SAPONIN ACTIVITY OF DIFFERENT PARTS OF ALFALFA SEEDLINGS

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Received October 20, 1984

Summary

The saponin content of alfalfa of different age was investigated by thin-layer-chromatographic densitometric method and by a biological method, by the *Trichoderma*-bioassay.

With the help of the *Trichoderma*-bioassay the highest level of saponin was determined in the cotyledons of the alfalfa seedlings, independently of the age of the seedlings.

Comparing the results obtained by the two methods it was found that the fungistatic activity or toxicity of the saponins of the cotyledons are more intensive than that of the leaves of developed plants.

Introduction

The presence of saponins in alfalfa has been recently reported (1). The main saponin constituent, the glucoside of medicagenic acid is a triterpene.

As aglucon, hederagenin occurs, too. Soyasapogenols could also be detected in smaller amounts in alfalfa (2).

Alfalfa saponins have haemolytic activity and toxic effect on animals (ruminants and poultry). Several fungi and germinating seeds are also sensitive to saponin (3).

Toxicity of extracted saponin to insects and plant pathogens suggests that it may be involved in the resistance mechanisms. This resistance depends on the age of the plant and the location where saponin is stored (4).

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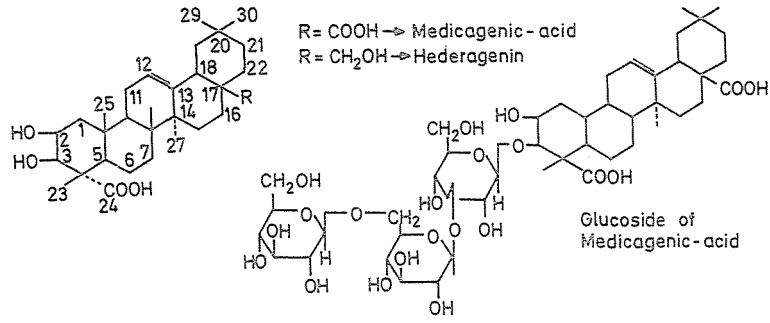

Fig. 1. The main saponin components of alfalfa

Materials and methods

The saponin content of alfalfa of different age was investigated by biological and thin-layer-chromatographic methods.

Alfalfa seedlings were grown in the light at 25 °C and 65% humidity during 42 days. The samples were gathered from the 7, 14 and 42 day-old seedlings, separately from the cotyledons, from primary leaves and from the consecutive leaves.

To extract the saponins we used hot methanol. After 3 repeated extraction of the wet leaves, the methanolic extracts were collected and the water traces removed with aceton. The extracts were evaporated to the same concentration referring to the dry leaf weight.

Thin-layer chromatography was used in two different solvent systems:
1. chloroform : methanol : water = 70 : 30 : 4.5 and
2. ethylacetate : acetic acid : water = 7 : 2 : 2 (5, 6).

For the detection of saponins we used a saturated solution of $SbCl_3$ in chloroform, or a 5% $Ce(SO_4)_2$ solution in a 20% aqueous H_2SO_4.

The quantitative evaluation of the chromatograms was performed after spraying the plates with the $SbCl_3/CHCl_3$ spray reagent and heating them at 100 °C for 5 min. The relative saponin content of the different leaves was measured by a scanning densitometer at 550 nm.

Trichoderma-bioassay was used for the determination of the toxicity of the saponin extracts from alfalfa leaves (7, 8). The agar-plates were inoculated with a spore suspension of *Trichoderma viride* G and thermostated in the dark at 30 °C. Diameters of the large colonies—growing on the plates—were measured after 48 and 60 hours. Calibration curves were obtained against standard alfalfa saponin extract. From each sample 3–5 different saponin concentrations, regarded as parallels, were measured.

Results

With the help of the *Trichoderma*-biossay the highest level of saponin was determined in the cotyledons, independent of the age of seedlings, whereas primary leaves contained smaller, but increasing concentration of toxic compunds. Table I shows the saponin activities measured by *Trichoderma*-bioassay.

Saponins from the cotyledons showed the highest fungistatic activity and the highest efficacy; their toxic effect decreased with dilution less than that of the standard alfalfa saponin or the saponins of the primary or normal leaves.

Table I
Saponin activity of alfalfa leaves measured by *Trichoderma viride*-bioassay

Alfalfa leaves		Saponin content mg/g d.w.
7 day old cotyledons	C7	6.4 ± 0.86
14 day old cotyledons	C14	6.3 ± 0.72
14 day old primary leaves	P14	2.2 ± 0.26
42 day old cotyledons	C42	6.4 ± 0.70
42 day old primary leaves	P42	4.9 ± 0.28
42 day old leaves	B42	1.0 ± 0.15

The same samples were analysed by TLC and determined densitometrically. Table II shows the relative percentage measured by the bioassay and TLC.

Table II
Relative saponin content of alfalfa leaves as measured by bioassay and TLC

Leaf sample	Saponin content (rel %)	
	TLC	Bioassay
C7	310	640
C14	225	630
P14	110	220
C42	247	640
P42	100	490
B42	100	100

Comparing the results obtained by the two methods we found that the fungistatic activity of the cotyledons is more intensive than that of the leaves of developed plants. This phenomenon is emphasized by the higher resistance of cotyledons to moulds.

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