Synthesis of

Kaempferol-3-O-(3'',4''-di-O- α -L-rhamnopyranosyl)- β -D-galactopyranoside and its Comparison with the Natural Ascaside

Isolated from *Astragalus caucasicus*

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The trisaccharide, 3,4-di-O-(α -L-rhamnopyranosyl)-D-galactose was synthesized using benzyl 2,6-di-O-benzyl- β -D-galactopyranoside as starting compound. Its acetobromo derivative was coupled with 4',7-di-O-benzyl-kaempferol. The structure of the synthetic kaempferol trioside was characterised by different spectroscopic methods (UV, IR, ^1H , ^{13}C NMR and MS). Chromatographic and mass spectrometric comparison of the synthetic product with ascaside precluded their identity.

Introduction

While the synthesis of naturally occurring flavonoid mono- and diglycosides are well documented and their synthesis is a routine process [1], the chemical preparation of flavonoid glycosides, containing a trisaccharide as sugar component seems to be more difficult and only few examples of such syntheses may be found in the literature [2, 4]. There seems to be a need for their syntheses, since the structure elucidation of such compounds by means of spectroscopic analysis, lacking distinct model compounds, proved to be equivocal [3].

Our results on the field of the structure elucidation of the xanthorhamnin series showed clearly, that the distinction between the different structure isomers by ^{13}C NMR or MS methods is only possible if there are synthetic models for the assignment on hand [4].

Here we wish to report on the synthesis of ascaside, a flavonol trioside isolated from *Astragalus caucasicus* [5], of which the structure was postulated with the aid of chemical degradation and spectroscopic investigations (UV, IR and ^1H NMR) to be a kaempferol-3-O-(3'',4''-di-O- α -L-rhamnopyranosyl)- β -D-galactopyranoside.

Some flavonol triosides with branched structures [6, 7] have been isolated, but the ascaside is the first representative among those which contain two rhamnoses and one galactose. The ascaside has an unique structure which prompted us to work out a synthetic route for its preparation. It is the first synthesis of a flavonoid trioside with a branched sugar moiety.

Result and Discussion

The key step in the ascaside synthesis is the preparation of its trisaccharide part. Generally, the synthesis of oligosaccharides requires two main operations: i) correct blocking to obtain the required aglycone, and ii) construction of the glycosidic linkage. In some cases the suitable protected aglycones are the bottle neck of the whole synthesis.

An unequivocal synthesis of ascaside required a suitable protected galactopyranoside derivative for the preparation of the trisaccharide component. For this reason we chose the recently prepared benzyl 2,6-di-O-benzyl- β -D-galactopyranoside [8] (1) which contains easily removable groups in all three positions. This aglycone was rhamnosylated by α -acetobromo-L-rhamnose in a modified Helferich procedure using a benzene-nitromethane (1:1) solvent system and $\text{Hg}(\text{CN})_2$ as acid acceptor to give compound 2. The high yield of the coupling (76%) and relatively

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short reaction time showed, that there was not any limited reactivity of the axial C₄-OH group, which was observed in the glycosylation reactions of 2,6-disubstituted galactopyranosyl derivatives [9, 10]. Perhaps the high yield of our reaction may be explained by the fact, that the reactivity of C₄-OH is increased by the glycosylation of C₃-OH.

The structure of compound **2** was verified by its ¹³C NMR spectrum. The chemical shifts for the anomeric carbons (102.9, 98.6 and 97.6 ppm) and their ¹J_{CH} values (160, 174 and 176 Hz) supported the suggested anomeric configurations; the two rhamnopyranosyl units have α-configuration. Compound **2** was readily hydrogenolysed over palladium on carbon and acetylated to give the crystalline nonaacetyl-trisaccharide (**3**). The ¹H and ¹³C NMR spectra of **3** showed the presence of both anomers of the reducing end in the galactopyranose unit in a ratio of α/β = 5/3. Deacetylation of **3** using the Zemplén method resulted in the trisaccharide (**4**) as an amorphous powder.

Treatment of **3** with HBr in acetic acid afforded the crystalline α-acetobromo derivative (**5**) in good yield. Condensation of the acetobromo-trisaccharide **5** with 4',7-di-O-benzylkaempferol [11] (**6**) in quinoline in the presence of silver carbonate and Drierite resulted in the crystalline **7** – after separation on silica gel column using a toluene-methanol, 8:2 solvent system as eluent –. The ¹H NMR and UV spectra of **7** were in good accordance with the proposed structure, indicating that the glycosylation took place in the position 3 of **6**. The β-anomer configuration of the new glycosidic linkage was suggested by the value of the optical rotation.

Deacetylation of **7** resulted in crystalline **8**, which after catalytic debenzylation gave the kaempferol-3-O-(3'',4''-di-O-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**9**). Conventional acetylation of **9** gave the crystalline peracetylated kaempferoltrioside (**10**) which also proved to be homogeneous. Though **9** was not crystalline, its purity was verified by TLC in different solvent systems and on different adsorbents. UV, IR, MS, ¹H and ¹³C NMR investigations confirmed the proposed structure. The β-glycosidic structure between the aglycone and the trisaccharide part is indicated by the presence of a low field signal at 101.3 ppm.

The TLC comparison of the synthesized compound **9** with the supplied natural product showed clearly that the latter is a mixture of at least two com-

pounds, one a kaempferol-, the other one an isorhamnetin- or tamarixetin-derivative.

The analysis of the mass spectrum of the permethylated isolated flavonoids showed the following characteristic fragments: *m/e* 189 for a rhamnose trimethyl ether, accompanied with two secondary fragments *m/e* 157 and 125. A fragment of a tetramethyl hexopyranosyl moiety could not be detected. The fragment M-205 = *m/e* 689 may be assigned to a permethylated kaempferol-(O-rhamnosyl)-O-galactoside structure. The *m/e* 719 is assignable to the permethylated quercetin analogue.

The fragments *m/e* 517 and 547 (A + S) belong to the kaempferol and quercetin-monomethyl ether-dimethyl galactoside skeletons, respectively. These facts seem to support the presence of a branched oligosaccharide moiety in ascaside. The absence of a *m/e* 362 (T + S₂) fragment rules out a linear carbohydrate chain. The spectrum of a peracetylated derivative of the mixture verifies the absence of a linear structure, too. Taking into account these fragments and the published data [5] (enzymatic degradation, diisopropylidene derivative) it may be assumed, that the isolated substance is a mixture of a kaempferol-3-O-(di-rhamnosyl)-O-galactoside and its tamarixitin or isorhamnetine analogue. The bond-types are most likely a 3'',6''- or a 4'',6''-di-O-rhamnosyl-galactoside.

- 2:** R = OBn; R' = H; R'' = Bn; R''' = Ac
3α: R = H; R' = OAc; R'' = R''' = Ac
3β: R = OAc; R' = H; R'' = R''' = Ac
4α: R = R'' = R''' = H; R' = OH
4β: R = OH; R' = R'' = R''' = H
5: R = H; R' = Br; R'' = R''' = Ac

- 7:** R = Bn; R' = H; R'' = Ac
8: R = Bn; R' = R'' = H
9: R = R' = R'' = H
10: R = R' = R'' = Ac

Bn = Benzyl
 Ac = Acetyl

Table. Assignment of the ^{13}C NMR chemical shifts and $^1J_{\text{CH}}$ data.

Compound	Galactosyl						Rhamnosyl						Rhamnosyl					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 ^a	102.5 (156)	79.3	73.6	69.4	74.6	69.1												
2 ^a	102.9 (160)	77.1	79.5	75.2	73.5	68.3	98.7 (174)	68.9	70.0	71.1	67.2	17.4	97.6 (176)	68.9	69.5	71.1	67.1	17.4
3 ^a	93.2	69.2	75.6	75.2	69.2	62.1	98.3	69.9	69.6	71.2	67.5	17.5	98.7	69.9	69.5	71.2	67.3	17.5
	97.9	73.2	78.4	75.4	74.4	62.1												
4 ^b	93.2	69.6	76.8	76.1	71.7	61.9	101.5	70.8	71.1	72.7	70.0	17.5	102.3	71.0	71.1	72.8	70.2	17.6
	97.2	73.1	80.0	75.7	75.5	61.9												
9 ^c *	101.3	71.6	78.2	73.3	75.6	60.4	100.7	70.3	70.8	71.9	68.9	17.9	101.8	70.5	70.9	72.0	68.9	17.8

* Chemical shift data for kaempferol skeleton of **9**: 156.6 (C-2); 133.1 (C-3); 177.5 (C-4); 161.2 (C-5); 98.8 (C-6); 164.3 (C-7); 93.7 (C-8); 156.7 (C-9); 104.0 (C-10); 120.8 (C-1'); 130.9 (C-2',6'); 115.2 (C-3',5'); 160.1 (C-4').

$^1J_{\text{CH}}$ coupling constants are given in brackets [Hz]. All chemical shifts are referred to TMS (i). Measured in ^a CDCl_3 ; ^b D_2O ; ^c $\text{DMSO}-d_6$.

The very little amount of isolated ascaside available precluded more detailed investigations, so that its structure requires further studies.

Experimental

Melting points were determined with a Kofler apparatus and are uncorrected. LEP A2 (Zeiss) polarimeter was used for measurement of specific rotations. UV and IR spectra were recorded with a Beckman DK-2A and a Beckman IR-8 spectrometer, respectively. ^1H NMR spectra were measured with a Varian A-60A spectrometer, and ^{13}C NMR spectra with a Bruker WP 200, for the given solutions (internal Me_4Si). Mass spectra were recorded with an AEI MS 30 spectrometer operating with direct-inlet system. T.l.c. was performed on precoated plates of silica gel F_{254} (Merck). Column and preparative layer chromatography were performed on silica gel HF (Merck).

Benzyl-2,6-di-O-benzyl-3,4-di-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)- β -D-galactopyranoside (**2**)

Benzyl-2,6-di-O-benzyl- β -D-galactopyranoside (**1**) [**8**] (4.50 g) was treated with α -acetobromo-L-rhamnose (7.04 g) for 2 h in 1:1 abs. benzene-nitromethane (400 ml) in the presence of $\text{Hg}(\text{CN})_2$ (5.04 g). After working up, the syrupy product was chromatographed on a silica gel column (350 g), using 9:1 dichloromethane-acetone as the eluent, yield: 7.56 g (76%), $[\alpha]_{\text{D}}^{25} -58.1^\circ$ (c 0.71, chloroform), R_f 0.70 (dichloromethane-acetone, 9:1).

Analysis for $\text{C}_{51}\text{H}_{82}\text{O}_{20}$

Found C 62.06 H 6.40,
Calcd C 61.55 H 6.28.

1,2,6-Tri-O-acetyl-3,4-di-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)- α,β -D-galactopyranose (**3**)

Compound **2** (5.0 g) was hydrogenated in ethanol (85 ml) in the presence of Pd on Carbon (400 mg) for 96 h. The reaction mixture was filtered, evaporated and acetylated in pyridine (30 ml) and acetic anhydride (30 ml) at room temperature. After the usual work up, the solidified **3** was crystallised from ethanol-water (8:2) (3.89 g, 91%), m.p. 93 to 96°, $[\alpha]_{\text{D}}^{25} +0.3^\circ$ (c 0.75, chloroform).

3,4-Di-O-(α -L-rhamnopyranosyl)-D-galactose (**4**)

The peracetylated trisaccharide **3** (0.60 g) was deacetylated with sodium methoxide (0.1 M, 0.5 ml) in methanol (20 ml) to give a foam after freeze-drying (300 mg, 90%), $[\alpha]_{\text{D}}^{25} -14.4^\circ$ (c 0.53, water), R_f 0.35 (*n*-butanol-methanol-water 3:1:1).

Analysis for $\text{C}_{38}\text{H}_{50}\text{O}_{23}$

Found C 51.18 H 5.89,
Calcd C 50.82 H 5.80.

α -Acetobromo-3,4-di-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)-D-galactose (**5**)

Compound **3** (500 mg) was dissolved in dichloromethane (5 ml) and treated with 40% hydrogen bromide in acetic acid (1.5 ml) for 1.5 h at 0°. The solution was diluted with dichloromethane, successively washed with saturated cold NaHCO_3 solution and ice-water, dried (Na_2SO_4) and evaporated. The residue was crystallised from ether-dichloromethane to give **5** (350 mg, 65%), m.p. 181–182°, $[\alpha]_{\text{D}}^{25} +68.2^\circ$ (c 0.585, chloroform), R_f 0.38 (toluene-ethanol, 9:1).

^1H NMR data (in CDCl_3): δ = 6.81 ppm (d, 1H, H-1; $J_{1,2} = 1$ Hz); 1.85–2.10 (m, 24H, 8 OAc); 1.22 and 1.20 (2d, 6H, 3CH₃; $J = 6$ Hz).

4',7-Di-O-benzyl-3-O-[3'',4''-di-O-(tri-O-acetyl- α -L-rhamnopyranosyl)-2'',6''-di-O-acetyl- β -D-galactopyranosyl]-kaempferol (7)

4',7-Di-O-benzyl-kaempferol [11] (6) (155 mg) was dissolved in fresh distilled chinoline (5 ml) and to this solution Drierite (300 mg) and silver carbonate (300 mg) were given at 0° in the dark. The reaction mixture was treated with 5 (300 mg) in two portions and stirred for 5 h at room temperature. It was diluted with dichloromethane and stirred with a KCl solution. After filtration the organic layer was cooled and washed with ice-cold 0.25 M sulfuric acid, and with water, dried (Na₂SO₄) and evaporated. The dark residue was successively chromatographed on a silica gel column and on a preparative layer using a toluene-methanol (8:2) solvent system as eluent to get 7 (130 mg, 31%), m.p. 135–136 °C (from ethanol-acetone), $[\alpha]_D^{25}$ –67.6° (c 0.519, chloroform), R_f 0.53 (toluene-methanol, 8:2) and 0.70 (dichloromethane-ethanol, 95:5).

UV (MeOH): $\lambda_{\max}$ (lg ϵ) 267 nm (4.41), 320 (sh), 343 (4.31).

¹H NMR data (in CDCl₃): δ = 12.38 ppm (br, 1 H, OH-5); 8.18 (H-2',6'; J = 2 and 9 Hz); 7.47 (Ph); 7.19 (H-3',5'; J = 2 and 9 Hz); 6.53 (d, H-8, J = 2 Hz); 6.42 (d, H-6; J = 2 Hz); 2.25–1.90 (m, 24 H, 8 OAc); 1.24 and 1.20 (2d, 2CH₃; J = 6 Hz).

Analysis for C₆₃H₆₈O₂₇

Found	C 59.99	H 5.48,
Calcd	C 60.19	H 5.45.

Kaempferol-3-O-[2'',6''-di-O-acetyl-3'',4''-di-O-(tri-O-acetyl- α -L-rhamnopyranosyl)- β -D-galactopyranoside] (8)

Compound 7 (150 mg) was hydrogenolysed in ethylacetate (20 ml) over Pd-on-carbon (20 mg) for 2 h. After filtration the solution was evaporated and the residue crystallised to give 8 (120 mg, 93%), 141–144 °C (from ethanol), $[\alpha]_D^{25}$ –88.7° (c 0.523, chloroform), R_f 0.40 (toluene-methanol, 8:2).

UV (MeOH): $\lambda_{\max}$ (lg ϵ) 266 nm (4.32), 295 (sh), 348 (4.26).

¹H NMR data (in CDCl₃): δ = 12.38 ppm (br, 1 H, OH-5), 7.95 (H-2',6'; J = 9 Hz); 6.92 (H-3',5'; J = 9 Hz); 6.33 (d, H-8; J = 2 Hz); 6.24 (d, H-6; J = 2 Hz); 2.35–1.92 (m, 24 H, 8 OAc); 1.24 and 1.20 (2d, 6H, 2CH₃; J = 6 Hz).

Kaempferol 3-O-[3'',4''-di-O-(α -L-rhamnopyranosyl)- β -D-galactopyranoside] (9)

Compound 8 was deacetylated in abs. methanol with sodium methoxide. After the usual work up

the residue was precipitated from methanol-isopropanol to get an amorphous solid, m.p. 180–190 °C (isol. Ascaside [5] m.p. 180–182 °C), $[\alpha]_D^{25}$ –52.9° (c 0.562, methanol).

HPTLC silica gel: R_f 0.46 (chloroform-methanol-water, 64:36:8), R_f 0.30 for Ascaside.

Polyamide TLC: R_f 0.82 (methanol-ethylacetate, 8:2), R_f 0.93 for Ascaside.

UV (EtOH): $\lambda_{\max}$ (lg ϵ) 266 nm (4.22), 300 (sh), 350 (4.12). For Ascaside: 268, 325, 335 and 350.

IR (in KBr): $\lambda_{\max}$ (cm^{–1}) 3400, 3000, 2950, 1650, 1600, 1550, 1530 and 1490.

For isol Ascaside: 3400, 3030, 1660, 1657, 1640, 1635, 1620, 1580, 1550, 1530 and 1515.

¹H NMR data (in DMSO-d₆ + CF₃COOD): δ = 8.12 ppm (H-2',6'; J = 9 Hz); 7.00 (H-3',5'; J = 9 Hz); 6.52 (d, H-8; J = 2 Hz); 6.29 (d, H-6; J = 2 Hz); 1.30 and 1.15 (2d, 6H, 2CH₃).

MS of 9 in permethylated form (ST 210°, PT 180°, 70 eV, R 1000/2 KV), m/e (% rel. int.): M⁺ 894(1), 749(2), 689(1), 671(1), 587(2), 566(3), 517(2), 460(8), 422(8), 402(6), 356(23), 342(40), 328 A + H (100), 314(73), 312(25), 299(55), 282(58), 189(38), 157(35).

MS of isolated Ascaside in permethylated form (ST 210°, PT 200°, 70 eV, R 1000/2 KV), m/e (% rel. int.): 719(1), 689(1), 547(1), 517(1), 432(1), 402(1), 386(2), 358(90), 328 A + H (100), 314(30), 312(15), 299(10), 285(10), 282(20), 189(65), 157(42).

4',5,7-Tri-O-acetyl-kaempferol-3-O-[2'',6''-di-O-acetyl-3'',4''-di-O-(tri-O-acetyl- α -L-rhamnopyranosyl)- β -D-galactopyranoside] (10)

Compound 9 was acetylated in abs. pyridine with acetic anhydride for 16 h at room temperature. After usual work up the residue was crystallised from ethanol-chloroform to give 10 (m.p. 158 to 159 °C, $[\alpha]_D^{25}$ –68.7° (c 0.526, chloroform), R_f 0.29 (toluene-methanol, 8:2).

UV (MeOH): $\lambda_{\max}$ (lg ϵ) 250 nm (4.32) and 310 (4.25).

¹H NMR data (in CDCl₃): δ = 8.13 ppm (H-2',6'; J = 9 Hz); 7.28 (H-3',5'; J = 9 Hz); 7.28 (d, H-8; J = 2 Hz); 6.80 (d, H-6; J = 2 Hz); 2.47–2.27 (m, 9H, OAc-5,7,4'); 2.25–1.93 (m, 24H, 8 OAc); 1.21 (2d, 6H, 2CH₃; J = 6 Hz).

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