SANTONIN—ITS DETECTION AND ESTIMATION

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Received December 14, 1950

The active principle of Levant wormseed was independently, and almost simultaneously, discovered in 1830 by Apothecary Kahler[1], of Dusseldorf and Alms[2], a candidate in pharmacy of Penzlin in Mecklenburg. Kahler sent his paper in April, 1830, to the editors of the *Archives des Apothekervereins*. Alms sent his paper late in August, 1830. Kahler's paper was laid aside, and the two articles were published side by side in the same number of the *Archives des Apothekervereins*. The priority of the discovery should, therefore, go to the credit of Kahler, who sent his paper earlier. In olden times, wormwood was known by the name of " Santonion " by Greek physicians Dioscorides, Pliny, Galen and their pupils. The Arabs called it " Afsantin." As a mark of regard for the old nomenclature, Oberdorfer[3] proposed the name " Santonin " for the newly discovered active principle of Levant wormseed. This name has been retained up to the present day. Santonin was commercially prepared by Merck[4] in 1832, and put on the drug market for the use of the medical profession.

Santonin is extracted from the leaves and unexpanded flower-heads of the santonin-containing species of *Artemisia*. Santonin-free artemisia, similar in appearance to the santonin-containing species, is also sometimes put on the drug market for sale.

Distinction Between Santonin-Containing and Santonin-Free Artemisia

There are no reliable botanical features, on the basis of which it is possible to distinguish the santonin-containing artemisia from the santonin-free drug. In the case of Asiatic species, the santonin-free artemisia in the fresh condition, possesses an intense unpleasant nauseating aroma, while the santonin-containing artemisia possesses a mild camphor-like aroma. With dried material from closely allied species, the two are indistinguishable on the basis of the intensity of the characteristic aroma.

Wallis and Mowat[5] have attempted to formulate diagnostic histological features differentiating the genuine santonin-containing artemisia from the santonin-free drug. According to them, the santonin-free santonica can be distinguished by (i) the presence of apical marginal hairs upon the involucral bracts, and (ii) the presence of hairs on the leaves. The scope of their work was limited. Their conclusions were based on the microscopical examination of a restricted number of specimens of Russian santonica, consisting of flowerheads and the linear-lanceolate leaves of

the flowering shoots. The foliage leaves present during the vegetative period were not given any consideration. The santonin-containing species from other sources were not examined. The generalisations as formulated by Wallis and Mowat cannot be generally applied and do not hold good in the case of santonin-containing species of *Artemisia* from India, Afghanistan and Iran. The Indian santonicas—*Artemisia kurramensis* Qazilbash[6] and *Artemisia brevifolia* Wall—have vegetative leaves with a dense coating of woolly hairs, and involucral bracts with marginal hairs.

The same species shows enormous variation in the santonin content, when growing in different localities under different climatic and soil conditions. In Gilgit, N.W. Kashmir, and the adjoining areas, *Artemisia brevifolia* Wall is very common. The plant grows abundantly and covers extensive areas, but there are only a few limited areas where artemisia containing a workable percentage of santonin grows in commercial quantities. *Artemisia brevifolia* Wall growing in extreme xerophytic conditions is usually devoid of santonin. Collections of santonin-containing artemisia from different areas show remarkable variation in their santonin contents. Morphologically they are all alike.

Artemisia Cina (Berg) Willk grows abundantly in Kurdistan, Turkistan, and parts of North-East Iran. Botanical specimens collected from different geographical areas are morphologically similar. They however show considerable differences in their santonin contents. Similar is the case of *Artemisia kurramensis* Qazilbash growing in the Upper Kurram and the adjoining Afghan territory. Collections of *Artemisia kurramensis* Qazilbash from different localities show considerable variation in their santonin content. Table I gives the percentage of santonin in samples of *Artemisia kurramensis* Qazilbash.

TABLE I

Locality	Date of collection	Santonin per cent.
Burki	3·9·'37	2·08
Kachkira	3·9·'37	2·38
Karakhila...	3·9·'37	1·16
Lalmi	3·9·'37	2·18
Nastikote...	3·9·'37	1·79
Kharlachi...	3·9·'37	2·41
Shalozan	27·9·'37	2·48
Dattakhel...	25·9·'37	1·40
Burki	20·10·'47	2·80
Kharlachi...	20·10·'47	2·70

In particular *Artemisia* species, such as *Artesimisia kurramensis* Qazilbash and *Artemisia brevifolia* Wall, there are no reliable botanical features, on the basis of which one could distinguish artemisia with higher santonin-content from artemisia with low santonin-content. With our

present knowledge it is not possible to evaluate the santonin value of artemisia on the basis of botanical characters alone. Chemical examination is always necessary.

Qualitative Chemical Examination

The genus *Artemisia* comprises a very large number of species, but the number of species containing santonin is very limited. Usually the santonin-containing species grow side by side with the santonin-free forms. Qualitative tests to distinguish between them have been proposed by different workers. These tests are based on either (i) sublimation or (ii) colour tests.

For sublimation tests, a small quantity of the drug is heated in a suitable apparatus, and the sublimate is tested for its santonin content. In view of the cost of the equipment, and the unreliability of the results, sublimation methods cannot be employed as rapid qualitative tests for the preliminary field work necessary for making commercial collections. In sublimation, santonin is accompanied by other related substances, including the resinous oily matter, and the sublimates do not give a reliable measure of the quantity of santonin present in the drug under examination.

The qualitative test with potassium methoxide proposed by the writer[7,8] is simple and reliable. It consists in shaking 0·5 g. of the powdered drug with 5 ml. of benzene for about 5 minutes, and filtering the mixture. The filtrate is evaporated to dryness in a porcelain dish on a steam-bath. 2 to 3 drops of potassium methoxide are added to the margins of the residue, and the dish again warmed on the steam bath. With material containing santonin, an orange-red, blood-red or carmine-red colour is produced, while in absence of santonin no such colour is observed. On treatment with this reagent, santonin-free artemisia gives an ochre, yellowish brown or burnt sienna colour. Potassium methoxide is prepared by gradually adding potassium 5 g., in small pieces, to 50 g. of methanol under a reflux condenser. A fairly large number of samples of different species of *Artemisia,* collected from different localities, were tested with potassium methoxide. Material devoid of santonin gave no orange-red, blood-red or carmine-red colour. In all cases quantitative determination of santonin confirmed the results of the qualitative tests. Table II gives the results of qualitative and quantitative examinations.

In addition to the samples recorded in Table II, a very large number of samples from various localities in Khyber, Tirah, Waziristan, Baluchistan, Swat, Dir, Bajaur, Chitral, Kaghan, Kurram, Gilgit and parts of Afghanistan were collected at different times. They were first tested qualitatively with potassium methoxide, and then assayed quantitatively. Samples showing negative results with potassium methoxide were found devoid of santonin. The potassium methoxide test has been found perfectly reliable and of general application in sorting out santonin-containing artemisias.

In the Gilgit agency there are extensive areas where *Artemisia brevifolia* Wall is abundant. In 1950 large commercial collections were made under the direction of the writer. Mass collections were made only

TABLE II

COLOUR REACTION OF VARIOUS SAMPLES OF ARTEMISIA WITH POTASSIUM METHOXIDE

Serial number	Locality	Date of collection	Colour with potassium methoxide	Santonin (per cent.)
1	Malikhel	16·8·'28	Burnt sienna	nil
2	Tirah	16·8·'28	,, ,,	nil
3	Taidah	21·9·'28	,, ,,	nil
4	Kharlachi	17·8·'26	Deep orange red	1·04
5	Nastikote	17·8·'26	,, ,,	1·15
6	Lalmi	27·9·'26	,, ,,	1·54
7	Laila danda	16·8·'26	Yellowish brown	nil
8	Kaghan	25·9·'28	,, ,,	nil
9	Chitral	16·9·'30	Burnt sienna	nil
10	Kharlachi	12·9·'28	Deep orange red	1·04
11	Dadder	19·9·'28	Light orange red	0·57
12	Landi Kotal	18·7·'30	Burnt sienna	nil
13	Hindu Bagh	20·6·'30	Yellowish brown	nil
14	Quetta	28·6·'30	,, ,,	nil
15	Kalat	12·7·'30	,, ,,	nil
16	Mingora	30·8·'30	Burnt sienna	nil
17	Jakdalik	12·9·'30		nil
18	*Spinwam*	15·9·'30	,, ,,	nil
19	Bajaur	30·9·'30	,, ,,	nil
20	Shirina	28·9·'30	,, ,,	nil
21	Khost	25·8·'32	Pale green	nil
22	Astor	27·8·'50	Ochre	negligible
23	Rattu	27·8·'50	Deep blood red	1·32
24	Gurhial	25·8·'50	,, ,,	1·25

from the areas where the artemisa gave a positive test with potassium methoxide. Samples were tested quantitatively and collections from different areas showed variation in santonin content, with 0·9 to 1·43 per cent. in the best areas. Samples giving negative results with potassium methoxide showed no santonin on assaying quantitatively.

Alcoholic potash has also been used. Potassium methoxide possesses certain advantages over alcoholic potash as a reagent for qualitative tests. It is more reactive and more sensitive and the colour reaction is more distinct and definite. Potassium methoxide remains unchanged in colour and viscosity for a long time. The test is very economical, and requires a small amount of material. Alcoholic potash in several cases has given

false results. Heyl and Tunmann[9], Eder and Schneiter[10], Rosenthaler[11] and Mouton[12] employed alcoholic potash. Heyl and Tunmann did not notice any colour differentiation between the santonin-containing and the santonin-free artemisias, whilst other workers noted remarkable differences in colour in the two cases. With santonin-containing artemisia a yellowish red and in the case of santonin-free artemisia a yellowish green colour was observed. According to Mouton, the intensity of the colour is not proportional to the amount of santonin present. Material with a low santonin content gave a deeper colour than material containing a higher santonin content. The writer has also tried alcoholic potash with a large number of samples of santonin-free artemisias from Baluchistan and Afghanistan. The colour reactions produced with material containing artemisin were not quite distinct from similar colour reactions produced with material containing santonin. With materials of low santonin content, the colour indications could not be relied upon.

Quantitative Chemical Examination

The commercial value of artemisia is determined on the basis of its santonin content. In the plant tissue, santonin is closely associated with oily and resinous substances and in consequence the quantitative separation of santonin is a task of considerable difficulty. The numerous suggested methods yield results of varying accuracy and there is no perfectly reliable exact method. The assay presents the following problems: (i) Complete extraction of the santonin from the plant-material, (ii) separation of the santonin from resinous and oily substances, (iii) purification of the santonin.

After trial of several recorded assay methods the following improvement upon the author's old assay method[13,14] was found to give very good results.

New Proposed Method

15 g. of finely powdered santonica is thoroughly shaken with 1·5 g. of powdered anhydrous sodium carbonate. The mixture is shaken in a 500-ml. separating funnel with 15 ml. of 15 per cent. ammonia solution for a few minutes, and 150 ml. of benzene is added, the mixture being thoroughly shaken at frequent intervals during 3 hours. After standing overnight the contents are thoroughly shaken for 15 minutes and allowed to settle for half an hour, and then carefully filtered through cotton wool previously moistened with benzene, into a 100-ml. measuring cylinder. During filtration the stem of the cylinder is covered to avoid the loss of benzene by evaporation. 100 ml. of the filtrate is transferred to a distilling flask. This represents 10 g. of the drug. The measuring cylinder is rinsed with a little benzene and the washing added to the distilling flask. The benzene extract is concentrated to about 15 ml. and the extract transferred to a beaker, the distilling flask being rinsed with 5 ml. of benzene two or three times. The combined benzene extract is evaporated completely to dryness. The residue is heated at 60° to 70°C. with

110 ml. of 5 per cent. w/v solution of barium hydroxide, freshly prepared and filtered, on a water-bath for about 20 minutes, the contents of the beaker being carefully stirred with a glass rod during this time. A yellowish green or dark green resinous solid separates. The hot solution of the barium salt is filtered through a double filter paper, previously moistened with water, the filter paper and the beaker washed twice with 10 ml. of hot water and the filtrate acidified with dilute hydrochloric acid. A slight excess of acid is then added and the mixture left in a water-bath at a temperature 60° to 70°C. for about 20 minutes and stirred gently at short intervals. After 15 minutes the solution is tested with congo-red paper, and if the acid reaction is not well marked, 1 or 2 ml. of acid is added. The acidified solution is allowed to cool, and when lukewarm is transferred to a separating funnel. The beaker which had contained the acid solution is rinsed with 25 ml. of chloroform, the washing is put into the separating funnel, and the contents shaken for 5 minutes. After separation the chloroform layer is passed through a pledget of cotton wool, moistened with chloroform, into an Erlenmeyer flask. The process is repeated with 3 successive quantities of 20, 15 and 10 ml. of chloroform. The combined chloroform solution is evaporated to dryness, the residue being freed from traces of chloroform by blowing dry air through it. The residue is boiled with 50 ml. of ethanol (15 per cent. w/w) under a reflux condenser for 15 minutes and filtered hot. The flask and the filter paper are washed 3 times with 5 ml. of warm 15 per cent. ethanol. The filtrate is heated with 100 mg. of a mixture of equal parts of animal charcoal and kieselguhr under a reflux condenser for about 10 minutes to remove resinous and colloidal impurities. It is then filtered hot into a crystallising dish. The residue and the filter paper are thrice rinsed with 5 ml. of the 15 per cent. ethanol. The filtrate is allowed to crystallise; the sides of the dish are scratched gently with a glass rod to hasten crystallisation. The dish is kept in the dark at 15° to 17°C. for 24 hours. The crystals of santonin are carefully collected on a weighed filter paper, washed twice with 5 ml. of the 15 per cent. ethanol, dried to constant weight at 100° to 105°C. and placed in a desiccator over sulphuric acid for 24 hours. The weight of santonin is determined and 0·0064 g. added as solubility correction. The total weight multiplied by 10 gives the percentage of santonin.

Consideration of the Proposed Method

Benzene is selected as the primary solvent, as it extracts the santonin efficiently but removes less resinous substances than do other solvents. The use of anhydrous sodium carbonate, and the ammonia solution, renders the acid resins insoluble in the benzene extracts and removes the fatty substances by saponification. Longer contact of the powdered drug pretreated with anhydrous sodium carbonate and ammonia enables the solvent to extract the santonin completely. A good deal of experimental work was carried out with the Kurram santonica (*Artemisia kurramensis* Qazilbash) and the Kashmir santonica (*Artemisia brevifolia* Wall), repre-

senting the fall crops of 1949 and 1950. The assaying was done in duplicate with the proposed gravimetric method and concordant results were obtained. A comparative study of the gravimetric methods of Eder and Schneiter[15]. Janot and Mouton[16], Janot and Esteve[17], Coutts[18], and Massagetov[19] was also carried on with the same materials, and the quantity and purity of the final santonin product determined in each case. The proposed method on the whole yielded better results as regards the amount and the purity of the final product as judged by the white colour and melting point (171° to 173°C.) . There is practically no loss of santonin, on account of the special technique employed for the complete extraction of santonin from the botanical material and the efficient separation of the product during final purification.

Summary

1. A short history of the discovery of santonin is given.

2. There are no reliable botanical features for distinguishing the santonin-containing artemisia from the santonin-free drug.

3. The qualitative test with potassium methoxide is quite reliable for distinguishing the santonin-containing artemisias.

4. A new gravimetric method for the estimation of santonin is described.

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