TRITERPENE SAPONINS FROM THE AERIAL PARTS OF *DIANTHUS CARYOPHYLLUS* VAR *REMONTANT* HORT.

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ABSTRACT

Triterpene saponins from the aerial parts of carnation (*Dianthus caryophyllus* var *remontant*) Hort. have been studied. Three gypsogenic acid glycosides including 3-*O*-glucopyranoside, 3,28-*O*-di-glucopyranoside and 3-*O*-glucopyranosyl,28-*O*-[glucopyranosyl(1 → 6)glucopyranoside] have been identified by means of LSI mass spectrometry and ¹H and ¹³C NMR. Inhibitory activities of isolated compounds against growth of the fungus *Trichoderma viride* and the growth of the roots of *Lepidium sativum* and *D. caryophyllus* seedlings were measured. None of the isolated compounds showed pronounced activity in *T. viride* test. Seedling root growth was affected severely at the presence of gypsogenic acid 3-*O*-glucopyranoside. Bidesmosidic form showed marginal stimulatory activity. The obtained data are discussed in relation to the activity of medicagenic acid 3-*O*-glucopyranoside, the compound differing just with 2-OH substitution from gypsogenic acid glycosides.

KEY WORDS: *Dianthus caryophyllus* var *remontant* Hort., carnation, triterpene glycosides, chemical structure, biological activity, allelopathy.

INTRODUCTION

The regress in plant growth, reduced resistance to diseases and lower quality of flowers can be observed when carnations are grown in a greenhouse on the seed bed planted previously to the same plant species (Gumnicka and Oleszek 1995). The reason for such a plant performance may be a "soil sickness" caused by the allelochemical introduced to the soil by leaching from the living plant or by liberating during decay of plant material. In the search of allelopathic principles present in *D. caryophyllus* aerial parts we were looking into one of the group of secondary metabolites such as saponins. Some groups of these compounds show strong allelopathic, antifungal and insecticidal activity (Oleszek et al. 1992, Oleszek 1993, 1995). To our best knowledge saponins in *D. caryophyllus* has not been researched and thus, the present work was undertaken to isolate, to elucidate the chemical structure and to evaluate a biological activity of some saponins from carnation aerial parts.

MATERIAL AND METHODS

Plant material

Plants of *Dianthus caryophyllus* var. *remontant* Hort. were grown in the greenhouse at University of Forestry and Wood

Technology, Lvov, Ukraine. Green aerial parts were collected, dried in the oven at 50°C and finely powdered.

General techniques

¹H and ¹³C NMR spectra in pyridine were taken with Bruker WH-250 Spektrospin and Bruker AMX-500 spectrometers. Negative ion LSI mass spectrometry was performed with Finnigan MAT 95 spectrometer with a glycerol as a matrix. Thin layer chromatography (TLC) was performed on a pre-coated silica gel (Merck, Si 60 F₂₅₄) plates. Saponins were detected by spraying with Liebermann-Burchard reagent followed by heating at 105°C for 3 min.

Extraction and isolation of saponins

Powdered material (200 g) was extracted in Soxhlet with chloroform to remove fats and pigments and then by boiling with 80% methanol. Methanol was evaporated and brown residue was dissolved in 25% methanol in distilled water, and the solution was loaded onto reverse phase C18 (5 x 10 cm) column. Column was washed with 200 ml of 30% methanol and then crude saponins were removed with methanol. Evaporation of the solvent yielded brown solid (4 g) which was redissolved in water and loaded on RP C18 (3 x 40 cm, 40 μm Merck). The column was washed with a linear gradient of

TABLE 1. ^{13}C NMR data of compounds 1-3.

C	1	2	3		1	2	3
1	35.6	35.7	35.6	C1'(Glc)	105.4	105.5	105.1
2	26.1	26.0	26.2	C2'	75.6	75.4	75.2
3	84.9	85.1	85.0	C3'	78.4	78.3	78.1
4	53.2	53.3	53.3	C4'	71.6	71.6	71.2
5	52.0	52.0	52.0	C5'	78.4	78.2	78.1
6	21.2	21.3	211.2	C6'	62.9	62.6	62.5
7	32.9	32.7	32.7	C1''		95.5	95.4
8	40.0	40.1	40.2	C2''		74.0	73.8
9	48.2	48.2	48.2	C3''		78.9	78.5
10	36.6	36.6	36.6	C4''		71.2	70.9
11	23.7*	23.6*	23.6*	C5''		79.0	78.5
12	122.3	122.3	122.4	C6''		62.3	69.1
13	144.8	144.7	144.7	C1'''			104.7
14	41.9	422.0	41.9	C2'''			74.9
15	28.2	28.1	28.1	C3'''			78.1
16	23.7*	23.6*	23.6*	C4'''			71.4
17	46.6	46.7	46.7	C5'''			78.1
18	42.1	42.0	42.0	C6'''			62.7
19	46.6	46.7	46.7				
20	30.9	30.8	30.8				
21	34.1	34.0	34.0				
22	33.2	33.4	33.4				
23	180.4	180.3	180.3				
24	12.7	12.6	12.6				
25	15.8	16.0	16.0				
26	17.2	17.2	17.2				
27	26.1	26.0	26.0				
28	180.1	176.4	176.5				
29	33.2	33.2	33.3				
30	23.7	23.7	23.7				

water and methanol. Fractions were collected with a fraction collector and analysed with TLC (chloroform-methanol-water; 63 : 32 : 5). Three of them, showing high concentrations of a one dominant component were further purified on Silica gel column chromatography (2.5 x 40 cm, Si 60 Merck) using n-BuOH saturated with H_2O (S1) or CHCl_3 -MeOH- H_2O (63 : 32 : 5) (S2) solvent systems. These way three single compounds were isolated with a following characteristics:

Compound 1: R_f : S1 = 0.53, S2 = 0.82; LSIMS (negative ion mode) m/z 647 $[\text{M}-\text{H}]^-$, 485 $[\text{M}-\text{hexose}-\text{H}]^-$, 423; ^1H NMR δ 0.88 (3H,s), 0.93 (3H,s), 0.98 (3H,s), 1.25 (6H,s), 1.58 (3H,s), 5.08 (1H, d, $J = 7$ Hz). For ^{13}C NMR see Table 1. Acid hydrolysis afforded aglycone and glucose as a sugar component (TLC).

Compound 2: R_f : S1 = 0.33, S2 = 0.64; LSIMS (negative ion mode) m/z 809 $[\text{M}-\text{H}]^-$, 647 $[\text{M}-\text{hexose}-\text{H}]^-$, 577, 485 $[\text{M}-2 \text{ hexoses}-\text{H}]^-$; ^1H NMR δ 0.88 (9H,s), 1.08 (3H,s), 1.24 (3H,s), 1.54 (3H,s), 5.08 (1H, d, $J = 7$), 5.42 (1H, br s), 6.29 (1H, d, $J = 7$ Hz). For ^{13}C NMR see Table 1. Alkaline hydrolysis afforded compound with TLC characteristics identical to 1 and acid hydrolysis yielded aglycone identical to that of compound 1 and glucose.

Compound 3: R_f : S1 = 0.11, S2 = 0.44; LSIMS (negative ion mode) m/z 971 $[\text{M}-\text{H}]^-$, 647 $[\text{M}-2 \text{ hexoses}-\text{H}]^-$, 537, 485 $[\text{M}-3 \text{ hexoses}-\text{H}]^-$; ^1H NMR 0.86 (9H,s), 1.09 (3H,s), 1.21 (3H,s), 1.56 (3H,s), 5.00 (1H, d, $J = 7$), 5.04 (1H, d, $J = 7$), 5.39 (1H, br, s). For ^{13}C NMR see Table 1. Alkaline hydrolysis afforded compound 1, acid hydrolysis gave glucose and aglycone identical to that of saponin 1.

Activity against *T. viride* fungus

Test were performed according to the procedure of Jurzysta (1979). Activity of compound 1 was compared to that of medicagenic acid 3-*O*-glucopyranoside, previously isolated from alfalfa roots (Oleszek et al. 1990).

Allelopathic activity

Activity of three isolated compounds and extracts from roots and aerial parts of carnation were tested together with 3-*O*-glucopyranoside of medicagenic acid against root seedling growth. Crest (*Lepidium sativum*) and carnation (*D. caryophyllus*) seeds were placed in Petri dishes lined with Whatman filter paper and moistened with the solutions of different concentrations of tested compounds. Seeds germinated in a distilled water were used as a control. After three days of germination the longest root was measured.

RESULTS AND DISCUSSION

Thin layer chromatography of carnation methanolic extracts showed a presence of several saponin components, strongly differing in their polarity. Combined column chromatography on reversed phase C18 followed by Silica gel separation afforded three pure dominant compounds, giving positive reaction with Liebermann-Burchard reagent. The structures were determined by a negative ion LSIMS spectra and ^1H , ^{13}C and ^{13}C DEPT NMR data (Table 1).

Compound 1 showed an $[\text{M}-\text{H}]^-$ ion at m/z 647 and a prominent fragment at 485, which were interpreted as a molecular ion and the aglycone part obtained by a cleavage of a hexose moiety. The hexose was identified as a glucose by means of TLC. This clearly showed 1 to be a monodesmoside possessing one glucose unit. The ^1H NMR spectrum of 1 exhibited six tertiary methyl groups and one anomeric hydrogen 5.08 (1H, d, $J = 7$ Hz). The ^{13}C NMR signals for the aglycone part of saponin were similar to that reported previously for 3-*O* substituted gypsogenic acid (Oshima et al. 1984). The presence of the β -glucose unit linked at the aglycone by an ether bond was suggested by the ^1H NMR (δ 5.08, 1H, d, $J = 7$ Hz, H-1') and by the ^{13}C NMR (δ 105.4) anomeric signals. Thus compound 1 was identified as the 3-*O*- β -D-glucopyranoside of gypsogenic acid (Fig. 1). The presence of this compound was previously reported in *Dianthus deltoides* (Bukharov et al. 1971) and was given a trivial name Diantoside A.

Compound 2 showed an $[\text{M}-\text{H}]^-$ ion at m/z 809 and prominent fragments at m/z 648 $[\text{M}-\text{H}-162]^-$ and 485 $[\text{M}-\text{H}-2 \text{ hexoses}]^-$, which correspond to the loss of one and two hexose units, respectively. Acid hydrolysis showed the presence of glucose, which suggested that two hexoses were both glucose units. Alkaline hydrolysis produced compound 1, indicating that one glucose unit was bound at the carboxyl group. This was further supported by the ^{13}C NMR anomeric signals (δ 105.5 and 95.5 ppm). One glucose unit was bound at carbon C3

- 1: R1 = Glc R2 = H
 2: R1 = Glc R2 = Glc
 3: R1 = Glc R2 = Glc(1→6)Glc

Fig. 1. Chemical formulas of isolated compounds.

(δ 105.5 ppm) by ether glycosidation and second one (δ 95.5 ppm) by ester linkage at C28. The other ^1H and ^{13}C NMR shift data were in agreement with those measured for compound **1** and remained in an agreement with the data reported by Oshima et al., (1984a) for 3,28-di-glucoside of gypsogenic acid, which was identified in *D. superbus* and was given a trivial name Dianoside A.

Compound **3** on acid hydrolysis furnished glucose and alkaline hydrolysis afforded compound **1**. This suggested that **3** is a bidesmoside with one glucose attached at the carbon 3C and second sugar chain attached to the carboxyl group. Its LSI mass spectrum showed intensive ion at m/z 971 $[\text{M-H}]^-$ and prominent fragment at 647 m/z $[\text{M-H-2 hexoses}]^-$ and 485 $[\text{M-H-3 hexoses}]^-$, indicating that **3** contains three glucose units, with ester linked chain composed of two glucoses. This was further supported by the presence of three anomeric carbons in ^{13}C NMR spectrum (δ 105.1, 95.4 and 104.7). The aglycone and sugar signals matched well to that in compounds **1** and **2** and were similar to the data presented by Oshima et al., (1984b) for the 3glucopyranosyl,28-gentobioside gypsogenic acid identified in *D. superbus*. This compound has been known under the trivial name of Azukisaponin IV isolated originally from *Vigna angularis* (Kitagawa et al. 1983). The presence of compounds **1**, **2** and **3** in *D. caryophyllus* have not been reported.

None of the isolated compounds showed inhibitory activity against fungus *Trichoderma viride*. Since the structure of

compound **1** differed from the medicagenic acid 3-*O*-glucoside just with the lack of -OH substitution at carbon C2 we deemed it of interest to compare antifungal activity of these two compounds (Fig. 2). It was shown that the lack of -OH at carbon C2 reduced antifungal activity to nearly zero level. This remains in a good agreement with a previous findings showing that blocking of the -OH groups in the medicagenic acid by acetylation led to a very significant lowering of antifungal activity (Oleszek et al. 1988). The present findings further support the importance of 2-OH functional group in determining the antifungal activity of triterpene saponins.

Two of the isolated compounds (**2** and **3**) showed no inhibitory activity against the growth of the roots of *Lepidium sativum* and *D. caryophyllus* seedlings (Table 2). Some stimulation of the growth at higher concentrations was observed. Strong inhibitory activity was expressed by the 3-*O*-glucopyranoside of gypsogenic acid (**1**) and the level of this activity was comparable to that showed by medicagenic acid 3-*O*-glucopyranoside; starting from the concentration of 100 ppm roots showed symptoms of browning and gradual decaying. This remains in a good agreement with a previous findings showing that generally all medicagenic acid glycosides, depending of sugar substitution showed divergent activities (Oleszek 1993).

There was some similarities found between plant growth inhibiting activities of medicagenic acid and gypsogenic acid glycosides. In both groups 3-*O*-glucopyranosides (monodesmosides) showed much higher activities than their bidesmosidic forms. However in the case of medicagenic acid glycosides, bidesmosidic saponins showed also pronounced activity, while for gypsogenic acid bidesmosides such activity was marginal. These results show, however, that saponins present in carnation may play an important allelopathic role in "soil sickness" in greenhouse monoculture. Since TLC of crude saponin extracts both from roots and tops showed over ten spots giving positive reaction with Liebermann-Burchard reagent it can be assumed that some of them have different aglycone e.g. dianic acid (29-hydroxygypsogenic acid), presence of which was reported in *D. superbus* (Oshima et al. 1984b). One extra hydroxyl group may like in medicagenic acid change activity substantially, so these saponins may show also some antifungal and plant growth inhibiting activities.

The data presented above disagree with analgesic and anti-hepatotoxic actions of saponins isolated from carnation. In these two tests gypsogenic acid glycosides showed higher activities than dianic acid glycosides. But the efficacy of substances in analgesic test was rather potentiated by increase in

TABLE 2. Root lenght (% control) of crest (*Lepidium sativum*) and carnation (*D. caryophyllus*) seedlings in the presence of saponins.

Saponin	Concentration (ppm)									
	50	100	250	500	1000	50	100	250	500	1000
	<i>Lepidium sativum</i>					<i>D. caryophyllus</i>				
1	103	77	26	14	12	83	54	27	23	na
2	97	105	108	104	100	106	100	111	103	105
3	102	106	112	140	157	114	126	128	126	110
Med. acid*	97	44	23	15	11	64	51	32	18	na
Aerial**	102	100	96	75	47	99	52	83	75	52
Roots***	115	108	80	42	36	120	107	61	47	28

* Medicagenic acid 3-*O*-glucopyranoside; ** Crude saponin mixture from the aerial parts of carnation; *** Crude saponin mixture from the roots of carnation; na – not analysed.

Fig. 2. Inhibition of the growth of a colony of *Trichoderma viride* fungus in the presence of medicagenic acid 3-*O*-glucopyranoside (Med. acid) and gypsogenic acid 3-*O*-glucopyranoside (1).

their hydrophilic character, while in the antihepatotoxic test activity decreased as the number of hydroxyl groups increased (Hikino et al. 1984). It seems that different activities are based on divergent mechanisms of action and particular tests can not be generalised for overall activity of particular triterpene saponins. More research is needed to draw some general conclusion and further work must be done to document to what degree saponins may play an allelopathic function in carnation production in greenhouse.

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SAPONINY TRITERPENOWE Z CZĘŚCI NADZIEMNYCH *DIANTHUS CARYOPHYLLUS* VAR. *REMONTANT* HORT.

STRESZCZENIE

Badano skład chemiczny saponin z części nadziemnych *Dianthus caryophyllus* var. *remontant*. Wyodrębniono i zidentyfikowano metodami spektralnymi LSIMS oraz ^1H i ^{13}C NMR trzy glikozydy kwasu gipsogenowego, w tym 3-*O*-glukopiranozyd, 3,28-*O*-di-glukopiranozyd i 3-*O*-glukopiranozylo,28-*O*-[glukopiranozylo(1 $\rightarrow$ 6)glukopiranozyd]. Zbadano aktywność wyodrębnionych związków w stosunku do *Trichoderma viride* i korzeni siewek *Lepidium sativum* i *D. caryophyllus*. Żaden z wyodrębnionych związków nie wykazywał aktywności w stosunku do *T. viride*. Wzrost korzeni siewek był silnie hamowany jedynie w obecności 3-*O*-glukopiranozydu kwasu gipsogenowego. Bidesmozydy tego kwasu wykazywały zdolność do niewielkiej stymulacji wzrostu korzeni siewek. Otrzymane wyniki są dyskutowane w odniesieniu do aktywności 3-*O*-glukopiranozydu kwasu medikagenowego, związku różniącego się od monodesmozydu kwasu gipsogenowego jedynie obecnością grupy OH przy atomie węgla C3 aglikonu.

SŁOWA KLUCZOWE: *Dianthus caryophyllus* var. *remontant* Hort., goździk, glikozydy triterpenowe, saponiny, budowa chemiczna, aktywność biologiczna, allelopatia.

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