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Triterpene saponins of *Trigonella monspeliaca* L.

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Abstract

The triterpene saponin fraction was isolated from *Trigonella monspeliaca* tops. It consisted of ten saponosides, three of which were determined to be medicagenic acid glycosides, the other seven — soyasapogenol glucosides. Acid hydrolysis of the saponin fraction yielded medicagenic acid, soyasapogenol B and its artifacts soyasapogenol C, D and F. These data consitute the first report on the occurence of medicagenic acid and soyasapogenol glycosides in the genus *Trigonella*.

Key words: Trigonella monspeliaca, saponins, soyasapogenols, medicagenic acid, isolation

INTRODUCTION

The genus *Trigonella* has been closely searched for the presence of steroid saponins mainly because of the usefulness of those compounds in steroid hormone drug production (Hardman and Fazli 1972, Bogacheva et al. 1976, Jain et al. 1977, Zambo and Szilagyi 1982). However, we could not find any papers in the available literature on the chemical structure of triterpene saponins in species of the genus *Trigonella*. For this reason the present paper describes the isolation and chemical characterization of triterpene glycosides of *Trigonella monspeliaca*.

MATERIAL AND METHODS

PLANT MATERIAL

The seeds of *Trigonella monspeliaca* were obtained from the Central Institute for Genetic and Crop Plant Research in Gatersleben, East Germany. The plant material was collected in the summer of 1985 from field-grown plants.

ISOLATION OF SAPONINS

Leaves and stems (110 g) were dried, powdered and defatted with chloroform then exhaustively extracted with 96% ethanol. The extract was concentrated under reduced pressure, 1 dm^3 of H_2O was added and saponins extracted into n-butanol. The butanol was evaporated in vacuo yielding 6 g of crude saponins (CS).

ISOLATION OF SAPOGENINS

5 g of CS were dissolved in 100 cm^3 of 2N HCl in 50% methanol and refluxed (4 h) to yield a mixture of sapogenins and prosapogenins (1.5 g). They were dried and extracted (Soxhlet) with chloroform for 24 h, and the chloroform fraction extracted with 5% NaOH. The basic fraction (BF) was saved and the chloroform fraction dried giving 670 mg of soyasapogenol mixture (SA). The residue remaining after chloroform extraction was further hydrolysed in the same manner as above for 36 h. Next, two volumes of water were added and the precipitate filtered, disolved in 5% NaOH and combined with the BF fraction. These combined fractions were extracted with ethyl acetate to remove some impurities. They were then acidified with HCl and the sapogenins extracted into ethyl acetate. This afforded 300 mg of crude medicagenic acid (CMa).

PURIFICATION OF AGLYCONES

The CMa was purified on a Si gel column (10 g) using ethyl acetate as the eluant. After evaporation of the solvent, medicagenic acid was crystallized from a dioxan-water mixture, yielding 20 mg of crystalline compound mp. 349-350°C (lit. (Boar and Allen 1973) 349-350°C); MS m/z (rel. int.) 502(3), 487(1), 457(3), 456(7), 253(9), 248(100), 235(10), 233(11), 203(88), 189(24), 133(26).

10 mg of this compound were dissolved in 2 cm^3 of C_5H_5N and 2 cm^3 of acetic anhydride were added. This mixture was heated (90°C, reflux) for 3 h. Then 10 cm^3 of water were added and extracted with ethyl acetate. Evaporation of solvent furnished the diacetyl derivative of medicagenic acid, white mp. 211-212°C, MS m/z 586(1), 543(4), 541(7), 526(2), 466(1), 248(99), 203(89), 189(29), 133(25), 253(22), 43(100).

Soyasapogenols were confirmed by comparing their R_f values (sapogenins and their acetyl derivatives) with these of authentic samples.

The melting points of the isolated compounds are uncorrected. EIMS were taken with a LKB 9000 spectrometer (70 eV).

THIN LAYER CHROMATOGRAPHY

2D-TLC of the CS was carried out on Si gel (Merck 60) using the following solvents: S_1: ethyl ecetate-acetic acid-water (7:2:2), S_2: n-butanol--acetic acid-water (4:1:1). Spots were visualized with Liebermann-Burchard reagent (Van Atta and Guggolz 1958) or by covering the plates with a gelatine-blood suspension.

Sapogenins were examined using S_3: petroleum benzin-acetic acid-chloroform (7:1:2) and S_4: benzene-methanol (92:8), their acetyl derivatives using S_5: hexane-benzene-acetone (50:45:5) and S_6: hexane-diethyl ether (65:35) (Jurzysta and Jurzysta 1979).

RESULTS AND DISCUSSION

The ethanol extracts of leaves and stems of *Trigonella monspeliaca* afforded a crude saponin fraction with a yield of 5% DM. As revealed by 2D-TLC, this fraction was comprised of ten dominant individual glycosides (Table 1). On the basis of the colour development of spots and their haemolytic activity three of them were found to be medicagenic acid glycosides. The seven remaining compounds possessed soyasapogenols in the genin parts.

This finding was further supported by the chemical investigation of the hydrolysis products in which several aglycones were indentified. One of them

Table 1

2D-TLC characteristics of *Trigonella monspeliaca* top saponins

Spot number	R_f value in solvent		Relative amount*	Colour after visualization with Liebermann--Burchard reagent		Haemolysis
	S_1	S_2		natural light	UV	
1	0.37	0.40	+	violet	pink	−
2	0.33	0.37	+	„	„	−
3	0.24	0.33	++	green	green	+
4	0.33	0.31	++	brown	pink-bricky	−
5	0.30	0.30	+++	„	„	−
6	0.24	0.28	+	„	„	−
7	0.27	0.24	++++	„	„	−
8	0.23	0.23	+	„	„	−
9	0.17	0.22	+++	green	green	+
10	0.11	0.15	+++	„	„	+

* Estimated by visual examination on TLC plates.

was isolated in crystalline form and on the basis of its melting point and the R_f and EIMS data of its natural and acetylated forms, was confirmed to be medicagenic acid. The occurrence of soyasapogenols in hydrolysis products was confirmed by thin layer chromatography. Acetylated and nonacetylated SA were chromatographed together with the appropriate standards of soyasapogenols and their acetyl derivatives. In this way, soyasapogenol B and its artifacts soyasapogenols C, D and F (Jurzysta 1984) were identified.

Thus, it was clearly proven that *Trigonella monspeliaca* triterpene saponins are the mixture of soyasapogenol and medicagenic acid glycosides. They seem to be qualitatively very similar to the saponin fractions of *Medicago media, Medicago falcata, Medicago sativa* (Jurzysta 1982) and *Medicago lupulina* (Górski et al. 1984) tops, which contained medicagenic acid, hederagenine and soyasapogenol glycosides.

Saponins are widely distributed in the plant kingdom. They have been found in at least 400 species belonging to more than 80 families (Birk 1969, Oakenfull 1981). However, in spite of the wide occurrence of saponins medicagenic acid glycosides appear quite seldom. So far they have been found in numerous species of the genus *Medicago* (*Papilionaceae*) (Jurzysta and Nowacki 1979), in two species of the genus *Herniaria* (*Caryophyllaceae*) (Bukharov and Shcherbak 1970, Klein et al. 1982) and in *Castanospermum australe* (*Papilionaceae*) (Hiller and Voigt 1977). There has been hitherto no data published on their occurrence in the genus *Trigonella*. Thus, our present paper is the first report on a new source of medicagenic acid glycosides in the plant kingdom.

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Saponiny trójterpenowe z Trigonella monspeliaca L.

Streszczenie

Z części nadziemnych *Trigonella monspeliaca* wyizolowano frakcję saponinową składającą się z dziesięciu dominujących składników glikozydowych. Trzy z nich były glikozydami kwasu medikagenowego a siedem pozostałych — glikozydami sojasapogenoli. Z hydrolizatu tej frakcji wyizolowano w postaci krystalicznej kwas medikagenowy a za pomocą TLC potwierdzono obecność sojasapogenoli B, C, D i F. Prezentowane dane są pierwszym doniesieniem dotyczącym występowania glikozydów kwasu medikagenowego i sojasapogenoli w rodzaju *Trigonella*.