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Lack of mutagenic activity of saponins in the Ames test

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Alfalfa saponins are multicomponent mixtures of oligosides of medicagenic acid, hederagenin and soyasapogenol B [1]. Chemical structures of the main glycosides of alfalfa saponins have been recently established [2 - 5]. Medicagenic acid glucosides but not Soyasaponin I, the major glucoside of soyasapogenol B show high haemolytic [6], allelopatic [7] and, especially, antifungal [2] activities. These activities characterize also hederagenin glycosides [3] which however occur in alfalfa saponins at extremely low concentration.

Antifungal activity has been initially demonstrated for medicagenic acid 3-*O*-glucoside [8, 9], and then for medicagenic acid and its natural glucosides and other derivatives [10, 11]. These findings suggested that these compounds could be used as active agents in the treatment of mycotic infections. Significant antimitotic [12] and cancerostatic actions [13] of several saponins as well as their antimutagenic activity towards B/a/P [14] have also been reported.

Limited absorption of cholesterol and bile salts in gut lumen and modification of cholesterol metabolism [15] represent another biological activity of saponins. These effects are attributed to formation of stable complexes between the saponins containing medicagenic acid and cholesterol [1, 16].

The above observations suggest that at least some alfalfa saponins could be used in medical treatment. Therefore, their possible mutagenic activity has to be precisely monitored. The aim of the present study was to estimate the mutagenic potential of the purified alfalfa saponins: medicagenic acid, medicagenic acid 3-*O*-β-D-glucopyranoside and Soyasaponin I.

Medicagenic acid and medicagenic acid 3-*O*-glucopyranoside were isolated from alfalfa roots and Soyasaponin I was obtained from clover *Trifolium incarnatum* seeds, in the Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation

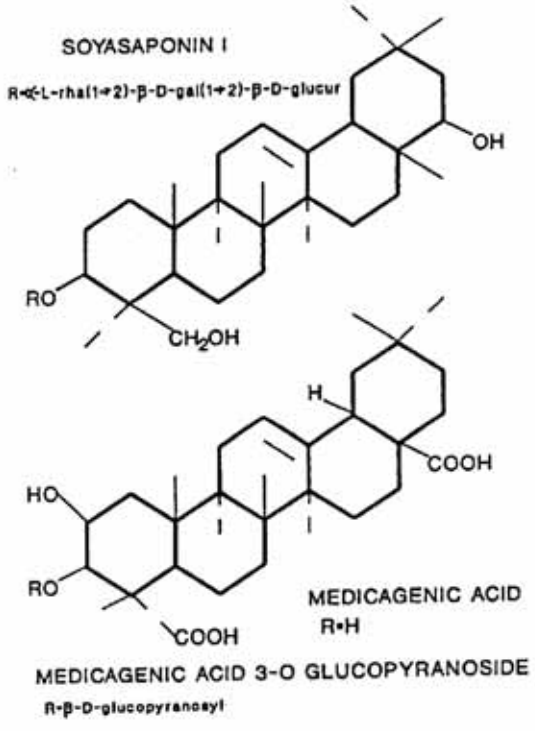

Fig. 1. Chemical structures of saponins

[1]Abbreviations: 2AF, 2-aminofluorene; MMC, mitomycin C; MMS, methyl methanesulfonate; 4-NQO, 4-nitroquinoline-*N*-oxide

Table 1

Revertant colony counts obtained using various strains of Salmonella typhimurium

Compound	Dose µg/plate		Number of revertants/plate ± S.D. [a]			
			TA97	TA98	TA100	TA102
Control			175 ± 24	38 ± 4	169 ± 25	291 ± 30
Medicagenic acid		−S9				
	50		183 ± 17	31 ± 4	151 ± 31	208 ± 31
	100		182 ± 41	41 ± 6	183 ± 42	198 ± 19
	200		203 ± 23	35 ± 3	193 ± 43	187 ± 26
		+S9				
	50		156 ± 16	28 ± 8	146 ± 9	204 ± 11
	100		146 ± 14	28 ± 6	135 ± 30	187 ± 19
	200		130 ± 10	30 ± 2	118 ± 23	197 ± 26
Medicagenic acid 3-*O*-β-D glucopyranoside		−S9				
	50		159 ± 33	28 ± 5	198 ± 19	344 ± 14
	100		132 ± 37	31 ± 7	186 ± 37	358 ± 16
	200		151 ± 38	36 ± 7	197 ± 50	326 ± 73
		+S9				
	50		160 ± 35	29 ± 8	169 ± 23	350 ± 26
	100		146 ± 27	31 ±10	178 ± 23	356 ± 28
	200		180 ± 24	25 ± 4	185 ± 13	297 ± 30
Soyasaponin I		−S9				
	50		117 ± 23	23 ± 9	106 ± 16	233 ± 29
	100		128 ± 33	29 ± 2	98 ± 21	296 ± 69
	250		119 ± 8	21 ± 3	120 ± 13	234 ± 47
	500		126 ± 31	18 ± 4	123 ± 11	230 ± 56
		+S9				
	50		165 ± 17	51 ± 6	126 ± 32	242 ± 22
	100		180 ± 38	56 ± 2	120 ± 12	215 ± 21
	250		155 ± 34	54 ± 3	112 ± 25	241 ± 21
	500		135 ± 34	41 ± 5	117 ± 26	189 ± 28

[a] The mutagenicity assays were carried out in triplicate, and the number of *his*$^+$ revertants was scored after incubation for 48 h at 37°C. The number of revertants per plate is an average number from at least 5 separate experiments ± S.D.

−S9, without metabolic activation; +S9, with metabolic activation (50 µl S9/plate). S9 was from Aroclor 1254-pretreated rat liver.

Positive controls: TA97 and TA98 without S9: 4-NQO (10 µg/plate) 920 ± 65 and 480 ± 50, respectively; with S9: 2-AF (10 µg/plate) 1520 ± 80 and 5380 ± 72, respectively.

TA100 without S9: MMS (1 µg/plate) 2450 ± 120; with S9: 2-AF (10 µg/plate) 2970 ± 150. TA102 without S9: mitomycin C (0.5 µg/plate) 6450 ± 120.

in Puławy (Poland). The structures of the compounds are shown in Fig. 1. The mutagenicity of isolated saponins was tested by the Ames method with *S. typhimurium* strains TA97, TA98, TA100 and TA102 with and without metabolic activation (S9 fraction). All strains were routinely checked for efficiency as recommended by Ames *et al.* [17, 18]. Positive mutagenesis controls with MMS[1] for TA100, MMC for TA102, 4-NQO and 2-AF for both TA97 and TA98 were also included. Liver microsomal fraction S9 was prepared from rats treated with Aroclor 1254 as described by Ames *et al.* [17, 18]. Fraction S9 was stored at –20°C and served as the source of soluble microsomal enzymes. The average concentration of protein in the S9 fraction was 38 mg/ml (36 - 42 mg/ml). Protein was determined according to Lowry *et al.* [19].

Studies on the mutagenic activity of alfalfa saponins were performed at their non-toxic concentrations, i.e. for Soyasaponin I up to 500 μg/plate, whereas for medicagenic acid and medicagenic acid 3-*O*-glucopyranoside up to 200 μg/plate (Table 1), due to a higher toxicity of both the latter compounds to bacteria.

None of saponins tested increased the number of *his*$^+$ revertants in strains TA97, TA98, TA100 or TA102 of *S. typhimurium*, either in the absence or in the presence of S9 fraction from rat liver (Table 1). Thus, according to the Ames criterion Soyasaponin I, medicagenic acid or glucoside of medicagenic acid were not mutagenic.

Bearing in mind a wide spectrum of biological activities of saponins and lack of mutagenic effects even at high concentrations (this report) one could expect that they might be of use in medicine.

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