

BERBERINE IN THE COMMON BARBERRY (*BERBERIS VULGARIS* L.).*BY E. R. SCHULZ.¹

The paper gives a historical review of studies on the isolation, chemistry, and uses in medicine of berberine and contains a detailed bibliography on the subject. It also contains results of an investigation on the precipitation of berberine as crystalline berberine acetone.

INTRODUCTION.

The barberry plant that spreads the black stem rust to cereals and grasses required considerable study before adequate means of eradication were found. Investigations on the optimum soil conditions, the habitat and the metabolism of the plant were begun before field experiments proved common salt and kerosene to be satisfactory means of eradication. The results of these investigations are of interest not only in further helping to solve the problem of getting rid of the harmful common barberry, but also in adding to the knowledge of plants. In this paper are reported the results of an investigation of the alkaloids, particularly berberine, in the barberry.

It has been reported in the literature that salts of this alkaloid have great influence upon animal and plant life. One-half per cent of berberine hydrochloride (34)³ checked the growth of *Bacterium coli*, stopped formation of indol and volatile fatty acids in protein decomposition, and acted as a strong poison to rabbits, frogs, hens and mice, causing leucocytosis and nephritis and affecting the nervous system. One per cent of berberine hydrochloride stopped the germination and development of the spores of mucedo; and 1/222 mol. (55) in 1 liter of water materially reduced the energy of germination of peas, wheat and rape. When this strength of solution was applied to the seedlings, growth was stopped. It has also been reported in connection with these experiments that partial neutralization of the effects of berberine was effected when 0.2 per cent of tannin solution had been applied to the plants treated with berberine hydrochloride solution.

The above-mentioned influences of berberine on plants suggests that better knowledge of the alkaloids and other little-known ingredients of the barberry might indicate a procedure in attacking the problem of immunity and susceptibility, such as exists in the case of the Japanese and the common barberries in relation to *Puccinia graminis*, the organism causing stem rust. With this point in mind, the following information is presented.

HISTORICAL REVIEW.

The yellow, bitter substance present in the barberry plant was known in the arts of medicine long before the chemists had a knowledge of the properties and the

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² This investigation was carried on in the laboratory of Dr. E. Kremers, Department of Pharmacy, University of Wisconsin. The writer is grateful to Dr. Kremers for many courtesies and many helpful suggestions.

³ Numerals in parentheses refer to papers in the "Bibliography" at end of paper.

chemistry of this substance. One finds in the literature on the subject that since the year 600 B. C. and extending to modern times, decoctions from the berries, bark and roots have been used as detersive agents, astringent stomachics, febrifuges and cures for venereal maladies. Occasionally the extracts were used also as dyes.

Isolation of Crystalline Berberine.

Not until the beginning of modern chemistry and the discovery by Sertürner in 1918 of the so-called alkaloids in plants, was the yellow substance in the barberry studied intensively. This discovery created a new outlook and stimulus, and attracted a number of investigators to the study of this interesting and complicated substance. As a result, we have at the present time satisfactory methods of extraction and rather definite knowledge of the chemistry of this substance.

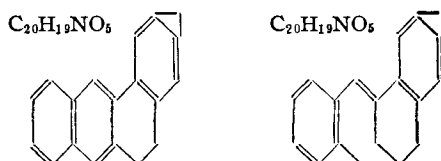
The first report on the isolation of the yellow alkaloid berberine from the root of the common barberry was published by Brandes (1) in 1825. He obtained it in an amorphous form of intense yellow color and was able to prepare from it derivatives with acids. The following year, 1826, Chevallier and Pelletan (2) for the first time obtained the substance in a crystalline form from the prickly ash (*Xanthoxylum clava-herculis*) and named it xanthopicrit. Buchner and Herberger (3), in 1830, obtained the same crystalline substance from the root of the barberry and they were the first to name it berberine. This name has been retained for the alkaloid by all subsequent investigators, although it is now known that this alkaloid is rather widely distributed in the plant kingdom and occurs not only in the Berberidaceae family, but in the Ranunculaceae, Papaveraceae and Rutaceae as well.

METHODS OF EXTRACTION IN USE.

The most successful methods of extraction that have been used are the hot-alcohol and hot-water methods. In case of the barberry, usually the bark and the roots have been used as the source of the berberine, although small amounts have been found also in the berries, leaves, and stem. The tissues are ground before extraction and then percolated with hot water, as done by Buchner (4) and others, or with hot alcohol, as reported by Lloyd (13), Stubbe (23), Gordin (27), and others.

CHEMISTRY OF BERBERINE.

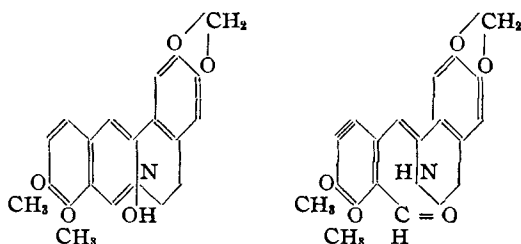
The isolated berberine and its salts of acids have been used by Schmidt (17, 18), Fleitmann (5), Henry (9), Perrins (12), Hesse (15), Flückiger (16), Gaze (19), Rüdél (24) and others for elementary analysis, which preceded the investigations of the structural composition of the molecule carried out by W. H. Perkin, Jr. (20, 22, 44) to whom our knowledge of the chemistry of the berberine is due. The latter's work supplemented by the investigations of Gadamer (35, 38), Faltis (41), and Tinkler (49) has established the following structural configuration of the molecule capable of existing in several forms:



Tinkler (49) has suggested that the aldehyde form exists as carbinol instead of an aldehyde, and it is agreed that the ammonium form is known only in solutions and not in solid state.

SYNTHESIS.

The synthetic preparation of tetrahydroberberine and oxyberberine by Pictet and Gams (46, 47) and of berberine by Haworth *et al.* (63), Buck *et al.* (62), and of oxyberberine by Perkin *et al.* (64) leave no doubt in the minds of most of the investigators as to the correctness of the above formulas in their major parts.



EXPERIMENTATION.

Extraction.

The hot alcohol method proved to be a good method for getting out the alkaloids of the barberry. Finely ground roots were extracted with hot alcohol until most of the color had disappeared from the drug. The residue was further extracted with hot water for long periods, but the water extract gave only traces of alkaloid, showing the alcohol to be an efficient extraction medium.

Preparation of Berberine Acetone.

The preparation of berberine acetone has been reported by Schmidt (18), Schreiber, and Gaze (19). According to Gordin (27, 32, 36), the isolation of berberine as berberine acetone seemed to give the best results. As details for obtaining the best yields were wanting, it was desirable to ascertain what degree of concentration of the alcoholic tincture and what amounts of acetone caused the best precipitation.

Small amounts of ground barberry roots were used to begin with. Three hundred grams were placed in a percolator and extracted successively with 3000 cc. of hot alcohol until most of the yellow color had disappeared from the drug. The alcohol was recovered by distillation from a water-bath until about 300 cc. of extract remained. The alcoholic distillate had a very striking odor. The residue was diluted with water to 1500 cc. The aqueous filtrate from the resinous precipitate resulting was made alkaline with 50 cc. of 10 per cent aqueous NaOH solution. The alkaline solution was warmed to 60° C., divided into three equal portions and 100 cc., 200 cc. and 300 cc. of acetone added, respectively. The crystalline precipitate of berberine acetone, which began to come down soon after cooling, was filtered off, dried at room temperature and weighed. The respective yields were 0.998 Gm., 0.958 Gm. and 0.860 Gm., or an average of 0.93 per cent of berberine acetone, corresponding to 0.77 per cent of berberine.

The experiment was repeated on a larger scale with 900 Gm. of the drug and 5 liters of alcohol. The alcoholic tincture was evaporated to 200 cc. and the ex-

tract subsequently diluted with water to 900 cc. To the filtrate, rendered alkaline and divided into three equal parts, 75 cc., 100 cc., and 200 cc., respectively, of acetone were added. The berberine acetone was separately dried and weighed as before. The respective weights were 3.529 Gm., 3.559 Gm., and 3.552 Gm. The addition of another 100 cc. of acetone to each respective mixture caused a further precipitation in the first but not in the second and third. This second precipitation was much purer and brought the total precipitate up to 4.594 Gm. Hence, the average yield was 1.3 per cent of berberine acetone, corresponding to 1.07 per cent of berberine. The melting point was at 165° to 175° C.

Inasmuch as the second and third reaction mixtures yielded about the same weight of precipitate, and as the third appeared slightly purer than the second, the correct conclusion appears to be that about 150 cc. of acetone may be expected to bring about the best precipitation under the degree of concentration used.

It was found that the mother liquor from the berberine acetone gave a strong alkaloidal test with Mayer's Reagent. Further investigations might have revealed in this liquor the presence of the companion alkaloids of berberine such as, berbamine, oxyacanthine, and others, which are known to be present in the barberry plant (see Stubbe, 23).

Preparation of Salts of Berberine.

Definite amounts of berberine acetone were mixed with water and heated over a water-bath, and calculated amounts of dilute acids were added in excess to replace the acetone in the berberine molecule. The addition of the acids to the berberine acetone in hot water caused the berberine acetone to dissolve. On cooling the acid salts precipitated. Hydrochloric acid formed beautiful yellow, silky crystals; nitric acid produced orange crystals; phosphoric, oxalic and salicylic acids formed yellow crystals.

Preparation of Pure Berberine.

Berberine sulphate was used for the preparation of pure berberine of the Gadamer (35, 38) aldehyde form. To the berberine sulphate was added enough Ba(OH)₂ to take care of the sulphate in the berberine molecule. After filtering off the solution, an excess of NaOH was added, when a light-colored precipitate formed. The precipitate was dissolved in ether and allowed to crystallize. The crystals were at first of light yellow color but on standing turned dark brown. They melted at 144° C.

SUMMARY.

The salts of the alkaloid berberine have been found to exert considerable influence on the physiological functions of plants and animals. This alkaloid has been used in medicine since before 600 B. C. and is still used to some extent.

Our knowledge of the chemistry of alkaloids dates from 1817, and berberine was first isolated in a pure form in 1826 from *Xanthoxylum*. In 1830, it was isolated from *Berberis* and named berberine. It has since been found that the same alkaloid occurs in several plant families.

Berberine is readily extracted from the ground plant tissue by hot water or hot alcohol. Its chemical formula is generally given as C₂₀H₁₉NO₆. It has been successfully synthesized in the laboratory.

Experiments reported in this paper showed almost complete extraction with hot alcohol. The addition of acetone to the extract precipitated the crystalline berberine acetone, giving yields as high as 1.3 per cent of the berberine acetone which is equivalent to 1.07 per cent of berberine.

Other crystalline salts were obtained readily by the addition of various acids to the hot berberine acetone water solution. Pure berberine in the form of light yellow crystals was prepared from berberine sulphate.

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A SIMPLE VISCOMETER FOR ABSOLUTE MEASUREMENTS.*

BY E. MONESS AND P. M. GIESY.

The laws of flow of liquids through capillaries have been worked out by Poiseuille and the formula as given by Bingham¹ is: $\eta = \frac{\pi g \rho R^4 t}{8 V l} - \frac{m \rho V}{8 \pi l t}$ where η is the viscosity, V the volume of flow in the time t , l and R respectively the length and radius of the capillary, g the acceleration of gravity, p the pressure in grams per sq. cm., ρ the density of the liquid and m a constant equal to 1.12.

Many of the viscometers used in industry, among these being the Saybolt, are based on simply allowing a definite quantity of liquid to flow from a standard container through a standard opening, the assumption being that the time of efflux of the liquid is a measure of its viscosity. The opening is usually a short tube to which the laws of flow above stated are not applicable, and the flow is allowed to take place by gravity thus giving a variable pressure as the flow proceeds. Such measurements are not capable of giving even relative viscosities of liquids, since the time of flow depends not only on the viscosity but also on the density of the liquid. If we wish to get absolute values for viscosity, the measurement must be made under conditions which yield data susceptible to mathematical treatment, according to the laws for the flow of liquids.

We have designed a rather simple viscometer² in which the liquid is allowed to flow through a capillary of known dimensions, and in which the pressure is kept constant throughout the run due to a constant hydrostatic head.

Figure 1 shows its construction. An inverted 8 L. bottle from which the bottom has been cut is used as the water-bath. A tube about 1 inch in diameter and about 10 inches long serves as the container for the viscous liquid. The upper end of the tube is closed by a rubber stopper through which passes an air tube, while the lower end is similarly closed by a rubber stopper through which passes the capillary, ending flush with the surface of the stopper. The lower end of the capillary passes through the rubber stopper closing the neck of the water-bath, and protrudes only about 2 mm. beyond the stopper. The air-tube has a piece of rubber tubing attached to the upper end, closed by means of a pinch cock.

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¹ "Fluidity and Plasticity," p. 18 (1922).

² Allen (*J. Soc. Chem. Ind.*, 1886, 131) and Schmid (*Chem.-Ztg.*, 1885, 1514) had designed viscometers in which flow took place under a constant head, but neither used a capillary tube for the flow, this taking place in Allen's viscometer from an orifice, and in Schmid's through a tube.