3. Objections have been found to all of the available methods, although the procedure of the U. S. P. is more rapid, more convenient to use, and gives results which are sufficiently accurate for the nature of the product.

4. Experiments are being conducted to determine whether some of the free alkali is gradually neutralized by the fatty acids in the soap.

REFERENCES.

(1) Simmons, W. H., "Soap, Its Composition, Manufacture and Properties," page 56.
(2) Oculus, *Seifensieder-Ztg.*, 60, 473–474 (1933).
(3) Herrmann, *Chem.-Ztg.*, 53 (1904).
(4) Poethke, W., *Chem. Umschau, Fette, Oele, Wachse, Harze*, 39, 121–126 (1932).
(5) Kling, A., Genin, V., and Florentin, D., *Bull. soc. chim.*, 15, 200–205 (1914).
(6) Bosshard, E., and Huggenberg, W., *Z. angew. Chem.*, 27, 11–20 (1914).
(7) Herrman, P., *Ibid.*, 27, I, 135–136 (1914).
(8) Bosshard, E., and Huggenberg, W., *Ibid.*, 27, I, 456 (1914).
(9) Dietrich, *Helfen. Ann.* (1886, 1887, 1889).
(10) Newington, F. H., *J. Soc. Chem. Ind.*, 35, 95–96 (1916).
(11) Davidsohn, J., *Chem. Umschau Fette, Oele, Wachse, Harze*, 33, 273–281 (1926).
(12) Davidsohn, J., *Ibid.*, 34, 260 (1927).
(13) Bergell, C., *Seifensieder-Ztg.*, 55, 327–328 (1928).
(14) Knigge, G., *Allgem. Öl-u. Fett-Ztg.*, 25, 603–605 (1930).
(15) Schuttig, C. L., *Chem. Analyst*, 29, 23–24 (1920).
(16) Banninger, Q., *Seifenfabr.*, 34, 264–266, 292–294 (1914).
(17) Hope, *Chem. News*, 219 (1881).
(18) Izmailski, V. A., *J. Russ. Phys.-Chem. Soc.*, 48, 411–432 (1916).
(19) Simmons, *Perfumery and Essential Oil Record*, 19, No. 19, 408–412 (1928).
(20) Knigge, G., *Allgem. Öl-u. Fett-Ztg.*, 26, 619–620 (1929).
(21) Schutte, O., *Seifensieder-Ztg.*, 57, 49–51 (1920).
(22) Divine, R. E., *J. Am. Chem. Soc.*, 22, 693 (1900).
(23) Dudley and Pease, *Engineering and R. R. Jour.*, 551 (1891).
(24) "Lewkowitsch," 5th Edition, III, 347 (1915).
(25) Allen, "Commercial Organic Analysis," 5th Edition, II, 590 (1924).
(26) *Circular of the Bureau of Standards*, Nos. 123, 124, 126, 127, 128, 129, 130, 131, 132 (1922).
(27) Hurst, Geo. H., "Soap, a Practical Manual of the Manufacture of Domestic, Toilet and Other Soaps," 339–341 (1907).

A NEW GLUCOSIDE FROM BLUEBERRY LEAF.*

BY N. KENNETH EDGARS.

The use of blueberry leaves or extracts made therefrom as a remedy for diabetes is not new to medicine. Wagner (1) in 1925 and Allen (2) in 1927 both experimented with extracts and concentrates made from these leaves and proved that at least one hypoglycemic, or blood sugar lowering, substance exists and can be extracted in a stable form. Their product, which was named Myrtillin erroneously, is an example of such an extract. It was not pure enough to give uniform results, but its discovery marked an important step in the history of the pharmacology of this plant. The name Myrtillin properly belongs to a galactoside which exists in the fruit of this genus and discovered by Willstätter in 1915 (3).

* New Era, Pharmaceutical Laboratory, Inc., Tenafly, N. J.

A new glucoside $C_{24}H_{36}O_{18}$, named Neomyrtillin, was first isolated and analyzed in 1929, but not placed in its proper chemical sequence until late in 1933. Neomyrtillin is the therapeutically active principle of blueberry leaves, having the distinct advantage over insulin of being effective as a hypoglycemic agent when administered by mouth.

The varieties of blueberry yielding the largest leaves are more important for the purposes here described. The "high bush" blueberry species have been found to have more leaf area per bush than the "low bush" species. Therefore only a brief botanical description of *Vaccinium corymbosum*, the most common "high bush" blueberry indigenous to New England and the east central states, wherein the bulk of all our commercial blueberries are found, will be attempted here.

A perennial shrub from one to three meters high, with numerous main stems originating from a common root stock. Main stems and secondaries both very much twisted and striated, from gray to brown in color. Leaves are ovate, 3 cm. wide by 7 cm. long, margin entire, both apex and base blunt, very short petiole and axillary bud. Texture herbaceous, from smooth to glabrous on the upper surface, lower surface much lighter in shade and pubescent. Leaves are alternate with internodes 2 or 3 cm. long. Flowers appear early in May as terminal or lateral clusters; the inflorescence generally assuming the corymbose shape; corolla oval-cylindraceous, dull white, 5-toothed, 8 mm. long by 5 mm. wide; calyx 5-lobed, saucer shaped; stamens 10, 6 mm. long, with whitish, hairy filaments and long, pointed or awned, amber-colored anthers, introrsely attached, which dehisce by terminal chinks. Fruit dark, blue-black spheres, 8 mm. across, soft, sweet and covered with a fine down of hairs. The interior consists of 5 cells, each partly divided by false partitions growing inward toward the placentæ.

The leaves are gathered in mid-summer and dried in special frames protected from direct sunlight. When so dried, they do not turn brown, but assume a dull, olive-green color. They are then transferred to a mill and reduced to powder, which is treated with volatile solvents in special extractors to remove wax, resins and chlorophyll. The marc is extracted with acidified methyl alcohol; the extract dried and placed in a Tannin Extracting Apparatus which removes tannins and non-specific proteins. The solute contains the Neomyrtillin, which is removed by precipitation with a carefully normalized and buffered solution of caustic soda and purified by washing first with ethyl alcohol and then with ether.

A proximate analysis of the air-dried and powdered blueberry leaf was made, using appropriate solvents for the several fractions. Standard methods were used whenever possible, otherwise original processes were devised. The fresh leaves lost 62% water in desiccator. The total ash of the air-dried leaf was 3.62%, of which 0.29% was acid-insoluble.

(Air-Dried Leaf.)

Moisture	8.58%	Water extract, sol. in alcohol and ether probably decomposition products (xanthophyll)	13.32%
Tannins	8.38%		
Chlorophyll	3.42%		
Wax, resin	3.55%		
Gum, mucin	5.10%	Crude fibre	12.72%
Carbohydrate	11.65%	Marc	30.80%
Neomyrtillin	2.00%		

Neomyrtillin obtained by the above process was a dull brown, amorphous powder with a slight alœ-like odor and a sweet astringent taste. It was not very soluble in cold water or alcohol but very soluble in both solvents when warmed slightly, yielding deep, red-brown solutions. An aqueous solution (1:100) was distinctly acid to methyl orange and, like most glucosides, was lævorotatory — $[\alpha]_D = -0.7$ (1:1000 H_2O). The melting point was 57° C. When hydrolyzed with zymase, the compound broke down into its component parts, among which glucose and tri-hydroxy benzoic acid were identified by standard qualitative tests (4).

Combustion analyses and molecular weight determinations both by freezing and boiling point methods (Beckmann), showed the molecular formula to be $C_{24}H_{36}O_{18}$. The compound responded to many of the reactions of galloyl glucose as described by Fischer, Bergmann, *et al.* (5) and yielded about 17% methoxyl groups in the Zeisel Reaction (4). Insoluble compounds were obtained with the salts and oxides of lead and mercury and with most alkaloids.

The compound would seem therefore to be a methoxygalloyl-glucose. As gallic acid is liberated on hydrolysis, it may be assumed that the methoxyl groups are attached to the glucose itself rather than to the galloyl side chain.

Tests for identity were devised, among which were the following: To 10 cc. solution of Neomyrtillin in water (1:100) add 5 to 10 drops of ferric chloride T.S. A greenish black color is produced, which changes to a reddish brown precipitate on the addition of 2 cc. ammonium hydroxide T.S.

Dissolve 0.5 Gm. Neomyrtillin in 25 cc. warm distilled water and mix with 5 Gm. ferric oxide in a mortar. Let stand for 20 minutes, filter and add 3 drops of *N*/10 NaOH to the filtrate. Evaporate the filtrate on a watch glass at 100° C. almost to dryness and set aside to dry in a warm closet. Examine under a microscope (x250). Long, colorless, or slightly yellow, polygonal, acicular crystals and similarly colored, characteristically shaped, stellar rosettes are present in large amounts (4).

Tests for purity include a modified heavy metals test and a test, devised by the author, for free tannin in the presence of gallates, using the Lamotte Block Comparator to match the colors exactly. In this test, carefully measured quantities of iron tannate (blue), iron gallate (green) and Neomyrtillin-iron are substituted for the colored solutions in the Block Comparator and the absence of free tannin in the sample ascertained colorimetrically. The ash does not exceed 0.05%.

In order to determine the blood sugar diminishing or hypoglycemic power of Neomyrtillin, a series of interesting pharmacological experiments were carried out, using rabbits as test animals. Rabbits are used almost exclusively in blood sugar experimental work because they are easy to handle and are especially responsive to piqûre.

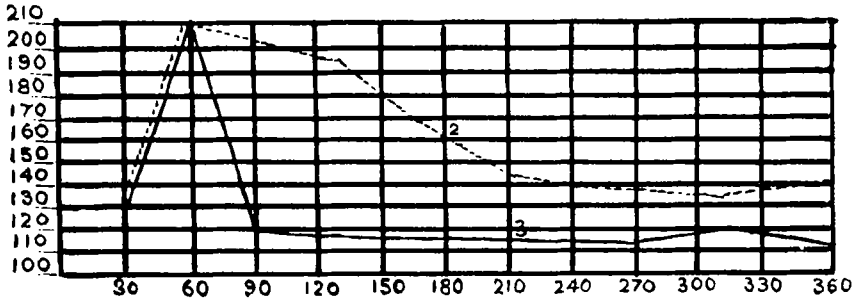

Fig. 1.

Artificial hyperglycemia (diabetes) was induced by the method of piqûre, as devised by Banting (6). This is a painless process in which the nerve center controlling the function of the pancreas is destroyed under anesthesia. The blood sugar level will remain high, usually over 200 mg. per 100 cc., until some hypoglycemic substance like insulin or Neomyrtillin is introduced hypodermically or orally; in which case the level will fall to a little over 100.

Substances to be administered hypodermically were brought as near to neutrality as possible, then sterilized and injected subcutaneously into either subscapu-

lar region or fed orally through a catheter. After a short period of rest and assimilation, blood for analysis was withdrawn from the ear lobe by severing one of the larger veinlets, which bulge on the outer surface, and allowing the blood to drop into a clean crucible containing a small amount of potassium oxalate to prevent coagulation. An exact amount of the oxalated blood was then pipetted from the dish and analyzed according to the method of Folin and Wu (7).

In the appended graph, the blood sugar reading is given at the left, in milligrams for each 100 cc. of blood, and the time in minutes from left to right. Dotted curve Number 2 represents the effect of feeding glucose hypodermically to starved rabbits. Solid line Number 3 shows the effect upon the same rabbits after administering Neomyrtillin hypodermically.

CONCLUSIONS.

1. The entire pharmacology of blueberry leaf has been studied.
2. The glucoside $C_{24}H_{36}O_{16}$ was isolated, purified, studied and the non-descriptive name Neomyrtillin given to it.
3. Neomyrtillin was found to possess hypoglycemic properties when administered to rabbits having induced alimentary hyperglycemia.

REFERENCES.

(1) Mark and Wagner, *Klin. Wochschr.*, 4, 1692 (1925).
(2) Allen, F. M., "Myrtillin in Pancreatic Insufficiency," *Can. M. A. J.*, 17, 1539 (1927).
(3) Willstätter and Zollinger, "Ueber die Farbstoffe der Heidelberre," *Ann.*, 408 (1915).
(4) Edgars, N. K., "The Pharmacology of Neomyrtillin from Blueberry Leaf with a Description of *Vaccinium Cæsarense*," N. J. (1934).
(5) Fischer, Emil "Untersuchunge ueber Depside und Gerbstoff," Berlin, Springer (1919).
(6) Banting, Best, *et al.*, "The Effects of Insulin on Experimental Hyperglycemia in Rabbits," *Univ. Toronto Stud., Physiol. Ser. No. 54* (1922).
(7) Folin and Wu, *J. Biol. Chem.*, 41, 367 (1920).

A STUDY OF THE ASSAY OF ACONITE AND THE STABILITY OF ITS PREPARATIONS.*[1]

BY GEO. BAKER[2] AND DEAN CHAS. B. JORDAN.

Aconite, though employed in medicine since the thirteenth century, has never had a completely satisfactory assay. Much confusion existed in the early investigation of the drug. It was not until about 1900 that the alkaloids existing in Aconite were definitely identified and their relative physiological action determined. The eighth revision of the United States Pharmacopœia (U. S. P. VIII) possessed a chemical assay for the drug and its preparations while the ninth revision carried an alternative physiological assay. The tenth revision of the U. S. P. dropped the chemical assay and made the bioassay official. The chemical assay method was unsatisfactory in that it determined total alkaloids. The alkaloids of *Aconitum Napellus* are aconitine, benzoylaconine, and aconine. Aconitine is the active agent and a practical assay must therefore determine the aconitine present in the drug and its preparations.

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] Based upon a thesis by Geo. L. Baker submitted to the Faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June 1935. (A fairly complete bibliography accompanies the thesis.)

[2] Eli Lilly & Co. Fellow, 1933–1935.