The aqueous liquids from the oxidation mixture were distilled and resulted in a clear, acid distillate and a yellow, acid residue from which nothing solid separated. The distillates were combined and neutralized with barium carbonate. The mixture was filtered and the residues tested for organic matter. None was found present. The aqueous portion was concentrated and the crystals obtained did not char. This excludes the possibility of volatile acids resulting from the oxidation.

The residues were combined and neutralized with barium carbonate. The excess barium carbonate was filtered off and gave no test for organic matter. The aqueous portion when evaporated to dryness was found to contain organic material. A positive test for picric acid could not be obtained.

SUMMARY.

It has been shown that azulene does not preëxist in the flowers of milfoil but is formed during the process of distillation in the preparation of the volatile oil.

The azulene-yielding compound is contained in the chloroformic extract from which the petroleum ether-soluble constituents (bulk of the so-called volatile oil but minus the blue azulene) have been removed.

The nature of this compound has not yet been determined.

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WASHINGTON BELLADONNA AND METHODS OF ASSAY.*

BY CLAIRE EVANS AND F. J. GOODRICH.

The somewhat unstable character of the active principles found in belladonna plants is well known and, in fact, the chemistry and structure of the important components have been thoroughly studied. The assay of the roots, as well as the leaves, of many belladonna plants for mydriatic alkaloids has been made, resulting in great variations with different samples. Some quantitative methods have been tried experimentally on prepared samples of the drug and many explanations offered as to the varying amounts of the alkaloids. Undoubtedly, numerous factors are responsible for the large differences in alkaloidal content of both roots and leaves.

It has been deemed of interest to investigate the alkaloidal content of belladonna roots collected over a period of years, using different, selected methods for making the determinations. Roots grown on the University of Washington campus were chosen because no assays, so far as could be learned, had been made on belladonna of western Washington. The purpose of the present study has been, therefore, to select a satisfactory method of assay and to determine the quantity of alkaloids from roots grown in the Pacific Northwest, collected in successive years and aged for varying periods.

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Many methods of analysis and modifications of these methods were tried. Those reviewed were the ones outlined by the United States Pharmacopœia since 1900, as well as the methods used by Mayer (1), Lyons (2), Gunther (3), Lefort (4), Thresh (5), Gerrard (6), Dunstan and Ransom (7), Kippenberger (8), Falieres (9), Beckurts (10), Thoms (11) and Rasmussen (12). Several of the methods were not deemed practical for the work, or did not give consistent results, and were not used in the examination of a selected number of samples of belladonna roots.

COMPARISON OF ASSAY METHODS.

In looking for a suitable method with which to assay the roots for alkaloids, many methods were reviewed and four of them were finally adopted: (*a*) the method of the U. S. P. X, (*b*) a modification of the latter, (*c*) a method by Lyons and (*d*) a method by Dunstan and Ransom.

After using the method of assay of the U. S. P. X on several samples and finding the results to agree only fairly well, a slight modification was tried. In place of a percolator, a separatory funnel was used to exhaust the drug. Since it seemed that the tenth-normal acid would cause too great an error in case the exact end-point was not obtained, the alkaloids were finally titrated with twentieth-normal sulphuric acid. The excess was then treated with fiftieth-normal sodium hydroxide, using methyl red as indicator, since the end-point given with it was more readily recognized than that given with cochineal.

Lyons suggested macerating about 10 Gm. of the drug with a mixture consisting of 1 cc. of stronger ammonia water, 4 cc. of alcohol and 5 cc. of chloroform-ether (1:6 by volume). The drug and solvent were thoroughly mixed, packed in a percolator and allowed to macerate for five or ten minutes before percolating with the appropriate solvent. From here the assay was carried on by shaking out with acid, then with chloroform and finally by titrating the alkaloids with twentieth-normal sulphuric acid.

According to the Dunstan and Ransom method about 20 Gm. of the dry, powdered root were exhausted by hot percolation with absolute alcohol. It was found that 60 to 80 cc. of the solvent were required. The percolate was diluted with water, acidified with HCl and repeatedly extracted with chloroform in order to remove fats and pigmented materials. The aqueous liquid was rendered alkaline with ammonia and the alkaloids removed with chloroform, which in turn was evaporated slowly on a water-bath. According to the original method, the alkaloids were determined gravimetrically at this stage.

In using the Dunstan and Ransom method, as specified above, it was found that the alkaloidal residue was never white. It would, therefore, be safe to conclude that there were still some impurities present which would give inaccurate results if the total weight of the residue from the final chloroformic extraction were calculated as alkaloids.

When the alkaloidal residue from the above was titrated with twentieth-normal sulphuric acid, and the excess of the latter titrated with fiftieth-normal sodium hydroxide solution, using methyl red as indicator, much lower results were obtained than by the gravimetric method. A comparison of the percentages obtained by using both the gravimetric and the volumetric methods, to determine the amount of alkaloids after extracting them by Dunstan and Ransom's method,

showed that samples which gave by the volumetric method 0.07, 0.14, 0.07 and 0.065 per cent of alkaloids, gave gravimetrically 0.27, 0.36, 0.27 and 0.14 per cent, respectively. The fact that the results from the gravimetric work were higher would tend to prove the presence of impurities in the residues. In this experimental work, it was therefore decided to titrate the alkaloidal residue.

TABLE I.—COMPARISON OF RESULTS—AIR-DRIED BELLADONNA ROOTS.

	Per Cent of Alkaloids.			
Sample.	U. S. P. X.	Modified U. S. P. X.	Lyons.	Dunstan-Ransom.
1	0.26	0.12	0.38	0.18
	0.30	0.15	0.26	0.32
	0.38	0.32	0.25	0.33
2	0.21	0.136	0.156	0.137
	0.23	0.16	0.190	0.137
3	0.16	0.138	0.096	0.14
	0.19	0.268	0.116	0.17
	..	0.133	0.028	..
4	0.28	0.099	0.053	0.30
	0.29	0.023	0.155	0.30
	..	0.128	0.104	..
	..	0.190	0.060	..
	..	0.092	0.158	..
5	0.18	0.134	0.189	0.13
	0.11	0.167	0.145	0.14
6	0.08	0.128	0.033	0.07
	0.10	0.110	0.109	0.07
	..	...	...	0.067
	..	...	...	0.139

From the accompanying table, reading from top to bottom in the column of results obtained by the U. S. P. X process, it is evident that the results yielded by that method are but fairly constant for each sample assayed. The official procedure also has the disadvantage of requiring much time and repeated testing before exhaustion of the drug is attained.

The figures obtained for the various samples by the modification of the U. S. P. X method show but little in favor of altering the official process in this way.

In the method suggested by Lyons, the solvent (chloroform and ether 1 to 6) proved of no special advantage. The various results obtained by this process, as shown by Table I, differed greatly for the same samples.

The data obtained by using the method suggested by Dunstan and Ransom, followed by titration, gave results which were the most consistent throughout the series of assays. Another point in favor of this method is that the actual attention required to exhaust the drug was less than with other methods, and the technique necessary in transferring the weighed sample of the drug to the Soxhlet was unimportant, whereas with the other assays there was always loss of time and much inconvenience at this stage of the work.

Reading across the table from left to right shows that the percentages obtained for the same sample, by various methods of assay are very discordant,

and the conclusion arises that there is still much room for improvement in the assays used.

Possible reasons for the lack of agreement between the above four methods, as indicated by the results in the first table, might be the absence of total exhaustion, caused by channeling, and insufficient maceration in the case of the total exhaustion processes. The possibility of hydrolysis might explain the lack of checks and the greater or lesser amount of hydrolysis accounts for variation by different methods. Since the final chloroformic solvent was evaporated on a water-bath, some of the volatile alkaloidal principles, claimed by some to be present, might have been dissipated. However, this last explanation could hardly be true, since the evaporation was the same by all methods and they did not all yield unusually low results.

Washington Roots after Storing.—Analyses were made of samples of belladonna roots which were harvested in the following years: 1922, 1923, 1924, 1925, 1926, 1927, from the University of Washington drug garden. No record was made as to exact dates of collections or age of the perennial roots. The collections were presumably made from plants grown prior to 1922, thus making an increase of one year in the growing age of each consecutive sample. These roots were all dried and stored until 1928, when they were assayed. The results were based on Dunstan and Ransom's method with addition of titration. The air-dried drug was used for analysis and the per cent of alkaloid then calculated on the basis of drug dried at 100 degrees C.

TABLE II.—ROOTS AFTER STORING—DRIED AT 100 DEGREES C.

Year of Collection.	Average Per Cent of Alkaloids.
1922	0.348
1923	0.166
1924	0.154
1925	0.318
1926	0.166
1927	0.104

As shown by Table II there was evidently no correlation between the length of time stored and the percentage of alkaloids, since the roots gathered in 1922 contained the largest quantity, while the 1927 roots contained the least. The variability between samples of different years might be accounted for by a possible difference in the total age of the root: *i. e.*, period of growth and time stored taken together. Another significant factor is that they were not collected at exactly the same time of the year.

SUMMARY.

Of the four methods used in this work, the assay by Dunstan and Ransom (with titration) seemed preferable.

In making a comparative analysis of the roots collected in successive years and stored over a period of time, it was found that there was no correlation between length of time stored and alkaloidal content.

The great variability between the samples of different years may be due

to the natural variation of different crops, to the total age of the roots, or to a possible difference in the time of year when they were collected.

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COLLEGE OF PHARMACY,
UNIVERSITY OF WASHINGTON,
SEATTLE, WASHINGTON.

A PHYTOCHEMICAL INVESTIGATION OF THE OLEORESIN OF PINUS MONTICOLA DOUGL.*

BY P. A. FOOTE[1] AND N. T. MIROV.[2]

Western White Pine (*Pinus monticola* Dougl.) is a five-needle pine growing on the "middle and upper slopes of northwestern mountains from the west side of the continental divide in northern Montana and British Columbia to Washington, Oregon and California" (1). It is a large source of timber in the West. The oleoresin herewith reported on came from trees growing in an environment far from their optimum; so much so that they offer more than ordinary phytochemical interest. Especially will this be true when the oleoresin from other localities has been analyzed. Unlike most spirits of turpentine, which usually consist of terpenes, sesquiterpenes and their oxygenated products, this oil contains about one per cent of a paraffin hydrocarbon, *n*-undecane, $C_{11}H_{24}$. This gives our investigation added interest since this paraffin has been identified only once before in the plant kingdom. This was found by Simonsen and Rau (2) in 1922 in the oleoresin of *Pincus excelsa* growing in India. In 1913 Schorger (3) isolated a paraffin hydrocarbon from *Pinus lambertiana*. This had physical properties close to

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[1] Prof. of Pharmacy, University of Florida.

[2] Formerly with the U. S. Forest Service.