GLYCOSIDES OF *Vaccaria segetalis*

V. VACCARIN

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Continuing a study of the glycosides of the seeds of Vaccaria segetalis (Neck.) Carcke (family Caryophyllaceae), we have isolated a new flavonoid compound which we have called vaccarin.

The positive cyanidin reaction [1], the chromatographic behavior, and the good solubility in water of this compound show its glycosidic nature. The almost identical intensities of the maxima of the short-wave and long-wave bands of the UV spectrum, and also the distance between them, which is between 50 and 60 nm permit it to be assigned to the flavones [2].

The acid hydrolysis of vaccarin gave a mixture of two substances of flavonoid nature and sugars (D-glucose and L-arabinose) which were identified chromatographically. The proportion of the combined flavonoid substances formed on hydrolysis amounted to 60% of the weight of the initial glycoside. Consequently, on acid hydrolysis one molecule each of glucose and arabinose was split off.

The flavonoid products of acid hydrolysis were separated on a column of polyamide sorbent. Both these substances (Table 1) also proved to be glycosides, but they were not cleaved by the usual hydrolysis with acids, undergoing mutual isomerization to form an equilibrium mixture after being heated with 10% hydrochloric acid for 3 hours. On hydrolysis with Kiliani's mixture, these compounds gave apigenin and, as sugars, D-glucose with a small amount of D-arabinose. On the basis of these properties, both flavonoids must be C-glycosides.

In order to determine the free hydroxy groups and the position of the carbohydrate substituent in the glycoside investigated, we performed a spectral investigation in the UV region using complex-forming and ionizing reagents [3, 4]. The values of the maxima and their shifts (Table 2) show that there are free hydroxy groups in positions 5 and 7. The azo-coupling reaction [5] for vaccarin and for the two flavonoid compounds (II) and (III) obtained by acid and alkaline hydrolysis of the glycoside (I) was positive. Consequently, only positions C_4' and C_6 remain for the carbohydrate substituents.

TABLE 1. Physicochemical Properties of Vaccarin and Its Derivatives

Substance	Composition	Mp, °C	$[\alpha]_D$, deg	R_f (15% CH_3COOH)
Vaccarin (I)	$C_{32}H_{38}O_{18}$	209–211	–49	0,71
Dearabovaccarin (II)	$C_{27}H_{30}O_{14}$	225–227	–25	0,55
Degluco(4')-vaccarin (III)	$C_{26}H_{28}O_{13}$	199–202	+42	0,66
Apigenin 6-C-anti-β-D-glucopyranoside (anti-IV)	$C_{21}H_{20}O_{10}$	230–231	+21,5	0,40
Apigenin 6-C-syn-β-D-glucopyranoside (syn-IV)	$C_{21}H_{20}O_{10}$	255–257	–43	0,19
Apigenin (V)	$C_{15}H_{10}O_5$	343–345		0,05

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TABLE 2. Spectral Characteristics of Vaccarin and Its Derivative in the UV Region (maxima and shifts, nm)

Substance	Ethanol		Ethanol + AcNa		Ethanol + EtNa		Ethanol + $ZrO(NO_3)_2$		
	λ	log ε	λ	Δλ	λ	Δλ	λ	Δλ	$\frac{\varepsilon A}{\varepsilon B} \cdot 100\%$
Vaccarin (I)	332 273	4,21 4,19	395	63	400	68	385 (A) 360 (B)	53	65
Dearabovaccarin (II)	335 271	4,20 4,18	392	57	400	65	385 355	50	55
Degluco(4')-vaccarin (III)	335 270	4,22 4,19	395	60	400	65	385 357	50	60
Anti form of compound (IV)	335 271	4,20 4,18	390	55	402	67	385 345	50	53
Syn form of compound (IV)	335 271	4,20 4,18	390	55	400	65	390 356	55	57
Apigenin (V)	336 270	4,40 4,38	380	44	400	64	390 355	54	103

TABLE 3. Values of the Chemical Shifts of the Protons in Vaccarin Derivatives (δ, ppm)

Substance	Protons					
	H_3	H_8	H_9	H_2H_6	H_3H_5	H_1''
Anti form of (IV)	6,75	—	6,55 (2,5)	7,90 (8)	6,95 (8)	4,75 (7,5)
Syn form of (IV)	6,65	6,15 (2,5)*	—	6,90 (8)	6,80 (8)	4,85 (7,5)
Desgluco(4')-vaccarin acetate	4''-OAc	3''-OAc	—	6''-OAc	2''-OAc	H-1''
	2,03	1,96	—	1,92	—	4,85 (7,5)

* The values of the spin–spin coupling constants (J), Hz are given in parentheses.

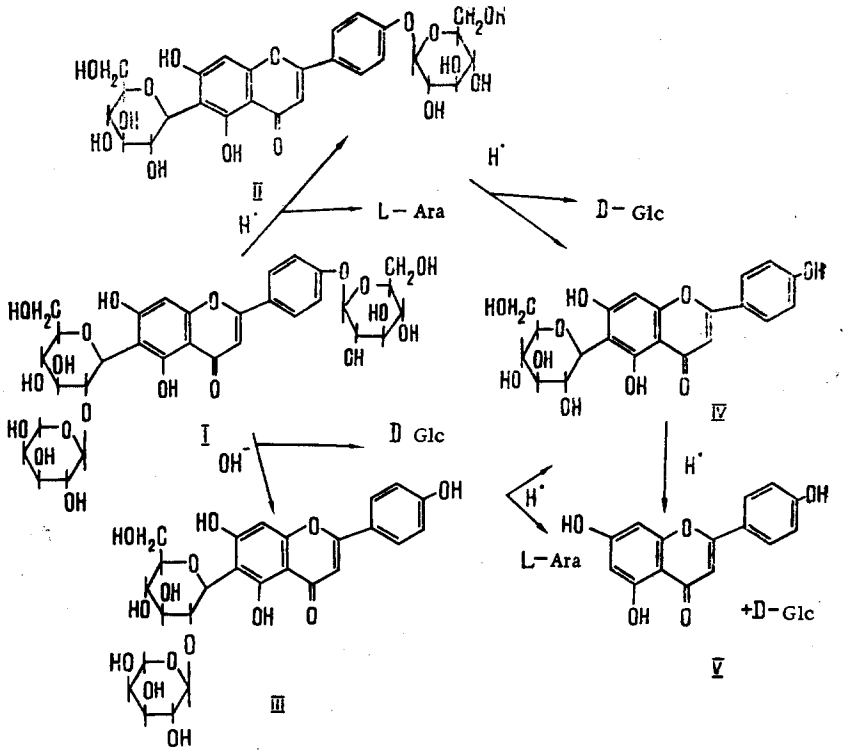
The results of an analysis of the zirconyl complexes in the UV region [6] show that the ratio of the intensities of the main (A) and the subsidiary (B) maxima in vaccarin and its derivatives is between 55 and 65%, which confirms the position of the carbohydrate substituent at C_6.

To determine the position and order of addition of the sugar residues we used the results of stepwise alkaline and acid hydrolysis. Stepwise hydrolysis with weak acid gave a diglycoside (II) and a C-monoglucoside (IV) (anti form). It was established by chemical and spectral investigations that (II) has the structure of 4',5,7-trihydroxyflavone 6-C-β-D-glucopyranoside 4'-O-β-D-glucopyranoside. This flavonoid, which we have called dearabovaccarin has the same composition as isosaponarin (isolated from the seeds of V. segetalis [7]) and is chromatographically identical with it.

The results obtained show that the L-arabinose may be attached to the glucose present at C_4' or that present at C_6. To investigate this, we performed the alkaline cleavage of the glycoside (I) [8]. This gave a bioside – degluco(4')-vaccarin (III) – the acid hydrolysis of which gave a mixture of the C-monoglycosides (IV) and L-arabinose.

The question of the position of attachment of the L-arabinose to the glucose at C_6 was solved on the basis of the results of the periodate oxidation of vaccarin and a study of the NMR spectra of the acetate of the bioside (III). When (I) was subjected to periodate oxidation and the oxidation products were hydrolyzed, no monosaccharides were found. Consequently, a 1→3 bond is excluded. Furthermore, the absorption of 5 moles of sodium periodate and the isolation of 2 moles of formic acid excludes a 1→6 bond.

The NMR spectrum of the acetate of compound (III) has signals at 2.03, 1.96, and 1.92 ppm (Table 3), corresponding to 4''-OAc, 3''-OAc, and 6''-OAc and has no signal at 1.77-1.82 ppm corresponding to 2''-OAc [9, 10]. Consequently, the L-arabinose is attached to the 2''-OH group of the D-glucose.



It was stated above that the acid hydrolysis of vaccarin, of dearabovaccarin, and of degluco(4')-vaccarin forms a mixture of two apigenin C-monoglycosides. The physicochemical properties of the substances obtained differ from those given in the literature [9] for saponaretin and vitexin. The products of the acid hydrolysis of vaccarin have a higher specific rotation (see Table 1). In addition, the positive azo-coupling reaction [5] and the ratio of the intensities of the maxima [6] in the UV spectra of the zirconyl derivatives (see Table 2) shows that both compounds, like vaccarin itself, are 6-C-monoglycosides of apigenin. According to the rotational theory of the structure of C-glycosides of flavones developed by one of us (V. I. Litvinenko) [6], the substance with mp 255-257°C must be apigenin 6-C-syn-β-D-glucopyranoside and the compound with mp 230-231°C must be apigenin 6-C-anti-β-D-glucopyranoside. The investigations performed show that the structure of vaccarin can be represented as 4',5,7-trihydroxyflavone 6-C-[O-α-L-arabopyranosyl-(1→2)-β-D-glucopyranoside] 4'-O-β-D-glucopyranoside. The configurations of the glycosidic centers and the sizes of the oxide rings of the sugars are given provisionally.

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with KSK silica gel containing 5% of gypsum and the following solvent systems: 1) chloroform–methanol–water (65 : 35 : 8); 2) 15% acetic acid; and 3) butan-1-ol–acetic acid–water (4 : 1 : 5). The glycosides were revealed with a 25% solution of phosphotungstic acid and a 2% solution of zirconyl chloride in methanol. The UV spectra were obtained on a SF-4A instrument and the NMR spectra on a JNM-4H-100 MHz spectrometer (with HMDS as internal standard) by G. M. Bulgakov. The results of the analyses of all the compounds corresponded to the calculated figures.

Isolation of the Glycoside. A solution of 30.0 g of the combined extractive substances from the seeds of V. segetalis in 150 ml of water was deposited on a column of ÉDÉ-10P (OH form), and the column was washed with water to eliminate saponins. The flavonoids were eluted from the column with 3% acetic acid. The acetic acid eluates were combined and extracted with butanol. The butanol extracts were evaporated and the residue was transferred to a column of silica gel (300 g) and chromatographed in system 1 with monitoring by TLC on silica gel in the same system. The fractions containing the vaccarin were combined, evaporated, and recrystallized from aqueous ethanol. This gave a crystalline powder with mp 209-211°C, $[\alpha]_D^{20}$ −49° (c 0.1; dimethylformamide). Yield 0.20% on the weight of the seeds.

Vaccarin Dodeca-O-acetate. A mixture of 50 mg of the glycoside, 1 ml of pyridine, and 1 ml of acetic anhydride was kept at room temperature for 48 h. Then the mixture was poured into 100 ml of

cold water, and the precipitate that deposited was recrystallized from ethanol. Mp 151-152°C, $[\alpha]_D^{27}$ −23.6° (c 0.93; chloroform).

Acid Hydrolysis of Vaccarin (I) to (IV). A solution of 2.0 g of the substance in 50 ml of 10% hydrochloric acid in 50% ethanol was hydrolyzed at 100°C for 3.5 h. After the end of hydrolysis, two volumes of water (100 ml) and 20 ml of ethanol were added to the mixture. After cooling, the hydrolyzate was deposited on a column of polyamide sorbent and this was washed with water to a neutral reaction. Then the flavonoids were eluted with 20% ethanol, the separation process being monitored on paper in system 2. The anti form of compound (IV) separated in the first fractions, and a mixture of syn and anti forms in the subsequent ones. After evaporation of the first fractions and recrystallization, we obtained a substance with mp 230-231°C, $[\alpha]_D^{20}$ +21.5° [c 0.1; dimethylformamide−methanol (1:1)].

By fractional crystallization, the concentrated fractions containing the mixture of isomers yielded the syn form of compound (IV) with mp 255-257°C, $[\alpha]_D^{20}$ −43° [c 0.08; dimethylformamide−methanol (1:1)].

The aqueous fraction of the hydrolyzate was neutralized with ÉDÉ-10P (OH form) and evaporated to small volume (0.5 ml). Paper chromatography in several systems of solvents showed the presence of D-glucose and L-arabinose.

Acid Hydrolysis of Vaccarin (I) to (II). A mixture of 0.2 g of the glycoside and 20 ml of a 0.2% solution of hydrochloric acid in 50% ethanol was heated in the water bath for 1 h. Compounds (II) and (IV), glucose, and arabinose were found in the hydrolyzate by paper chromatography. On standing for several days, the diglycoside (II) deposited in the form of a lustrous yellow crystalline powder with mp 225°C, $[\alpha]_D^{20}$ −25° (c 0.1; dimethylformamide). On acid hydrolysis (2% sulfuric acid, 100°C, 3 h), compound (II) gave glucose and both forms of compound (IV).

The mother liquors after the separation of the (II) were neutralized and evaporated, and the anti form of compound (IV) was isolated by preparative PC in system 2.

Alkaline Hydrolysis of Vaccarin (I) to (III). A solution of 0.7 g of the substance in 50 ml of 0.5% aqueous caustic potash was heated in the water bath for 6 h. After cooling, the reaction mixture was neutralized with a dilute solution of sulfuric acid and extracted with butanol. The butanolic extracts were distilled, and the residue was chromatographed on a column of silica gel, with elution in system 1. Fractions containing the (III) were combined and evaporated. This gave a flavonoid with mp 199-202°C, $[\alpha]_D^{20}$ +42° (c 0.1; 70% methanol); on hydrolysis with 2% sulfuric acid, compound (III) yielded L-arabinose and a mixture of the two forms of (IV).

The nonaacetate of the bioside (III) had mp 153-155°C, $[\alpha]_D^{27}$ −58.8° (c 0.18; chloroform).

Periodate Oxidation of Vaccarin. The glycoside (I) (80 mg) was oxidized with a 1% solution of sodium metaperiodate. A blank experiment was performed in parallel. The consumption of periodate was determined by titration with a 0.1 N solution of sodium thiosulfate, and the formic acid liberated was titrated with 0.01 N caustic soda. Each mole of glycoside consumed 5 moles of sodium periodate and formed 2 moles of formic acid.

The amount of residual sugars was determined in a separate part of the reaction mixture after the destruction of the excess of sodium periodate and subsequent hydrolysis. No sugars were detected by paper chromatography in various systems.

Acid Hydrolysis of the Two Forms of Compound (IV). Each of the C-monoglycosides (the syn and anti forms of (IV)) (0.02 g) was hydrolyzed with Kiliani's mixture for 20 h in the water bath. The two forms gave the same aglycone with mp 343-345°C. On chromatography in system 2, the substance was identified as apigenin. Paper chromatography of the aqueous fraction of the hydrolyzate after neutralization with ÉDÉ-10P (OH form) showed the presence of D-glucose and a small spot of D-arabinose.

CONCLUSIONS

The structure of the flavone C-glycoside vaccarin, isolated from the seeds of Vaccaria segetalis (Neck.) Carcke, has been determined.

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