1 Thermal curves of tomatoside in air atmosphere

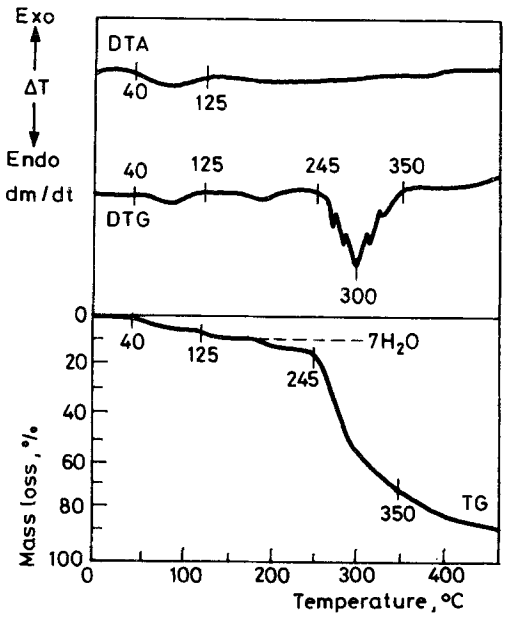

Fig. 2 Thermal curves of tomatoside in nitrogen atmosphere

245° to 325°. The presence of a few effects is also evidenced by the DTG peak bifurcation, corresponding to this process.

Investigation of the same substance in nitrogen atmosphere (Fig. 2) shows water release within the temperature range of 40-125° with 10% loss. Some 3.5% of the substance is lost without any visible effects on the DTA curve within the temperature range of 140-185°. Furthermore, within the temperature range of 245-350°, the mass loss constitutes about 55%, without clearly expressed thermal effects on the DTA curve.

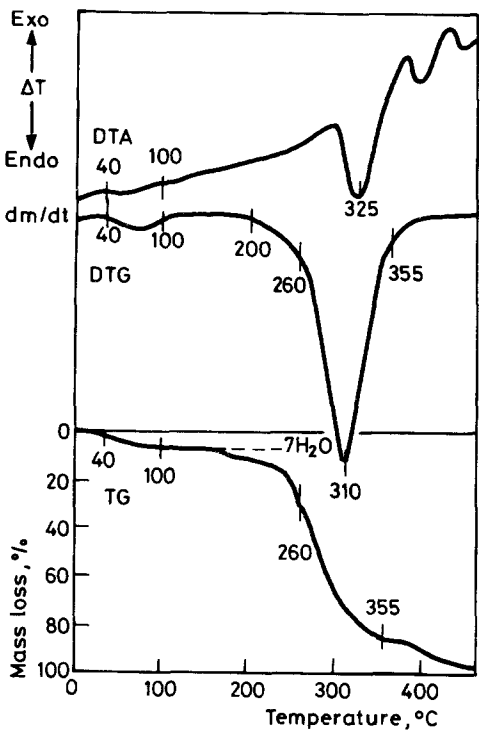

Fig. 3 Thermal curves of tomatoside in air medium on a 6-plate sample holder

For further study of effects of the experimental conditions on TG, DTG and DTA curves, we used a platelike sampleholder in air atmosphere instead of conic ones. In this case, the general shape of the TG curve was found to change insignificantly (Fig. 3). However, the exothermic peak on the DTA curve disappears at 175° (Fig. 1), and a bend appears instead. A clear endothermic peak could also be seen on the DTA curve with minimum at 325°, which could not be manifested in the case of conic crucible. Since the use of the plate sampleholder makes it possible to increase maximally

the surface of the sample and minimize layer thickness, the gas pressure of the reaction product differs from the usual oven gas pressure, it may be concluded from the comparison of thermal curves (Figs 1, 2 and 3), that the surface to thickness ratio of the tomatoside sample layer substantially affects DTG and DTA curve shapes.

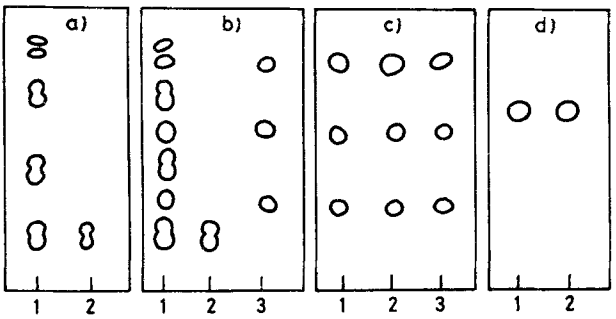

Fig. 4 TLC of the tomatoside thermolysis products: a, b and c - in a glycoside solvent system, d - in a sapogenin one. Spot development: a - by Ehrlich's reagent, b - by concentrated H_2SO_4, c - with Sanie's reagent.
1a, 1b, 1c - the tomatoside heated up to 165°; 2a, 2b - negative tomatoside; 2c, 2d, 3b - products of the tomatoside, heated up to 250°; 3c - partial acid tomatoside hydrolysis products (neotigogenin monoside, bioside and trioside); 1d - neotigogenin

TLC investigations (Fig. 4) give a similar chromatograms of the thermolysis products obtained in air and nitrogen atmosphere, and show the appearance of at least two (Fig. 7: a, d) new substances, less polar than the original one, both at 165° and at 230°, giving a positive reaction with E-reag. (red colour), specific for furostanol glycosides, as well as of three substances, also less polar than the original one, giving a positive reaction in TLC with Sanie's reagent (orange colour), specific for spirostanol compounds (Figs 7: b, c, e).

TLC development with a universal developer, concentrated sulphuric acid, allows finding six compounds as basic thermal degradation products of tomatoside. The differences between the chromatograms of the samples, heated up to 165° and 250°, concern only quantitative relation between substances in a mixture. The portion of compounds which do not give positive reaction with E-reag., is increased at higher temperature. In a sample obtained at 250°, there are almost no compounds left to react positively with E-reag. It indicates the beginning of carbohydrate chain decomposition at a temperature of about 165°, both at C_3 and at C_{26}, with the formation of

spirostanol and furostanol glycosides, containing various monosaccharide quantities at C_3.

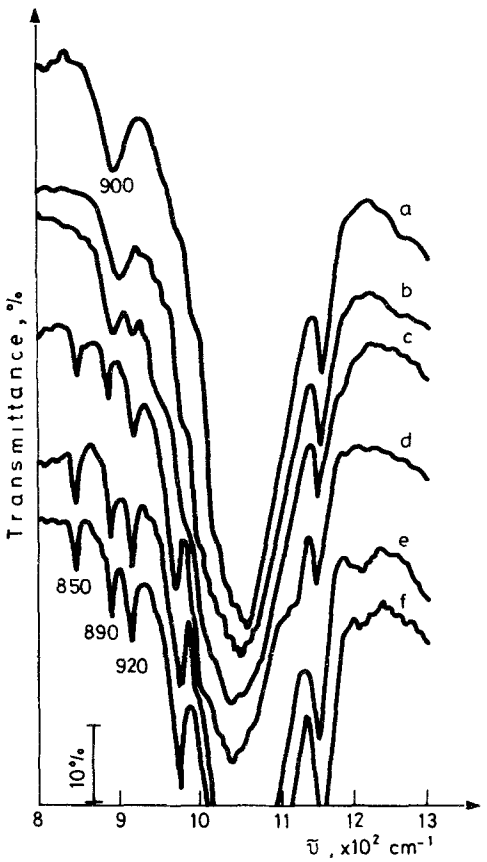

Fig. 5 IR spectra of tomatoside. Tomatoside: a) native, b) 120°-heated, c) 165°-heated, d) 250°-heated. Tomatonine: e) native, f) heated up to 200°

It is also supported by IR-spectra (Fig. 5), showing within the temperature range up to 165° the maintenance of the absorption region at 900 cm^{-1}, featuring furostanols. Other absorption bands have not been observed within the range of 1000-800 cm^{-1}. However, on heating tomatoside over 165°, absorption regions characteristic of the spiroketal flanking group of steroidal aglycones appear in the IR-spectrum at 850, 890 and 920 cm^{-1}, supporting TLC data indicating that the furostanol genin is converted into the spirostanol one at temperatures above 165°. The great absorption intensity

shows, that, in this case, a 25S-series sapogenin is formed, which is likely to be neotigogenin. It must be noted that neotigogenin will also be formed during the acidic splitting of glucose residues from C-26 tomatoside aglycone position.

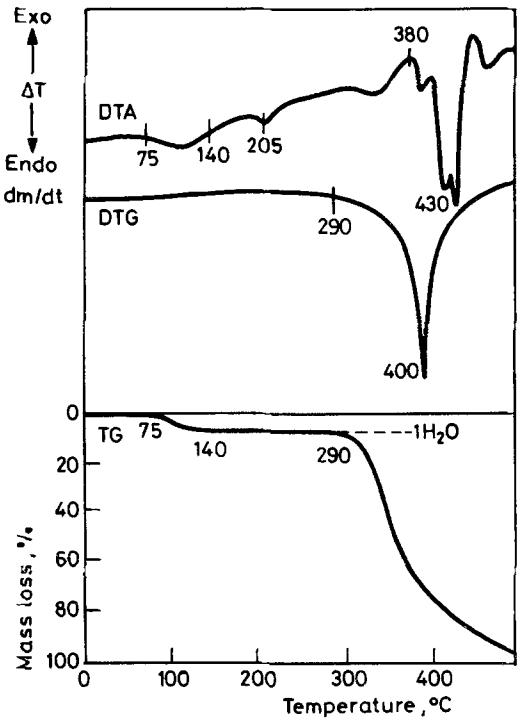

Fig. 6 Thermal curves of neotigogenin in air atmosphere

To confirm TLC evidence of the formation of new furostanol and spirostanol glycoside with a decreased carbohydrate chain at aglycone C_3, a series of transitional decomposition products were isolated. It was sapogenin, which turned out to be neotigogenin by IR- and mass spectra, as well as by physico-chemical characteristics, bioside, monoside and neotigogenin trioside, which in TLC coincide in the R_f value (Fig. 4) with the corresponding products, obtained by a partial acid hydrolysis of tomatoside [3]. Identity with the latter ones was proved after a full acid hydrolysis of each one. All of them released neotigogenin during acid hydrolysis. Galactose (in the case of the monoside), galactose and glucose (in the case of bioside and trioside) have been detected by PC as monosac-

charide residues in the presence of authentic samples. GLC of acetates of the aldonitrile derivative monosaccharides, obtained after the hydrolysis of the product coincide in retention time (R_t) with the same derivatives of galactose and glucose. Peak area ratio of the substances chromatographed turned out to be 1.00:1.07 in the case of bioside and 1:2.20 in the case of trioside, which indicates their monosaccharide composition (Fig. 7, formulae b, c). These records support the formation of products in the process of furostanol glycoside heating, which are released during its partial and full acid hydrolysis.

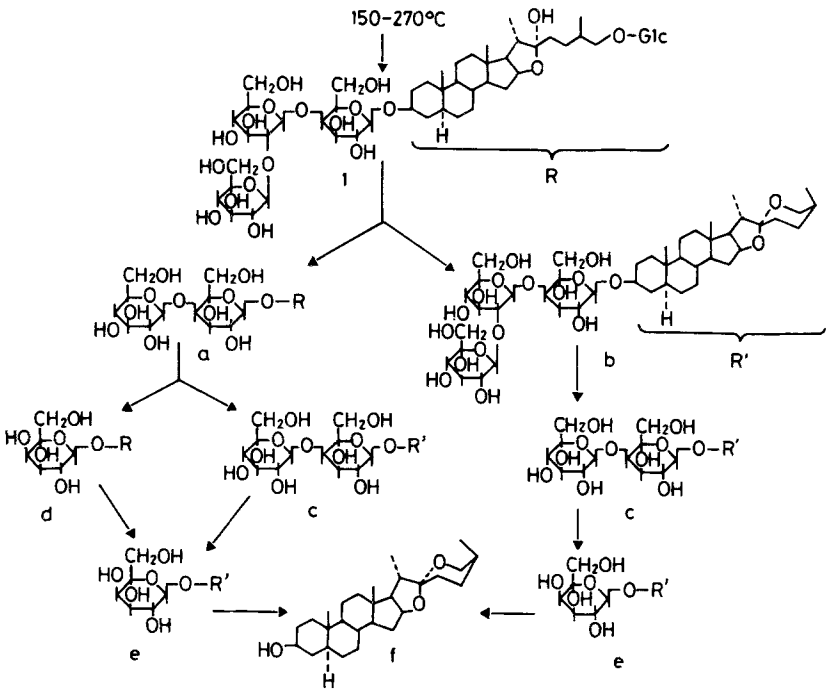

Fig. 7. The ways of the thermal decomposition of tomatoside and the main intermediate products

The appearance of two new substances, positively reacting in TLC with E-reag., shows that in contrast to the acidic decomposition, during thermal decomposition furostanol compounds with shortened carbohydrate chain at C_3 are formed. After chromatographic isolation the acid hydrolysis of each of them yielded the same products (in the order of polarity increment) as

neotigogenin monoside and bioside. This suggests that the main furostanol glycoside decomposition stages are as shown in Fig. 7.

It is clear from TLC data that the sapogenin formed is more stable thermally than the carbohydrate component and aglycone of the furostanol glycoside. TA of the neotigogenin obtained supports it. It is concluded from the thermal curves of the neotigogenin sample studied (Fig. 6) that the sample contains about 4% of water of crystallization which corresponds to one mole of water per one mole of sapogenin. This water is lost in the temperature range of 75-140° with an endoeffect on the DTA curve, which indicates its crystallization character.

At the temperature of 205°, a sharp endothermic peak is observed, with no accompanying mass loss on the TG curves. It may be concluded that it is connected with a phase transition (solid - liquid), the sample melting, as the temperature coincides with the melting point determined by other methods.

An analysis of the TG, DTG and DTA curves shows that the initial temperature of sapogenin decomposition is about 275°, and the degradation process consists of a few overlapping endo- and exoeffects. In the TLC of the sample heated to 250°, traces of other, less polar, compounds occur, while the sample heated up to 250° maintains its nativity. It means that the beginning of sapogenin decomposition coincides with the beginning of the mass loss on the TG-curve, unlike the case of the native glycoside, tomatoside, where the beginning of the carbohydrate portion splitting does not take place with marked mass losses, and hence, there is no possibility to record the beginning of a sample decomposition by means of the TG curve.

It is confirmed by the fact, that a glycoside, heated up to 265°, releases neotigogenin, which accumulates when a sample is maintained under these conditions.

Conclusions

For the first time, thermal curves of steroidal furostanol glycoside and spirostanol sapogenin have been obtained. It is shown that by means of TA it is possible to determine the quantity of water contained in a glycoside and sapogenin sample, the melting point and the initial temperature of a sapogenin sample decomposition.

According to the TG curves, the mass loss of these steroids begins at the temperature over 200° in the case of the glycoside and above 270° in the case of sapogenin, but the glycoside degradation begins earlier with

breakage of links between monosaccharide residues of a carbohydrate component of glycoside.

A study of the intermediates showed that in the end the thermal degradation of furostanol glycoside consists in the shortening of the carbohydrate portion at C_3, splitting of glucose from C-26 position with F ring closure and in a conversion of the furostanol aglycone into the spirostanol one - the process being identical with the gradual acid hydrolysis. In contrast to it, new furostanol glycosides are also formed in the course of the thermal decomposition.

Spirostanol sapogenin release is the last degradation stage with maintaining the C-27 steroidal character of a sample. At temperatures higher than 270° sapogenin degrades.

By regulating the time and temperature, one can obtain from furostanol glycosides a series of new furostanol and spirostanol glycosides and sapogenin.

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Zusammenfassung — Mittels Thermoanalyse (TA), Dünnschichtchromatographie (TLC) und IR-Spektroskopie (IR) wurde die thermische Zersetzung von Furostanolglycosid (Tomatosid) untersucht. Die thermoanalytischen Kurven von Tomatosid wurden unter verschiedenen Bedingungen erstellt und die Struktur der wichtigsten Zwischenprodukte ermittelt. Es wurde festgestellt, daß Furostanolglycosid beim Erhitzen über 150 in das Spirostanolglycosid übergeht. Gleichzeitig erfolgt ein schrittweises Aufspalten der Kohlenwasserstofffraktion bei Aglycon C-3. Der Steroidteil des Glycosides wird bei Temperaturen oberhalb 270 abgebaut. Die beschriebenen Vorgänge verlaufen in Luft bzw. in Stickstoff gleichermaßen.