Thermal Degradation of Glycosides, I

Degradation of Typical Triterpenoid and Steroid Glycosides

Ryuichi Higuchi, Yoichi Kitamura, and Tetsuya Komori*

Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812 Japan

Received August 8, 1985

The thermal degradation of triterpenoid and steroid glycosides is described. By mere heating on a melting point apparatus, typical triterpenoid and steroid glycosides (involving cardiac and basic steroid glycosides) afford their aglycones and prosapogenins with the cleavage of their sugar-aglycone and sugar-sugar linkages similar to acid and enzymatic hydrolysis. Some genuine aglycones which were labile towards acids were also obtained by this procedure. The pyrolyzed products were isolated by chromatography, and the structures were elucidated by spectroscopic evidences.

Thermische Zersetzung von Glycosiden, I. — Zersetzung typischer Triterpenoid- und Steroidglycoside


In the reports 1) on structure elucidations of triterpenoid and steroid glycosides, their melting points were described, and it has not been examined whether the glycosides were only melted or melted with a chemical reaction at their given melting points.

A study aiming at the solution of the chemical behavior of such glycosides at the melting point has been conducted in this laboratory, and we wish to report the results in this paper.

Hederagenin 3-O-glycoside, a typical triterpenoid glycoside, was examined first. The melting point of hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside 2) (1) [256—259 °C] was measured, and the fused residue (pyrolysate) was examined by TLC to show two spots corresponding with the prosapogenin [hederagenin 3-O-α-L-arabinopyranoside 3) (2)] and the aglycone [hederagenin 3)] (3)] of 1 together with the spot of 1. When 1 was heated to 280 °C in
the same manner, the yields of the products were increased as was expected. The pyrolysate at 280°C was subjected to column chromatography to give an aglycone and a prosapogenin, and they were identified with 3 and 2, respectively, by comparison of m. p., [α]D, IR, and 13C NMR spectra.

Scheme 1

Diosgenin 3-O-glycoside, a typical steroid glycoside, was investigated next. The melting point (272–278°C) of diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-β-D-glucopyranoside (4) was taken, and the pyrolysate showed in TLC three spots together with that of 4. Two of the products were identical on TLC with diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (5) and diosgenin 3-O-β-D-glucopyranoside (6), respectively. Heating of 4 to 290°C afforded one additional spot on TLC corresponding with that of diosgenin (7). The three pyrolysis products 5, 6, and 7 were identified by m. p., [α]D, IR, and 13C NMR.

Scheme 2

Digitoxin [digitoxigenin 3-O-β-D-digitoxosyl-(1→4)-β-D-digitoxosyl-(1→4)-β-D-digitoxoside (8)] was examined as an example for cardiac glycosides. The pyrolysate of 8 at melting point (250–255°C) revealed on TLC three spots except
R. Higuchi, Y. Kitamura, and T. Komori

for that of 8. One of the three spots shows the same $R_F$ value as digitoxigenin\(^6\) (9), and another two spots must be of prosapogenins. Further heating of 8 to 275°C caused increase of thermolysis products, and they were separated by column chromatography to afford an aglycone and two kinds of prosapogenins, 10 and 11. The aglycone was identical (m. p., $[\alpha]_D$, IR, \(^{13}\)C NMR) with 9. The compounds 10 and 11 were determined as digitoxigenin 3-O-$\beta$-D-digitoxoside and 3-O-$\beta$-D-digoxosyl-(1→4)-$\beta$-D-digitoxoside, respectively, by \(^{13}\)C NMR and FABMS data.

![Scheme 3](image)

As an example for basic steroid glycosides, a 3-O-glycoside of solanidine was investigated. TLC of the pyrolysate at the melting point (236–241°C) of solanidine 3-O-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\alpha$-L-rhamnopyranosyl-(1→4)]-$\beta$-D-glucopyranoside\(^7\) (12) showed two spots which are thought to be prosagonenins together with that of the original compound 12. When 12 was heated to 300°C, another two spots appeared on TLC. The pyrolysate at 300°C was separated by column chromatography to give an aglycone (13) and three prosapogenins, 14, 15, and 16. The aglycone 13 was identified by direct comparison (m. p., $[\alpha]_D$, IR, \(^{13}\)C NMR) as solanidine\(^8\). The compounds 14, 15, and 16 were elucidated by FDMS and \(^{13}\)C NMR as solanidine 3-O-$\beta$-D-glucopyranoside, 3-O-$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranoside and 3-O-$\alpha$-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranoside, respectively.

As mentioned above, at the melting point of typical triterpenoid and steroid glycosides, the sugar—aglycone and the sugar—sugar linkages (glycosidic linkage) are cleaved, partially, to give the corresponding prosapogenins and aglycones. Heating higher than melting point facilitates the fission of glycosidic linkages and increases the yields of pyrolysis products. Sugar moieties of the glycosides were

---

Liebigs Ann. Chem. 1986
not detected in all the cases. They must be decomposed to yield smaller fragments or polymerized\(^9\) by the effect of high temperature.

Meanwhile, the isolation of digitoxigenin (9) from digitoxin (8) by means of pyrolysis has attracted considerable attention for the following reason. It is a matter of common that the 14-hydroxy group in the aglycone of cardiac glycosides is easily eliminated during acid hydrolysis and it is difficult to obtain the genuine aglycone. In fact, on hydrolysis with acid, 8 affords the dehydroxy derivative 17, but the genuine aglycone 9 cannot be obtained.

As the condition of pyrolysis for glycosides was thought to be milder than that of acid hydrolysis, thermal degradation of a glycoside of an acylated triterpenoid was examined with the object to gain the genuine aglycone. It was reported\(^10\) that 22-O-acyl-A\(_1\)-barrigenol 3-O-β-D-glucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucuronopyranoside (18) was hydrolyzed with crude hesperidinase to yield genuine aglycones, the 22-O-acyl-A\(_1\)-barrigenols 19 and 20, but, on acid hydrolysis, 18 afforded the 28-O-acyl-A\(_1\)-barrigenol 21, 22, and A\(_1\)-barrigenol (23),

Liebigs Ann. Chem. 1986
which were secondarily produced by migration and elimination of the acyl groups, and traces of the genuine aglycones. On the other hand, when 18 was heated to 260°C (m. p. of 18: 228–231°C), the sugar–aglycone linkage was completely cleaved\(^1\) to give only aglycones. The aglycones showed on TLC the spots corresponding to those of 19, 20, 21, and 22, and they were isolated by means of column chromatography and preparative TLC and identified (IR, \(^1\)H NMR, and \(^1\)C NMR).

Accordingly, thermal degradation of triterpenoid and steroid glycosides is expected to be one of the methods for providing genuine aglycones which are unstable to acids.

Finally, pyrolysis of an ester glycoside also was examined. A hederagenin 3,28-O-bisglycoside, the 3-O-α-L-arabinopyranosyl hederagenin 28-O-gentiobioside\(^2\) (24), was heated to 223°C (melting point) and the pyrolysate examined on TLC to show the spots of 2 (3-O-glycoside) and 24. The general method for preparation of 3-O-glycosides from 3,28-O-bisglycosides is alkaline hydrolysis, but pyrolysis also is expected to be the method of providing 3-O-glycosides if the procedure is applied to such bisglycosides as 24.
It was found that typical triterpenoid and steroid glycosides give, by mere heating, their prosapogenins and aglycones just as on acid and enzymatic hydrolysis \(^{13}\), and that some genuine aglycones, which are labile towards acids, are obtained by this procedure. This method is also applicable to the selective cleavage of sugar—aglycone linkages of ester glycosides. On thermal degradation, sugar moieties of the glycoside are not obtained. This procedure, however, is easy and simple, especially need no solvent and catalyst, and is hoped to be a useful method for structure determination of triterpenoid and steroid glycosides. Concerning the thermal degradation of glycosides, there have been reported the degradations of cellulose \(^{16}\), methyl glycosides \(^{15}\), phenyl glycosides \(^{16}\), and flavonoid glycosides \(^{17}\).

The authors are grateful to Prof. T. Nohara of Kumamoto University for kindly providing the samples of diosgenin glycosides. Thanks are also due to Mr. R. Isobe, Mr. A. Tanaka and Miss K. Soeda of the Faculty of Pharmaceutical Sciences, Kyushu University, for FAB, FD and EIMS, \(^{13}\)C NMR and \(^{1}H\) NMR data, respectively, and Miss Y. Noguchi and Miss R. Yamada for technical assistance.

**Experimental**

Optical rotations were measured at 26—29°C with a Jasco DIP-SL automatic polarimeter. — IR spectra were obtained with a Jasco IR-G spectrometer. — \(^{1}H\) NMR spectra were taken at 100 MHz with a Jeol C-100H spectrometer in CDCl\(_3\) unless otherwise specified and chemical shifts are given in \(\delta\) values with tetramethylsilane (TMS) as internal standard (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). — \(^{13}\)C NMR spectra were taken at 25.05 MHz with a Jeol FX-100 spectrometer with TMS employing the FT mode in C\(_6\)D\(_6\)N unless otherwise specified. — EIMS were measured with a Jeol D-300. FAB and FDMS were recorded using a Jeol DX-300. FABMS were taken at 2 kV for ion source and 6 kV for argon atom beam, and the spectra were obtained from glycerol solutions. FDMS were measured at +1 to ca. +3 kV for the field anode and —5 kV for the slotted cathode plate and an emitter-heating current of 17—20 mA. — TLC was performed on Kieselgel \(60\ F_{254}\) (Merck) using 5% \(H_2SO_4/CH_3OH\) solution for detection. Silica-gel and sephadex column chromatography were carried out with Kieselgel 60 (0.06—0.2 mm) [Merck] with a sample/adsorbent ratio of 1:30—40 and Sephadex LH-20 (25—100 \(\mu\)) [Pharmacia Fine Chemicals] with the ratio of 1:150—200 using CH\(_3\)OH as solvent. HPLC were performed on Waters Model ALC/GPC 244 using RI detector, the conditions were as follows: column Radial Pak A C\(_{18}\) (Waters), flow rate 1.0 ml/min.

**General procedures for melting-point measurements and thermal degradations:** 1—10 mg of samples were helded between two cover glasses (a cover glass: 18-mm square and 0.13—0.17-mm thickness) for microscope, and the samples were heated at a rate of 15—20°C/min on the hot stage of a Yanagimoto micro melting-point apparatus, and the temperatures recorded were uncorrected. In each case the residues between the glasses were extracted with CH\(_3\)OH or CHCl\(_3/CH_3OH\) (1:1) mixture, and the extracts were concentrated to give crude thermal degradation product (pyrolysate).

**Thermal degradation of I:** The glycoside I (260 mg) was heated to 280°C. The pyrolysate showing three major spots (\(R_f\) = 0.56, 0.33, and 0.17) on TLC [solvent CHCl\(_3/CH_3OH/H_2O\) (8:2:0.2)] was chromatographed on silica gel [eluant CHCl\(_3/CH_3OH/H_2O\) (9:1:0.1)]

Liebigs Ann. Chem. 1986
to give fraction 1 \((R_F = 0.56; 12 \text{ mg})\), fraction 2 \((R_F = 0.33; 29 \text{ mg})\), and 1 \((R_F = 0.17; 71 \text{ mg})\). Fraction 1 was crystallized from \(CH_3OH\) to give colorless prisms, m. p. >300°C, \([\alpha]D = +78.2 \text{ (c = 0.69 in pyridine)}\), which are identified with 3 \((\text{Lit.}3)\): m. p. >300°C, \([\alpha]D = +81 \text{ (pyridine)}\) by direct comparisons \((\text{IR}3)\) and \(^{13}\text{C NMR}18\)).

- Fraction 2 was passed through a sephadex column to give a colorless powder, m. p. 227–230°C, \([\alpha]D = +48.7 \text{ (c = 2.50 in pyridine)}\), which was identified with 2 \((\text{Lit.}3)\): m. p. 228–229°C, \([\alpha]D = +49 \text{ (CH}_3\text{OH)}\) by direct comparisons \((\text{IR}3)\) and \(^{13}\text{C NMR}18\)).

**HPLC of pyrolysates of 1:**

- \(t_R[\text{min}]\) of peaks were 7.8 \((1)\), 9.0 \((2)\), and 11.6 \((3)\) \((\text{solvent 85-\% CH}_3\text{OH})\); ratio of peak areas was 1:2:3 = 57:5:1 \((259°C)\) and 6:3:2 \((280°C)\).

**Thermal degradation of 4:**

The glycoside 4 \((480 \text{ mg})\) was heated to 290°C to afford a pyrolysate which showed five spots on TLC \((\text{solvent CH}_3\text{Cl}/\text{CH}_3\text{OH}/\text{H}_2\text{O (8:2:0.2)})\) of \(R_F = 0.77, 0.33, 0.24, 0.17, \text{ and 0.12}\). The pyrolysate was passed through a silica-gel column \((\text{eluant CH}_3\text{Cl}/\text{CH}_3\text{OH}/\text{H}_2\text{O (8:2:0.2)})\) to give fraction 1 \((R_F = 0.77; 15 \text{ mg})\), fraction 2 \((R_F = 0.33; 21 \text{ mg})\), fraction 3 \((R_F = 0.24; 5 \text{ mg})\), fraction 4 \((R_F = 0.17; 69 \text{ mg})\), and 4 \((R_F = 0.12; 234 \text{ mg})\). Fraction 1 was crystallized from \(CH_3OH\) to give colorless needles, m. p. 205–207°C, \([\alpha]D = -126.1 \text{ (c = 0.62 in CHCl}_3\text{)}\), which were identified with 7 \((\text{Lit.4})\): m. p. 204–207°C, \([\alpha]D = -129 \text{ (CHCl}_3\text{)}\) by direct comparisons \((\text{IR}4)\) and \(^{13}\text{C NMR19}\)).

- Fraction 2 was precipitated from \(CH_3OH\) to give a colorless powder, m. p. 251–253°C, \([\alpha]D = -123.8 \text{ (c = 0.71 in CH}_3\text{OH)}\), which was identified with 6 \((\text{Lit.4})\): m. p. 254–256°C, \([\alpha]D = -132 \text{ (CH}_3\text{OH)}\) by direct comparisons \((\text{IR}4')\) and \(^{13}\text{C NMR19}\)).

- Fraction 3 was passed through a sephadex column to give a colorless powder which was regarded as a mixture as mentioned in the text.

- Fraction 4 was passed through a sephadex column to give a colorless powder, m. p. 232–235°C, \([\alpha]D = -155.7 \text{ (c = 1.23 in CH}_3\text{OH)}\), which was identified with 5 \((\text{Lit.4})\): m. p. 233–237°C, \([\alpha]D = -118.2 \text{ (CH}_3\text{OH)}\) by direct comparisons \((\text{IR}4)\) and \(^{13}\text{C NMR19}\)).

**Thermal degradation of 8:**

The glycoside 8 \((400 \text{ mg})\) was heated to 275°C, and the pyrolysate showing four spots \((RF = 0.65, 0.50, 0.33, \text{ and 0.22})\) on TLC \((\text{solvent AcOEt})\) was chromatographed on silica gel \((\text{solvent AcOEt})\) to give fraction 1 \((RF = 0.65; 32 \text{ mg})\), fraction 2 \((RF = 0.50; 25 \text{ mg})\), fraction 3 \((RF = 0.33; 38 \text{ mg})\), and 8 \((RF = 0.22; 116 \text{ mg})\). Fraction 1 was crystallized from 40-% \(C_2H_5OH\) to give colorless prisms, m. p. 248–253°C, \([\alpha] = +14.3 \text{ (c = 0.77 in CH}_3\text{OH)}\), which were identified with 9 \((\text{Lit.6})\): m. p. 249–254°C, \([\alpha] = +19.1 \text{ (CH}_3\text{OH)}\) by direct comparisons \((\text{IR}6)\) and \(^{13}\text{C NMR20}\)).

- Fraction 2 was precipitated from \(CH_3OH/H_2O\) to afford 10 as colorless powder, m. p. 170–173°C, \([\alpha]D = +1.0 \text{ (c = 1.25 in CH}_3\text{OH)}\). FABMS: \(m/z = 505 \text{ [M+H]+}\). \(^{13}\text{C NMR: The signals due to the aglycone part were in good agreement with those of 8, and the signals of the sugar part were assigned by comparison with those of methyl digitoxoside as shown in Scheme 3.}"

- Fraction 3 also was precipitated from \(CH_3OH/H_2O\) to give 11 as a colorless powder, m. p. 140–142°C, \([\alpha]D = +1.0 \text{ (c = 1.25 in CH}_3\text{OH)}\). FABMS: \(m/z = 635 \text{ [M+H]+}\). \(^{13}\text{C NMR: The aglycone signals corresponded with those of 8, and the sugar signals were assigned by taking the glycosidation shift21 into account and comparing with the spectrum of methyl digitoxoside as shown in Scheme 3.}"

**HPLC of pyrolysates of 8:**

- \(t_R[\text{min}]\) of peaks were 22.2 \((8)\), 15.0 \((11)\), 10.8 \((10)\), 8.0 \((9)\) \((\text{solvent 70-\% CH}_3\text{OH})\); ratio of peak areas was 8:11:10:9 = 92:7:4:8 \((255°C)\) and 18:6:4:5 \((275°C)\).

**Thermal degradation of 12:**

The glycoside 12 \((480 \text{ mg})\) was heated to 300°C. The pyrolysate showed five major spots \((RF = 0.81, 0.58, 0.47, 0.31, \text{ and 0.18})\) on TLC \((\text{solvent CH}_3\text{Cl}/\text{CH}_3\text{OH}/\text{H}_2\text{O (7:3:0.3)})\). Silica-gel column chromatography of the pyrolysate using \(\text{CHCl}_3/\)
CH₃OH/H₂O (10:2:0.2 → 8:2:0.2) as solvent afforded fraction 1 (RF = 0.81; 16 mg), fraction 2 (RF = 0.58; 29 mg), fraction 3 (RF = 0.47; 20 mg), fraction 4 (RF = 0.31; 32 mg), and 12 (RF = 0.18; 47 mg). Fraction 1 was passed through Amberlite IRA-410 column (eluant CH₃OH), followed by sephadex column, to give a colorless powder (15 mg), m. p. 219–221 °C, [α]D = −35.7 (c = 0.34 in CHCl₃), which was identical (IR and ¹³C NMR) with 13 (Lit. m. p. 218–219 °C, [α]D = −29 (CHCl₃)). Fraction 2 was treated as fraction 1 to give 14 (27 mg) as colorless powder, m. p. 226–230 °C, [α]D = −44.5 (c = 1.73 in pyridine). FDMS: m/z = 559 [M⁺], 728 [M + Na]⁺. ¹³C NMR: The signals due to aglycone and sugar part were in good agreement with those of 13-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside. Fractions 3 and 4 were treated in the same way as fraction 1. Fraction 3 afforded 15 (19 mg) as colorless powder, m. p. 195–200 °C, [α]D = −93.9 (c = 0.18 in pyridine). FDMS: m/z = 705 [M⁺], 728 [M + Na]⁺. ¹³C NMR: The signals corresponded with those of solanidine 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Fraction 4 afforded 16 (31 mg) as colorless powder, m. p. 190–195 °C, [α]D = −68.2 (c = 0.43 in pyridine). FDMS: m/z = 705 [M⁺], 728 [M + Na]⁺. ¹³C NMR: The signals corresponded with those of solanidine 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Acid hydrolysis of 8: The glycoside 8 (100 mg) was heated with 5-% HCl in 90-% CH₃OH (10 ml) for 1 h. The reaction mixture was diluted with H₂O, neutralized, and extracted with CHCl₃. The organic layer was washed, dried, and evaporated. The residue showing no spot of 9, but one major spot (RF = 0.77) on TLC (solvent AcOEt) was passed through a silica-gel column [eluant n-hexane/AcOEt (3:2)] to give 17 (32 mg) as colorless needles (from n-hexane/AcOEt), m. p. 188–192 °C. EIMS: m/z = 356 (M, C₃₂H₃₂O₈). ¹H NMR: 6.13 (q, J = 6 Hz, 18-H), 6.15 (q, J = 6 Hz, 22-H), 3.64, 4.17 (each d, J = 11 Hz, 28-H₂), 3.71, 4.10 (each d, J = 11 Hz, 28-H₂). ¹³C NMR (CDCl₃): The signals were assigned by comparing with the spectrum of 9 as shown in Scheme 5.

Thermal degradation of 18: The glycoside 18 (220 mg) was heated to 260 °C, and the pyrolysate was extracted with hot AcOEt (30 ml). The organic layer was concentrated to crude glycoside mixture (85 mg). The mixture showing three spots (RF = 0.60, 0.38, and 0.35) on TLC [solvent CHCl₃/CH₃OH (20:1)] was passed through a silica-gel column [solvent CHCl₃/CH₂COCH₃ (6:1)] to give fraction 1 (RF = 0.60) and fraction 2 (RF = 0.38 and 0.35). Fraction 1 showed two spots (RF = 0.33 and 0.30) on TLC [solvent n-hexane/AcOEt (3:2)] and was separated by preparative TLC (solvent as above) to afford 19 (RF = 0.33; 12 mg) [identified by IR and ¹³C NMR] as colorless powder, m. p. 221–224 °C, and 20 (RF = 0.30; 9 mg) [identified by IR and ¹³C NMR] as colorless powder, m. p. 237–240 °C. Fraction 2 was also separated by preparative TLC [eluant CHCl₃/CH₂OH (20:1)] to afford 21 (RF = 0.38; 6 mg) [identified by IR, ¹H and ¹³C NMR] as colorless powder, m. p. 144–146 °C, and 22 (RF = 0.35; 13 mg) [identified by IR, ¹H and ¹³C NMR] as colorless powder, m. p. 248–251 °C.

<table>
<thead>
<tr>
<th>¹H NMR (C₅D₅N)</th>
<th>¹³C NMR (C₅D₅N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>6.13 (q, J = 6 Hz, 12 Hz, 22-H)</td>
</tr>
<tr>
<td>20</td>
<td>6.15 (q, J = 6 Hz, 12 Hz, 22-H)</td>
</tr>
<tr>
<td>21</td>
<td>3.64, 4.17 (each d, J = 11 Hz, 28-H₂)²</td>
</tr>
<tr>
<td>22</td>
<td>3.71, 4.10 (each d, J = 11 Hz, 28-H₂)²</td>
</tr>
</tbody>
</table>

² In CDCl₃.

Liebigs Ann. Chem. 1986
Thermal degradation of 24: The ester glycoside 24 was heated to 223°C (melting point). On TLC [solvent CHCl₃/CH₃OH/H₂O (7:3:0.3)] the pyrolysate showed the spots of 24 (Rₜ = 0.12) and of 2 (Rₜ = 0.59).

CAS-Registry Numbers

5) One product was not homogeneous from the ¹³C NMR spectroscopic evidence. Purification of the product was difficult and further investigation was not carried out.
6) Commercial product.
7) 12, colorless needles (from CH₃OH/H₂O), m. p. 236-241°C, [α]D = -80.7 (c = 1.14 in pyridine), was obtained from the methanol extract of potato shoots together with solanine in this laboratory. The structure of 12 was determined by identification of the acid-hydrolysis products and detailed assignment of the ¹³C NMR signals.
11) Heating at temperatures 30°C higher than the melting point usually affords aglycone, prosapogenins, and starting material. The gluconic acid—aglycone linkage must be labile towards heating compared with the other sugar—aglycone linkage.
13) It is commonly known that 3-O-glycosides of triterpenoids and steroids afford the corresponding prosapogenins and aglycones on acid or enzymatic hydrolysis.
17) H. Geiger and G. Schwinger, Phytochemistry 19, 897 (1980).

Liebigs Ann. Chem. 1986