The analysis was carried out by the Dumas method:

1. 0.1015 Gm. gave 2.9 cc. N at 738.6 mm. and 27.5° = 3.06 p. c. N.
2. 0.1005 Gm. gave 2.9 cc. N at 738 mm. and 28° = 3.04 p. c. N.

Calculated for $C_{18}H_{21}O_3N.C_6H_6(OH)_3COOH$..... 2.96 p. c.

Strychnine Shikimate.—This was prepared from the strychnine base and aqueous shikimic acid as carried out in the preparation of the quinine salt. The salt crystallized out as colorless long plates, m. p. 234–236° (sintered at 154°).

Analysis: This was carried out as for quinine shikimate except that chloroform was used instead of ether as the extracting solvent.

1. 0.1075-Gm. sample yielded 0.078 Gm. strychnine corresponding to 72.5 p. c.

Calculated for $C_6H_6(OH)_3COOH.C_{21}H_{22}O_2N_2.2H_2O$ is 75.63 p. c.

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TOXICITY IN THE LEAVES OF RHODODENDRON CALIFORNICUM, HOOK. I.*

BY F. A. GILFILLAN AND CHIEKO OTSUKI.[1]

Since the days of Xenophon, in the fifth century B. C., it has been known that certain rhododendrons were poisonous, but all systematic investigation (1) of this toxicity dates since 1885, during which period research has been carried out on 15 species, of which 13 were found to contain poison.

Rhododendron californicum, which abounds in the forests of northern California and of western Oregon, Washington and British Columbia, was not one of the 15 species investigated. Although various botanical handbooks (15) state that the plant is said to be poisonous, no reference was found in the literature to any research on that particular species. The following report represents a preliminary investigation on the toxicity of this rhododendron.

EXPERIMENTAL.

Materials.—Leaves were collected in June 1935, and also in December 1936, along the Oregon coast near Newport and near Florence. Both samples were immediately air-dried indoors, losing about 58% of their weight during the curing. The dried leaves still contained about 16.4% of moisture, corresponding to 64.9% in the green leaves. The ash content was about 2.9% on the cured leaves, corresponding to about 1.2% in the green state.

Extraction with Alcohol.—Three different concentrations of alcohol, 15%, 45% and 95% were employed in extracting the ground dried leaves. The yields were 37%, 44.5% and 35%, respectively, but from the dried extract so produced, no crystalline substance could be obtained, nor other material with marked physiological activity, wherefore the method was abandoned. It is

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[1] Abstracted from a thesis presented by Chieko Otsuki in partial fulfilment of the requirements for the degree of Master of Science from the Oregon State College.

possible that the procedure might have been successful had it been employed on larger quantities than were used.

Extraction with Water.—Of the various aqueous extraction methods tried, the following appeared to be the best procedure. About five gallons of water were brought to boil in a large kettle, and 1000 Gm. of dried leaves were slowly added, boiling being maintained, and continued for half an hour after all leaves were added. After cooling, the liquor was decanted and evaporated to dryness, yielding 51.9% of extract "L," based on dried leaves.

Purification with Lead Acetate.—Of the above dried extract "L," 78 Gm. were dissolved in water, treated with excess 10% neutral lead acetate solution, filtered, saturated with hydrogen sulfide gas, again filtered and the filtrate concentrated. From the thick greenish liquid there separated a crop of greenish yellow crystals, "N." After washing with water, these weighed 1.827 Gm., representing 1.22% of the weight of the dried leaves, or 0.51% of green leaves. Concentrated sulfuric acid turned these crystals red, the color becoming more intense on warming. When diluted and made alkaline, the color disappeared, but reappeared on acidification. The crystals did not reduce Fehling's solution, before or after hydrolysis. When recrystallized, either from water or from normal butyl alcohol, they showed a melting point of 183.4° C. (corr.). They contained no nitrogen. Combustion showed them to consist of 55.7% of carbon and 7.34% of hydrogen, indicating an empirical formula of C_2H_3O. The molecular weight determination on this substance "N" is reported below.

Purification with Magnesium Oxide.—With 295 Gm. of the dried extract "L" were mixed 300 Gm. of heavy magnesium oxide and sufficient water to form a paste. After drying and pulverizing, the resultant 624-Gm. mix was extracted with two liters of 95% alcohol, warming and stirring for two hours. After standing over night, the alcohol was decanted, and 750 cc. additional alcohol were added to the powder, this second extraction being handled as before. When the combined alcoholic extracts were concentrated, amber-colored crystals were produced, weighing 8.8 Gm. and representing 1.55% of the dried leaves, or 0.65% of green leaves. Color reactions with various reagents were similar to those of the "N" crystals, but there was a slight reduction of Fehling's solution, due possibly to some impurity. After recrystallization from water, leaving the mother liquor "O," the crystals were almost white. Several determinations of the melting point of these "M" crystals, alone and in mixtures with the "N" crystals, showed them to be identical.

Molecular Weight Determinations.—It was first attempted to determine the molecular weight of the crystals "N" by the depression of the freezing point of water, but inconclusive results were obtained, varying from 199 to 319. Employing the boiling-point method in absolute alcohol for crystals "M" encountered more difficulty, as the samples were too small, and the results obtained were 321 and 401. When all crystals of "M" and "N" were mixed so as to secure a larger sample, results with 1.3488 Gm., 1.7865 Gm. and 2.2473 Gm. were 362, 365 and 349, respectively. If the value 344 be tentatively accepted, the formula for this substance would be $C_{16}H_{24}O_8$.

Toxicity.—A kilogram guinea pig was fed 4.8 Gm. of the extract "L," representing about 25 Gm. of green leaves. The animal died in 12 hours.

The crystals "M" were injected subcutaneously into two guinea pigs in amounts up to 33 mg., also the crystals were fed to three rabbits in amounts up to 150 mg., but none of the animals exhibited any toxic symptoms.

The mother liquor "O" from which these crystals had been obtained was next investigated. On evaporation of 1 cc. of this solution, there was obtained 0.1337 Gm. of a varnish-like residue. This amount, injected subcutaneously into a 435-Gm. guinea pig, caused retching and salivation in two minutes, and in 12 minutes, death. The heart was in diastole. Another guinea pig, weighing 475 Gm., when injected with 33 mg. of the material, showed the same symptoms, and died in 42 minutes. A rabbit weighing 1350 Gm. was fed 150 mg. of the substance, but showed no symptoms within 24 hours. It was then given 150 mg. subcutaneously, which produced convulsions in 14 minutes and death in 33 minutes.

SUMMARY AND CONCLUSIONS.

1. The dried leaves of *Rhododendron californicum*, Hook. were extracted with boiling water, and the extract purified by either of two processes.

2. From this poisonous extract was isolated a crystalline substance, analyzing as $C_{16}H_{24}O_8$, but devoid of toxic properties.

3. From the mother liquor of the crystals was obtained a resinous substance of high toxicity.

The investigation is being continued.

The authors are indebted to Mr. Claude Huggins of Springfield, Oregon, who provided most of the rhododendron leaves for this investigation, and to Mr. L. C. Britt, Chemist of the Oregon State Board of Pharmacy, who suggested several of the procedures used.

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AN ASSAY METHOD FOR TABLETS OF BELLADONNA EXTRACT.*

BY DALE T. WILSON.

Because of manipulative difficulties, the U. S. P. XI assay method for Extract of Belladonna is not applicable to tablets containing this extract. A modification of the U. S. P. XI assay method, involving the preliminary extraction of the alkaloids according to the shake-out method of the U. S. P. X, is somewhat more workable. However, the nature of tablet mixtures is such that rather stable emulsions are formed during the shake-out, with the result that assays are tedious and time-consuming. Furthermore, the final end-point in the titration is usually masked by the presence of chlorophyl and other colored extractive matter.

In an attempt to overcome these objectionable features the author has developed a method which appears to be quite satisfactory for the estimation of total alkaloids in Tablets of Belladonna Extract. The details of the procedure are given as follows.

Weigh not less than twenty tablets and determine the average weight. Powder and weigh accurately a sample equivalent to 25 grains of belladonna extract and transfer it to an extraction thimble having approximately a length of 94 mm. and an internal diameter of 33 mm. Place the extraction thimble in a medium size Soxhlet extractor and add about 125 cc. of ether in

* Chemical Control Laboratories, Eli Lilly and Company, March 10, 1938.