

Isolation and chemical characterization of saponins from lucerne seeds (*Medicago media* Pers.)

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(Received: September 27, 1972)

Abstract

Saponins of lucerne seeds have been prepared by two different methods. It was found by thin-layer chromatography that these glycosides consist of at least four components with one dominant. Soyasapogenols B, C and E were identified in acid hydrolysate of these saponins and in carbohydrate moiety glucose, galactose, rhamnose, small quantity of arabinose and xylose and glucuronic acid. It was established that saponins of lucerne seeds do not haemolyse red blood cells, contrary to top, root and blossom saponins. That may be due to the lack of medicagenic acid glycosides.

INTRODUCTION

Isolation and chemical characterisation of saponins found in lucerne tops (Walter et al., 1954; Lindahl et al., 1957; Shany et al., 1970), roots (Morris et al., 1961; Shany et al., 1970) and flowers (Morris and Hussey, 1965) have been reported but lucerne seed saponins have so far not been investigated, and are the object of the present work.

EXPERIMENTAL

Preparation of lucerne saponin extract

1 kg of ground lucerne seeds of Miechowska variety were defatted with ethyl ether and extracted 5 times with 3-litre portions of boiling methanol for 2 hours. The combined extracts were concentrated in vacuo to 250 ml. Grey and green precipitate obtained was discarded and crude saponin solution was purified with lead acetate or cholesterol.

Purification of saponins

Lead acetate method. 500 ml of crude saponin extract was concentrated to about 250 ml, diluted twofold with water and 80 ml 1 N lead acetate solution was added. The precipitate was filtered off, washed with water, dispersed in 500 ml methanol and H_2S was passed through it. The PbS precipitate was discarded and the filtrate was evaporated in vacuo. The obtained saponins were dried at 70°C .

Cholesterol method (Walter et al. 1954). 500 ml of crude saponin extract was concentrated in vacuo to a sirup, diluted with 500 ml of water and 40 g powdered cholesterol was added. The mixture was kept for one hour on a boiling water bath and left to stand overnight at room temperature. The precipitate was filtered, washed with water until the filtrate was colourless and dried for 24 hours at 40°C . Dried precipitate was dissolved in 50 ml anhydrous pyridine and a tenfold amount of ether was added. Saponins were filtered, washed with ether and dried in vacuo at 40°C .

Chromatography of saponins

Paper chromatography. 20 μl portions of 2% saponin solution were applied on Whatman No. 1 paper strips. The chromatograms were developed by the ascending technique along 40 cm in two solvent systems: n-butanol-ethanol-25% ammonia (10:2:5), (Khorlin et al. 1963); n-butanol-pyridine-water-benzene (5:3:3:1), upper phase. Then the chromatograms were dried for 30 min. at 100°C and detected with the following tests: 20% SbCl_3 in chloroform (Coulson, 1958), 25% phosphotungstic acid (Pasich, 1958), Liebermann-Burchard reagent (Van Atta and Guggolz, 1958).

Thin-layer chromatography. Plates with a 0.3 mm layer of Silica gel G acc. to Stahl were activated for one hour at 110°C . 3 μl of 2% saponin solution were applied and developed on the distance of 16 cm in the system n-butanol-pyridine-water (3:1:1). The developed plates were dried for 30 min. at 110° and detected with a mixture of conc. sulphuric acid-acetic anhydride-anhydr. ethanol (1:1:10), (Wilson et al., 1967) and heated for 5 min. at 110°C .

Acid hydrolysis of saponins

Saponins (200 mg) were dissolved in 100 ml of methanol and 200 ml of 7% HCl was added. The solution was heated on a boiling water bath for 18 hours. The precipitated aglycones were filtered, washed with water until neutral washing and dried. The filtrate containing carbohydrates was neutralised with Ag_2CO_3 .

Identification of the aglycones

Aglycones were analysed by thin-layer chromatography already described for saponins with the use of soyasapogenols and medicagenic acid as markers. Authentic soyasapogenols A, B, C and D were obtained from Prof. Dr. O. Jeger from Zürich. Soyasapogenol E was separated from soybeans (Wilner et al. 1964) and medicagenic acid from lucerne roots (Morris et al. 1961). Chromatograms were developed in the following solvent systems: petroleum ether—chloroform—acetic acid (7:2:1), (Shany et al. 1970); di-isopropyl ether—acetone (5:2) acidified with two drops of acetic acid (Gestetner et al. 1970). For the detection of aglycones the same tests as for saponins were used.

Identification of carbohydrate components

Carbohydrate components of hydrolysates were identified by ascending chromatography on Schleicher and Schüll 2043 b paper using standard sugars as markers. Chromatograms were developed three times with the following solvent mixtures: n-butanol—pyridine—water—benzene (5:3:3:1), (De Whalley et al. 1951); n-butanol—acetic acid—water (4:1:5), (Partridge 1948); isopropanol—pyridine—acetic acid—water (8:8:1:4), (Gordon et al. 1956). For the detection of carbohydrates aniline phthalate (Partridge 1949) and ammonium silver nitrate (Trevelyan et al. 1950) were used.

RESULTS AND DISCUSSION

Many attempts have been made to select a suitable procedure for extraction and purification of seed saponins. Each fraction obtained was examined for foaming ability. The fractions containing saponins were then analysed by chromatography. It was evident from the initial attempts that extraction of saponins is easy when aqueous methanol or ethanol is used but the extract contains large amount of balast substances. Therefore it was decided to use repeated extraction with anhydrous methanol.

The generally used procedure for purification of saponins by precipitation with acetone or ether proved unsuccessful in this case, because the products obtained in this way contained a lot of impurities such as carbohydrates, flavonoids and unidentified components which gave a positive reaction in some of the terpenoide tests. Attempted ion-exchanger purification failed. Good results were obtained with cholesterol and lead acetate for purification.

In the first case advantage was taken of ability of saponins to form a poorly soluble complex with cholesterol which may be decomposed

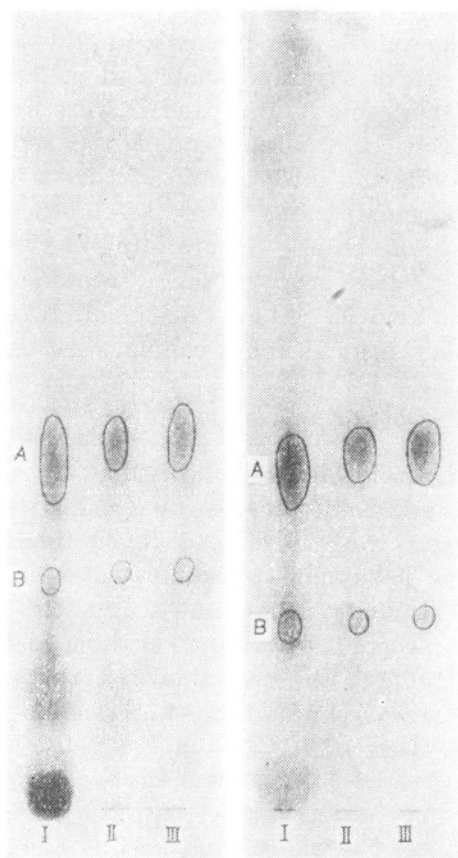


Fig. 1

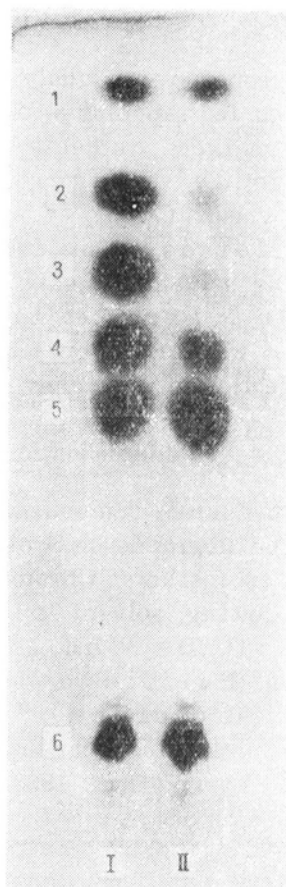


Fig. 2

Fig. 1. Paper chromatograms of saponins (A, B) developed with solvents: n-butanol-ethanol-25% ammonia (10:2:5), on the left; n-butanol-pyridine-water-benzene (5:3:3:1), (right), detected with antimony (III) chloride. I - The crude saponin extract, II - Saponins purified with lead acetate, III - Saponins purified with cholesterol.

Fig. 2. Paper chromatogram of carbohydrates developed three times with n-butanol-pyridine-water-benzene (5:3:3:1) and detected with ammonium silver nitrate. I - Sugar standard of rhamnose (1), xylose (2), arabinose (3), glucose (4), galactose (5), glucuronic acid (6). II - Sugar components of hydrolyzate of saponins.

with pyridine. Saponins obtained in this way are powder-like and contain traces of flavonoid components.

By the second method of purification, the saponins were precipitated with lead acetate and the lead-saponine complex was decomposed by treatment with H_2S . Although flavonoids were still present, the dominant

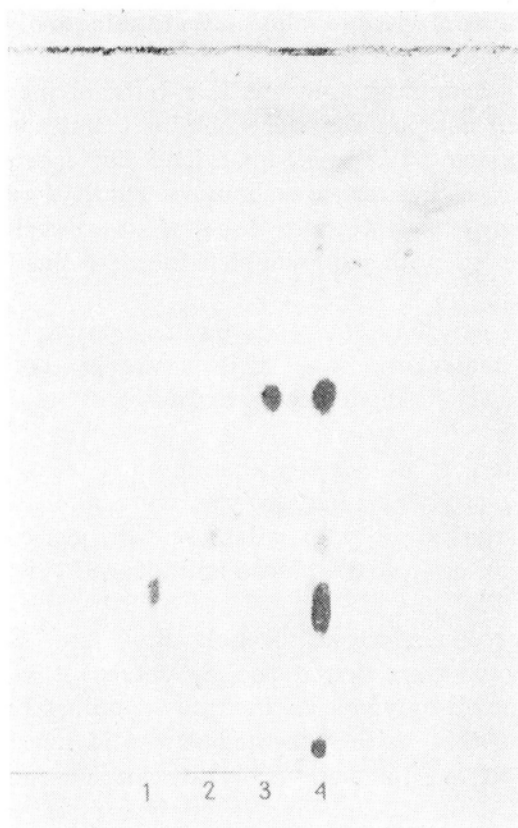


Fig. 3. Thin-layer chromatogram of aglycones developed with petroleum ether - chloroform - acetic acid (7:2:1), detected with Liebermann-Burchard reagent. 1 - Soyasapogenol B, 2 - Soyasapogenol E, 3 - Soyasapogenol C, 4 - Aglycone components of saponin hydrolyzate.

saponin was obtained in crystalline form (this will be the subject of a future report).

Saponins occurring in plants are a complex mixture of glycosides differing in carbohydrate and aglycone moieties. Many developing solvent systems for paper and thin-layer chromatography have been used to find appropriate conditions for separation. The best separation by paper chromatography was achieved by the following solvent mixtures: n-butanol - ethanol - 25% ammonia (10:2:5) and n-butanol - pyridine - water - benzene (5:3:3:1), by multiple development (Fig. 1).

With both the solvent systems lucerne seed saponins were separated into two components. In the first system flavonoids remain at the bottom and in the second they are located near the solvent front. Differences in the migration speed of saponins and impurities when using the two

solvent systems make possible chromatographic analysis of crude or little purified saponin extracts.

By thin-layer chromatography with n-butanol-pyridine-water (3:1:1) four saponin components were obtained, but two additional constituents were represented in small quantities. Differences in the number of spots obtained by these two techniques result from the separation and possibly from using stronger reagents to develop the spots on silica gel as compared with paper, which increase the sensitivity of this method.

It was found then that lucerne saponins consisted of at least four components. The main one ($R_f = 0.51$) occurs in dominant quantities, the second ($R_f = 0.34$) in moderate quantities and two others ($R_f = 0.68$ and 0.82) in traces.

Saponins isolated by the cholesterol method were then hydrolyzed with acid and the products of hydrolysis were analysed. In the carbohydrate moiety large quantity of galactose, less glucose and rhamnose, small amounts of xylose and arabinose and a large amount of glucuronic acid were found (Fig. 2).

As regards aglycones, soyasapogenols B, C and E were identified and other terpenoids were found the R_f values of which do not correspond to any known aglycone of lucerne saponins (Fig. 3).

It is interesting that neither medicagenic acid nor soyasapogenols A and D were present in saponin hydrolyzates of lucerne seeds. All those compounds were identified in saponin hydrolyzates of roots and tops (Shany et al. 1970) and medicagenic acid glycoside was also isolated from the flowers of lucerne (Morris and Hussey 1965).

On the other hand it is interesting that lucerne seed saponins do not haemolyse the red blood cells. Shany et al. (1970) are of the opinion that the haemolytic activity depends on the sapogenin to carbohydrate ratio. It seems, however, that the absence of haemolytic activity of lucerne seed saponins may be due to the lack of medicagenic acid glycosides.

The author wishes to thank Prof. O. Jeger of the Federal Institute of Technology, Zürich, for authentic soyasapogenols.

REFERENCES

- De Whalley H. C. S., Albon N. and Gross D., 1951, Applications of paper chromatographic methods in the sugar and applied industries, *Analyst* 76:287-300.
Gestetner B., Shany S., Tencer Y., Birk Y. and Bondi A., 1970, Lucerne saponins II. — Purification and fractionation of saponins from lucerne tops

- and roots and characterisation of the isolated fractions, *J. Sci. Fd Agric.* 21: 502—510.
- Gordon H. T., Thornburg W. and Werum L. N., 1956, Rapid paper chromatography of carbohydrates and related compounds, *Anal. Chem.*, 28: 849—855.
- Khorlin Ya., Bakinovskiy L. V., Vas'kovskiy V. E., Van'vaminova A. G. and Ovadov Yu. S., 1963. Triterpenic saponins 6. Distributive chromatography of triterpenic saponins, *Izv. Akad. Nauk, U.S.S.R., ser. chem.* 11: 2008—2011.
- Morris R. J., Dye W. B. and Gisler P. S., 1961, Isolation, purification and structural identity of an alfalfa root saponin. *J. Organ. Chem.* 26: 1241—1243.
- Morris R. J. and Hussey E. W., 1965, A natural glycoside of medicagenic acid. An alfalfa blossom saponin, *J. Organ Chem.* 30: 166—168
- Partridge S. M., 1948, Filter-paper partition chromatography of sugars, *Biochem. J.* 42: 238—253.
- Partridge S. M., 1949, Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars, *Nature* 164: 443.
- Pasich B., 1958, Detection of triterpenoid acids on paper chromatograms, *Nature* 181: 765.
- Shany S., Birk Y., Gestetner B. and Bondi A., 1970, Preparation, characterisation and some properties of saponins from lucerne tops and roots, *J. Sci. Fd Agric.* 21: 131—135.
- Van Atta R. G. and Guggolz J., 1958, Detection of saponins and sapogenins on paper chromatograms by Liebermann-Burchard reagent, *J. Arg. and Food Chem.* 6: 849—850.
- Walter E. D., Van Atta G. R., Thompson C. R. and McClay W. D., 1954, Alfalfa saponin, *J. Amer. Chem. Soc.* 76: 2271—2273.
- Willner D., Gestetner B., Lavie D., Birk Y. and Bondi A., 1964, Soya bean saponins. Part V. Soyasapogenol E, *J. Chem. Soc., Lon.*, 5885—5888.
- Wilson W. E., Johnson S. A., Perkins W. H. and Ripley J. E., 1967, Gas chromatographic analysis of cardiac glycosides and related compounds, *Anal. Chem.* 29: 40—44.
- Trevelyan W. E., Procter D. P. and Harrison J. S., 1950, Detection of sugars on paper chromatograms, *Nature* 166: 444.

Wyodrębnienie i charakterystyka chemiczna saponin nasion lucerny mieszańcowej (Medicago media Pers.)

Streszczenie

Za pomocą dwóch różnych metod wyodrębniono saponiny z nasion lucerny mieszańcowej. Technika chromatografii cienkowarstwowej wykazano, że glikozydy te składają się co najmniej z czterech komponentów, z których jeden występuje w ilości dominującej. W kwaśnym hydrolizacie wyodrębnionych saponin stwierdzono obecność sojasapogenoli B, C i E, a w części cukrowej zidentyfikowano glukozę, galaktozę, ramnozę, nieznaczne ilości arabinozy i ksylozy oraz kwas glukuronowy. Wykazano, że saponiny nasion lucerny w odróżnieniu od saponin liści, kwiatów i korzeni nie wywołują hemolizy czerwonych ciałek krwi, co może być związane z nieobecnością glikozydów kwasu medikagenowego.