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Calcium-Magnesium salt of saponins from bird's foot trefoil seeds (*Lotus corniculatus* L.)

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Abstract

A calcium-magnesium salt of saponins was isolated from bird's foot trefoil seeds with a yield of 0.04%. The salt contained: 1.37% — Ca and 0.04% — Mg. The saponin salts were deionized on IR-120 Amberlite and free crystalline saponin was obtained. In both cases the presence of one major component and of three trace ones was found, using TLC. In acid hydrolysates of the saponin salts and of the free saponin the following sugars were identified: rhamnose, xylose, glucose, galactose, glucuronic acid. From among aglycones soyasapogenols B, C, traces of E, and of one unidentified in both cases were found. The Ca-Mg salt obtained seems to be a natural form of saponins in bird's foot trefoil seeds.

INTRODUCTION

In our previous report we found saponins to be present in bird's foot trefoil seeds (Jurzysta et al., 1973). The objective of the this paper is isolation and chemical characterization of the natural form in which these saponins occur.

EXPERIMENTAL

Isolation of saponins

1 kg of ground bird's foot trefoil seeds of cv. Skrzeszowicka were defatted with petroleum ether in a Soxhlet apparatus. 330 g portions were extracted 5 times with 1 l of 90% ethanol in water bath for an hour. The combined extracts were concentrated in vacuum until all alcohol

was removed. The water residue was first several times extracted with 250 ml portions of diethyl ether and then a few times with 250 ml portions of ethyl acetate in a separatory funnel. The petroleum-, diethyl ether, and ethyl acetate extracts were discarded. The aqueous layer was kept in a refrigerator for three weeks. A white shimmering precipitate S crystallised spontaneously in the aqueous layer. The precipitate was centrifuged and washed several times with water, and finally with acetone.

Ash

Precipitate S (100 mg) was burned and the ash was weighed, and analysed in Absorption Spectrophotometer UNICAM SP-90 for Ca and Mg.

Amberlite IR-120 treated saponins

Precipitate S (100 mg) was dissolved in 100 ml 50% ethanol and poured through a 10 × 1,5 cm column with Amberlite. Amberlite IR-120 (Serva) was in H form. The filtrate was concentrated in a vacuum rotary evaporator to remove all alcohol. A white precipitate C crystallyzed in the aqueous residue. Precipitate C was centrifuged and crystallyzed two times from water-ethanol solution.

Chromatography of saponins

The 4 μl of 2% water-ethanol solution of precipitates S and C were applied to plates of Silica gel G (Merck) and developed in ethyl acetate — acetic acid — water (3 : 1 : 3) and n-butanol — acetic acid — water (4 : 1 : : 5) solvent systems. The developed plates were dried for 30 min at 110° C, sprayed with Liebermann-Burchard reagent (Wilson et al., 1967) and heated for 5 min at 110° C.

Acid hydrolysis of saponins

Precipitates S and C 50 mg each, were hydrolysed for 5 hr. in H_2SO_4 in dioxan-water solution (Shany et al., 1970). Aglycones were precipitated with water, filtered, washed with water, and dried. The filtrate containing sugars was neutralised with $BaCO_3$.

Identification of the aglycones

The aglycones and soyasapogenol standards were chromatographed on Silica gel G plates. Chromatogrames were developed in the following solvent systems: petroleum ether—chloroform—acetic acid (7 : 2 : 1) (Shany et al., 1970), and benzene — 96% ethanol (92 : 8). The chromatogrames were detected in the same way as for the saponins.

Identification of carbohydrate components

The neutralised hydrolysate (20 ml) was evaporated until dry, and dissolved in 0.4 ml of 10% isopropanol. The solution (10 μl) and sugar standarts were applied to Schleicher and Schüll 2043b paper strips. The chromatogrames were developed three times in the following solvent system: n-butonal—pyridine—water—benzene (5 : 3 : 3 : 1) (De Whalley et al., 1951). For the detection ofthe sugars ammonium silver nitrate was used (Trevelyan et al., 1950).

RESULTS AND DISCUSSION

White fine crystalline precipitate S (400 mg) was obtained with a yield of 0.04% dry weight of bird's foot trefoil seeds. The precipitate was sparingly soluble in water, and unsoluble in non-polar solvents. It gave red colour with sulfuric acid which is characteristic of triterpenoids. The precipitate had m. p. of 261—265° C and it contained 3.7% ash, 1.37% Ca, and 0.04% Mg. The compound obtained was subjected to acid hydrolysis and analysed for sugars and aglycones using TLC. From among sugars there were identified: rhamnose, xylose, glucose, galactose and glucouronic acid (Fig. 1) Among aglycones there were found: soyasapogenols B, C, traces of E and one aglycon unidentified (Fig. 2).

For deionization a water-ethanol solution of saponin salts was poured through an ionite column. The free saponin (precipitate C) was obtained from a concentration filtrate with a yield equivalent to 90% weight of the salt. The free saponin was readily dissolved in polar solvents and sparingly soluble in water. The free saponin readily crystallysed from water-ethanol solution in the form of white needle-shaped crystals. It melted at 228—231° C.

The free saponins content the same sugars and aglycones as their salts. The free saponin and the salts were chromatographed using TLC. In both cases the presence of one major component, Rf 0.22) and of three traces ones *Rf* 0.17; 0.23; 0.26 in n-butanol—acetic acid—water) (4 : 5 : 1) was established.

The results obtained give evidence that bird's foot trefoil seed sapoins occur in the form of calcium-magnesium salt.

Saponins of the same form were isolated (Walter et al., 1955) from Ladino clover and alfalfa using a similar procedure. The saponin isolated from bird's foot trefoil seeds shows some similarity to that obtained by Walter. It differs from the Walter's saponin by presence of an unidentified aglycon X, traces amounts soyasapogenol E in the place of A, and by a higher melting point.

Fig. 1. Paper chromatogram of sugars developed three times with n-butonal—pyridine—water—benzene (5 : 3 : 3 : 1) and detected with ammonium silver nitrate

I — Sugar components of hydrolysate of the saponin salt.

II — Sugar standarts of rhamnose, (1), xylose (2), glucose (3), galactose (4), glucouronic acid (5).

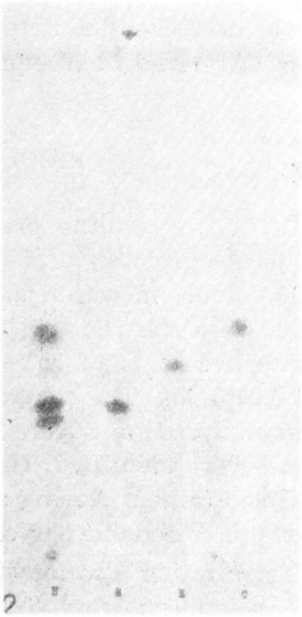

Fig. 2. Thin layer chromatogram of aglycones developed with benzene — — 96% ethanol (92 : 8), detected with Lieberman-Burchard reagent

H — aglycone components of the saponin salt hydrolysate.

B — soyasapogenol B, E — syasapogenol E, C — soyasapogenol C.

The crystalline saponin isolated from bird's foot trefoil seeds by lead acetate method (Jurzysta et al., 1973), appeard to be identical with the free saponin obtained now. Obtaining the free saponin in the lead acetate method was probably due to treating the saponin salts with lead acetate and H_2S, which caused disintegration of the natural linkage of the saponin with Ca and Mg.

The results obtained previously and those reported now suggest that the calcium-magnesium salt is the natural and probably the only form of saponins in bird's foot trefoil seeds.

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Sól wapniowo-magnezowa saponiny z nasion komonicy rożkowej (Lotus corniculatus L.)

Streszczenie

Z nasion komonicy uzyskano sól wapniowo-magnezową saponiny z wydajnością 0.04%. Sól saponiny zawierała 1.37% Ca i 0.04% Mg. Sól saponiny poddano dejonizacji na jonicie IR-120 Amberlite, otrzymując wolną saponinę w stanie krystalicznym. Metodą chromatografii cienkowarstwowej stwierdzono w obu wypadkach obecność jednego składnika głównego oraz trzech śladowych. W kwaśnych hydrolizatach soli saponinowych i wolnych saponin nie stwierdzono różnic. W obu wypadkach zidentyfikowano cukry: ramnozę, ksylozę, glukozę, galaktozę, kw. glukouronowy; oraz aglikony: sojasapogenole B, C, ślady E i jeden aglikon nie zidentyfikowany. Wydaje się, że otrzymana sól Ca-Mg jest naturalną formą saponiny w nasionach komonicy.