FLAVONOID-C-GLYCOSIDES FROM *TRITICUM AESTIVUM* L.*

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ABSTRACT.—From the seedlings of *Triticum aestivum* some new apigenin-di-C-acylglycosides have been isolated in addition to vicenin-1, isoschaftoside, and schaftoside or their galactosyl isomers. One of the new glycosides was identified as sinapoyl-8-D-galactosyl-6-C-arabinosylapigenin; two other new glycosides were derived from the same di-C-glycoside or its isomer and contained ferulic acid.

The flavonoid composition of *Triticum aestivum* has been the subject of several chromatographic and chemical investigations. Harborne and Hall (1) identified in the leaf extracts of *Triticum-* and *Avena* species several flavon-C-glycosides such as saponarin iso-orientin and 8-C-arabinosylhexoside of apigenin. Julian *et al* (2) showed that the flavonoid composition is more complex and supplemented the list with the C-glycosides lutonarin, lucenin-3, and vicenin-2. In addition, two new glycosides, orientin-7-0-rutinoside (Wyomin) and isoswertisin-4'-0-glucoside were structurally elucidated. The seedlings of *Triticum aestivum* were investigated for the first time by King (3, 4). He described two flavonoids, A and B, as derivatives of members of the 6,8-di-C-glycosylapigenin-type. Hydrolysis of flavonoid A led to flavonoid B and sinapic acid; flavonoid B, on hydrolysis, gave a third flavonoid. A full structure determination was not achieved.

RESULTS AND DISCUSSION

The residue of a methanol extract was extracted with ethyl-acetate and then fractionated by column chromatography on cellulose with ethyl acetate as solvent into two main flavonoid fractions: Fraction I showed on cellulose tlc a mixture of 5 compounds (A–E) in the Rf-range 0.1–0.55, while fraction II turned out to be a mixture of two flavonoids (F, G). The flavonoids of fraction I were separated by preparative tlc on cellulose plates and identified by cochromatography with test substances and ir-spectroscopic comparison. Compound A was identified as a schaftosid-isomer, presumably identical with the flavonoid C of King (3, 4), compound B as isoschaftoside (8-glucosyl-6-arabinosylapigenin), compound C as vicenin 1 (8-glucosyl-6-xylosylapigenin) and compound D as schaftoside (8-arabinosyl-6-glucosylapigenin). Flavonoid E corresponded with a compound which was obtained by isomerization of schaftoside besides isoschaftoside. On the basis of the ^{13}C-nmr-spectroscopic studies of the main glycoside of fraction I, it cannot be completely excluded that the hexose in B and E is galactose rather than glucose.

Fraction II, which showed two spots on cellulose-tlc (F, G), could be separated by polyamid chromatography into four compounds (α, β, γ, δ). Only glycoside α could be obtained in pure form. The other glycosides were not separable even by hplc on a reversed phase column. The glycoside (mp = 205–207°) gave, after alkaline and acidic hydrolysis, sinapic acid and a flavonoid-C-glycoside with the chromatographic behaviour of isoschaftoside. The permethylated deacylated glycoside showed a ms fragmentation pattern which was nearly identical with that of isoschaftoside (5). The di-isopropylidene derivative obtained with $CuSO_4$ as a

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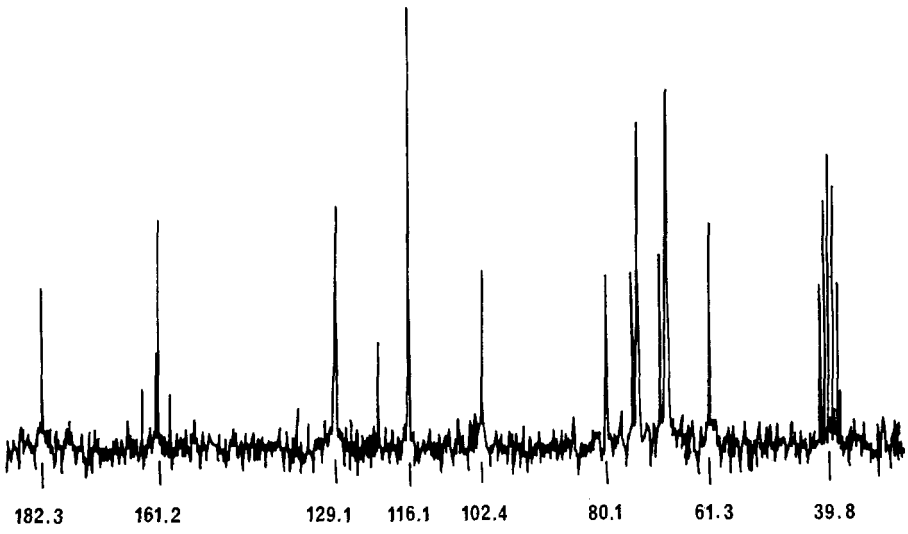

Fig. 1

^{13}C-NMR-spectrum of 8-C-galactosyl-6-C-arabinosyl-Apigenin in DMSO-d_6
(pr. broadband decoupled)

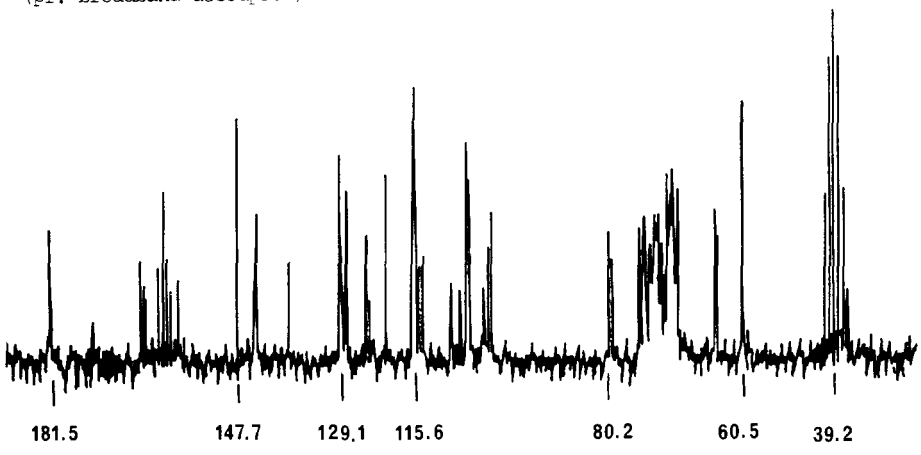

Fig. 2

^{13}C-NMR-spectrum of sinapoyl-8-C-galactosyl-6-C-arabinosyl-Apigenin in DMSO-d_6
(pr. broadband decoupled)

catalyst, however, was not consistent with an arabinose- and glucose moiety. When $CuSO_4$, is used as the catalyst instead of toluensulfonic acid from hexopyranosides no 4,6-O-IP-derivatives are formed. The intensity of the molecular ion peak of the permethylated isopropylidene derivative ($M^+ = 728$) was in better agreement with a 6-C-bound arabinose (3,4-O-IP) and a 8-C-bound galactose (3,4-O-IP).

These results correspond with the ms data of a 6-C-galactosyl-8-arabinosyl-apigenin, isolated by Chopin et al. (7) from *Polygonatum multiflorum*, and a 8-C-galactosyl-apigenin from *Briza media* (8). Confirmation of the galactose unit came from the ^{13}C-nmr-spectrum, which showed the signals at $\delta = 80.1$, 74.4, 74.4, 69.1 and 61.3. Glucose would have given rise to another signal pattern as is shown in the table 1.

TABLE 1. δ-values (ppm) of sugar-C-signals.

(Schaftoside) apigenin-6-C-glu-8-C-ara		apigenin 6-C-gal-8-C-ara (5)		apigenin 8-C-gal-6-C-ara	
81.5		79.0		80.1	
78.7			74.8		75.4
	75.1		74.5		74.4
	74.5	74.4		74.4	
73.7		73.5		74.4	
	71.1		71.0		70.3
71.1			69.4		69.1
70.2			68.9		69.1
	69.2	68.3		69.1	
	68.9	68.2		69.1	
61.0		60.7		61.3	

The position of the sinapoyl residue could be deduced from the ms, the ^{13}C-nmr spectrum, and the isopropylidene derivative. Since the permethylated acyl-glycoside ($M^+ = 910$) showed the unchanged fragmentation of the 6-C-arabinose M–131 > M–119 > M–245, the acyl residue must be attached to the galactose unit. Formation of a PM–IP with the $M^+ = 922$ confirmed this hypothesis. Of the four possible positions, the C_6-OH-group in the galactosylresidue could be excluded since the ^{13}C-nmr-spectrum showed two nonshifted signals at $\delta = 60.5$ and 60.8.

The finding that the actylglycoside showed not the expected 11 but 18 ^{13}C-sugar-signals indicates that the isolated glycoside is a mixture of 2,3 or 4-OH acyl-glycoside isomers. The signal shifts for the sinapoyl residue are in good accordance with the data given by Ternai and Markham (9).

On the basis of these results, the structure in figure 3 can be proposed for the acylglycoside.

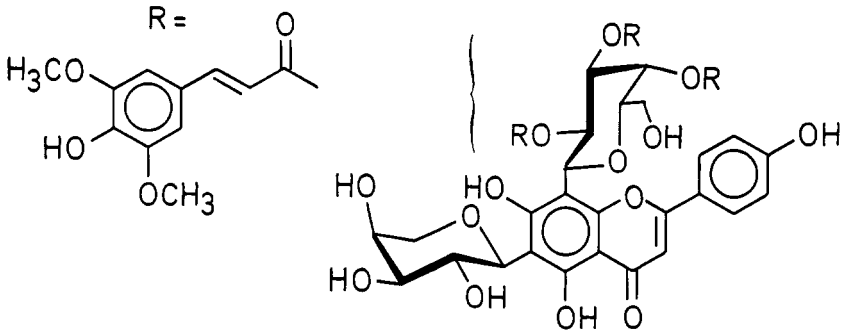

Fig. 3

The three other glycosides of fraction II are acyl derivatives of the former 8-C galactosyl-6-C-arabinosyl-apigenin or its isomer. One glycoside (γ) contains also sinapic acid, while the other two (β,δ) bear a feruoyl residue.

EXPERIMENTAL[1]

ISOLATION PROCEDURE.—Ten kg of the dried seedlings were defatted with petroleum ether in a Soxhlet (2 days) and then extracted with methanol (10 days) until the extract was colorless. The methanol-extract was concentrated to a brown syrup, the residue dissolved in 5 liters of water and filtered after several days of storage at 0°. After filtration the brown solution was extracted once more with petroleum ether for 24 days on a perforator and then extracted with ethyl-acetate for 14 days. The ethyl acetate-phases were combined, concentrated and, after storage for one week at −5°, filtered to remove precipitated impurities. The separation of components of the purified ethyl acetate-phase was performed on five cellulose (Ederol) columns (diameter, 9 cm; height, 50 cm) with 1% acetic acid as the solvent. The separation was monitored by tlc. Fractions 1–27 were nearly free of flavonoids. Concentration of the combined fractions 28–39 produced 1.5 g of a flavonoid mixture (fraction I). Fractions 45–70 resulted in about 2.3 g of a second flavonoid mixture (fraction II).

Fraction I was separated into 5 bands (A–E) on preparative cellulose plates with 15% acetic acid as the solvent. The bands were eluted with methanol, the solutions evaporated, and the residues subjected to uv, ir-spectroscopy and mp determination.

Band A: (R_f=0.12)=Isomer of schaftoside=same R_f-value as an isomerization product of auth. schaftoside
Band B: (R_f=0.34) Isoschaftoside, identified by co-tlc
Band C: (R_f=0.34)=Vicenin 1, identified by co-tlc and ir
Band D: (R_f=0.46) Schaftoside, identified by mp
Band E: (R_f=0.51)=Isomer of schaftoside=same R_f-value as a isomerization product of authentic schaftoside

Fraction II was separated on 8 polyamide columns (mesh size 20–60 μ; diameter 1.5 cm; height, 30 cm) with 50% methanol as the solvent. The separation was monitored by tlc (silica gel 60 G_{254}, Merck, *n*-butanol-ethylacetate-water/4:1:5), Reagent: basic lead acetate. Fr. 1–3 (glycoside α=R_f=0.32); Fract. 4–8 (glycoside α and γ=R_f=0.32 and 0.40); Fract. 9–15 (glycoside β and δ=0.36 and 0.44). Glycoside α was purified on Sephadex LH 20.

SINAPOYL-8-C-GALACTOSYL-6-C-ARABINOSYL-APIGENIN (GLYCOSIDE-α).—The compound gave the following physical data: mp=205–207° (ETOH); $[\alpha]^{26}D$ −136.8° (pyridine, C=0.103; uv: MeOH λ max=237 (log ϵ=4,334), 330 (log ϵ=4,533) nm; MeOH+NaOMe 282 (sh), 338 (sh), 392; MeOH+NaOAc 282, 338 (sh), 390; MeOH+$AlCl_3$ 282, 309, 342, 339 (sh); MeOH+$AlCl_3$ HCl 281, 306, 340, 393 (sh); ir: ν max KBr cm^{-1}: 3300, 2930, 1680, 1615, 1560, 1495, 1430, 1340, 1265, 1235, 1205, 1160, 1140, 1085, 1000, 830; ^{13}C-nmr: (DMSO-d_6, t=90°, 25,03 MHz, ppm)—*apigenin*:=101.7 (C-10), 102.9 (C-3), 105.7 (C-8), 106.2 (C-6), 115.6 (C-3', C-5'), 120.8 (C-1'), 127.9, 129.1 (C-2', C-6'), 158.2, 159.6, 160.7, 162.0 (C-4', C-9, C-7, C-5, C-2), 181.5 (C-4); *C-sugar*: 60.5, 60.8, 67.8, 68.7, 69.1, 69.7, 70.7, 71.3, 72.2, 72.9, 73.5, 74.0, 74.6, 79.5, 80.2 (sugar-C-atoms not assigned); *sinapoyl-residue*: 55.8 (OMe), 107.2, 108.9 (C-2, C-6), 114.2, 114.7 (C-β), 123.9, 124.3 (C-1), 138.1 (C-4), 144.1 (c-α), 147.7 (C-3, C-5), 164.1, 164.5, 165.3 (C-γ); ms: (permethylether, ST 220°, PT 180°, 70 eV, (rel. int.)) 910 M^+ (1), 895 M-15 (3), 897 M-31 (14), 863 M-47 (2), 791 M-119 (6), 779 M-131 (8), 765 M-145 (5), 735 M-175 (1), 407 (9), 365 (10), 351 (11), 339 (12), 335 (10), 325 (10), 323 (14), 321 (13), 312 (12), 311 (10), 309 (17), 238 (95), 223 (70), 221 (100).

ISOPROPYLIDENE-DERIVATIVE OF GLYCOSIDE.—One mg of the glycoside was dissolved in 10 ml of anhydrous acetone and, after addition of about 1 mg of anhydrous Cu SO_4, the mixture was kept for 5 hours at room temperature. It was then filtered and evaporated to dryness. The permethylated glycoside, prepared according to Brimacombe (10), was subjected to mass spectrometry at St 210°, PT 2000, 70 eV, R 1000/2KV; M^+=922 indicated a 3,4-O-IP-arabinosyl-PM-derivative.

ALKALINE HYDROLYSIS.—Glycoside α (30 mg) was hydrolyzed with 5 ml of 0.1 n NaOH, 5 minutes at 70°. After neutralization, the solvent was evaporated to dryness and then dissolved in *n*-butanol-ethylacetate-water (4:1:5) and chromatographed on a cellulose column, (Cellulose-Serva, diameter 6 cm; height, 50 cm) with the same solvent.

The first fraction contained sinapic acid (bright blue fluorescence), R_f=0.18 in toluene-acetic acid-water (4:1:5). The second fraction was the deacylated product; it was purified on

[1]The nmr and ms were recorded on a Jeol FS-100 at 90° and an AEI ms 30 instrument. The mp are uncorrected. The seedlings of *Triticum aestivum* were purchased from the firm Grandl, Augsburg (1976).

Sephadex LH-20- to give 18 mg of *6-C-arabinosyl-8-C-galactosyl-apigenin*, mp 208-214° (ethanol). The compound gave the following physical data: uv: MeOH λ max=275, 330 nm; ^{13}C-nmr: (DMSO-d_6, τ=90°, 25,03 MHz, ppm)—*apigenin-moiety*: δ=102.4 (C-10), 103.3 (C-3), 103.9 (C-8), 107.0 (C-6), 116 (C-3', C-5'), 121.4 (C-1'), 129.1 (C-2', C-6'), 156.3 (C-9), 159.2 (C-7), 161.2 (C-5), 161.6 (C-4'), 163.6 (C-2), 182.3 (C-4) 8-C-galactose: 61.3 (C-6"), 69.1 (C-4"), 74.4 (C-1"), 74.4 (C-3"), 80.1 (C-5"): *6-C-arabinose*: 69.1, 69.1, 70.3, 74.4, 75.4; ms: (permethyl-ether, ST 220°, PT 180°, 70 eV, *m/e* (rel. int.)) 704 M^+ (8), 689 M-15 (25), 674 M-30 (307, 673 M-31 (100), 659 M-45 (8), 658 M-46 (7), 657 M-47 (10), 644 M-60 (10), 643 M-61 (20), 627 M-77 (16), 585 M-119 (24), 573 M-131 (31), 559 M-145 (19), 514 M-163 (10), 529 M-175 (15) (Di-isopropyl-permethyleter M^+=728, ST 210°, PT 200°, 70 eV).

GLYCOSIDE *β*, *γ*, *δ*.—These could be obtained only unpurified with glycoside *α*. After hydrolysis of the first mixture (*α*, *γ*), only sinapic acid could be identified. From the second mixture (*α*, *β*, *δ*) only ferulic acid could be obtained. The R_f of ferulic acid was 0.25 (toluene-acetic acid-water, 4:1:5).

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