Preliminary Phytochemical Study of *Lychnis alba* Saponin

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Distribution of *Lychnis alba* saponin in the plant is determined. A method for the isolation of the saponin by column chromatography and the detection of the saponin on paper chromatograms with a gelatin blood suspension gel is reported.

SAPONIN containing plants have been used in therapeutics and in the household for centuries. In recent years, these plants have become a focal point in pharmaceutical research. Discovery of the adrenal cortical steroids resulted in a large scale and systematic investigation of plants containing sterol saponins in the United States as possible sources of the precursors essential to the synthesis of cortisone and its derivatives. U. S. Department of Agriculture research groups (1) have tested about 5,000 samples representing a large number of the genera and plant families of the United States and abroad for their saponin, alkaloid, flavanoid, and sterol content. In an earlier phytochemical study (2), 21 wild growing plants in Latvia were tested for the presence of saponins.

In the phytochemical studies of wild growing plants in Michigan, *Lychnis alba* Mill., a member of the family *Caryophyllaceae*, seemed to deserve a closer investigation due to its hemolytic properties, abundance, and relationship to *Saponaria officinalis* L., a well-known source of a saponin. *Radix saponariae* is used in Germany as an expectorant (3).

EXPERIMENTAL

Plant.—*Lychnis alba* Mill. (Fig. 1) known also as *Melandrium album* Rydb. was originally described by Miller. This plant is also known as white cockle, campion, and evening lychnis (4).

The morphological description of the plants collected for the investigation were checked (4, 5) and identification of the plants was confirmed by Drs. Virginia and Harold E. Bailey of Wayne State University, Detroit, Mich.

Lychnis alba Mill. is a naturalized plant from Eurasia growing on the waste places, along the roadsides and borders of the fields, and on the recently idled farm lands. The Northern boundary of its distribution extends from Quebec to British Columbia, and to the South as far as North Carolina, Ohio, Illinois, Colorado, and California.

It is a dioecious annual or short-lived perennial plant, 2 to 3 feet tall, with a stout root system. Inflorescence is usually branched. Flowers are white, fragrant, and opening in the evening. Fruit is an ovoid capsule.

Fig. 1.—*Lychnis alba* Mill. staminate and pistillate plants.

Material and Method.—The plant material was collected in Mecosta County, Mich., in June and October 1959, dried in a drying oven, and powdered to pass through a No. 40 mesh.

Distribution of the saponin in the plant was determined by Kobert's hemolytic index (6) see Table I. Citrated rat blood was used. Plant extracts were prepared in a normal saline solution on a steam bath.

In this study 2 Kg. of the dried roots were used. Fatty and waxy substances were removed with petroleum ether (b.p. 30–60°) and ether. Hemolysis was used as a test to follow the course of the isolation of the saponin.

Solubility and heat stability tests of *L. alba* saponin indicated that the saponin could be ex-

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TABLE I.—HEMOLYTIC INDEXES OF PARTS OF *L. alba*

Part Used	Concn. of Saline Extract Prepared	Hemolytic Index
Root	1:250	1250
Seeds	1: 50	166
Leaves	1: 50	166
Flowering tops	1: 50	did not hemolyze

tracted by hot water or hot alcohol. From the hot 95% alcoholic extract, a part of the saponin precipitates as a white amorphous substance. Preliminary adsorption tests on alumina and Florisil columns revealed that *L. alba* saponin is adsorbed on the alumina as well as on the Florisil when water or 95% alcohol is used as a solvent. From developed chromatograms the saponin could be eluted with a 25% alcohol.

Three fractions of *Lychnis alba* saponin (fractions A, B, and C) were prepared in the following manner:

Fraction A.—A 1-Kg. portion of the dried powdered root was extracted with water on a steam bath four times for 4 hours each time. Saponin fraction was adsorbed on a Florisil column and eluted with 25% alcohol. After removal of the solvent in a flash evaporator, an amorphous, slightly yellowish substance was obtained, fraction A. Hemolytic index of this fraction was 13,300.

Fraction B.—Dried powdered root, 1 Kg., was extracted with 95% alcohol on a steam bath six times for 4 hours each time. From the hot alcoholic extract, a part of the saponin precipitated out, fraction B. Hemolytic index of this fraction was 6,600.

Fraction C.—That saponin fraction remaining in the solution of 95% alcohol was again adsorbed on a Florisil column and eluted with 25% alcohol. After removal of the solvent, an amorphous yellowish substance was obtained, fraction C. Hemolytic index of this fraction was 6,600.

All efforts to crystallize the saponin fractions failed. The same was true in using cholesterol for purification.

Paper Chromatography.—In available literature glycosides as well as saponins have been detected on the paper chromatograms by using reagents forming colored spots with the glycone of the glycoside, or specific reagents are used to detect the aglycone.

Bliss and Ramstad (7) used paper chromatography for the glycoside separation and located the glycosides on the chromatograms after hydrolysis with carbohydrases and spotting the sugars with an aniline hydrogen phthalate reagent. Dutta (8) has used a sodium *meta*-periodate in alkaline potassium permanganate solution to identify the saponin spots on paper chromatograms.

Gelatin blood suspension in an isotonic solution has been used for a long time for the microchemical detection of the saponins in the plant materials as indicated by Fischer (9).

In this study a gelatin blood suspension gel was used to reveal the saponin spots on the paper chromatograms.

Strips of Whatman filter paper No. 1 were spotted with a 2% solution (5 λ) of saponin fractions A, B, and C, then dried and placed in a chromatographic chamber. When the equilibrium in the chamber was reached, chromatograms were developed with several developers. Gelatin blood suspension (5 Gm. gelatin, bacteriology grade, was dissolved in an isotonic solution containing 0.7% sodium chloride and 0.6% secondary sodium phosphate; to 100 ml. of such a solution, 5 ml. of citrated rat blood was added) was poured through muslin, to retain the air bubbles, on a leveled glass plate on which the desired area was enclosed with melted paraffin.

In order to provide a closed chamber around the blood gelatin suspension, another glass plate was placed on rubber tubing surrounding the designated area. When gel was formed, paper chromatograms were placed on the gel. After 2 to 4 hours, red blood cells hemolyzed under the saponin spots on paper chromatograms and the filter paper in that area stained reddish-brown. See Table II and Fig. 2.

TABLE II.—R_f VALUES OF THE SEPARATE FRACTIONS OF *L. alba* SAPONIN USING ASCENDING CHROMATOGRAPHY

Developer	Fraction A	Fraction B	Fraction C
No. 1 *n*-Butanol, 60 ml. Ethanol, 13 ml. 1 *N* Ammonia, 27 ml.	0.33	0.33	0.33
No. 2 *n*-Butanol saturated with 1 *N* HCl	0.14	0.14	0.14
No. 3 *n*-Butanol, 4 ml. Acetic acid, 1 ml. Distilled water, 5 ml.	0.15	0.16	0.16
No. 4 *n*-Butanol saturated with water	0.09	0.11	0.10

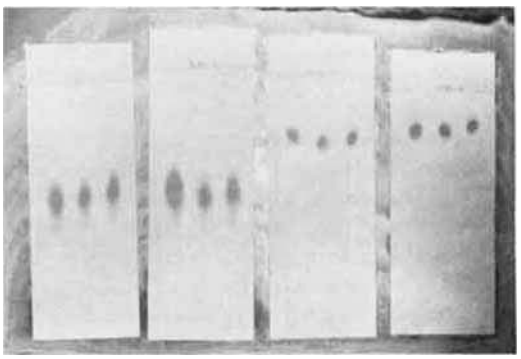

Fig. 2.—Saponin spots on paper chromatograms after hemolysis. The first two chromatograms are digitonin, the second two chromatograms are *Lychnis alba* saponin.

As long as developer No. 1 provided the highest R_f value for *L. Alba* saponin, several experiments were performed by spotting wide paper strips with all three saponin fractions and developing by developer No. 1. According to the results obtained, each of the three fractions contains one saponin and it is likely the saponin in each fraction is the same compound.

SUMMARY

Lychnis alba Mill. is a plant which contains a saponin. Most of the saponin is in the root.

Saponin can be extracted with hot water or hot alcohol, concentrated and purified on a Florisil column, and eluted with 25% alcohol.

Three fractions of *L. alba* saponin have been prepared and it is very likely that they contain just one saponin.

Gelatin blood suspension gel is useful in the detection of saponin on paper chromatograms.

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Kinetics of Thiamine Hydrolysis

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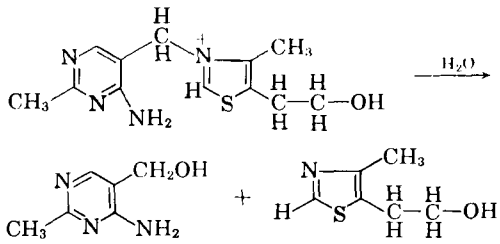
The pH-rate profile of the hydrolytic cleavage of thiamine determined from kinetics measurements has been found to be unusually complex. Under essentially buffer-free conditions, the overall behavior suggests existence of at least four separate reactions: (*a*) acid catalyzed mechanism leading to the formation of oxythiamine at pH below 1; (*b*) water cleavage of protonated thiamine yielding a pyrimidine and a thiazole fraction between pH 1–6; (*c*) at pH 2–6.5, hydroxyl ion catalysis of protonated thiamine producing the same products found in reaction (*b*); (*d*) above pH 6.5, water cleavage of thiol thiamine to yield a pyrimidine diamine and other undetermined products. The ionic strength and temperature dependency of the reaction has also been determined. Contrary to earlier works, the hydrolytic reaction appears not to be subject to major negative or positive catalysis by amino acid species. The rate, however, is pronouncedly influenced by what appears to be general base catalysis by several buffer species.

ALTHOUGH hydrolytic cleavage of thiamine, an important pharmaceutical product, represents the major mode of anaerobic degradation of the vitamin, the kinetic dependency of this reaction on pH has not previously been reported in terms of the reactive species present. The present contribution is concerned with the results of an investigation designed to establish the pH profile of this important reaction, to reconfirm the degradation products under the conditions of the experiments and to correlate them with the kinetic observations, to determine whether agents other than hydroxyl and hydrogen ions can accelerate or inhibit the cleavage, and to establish its dependency on other kinetic variables.

Some doubt has been expressed previously on the question of whether the cleavage followed a strictly first-order kinetic expression with respect to the vitamin (1–3), although it appears generally agreed that the rate of hydrolysis was enhanced by increasing pH and the temperature (4–8). Others (9, 10) have also noted that buffers increased the rate of the reaction, although no attempts were made to evaluate the specific effects. McIntire and Frost (2), studying the effects of various compounds on the hydrolytic rate, claimed to have found inhibitory and catalytic effects but did not establish mechanisms for the observed results.

Several cleavage products have been recovered and identified. Watanabe (11), working with the reaction products resulting from the hydrolysis of a 0.5% thiamine solution heated at 140° for 12 hours at approximately pH 3.5, the pH resulting from a solution of the salt itself, was able to isolate and identify the degradation products and proposed the following reaction



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