A NOTE ON THE GRAVIMETRIC DETERMINATION OF SANTONIN

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SANTONIN is extracted from the leaves and unopened flower-heads of a number of santonin-containing species of Artemisia. The most important santonin-containing species are :—*Artemisia Cina* (Berg) Willkomm, *Artemisia pauciflora* Weber, *Artemisia brevifolia* Wall and *Artemisia kurramensis* Qazilbash.[1] They are confined to different geographical areas, and differ from one another as regards ecological, morphological and physiological features. Santonin in the plant tissue is closely associated with resinous, oily and fatty substances. The relative proportion of santonin, oils, fats and resins varies remarkably in different species. The synthesis of chemical constituents in the plant is dependent upon its physiological activities. The normal physiological functions are greatly influenced by various ecological factors such as temperature, solar radiation, altitude, atmospheric humidity, soil composition and water-availability. Prolonged influence of abnormal ecological conditions bring about remarkable changes in the nuclear structure of the cells, and these changes invariably are followed by notable morphological and physiological changes. Under the influence of ecological factors, a particular santonin-containing species may show a remarkable variation in its santonin content. Individuals of the same species, when growing under different ecological conditions, show great differences in the relative proportions of santonin, oils and resins. Some of them contain a very high proportion of resinous and oily substances. The complete separation of santonin from these substances is rather a difficult task for which different workers have employed special techniques. The important gravimetric methods fall into two main groups:—(1) methods by which a soluble santonate is formed; (2) methods using a solvent for the primary extraction of santonin. The numerous suggested methods yield results of varying accuracy as judged by the amount and the purity of the final santonin product. The purity of the final product is determined by the colour and the melting-point. Pure santonin is white, and the m.pt. varies from 171° to 174° C. The efficiency of a particular method can easily be ascertained by taking into consideration the weight and purity of the final santonin-product. The weight should be consistent with the theoretical values of the control experiments. The purity of the final product can be determined by noting the colour and the m.pt. Impure and contaminated product is a coloured material, with m.pt. lower than 171° C.

METHODS BY WHICH A SOLUBLE SANTONATE IS FORMED

Trommsdorff,[2] Miahle and Claud,[3] Calloud,[4] Wettstein,[5] Grosschopff,[6] Hirsch,[7] Busch,[8] Soteria,[9] Sestine[10] and Massagetov,[11] extracted santonin from the plant as calcium santonate after treating it with a mixture of calcium

oxide or calcium hydroxide in water, dilute ethanol or ethanol. The solution is acidified with hydrochloric acid, acetic acid, or carbon dioxide and the santonin liberated. Massagetov, Sestine and Soteria extracted the liberated santonin from the acidified solution with chloroform. Other workers dissolved the liberated santonin in ethanol, and the ethanolic solution, after treatment with a suitable purifying reagent (animal charcoal, lead acetate, sodium carbonate, or zinc sulphate), was filtered, the fatty and resinous substances being removed. Santonin crystals were recovered from dilute ethanolic solution.

Sestine pretreated the drug with light petroleum, which not only removes the oily substances but also extracts a portion of the santonin. If the light petroleum extract is evaporated in a porcelain dish on a steam bath, and the residue is treated with a few drops of potassium methoxide, a deep orange red or carmine red colour is produced. This indicates an appreciable amount of santonin present in the light petroleum extract.

Massagetov and Soteria removed the oily and resinous substances by shaking the chloroform extract with 4 per cent. sodium hydroxide solution and dilute ammonia solution respectively. Soteria obtained a syrupy residue of santonin by evaporating the chloroform. Massagetov dissolved the dried chloroform extract in 1 or 2 ml. of ethanol, to which was added 100 ml. of boiling water, the mixture was evaporated to 50 to 70 ml., and then placed in a cool place for crystallisation. Santonin crystals were collected after 16 to 24 hours, and a solubility correction was applied.

Dragendorff[12] and Neuman[13] used sodium hydroxide and extracted the santonin in the form of sodium santonate. On treatment with hydrochloric acid, santonin is liberated and extracted with chloroform.

The oldest methods of extraction of santonin are included in this group. These methods suffer from the following disadvantages:—(i) the methods require several manipulations, are long and time consuming; (ii) large volumes of liquids are used; this is definitely a great disadvantage, when dealing with large quantities of material; (iii) the santonin finally obtained is not quite pure. The different methods yield final products of varying purity. In this group, Massagetov's method on the whole gives very good results, especially when dealing with artemisia containing a high percentage of santonin. It must, however, be pointed out that some santonin is also removed along with resinous substances when chloroform extract is shaken with 4 per cent. sodium hydroxide solution. The presence of santonin in the sodium hydroxide solution can easily be detected with potassium methoxide.

Methods Using a Solvent for the Extraction of Santonin

A number of solvents have been used for this purpose. The more important ones are: chloroform, ether, benzene, ethanol, acetone.

(i) *Chloroform.* In the methods of Fromme,[14] Van den Berg,[15] Nelson,[16] Katz-Nelson[17] and Qazilbash[18] chloroform is used as the primary solvent. The chloroform extract is treated with an aqueous solution of barium hydroxide and the santonin is converted into barium santonate.

The latter is acidified and the liberated santonin is extracted with chloroform. The residue obtained from the chloroform solution is dissolved in ethanol and the santonin is crystallised from 15 per cent. ethanol. The methods employed suffer from the following disadvantages:—(i) numerous extractions are necessary; (ii) chloroform is not a suitable primary solvent; under the action of light and moist air, chloroform readily undergoes decomposition into phosgene and hydrochloric acid and the decomposition is accelerated during the refluxing process for extraction, also during the distillation for recovery of the solvent; (iii) the santonin crystals finally obtained contain impurities, as is shown by the colour and the m.pt.; (iv) a definitive solubility correction is applied, but the solubility is variable at different temperatures.

(ii) *Ether.* Thatter,[19] Katz,[20] Goerlich,[21] Caspari[22] and Feldhoff[23] employed ether as the primary solvent. Kariyone and Kimura[24] also employed ether as the primary solvent in their volumetric method. Lime, barium hydroxide, and carbonic acid, aluminium acetate and magnesium oxide, or basic lead acetate were employed as decolorising and purifying agents and for the removal of resinous impurities. The santonin is finally crystallised from 15 per cent. ethanol. Thatter's method gives an impure final product, which is contaminated with some aluminium acetate and magnesium oxide. Katz's method is very long and tedious, and the final santonin product is impure and contaminated with resinous impurities. Goerlich adopted Katz's method of extraction but modified his method of purification. Goerlich's method requires several manipulations and is very lengthy and on this account is not of much practical utility. The final product is impure and contaminated with resinous impurities. Feldhoff's method gives fairly good results. The final product is slightly coloured on account of the presence of some resinous impurities. The percentage of santonin is also somewhat lower than that obtained by the other reliable gravimetric methods.

(*III*) *Ethanol.* Burlage and Smith[25] employed ethanol for the extraction of santonin from the drug. The powdered drug is first defatted by boiling with light petroleum saturated with santonin. The drug thus treated is extracted with ethanol, the combined extracts are boiled with lead acetate solution and filtered hot. The filtrate is allowed to stand for 24 hours and the crystals are collected and dried to constant weight at 100° C. A solubility correction is applied.

The use of light petroleum saturated with santonin is objectionable, since some additional santonin is introduced into the plant tissues, and is not removed prior to extraction with ethanol; this leads to high results. The final product of santonin is contaminated with resinous and other impurities from the lead acetate. Light petroleum removes the oily substances but very little of the resinous substances is eliminated. The m.pt. of the final product varies from 166° to 168° C.

(*IV*) *Acetone.* Palkin[26] used acetone for the extraction of santonin. The concentrated extract is treated with alkali, the santonin is converted to the alkali salt, the resins are precipitated by calcium chloride and the solution is filtered. The filtrate is acidified, the acidified solution is

extracted with chloroform, the chloroformic extract is washed with alkali and evaporated to dryness. The residue is converted to calcium santonate and the solution is then acidified and the liberated santonin is dissolved in chloroform. The residue on evaporation represents the yield of santonin.

Palkin's method is long and involves several time-consuming manipulations, and the results are not concordant. The residue of santonin is contaminated as judged by its colour and m.pt. The m.pt. varies from 165° to 168° C. Palkin's method therefore cannot be employed as a reliable practical method.

(*V*) *Benzene.* Eder and Schneiter,[27] Mouton,[28] Janot and Mouton,[29] Janot and Esteve,[30] Coutts,[31] Farnandez and Socias[32] and Qazilbash[33] employed benzene for the extraction of santonin from the plant. Butzelman[34] used a mixture of benzene 90 per cent. and chloroform 10 per cent. for the primary extraction. Benzene is given preference over chloroform and ether, as it dissolves less of the resinous substances. This group includes the latest gravimetric methods; the more important are given below in outline:—

(i) *Eder and Schneiter's method.* The finely powdered drug is shaken frequently during half an hour with benzene. An aliquot portion of the filtered extract is evaporated to dryness. The dry residue is boiled with 15 per cent. ethanol under a reflux condenser for 15 minutes and filtered. The cooled filtrate is boiled with kaolin, and filtered hot. Santonin crystallises on cooling and standing for 24 hours. A solubility correction is added.

(ii) *Janot and Mouton's method.* The powdered drug is made into a paste with ammonium hydroxide solution. The paste is dried and powdered. The powdered material is shaken with benzene for half an hour and an aliquot portion of the extract is evaporated and dried at 100° C. The residue is boiled with 15 per cent. ethanol. The santonin separates from filtrate on cooling and standing for 24 hours. A solubility correction is applied.

(iii) *Janot and Esteve's method* This is an adaptation of Janot and Mouton's method. The dry residue obtained from the benzene extract is treated with a saturated solution of barium hydroxide, and filtered; the filtrate is acidified with dilute hydrochloric acid and after 48 hours the crystals of santonin are collected.

(iv) *Coutts's method.* The coarsely powdered drug is shaken frequently during 6 hours with benzene and the extract is agitated with 8 per cent. sodium carbonate solution. An aliquot portion of the benzene extract thus obtained is evaporated to dryness on a water-bath. The residue is heated with a saturated solution of barium hydroxide, filtered and the cold filtrate acidified with dilute hydrochloric acid. The santonin crystals are collected after 24 hours from the acid solution, and dried to constant weight.

(v) *Butzelman's method.* The powdered material is first treated with 10 per cent. hydrochloric acid and then shaken with a mixture of benzene 90 per cent. and chloroform 10 per cent. After 12 hours the extract is boiled with 5 g. of barium hydroxide and 100 ml. of water and filtered; the filtrate is acidified with hydrochloric acid. The solution is shaken with

chloroform, the extract washed with 1·5 per cent. sodium hydroxide solution and water, and evaporated to dryness. The residue is boiled with 15 per cent. ethanol, filtered and allowed to cool. After 24 hours the crystals of santonin are collected, washed with 15 per cent. ethanol alcohol and dried at 105° C.

(vi) *Qazilbash's method.* The finely powdered drug is thoroughly mixed with anhydrous sodium carbonate, and the mixture shaken with 15 per cent. ammonia solution. Benzene is added and the mixture thoroughly shaken at frequent intervals during 3 hours. After 24 hours, an aliquot portion is dried, and then heated at 60° to 70° C. with 5 per cent. w/v solution of barium hydroxide, and filtered. The filtrate is acidified with dilute hydrochloric acid. The cooled solution is shaken with chloroform, and the extract evaporated to dryness. The residue is boiled with 15 per cent. ethanol under a reflux condenser and filtered. The filtrate is heated with a mixture of equal parts of animal charcoal and kieselguhr, and filtered. The filtrate is allowed to crystallise in the dark at 15° to 17° C. for 24 hours. The santonin crystals are dried at 100° to 105° C. and placed in a dessicator over sulphuric acid for 24 hours. A solubility correction is applied.

The method of Eder and Schneiter needs very little material and the number of manipulations is much reduced. The method is simple, precise and rapid, and on the whole gives very good results, when dealing with *Artemisia Cina* (Berg) Wilkomm and *Artemisia kurramensis* Qazilbash, containing more than 1·5 per cent. of santonin. The final product is lightly coloured, and is not quite pure as judged by the colour and the m.pt. The m.pt. of santonin, obtained from artemisias containing more than 1·5 per cent. varied from 166° to 168° C. With *Artemisia brevifolia* Wall, and the induced polyploids of *Artemisia kurramensis* Qazilbash, the results are unsatisfactory. With artemisias containing a low percentage of santonin, the final product is much contaminated with resinous impurities. In Eder and Schneiter's method, santonin is not completely extracted during the period of half an hour, the final result on this account is somewhat lower than the result obtained by the methods of Massagetov and Qazilbash. Eder and Schneiter agitated the powdered drug for half an hour. Coutts considered 6 hours' agitation necessary for adequate extraction of santonin. Farnandez and Socias digested the drug with benzene for 24 hours. Coutts's method gives low results. Some santonin is also removed with the sodium carbonate solution used to remove the oily and resinous substances. Moreover all the santonin is not crystallised out during a period of 24 hours. Complete separation of all the santonin as crystals takes a much longer time. Janot and Esteve collected the santonin crystals after 48 hours. The acidified solution, however, still contained some santonin. The santonin crystals finally obtained by the methods of Coutts and Janot and Esteve are contaminated.

The methods of Janot and Mouton, and Buzelman give satisfactory results when dealing with artemisias containing a good percentage of santonin. With artemisias containing a high proportion of resinous and oily substances, the final product of santonin is impure. Various forms

of *Artemisia brevifolia* Wall, growing at high altitudes under extreme xerophytic conditions in the Himalayas, contain a high proportion of oily and resinous substances.

Qazilbash's method is of general application. Santonin is adequately extracted, and the final product of santonin is pure as judged by the colour and the m.pt. The m.pt. is 171° to 173° C. and the product is white.

SUMMARY

1. The important santonin-containing species of Artemisia are: *Artemisia Cina* (Berg) Willkomm, *A. pauciflora* Weber, *A. brevifolia* Wall and *A. kurramensis* Qazilbash. They represent distinct species, differing from one another ecologically, morphologically and physiologically.

2. Santonin is closely associated with oily and resinous substances in the plant tissue. The complete separation from these oily and resinous substances is a difficult task.

3. A review of important gravimetric methods is given.

4. The gravimetric methods are useful in determining santonin in artemisias containing a good amount of santonin. Manufacturers are not interested in artemisias with low santonin-content.

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