A Thesis on

The Seasonal Variation in Santonin Content of the Indigenous Halophytic Artemisias

submitted in partial fulfilment of the requirements for the degree of

Doctorate of Philosophy
of the
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P R E F A C E .

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CONTENTS

INTRODUCTION

Santonin is at present extracted commercially from the unexpanded flowerheads of Artemisia cina, (Berg), Willkomm, and certain other species of Artemisia. This part of the herb is considered to contain the highest proportion of this principle and it is believed to be present in maximum amount just before the flowerheads expand. It is known, however, that the leaves of certain other species contain santonin and may be used for extraction of it. There is no evidence regarding its occurrence in the leaves of A. cina, or in the flowerheads of the other species.

It was thought that the behaviour of santonin might be analogous to that of certain alkaloids, etcetera, which have been shown to be formed in the leaves and to be translocated to the younger parts of the plant, such as the growing points. It was decided therefore to carry out an investigation of the different parts of successive batches of Artemisia maritima, Linn., and A. gallica, Willd., which contain santonin and which are to be found growing wild on the east coast of Scotland. The seasonal variation in the santonin content would thus be ascertained.

This was carried on over a period of three seasons and a regular periodic variation was found to exist. A probable explanation of the variation was formulated, correlating it with respiratory processes in the plant.

Batches of the two species were also collected from a large number of localities all round the coasts of England and

Wales. On examination these were also found to contain santonin, although the proportion was not so high as in the Scottish plant. Wherever possible a few successive batches of the species were obtained from these other localities and a similar seasonal variation in the santonin content was found. Cultivated plants also showed a similar variation.

A new assay process was formulated, and was found to be reliable in yielding consistent results and can be applied to drugs of low santonin content. Most of the existing methods were tried out and observations on their use are made.

At the beginning of the research it was not known whether the two species were sufficiently delimited and Dr. J.W. Gregor of the Scottish Society for Research in Plant-Breeding, was asked by the writer for his opinion on the advisability, and desirability for the present research, of carrying out a study of the range of variation within the species of these plants. He replied as follows :-

"The experimental study of species, and of the ecological and geographical races which make up species, promises ultimately to bring the results of taxonomic studies into line with the requirements of research workers in other fields of science"

"Until such time, however, as the results of the combined efforts of geneticists, ecologists, cytologists and morphologists regarding the status of the specific and sub-specific units of our flora become available the research worker

who is desirous of having his experimental material identified, be it plant or animal, has no alternative but to accept the opinions, based on morphology, of competent systematists. While "identification by observation" must be relied upon at the present time, it should be remembered that experimentation has shown that races morphologically indistinguishable may be physiologically dissimilar."

"To attempt an analysis of the sub-specific units contained in A. maritima of Linné is obviously outside the scope of your investigation and in my opinion the only course open to you is to accept the diagnosis of the Kew botanists and mention the habitat from which each collection was taken."

The two species are sufficiently well distinguished without referring to the sub-specific characters. This work is more concerned with the seasonal variation in santonin content, which is probably similar in all similarly situated variants of the species, than with the purely botanical characters of the plant. In accordance with Dr. Gregor's advice and in view of the futility of an examination of the sub-specific characters of plants from only one or a limited number of localities, a comprehensive study of these characters was not made. Certain characters which have been shown to be of value in identifying certain other species of Artemisia, when the flowerheads alone are available for this purpose, were, however, thoroughly examined. The author's identification of the two species was confirmed by authorities on systematy, to whom sample batches were sent.

PART I.

HISTORICAL

CHAPTER I.

EARLY HISTORY

The botanical name, Artemisia, is a very ancient one. Its origin is attributed by some authors to Artemisia, wife of Mausolus, and later Queen of Caria, who died in 348 B.C., and who gave it her own name in honour of its virtues. Others consider it to be derived from Artemis, the Greek name of Diana, Goddess of the Moon, who was regarded as presiding over the diseases of women.

The use of the plant as a drug can be definitely traced, under the name of "santonica" as far back as the time of Dioscorides (1) in 77 A.D. He mentions two varieties, one of which grew in Gaul in the country of the Santones, the modern Saintonge, and it seems probable that the name, "santonica", was derived from this. Pliny (2) also speaks of the plant as the current remedy for intestinal worms. The Romans during their conquest of Gaul in the first century A.D. found the plant growing there, and used it as an effective vermifuge. The species found growing in Saintonge was probably A. gallica, Willd.

The second variety mentioned by Dioscorides came from Cappadocia in N.E. Asia Minor and has been regarded through the ages as a valuable drug. The French plant soon passed into disuse and it is not mentioned as a source of the drug in later literature; nevertheless its local use has apparently continued. Later references always indicate a drug coming from further east. Saladinus (3) in 1450 refers to a remarkable vermifuge for children "semen ~~contra~~ sanctum vel Alexandr~~etta~~inum" which came from the

east, although the geographical source is not definitely stated. The drug has been known by several names. The "semen sanctum" of Saladinus, is stated by some authors to have been applied to the herb because the priests were wont to carry twigs of this plant in preference to branches of the Olive, in their ancient religious ceremonies. Others ascribe this name to the fact that it was believed to originate in the Holy Land or Palestine. Another name which was much used, "semen contra", is a contraction of the descriptive Latin "semen contra vermes". Other names given by Lemery (4) as synonyms of santonica are "semenzine", diminutive of the Italian "semenza" (seed); "semen santonicum"; "semen Zedoaria" and "Hagiospermum"; other names common in France are "santoline"; "Barbotine" and "cantoline. In connection with "santoline" it is interesting to note that "santolina" also means the Holy Herb, and that the name "semen sanctum" may have been derived from this. This name is also applied to the plant Santolina Chamaecyparissias, which was also used as a vermifuge and still enjoys (5) some local reputation, but Lemery refers to this plant by the quite distinctive name of "garderobe". Most of these synonyms of santonica are still used today, although the drug has been shown (6) to consist of flowerheads and not of seeds.

The geographical source of the drug is not mentioned by any of the earlier authors and in the fifteenth century writings the references to it are very vague. The first author to state the geographical source with any certainty was Pomet (7) (1694), who described three varieties coming from different localities. The first and most highly esteemed variety came from Bucharia

through Persia by caravan to Alexandretta, Aleppo and Smyrna, and was shipped from these ports to England and France by way of Holland. Tavernier (8) in 1677 had mentioned this variety and stated that it came from the Province of Kerman in Persia, but it is probable that it only passed through Kerman on its way from Bokhara to Alexandretta. At that time Bokhara, the capital of Bucharia in eastern Turkestan, was the centre of all the commercial routes from east and west Asia to Europe. From Bokhara, goods were conveyed by caravan through Persia to Meshed and then to Baghdad in Iraq. From Baghdad they were taken by caravan to Aleppo and shipped from Alexandretta to European ports. Goods originating in eastern Turkestan would, therefore, have been conveyed through Persia on their way to Alexandretta and European ports, and the early plants probably came from the same localities as they are obtained from at the present day. From the description of the Aleppo variety it seems certain that it is the variety which occurs in commerce at the present day and is recognised as A. cina, (Berg), Willkomm (9) distinguished, as the earlier authors say, by its smoothness and the absence of hairs. Other varieties were in common use at that time also, but this variety has always been regarded as the best and by the beginning of the twentieth century it alone was recognised as the genuine drug.

The other two varieties of the drug described by Pomet are stated to come from Bhutam, North of Assam, and from the confines of Russia near the Don and Volga rivers, the latter being composed of large hairy grains. It is probable that this was the

A. maritima, var Stechmanniana, Besser, which was collected by the German colony at Sarepta up until 1869 and the collection of which has recently been revived. Planchon and Collin (10) (1896) describe this variety in detail. Aufrecht (11) (1923) had, a few years ago, occasion to examine some samples of santonica from this source and found about 1.6 per cent of santonin in them.

Pomet in his dissertation on the subject recommends the choice of well-developed, green and good smelling "semen contra" and that it should be free from foreign substances, particularly the seeds of Auronne, which are often substituted, a description which shows that he was familiar with the drug and that its use was most beneficial at a season of the year when, it is now known, it would contain the most santonin, although the existence of this principle was not at that time known.

In the year following the description of the drug by Pomet, Paul Hermann (loc.cit.) pointed out that the drug was composed of small flowerheads and not of seeds, as had previously been thought, and as many of the older names would indicate.

During the eighteenth century no appreciable increase in the knowledge concerning the drug appears to have been made, although it seems to have become much more widely used during that time. Mention is made of it in some of the older pharmacopoeias published in the eighteenth century, and in these it is usually associated with such other vermifuges as Tansy and Santolina which were in use at that time and were to be found as adulterants of, or substitutes for, the higher priced santonica.

Early in the nineteenth century Kahler (12), a Dusseldorf pharmacist, isolated the active principle of the drug from an ethereal extract of the plant. This was in 1830 and later in the same year Alms (13) independently and without previous knowledge of Kahler's work, also discovered its presence. Alms gave to this white crystalline principle the name santonin. Following this discovery a number of chemical analyses of the plant were carried out and the presence of santonin confirmed, but it was not until 1894 that a second crystalline principle, artemisin, was isolated by Merck (14), from the mother liquors from the assay of santonin, and named thus by him.

Detailed descriptions of the drug and of its geographical source, followed, and Planchon and Collin (loc.cit.) in 1896 published a description of these varieties to be found in commerce at that time. These were described under the names of (1) semen contra of Aleppo or Alexandria; (2) semen contra of Russia or Sarepta; and (3) semen contra of Barbary.

(1) They state that the botanical origin of the Alexandrian, which was considered to be the best and only official variety, was still then a matter of controversy. The description which they give corresponds to that of A. cina, now known to be the source of the genuine drug from Turkestan. They describe it as follows :- The drug consists of the small unexpanded flowerheads mixed with a variable proportion of leaves and stalks and freed from sand by sifting. The flowerheads are 3 mm. long and 1 mm. broad; when fresh they have a yellowish-green tint, which becomes

brown on keeping. The involucre is formed of about a dozen contiguous bracts; the lower ones are small, distant ovate and the upper or interior are elongated, strongly keeled at the back and covered with small resinous glands, which are yellowish and shining, the margins are membranous, colourless, transparent and marked with fine striae and are quite glabrous. In the young state the median nerve bears a few colourless, woolly hairs, which disappear on maturity. The whole flowerhead then becomes smooth and nearly glabrous; this distinguishes the genuine from other varieties in which the flowerheads are rendered adherent by the presence of woolly down. The involucre encloses 3 to 5 florets inserted on a glabrous receptacle. The coralla is tubular, narrowed at the base and expanded at the tip into 5 short triangular teeth. The debris of the flower stalk is very slender, rigid and grooved, with swellings here and there indicating the points of insertion of the leaves. The taste is bitter and camphoraceous.

(2) The Russian or Sarepta variety is described and is distinguished by the more or less coherence of the flowerheads due to the presence of hairs taking the form of a white webby down visible under a hand lens. The flowerheads are also smaller, brown, and often partially expanded. The inner bracts of the involucre are narrower, shiny, and strongly keeled, and the oil glands which cover them are larger and more orange in colour. The expanded flowers have a fine red tint and there is much debris of branchlets and foreign matter present. There was more than one variety of Russian "semen contra". Before 1872, according

to the authors, the variety just described, probably A. maritima var. Stechmanniana, Besser, had ceased to be collected, but another variety which is described as being distinguished by the white cottony appearance of the flowerheads, had come simultaneously and continued to come from the same locality. This later variety is referred by the authors to A. Lercheana, Stechm. which is given in Index Kewensis as a synonym of A. fragrans, Waldst. and Kit. The earlier one has been referred to A. pauciflora, Stechm. which is synonymous with A. Lercheana Karel and Kirel, and to A. monogyna, Waldst. and Kit. (A. fragrans, Willd.).

The source from which they were derived is, however, a matter for conjecture and our knowledge of the plants growing in that locality suggests that the drug was most probably derived from A. maritima, var. Stechmanniana, Besser and A. pauciflora, Stechm.

(3) The "semen contra" of Barbary was not held in very high esteem and became later to be regarded as a substitute for, or adulterant in, genuine santonica. It came from Morocco and N.W. Africa, and was attributed to A. Sieberi, Besser, which is given in Index Kewensis as a synonym of A. Herba - alba, Asso. Marie (15) considered it to be derived from the latter plant, which has never been reported to contain santonin, although a number of negative reports have appeared. Planchon and Collin described it as having the appearance of a mixture of a brown and greyish white colour and the flowerheads adhere to one another by their hairy covering. The flowerheads are rounded or ovoid and attached

to tiny branchlets. The bracts of the involucre are obtuse, and the lower end rounded, the upper oval, the debris of linear floral leaves is very short and the stunted summits have three or four flowerheads crowded together. A sample of drug agreeing with this description and recently imported from Morocco was examined by the present author, and was found to contain no santonin.

It is evident from the literature that varying results were obtained by the use of the crude drug, but it is also evident that closely allied drugs containing no santonin had some anthelmintic action.

Before the discovery of santonin it was customary for the drug to be administered in the form of a decoction or infusion, and since santonin is only very slightly soluble in water the pronounced anthelmintic action of these galenicals can hardly be attributed to this principle. Such drugs as A. Herba - alba, which have been shown to contain no santonin or only a very little, also have some action, and it is apparent therefore that some other constituent, or constituents, of these plants serve the purpose equally well. Another form in which the plants was used was as a confection, and the flowerheads alone covered with honey or sugar, or cooked with meat or in soup were also used. The use of these preparations still persists in some country districts in Britain, and in some districts of Scotland and Wales where the plants do not grow wild, A. gallica is cultivated as a garden plant and harvested just before the flowerheads begin to expand, to be used in the form of an infusion for various complaints, as required.

Despite the obvious activity of these galenical preparations it has been customary, since the discovery of santonin, to evaluate a drug on its santonin content and it has even been suggested that a standard santonica should be included in the pharmacopoeias. This is done in a few of the foreign pharmacopoeias, but unless galenical preparations are also included this would appear to be unnecessary, since the only object of an official standard is to ensure a uniform drug for the preparation of galenicals. Extractors of santonin would continue to use such drugs as they profitably could, for the extraction of this principle, even if a standard was included in the pharmacopoeia.

Extraction of Santonin

London is at present the centre for the extraction and distribution of santonin. Supplies of genuine santonica guaranteed to contain not less than 2 per cent. of santonin are sent from Tschimkent in Russia by rail to Orenburg and thence to Leningrad by way of Moscow. From Leningrad it is brought direct by steamer to London, transactions being under the direction and control of the Soviet Government.

Ninety-eight per cent. of the weight of the plant is valueless and at one time it was customary for the active principle to be extracted nearer the locality in which the plant grows. Very little is known regarding the extraction of santonin prior to 1880, when Russia took over the government of Turkestan. At that time two German factories existed near Tschimkent for the extraction of the drug, and in 1882 a Russian factory was also

erected there, for the purpose of producing and purifying santonin. Considerable competition from the German factories, who secured monopolies on the collecting, caused the Russian factory to be closed down in 1889, and the production and distribution of santonin was controlled by Germany for some years. In 1895 the Russian factory was reopened and continued in operation until the outbreak of war in 1914. The German factories had meanwhile been closed down. At this time santonin was being extracted only, and not purified, in the Russian factory, and the crude product was being sent to Germany to be purified. Hamburg was the centre of purification and subsequently the centre of distribution. Russia therefore had at that time a monopoly of production and Germany a monopoly of distribution. With the outbreak of war the monopolies were destroyed. The Russian factory continued to extract santonin until 1917, but the purification and distribution had been transferred to London. By 1917 the Russian factory could no longer obtain the necessary chemicals from European sources and it was finally closed down. During the following two years no santonin appears to have been extracted, but accumulated stocks were sent to England by the Soviet Government, and kept the markets supplied. The Russian factory has now been demolished, and although it would appear to be a considerable saving to have the extraction done near the locality in which the plant grows, no other factory for this purpose has been erected. The crude drug is sent, under strict control of the Soviet Government, to London, which, therefore, remains the centre of extraction and distribution of santonin.

CHAPTER II.

PRESENT DAY SOURCES OF SANTONIN

Until quite recently practically all the Artemisia of commerce was obtained from the one species, A. cina, growing in Russian Turkestan. Since A. brevifolia, Wallich growing in India has been shown (16) to contain quite an appreciable amount of santonin, supplies of this plant have been imported into this country and commercial extraction has been undertaken. The total amount of santonin extracted from this source is, however, small compared with that from the Russian plant.

Cultivation of an unidentified Artemisia is being undertaken in Holland under the direction of Prof. van der Wielen (17), with a view to producing santonin commercially from this source, but, although the cultivation was commenced in 1921, the extracted principle is apparently not yet on the market. Within more recent years it has been reported that an unnamed variety of A. maritima, Linn, containing santonin and growing on the shores of Germany is being fostered. It is intended to propagate this plant until sufficient is obtained to make commercial extraction possible. From this it will be seen that other possible sources of the drug have still to be developed and that the Russian-Turkestan, A. cina, is still the chief source of present day supplies.

A. cina, occupies large stretches of territory in the north-eastern district of the province of Turkestan on the right bank of the Syr-darya river, and on the adjoining steppes. Attempts have been made to extend the cultivation in Turkestan but

the character of the soil appears to be a controlling factor, determining the area of growth and the attempts have not been successful. Geological characters indicate that the steppes whereon A. cina, now grows were very probably at one time salt lakes. The soil is certainly very saline and this species, like a number of others of the same genus, favours such a soil.

Botanical Sources of Genuine Santonica.

All the twentieth century pharmacopoeias include santonin, but identification of the species from the flowerheads alone, which constitute the drug, is very difficult, and it is not surprising that, although practically all of the drug is obtained from the one source, the different pharmacopoeias are not in agreement regarding the name of plant from which it is obtained. Thus the British Pharmacopoeia 1914 gave the source as A. maritima var. Stechmanniana, Besser.; the United States Pharmacopoeia refers it to A.pauciflora (Ledebour) Weber, and others give it variously as A. cina, Berg; A. pauciflora, Stechm.; A. maritima, Linn. var. pauciflora, Ledebour, etcetera.

Realising the difficulty of identifying the species by the flowerheads alone, Wallis and Mowat (18) in 1925 carried out an investigation of the microscopic characters of flowerheads obtained from authentic specimens of the plants named in the various Pharmacopoeias. By this means they were able to state that a number of the names were synonymous, and to describe characters of the flowerheads by which the species could be distinguished. The results of their investigation led them to conclude that A. cina, (Berg), Willkomm, is the plant from which the commercial Russian

drug is obtained. The characters by which the commercial drug from this species may be identified ~~is~~ are summarised by them as follows :-

Foliage leaves; are linear lanceolate with a rounded apex and an apiculus. The leaves have a midrib with numberous pinnately arranged branches connecting it with the two marginal veins, which run parallel to the margin at about one-third the distance from it to the midrib. There is a complete absence of long hairs but sessile glands are present.

Bracts of the flowerheads. The bracts vary in number from 14 to 20, usually 16. The midrib branches freely and the veinlets are contorted and frequently anastomose. The number of cottony T.shaped hairs is small and apical marginal hairs are always absent.

The Flowers. The apices of the corolla lobes are never more than slightly papillose and bear no trichomes.

The investigation enabled them to describe the characters of a number of other Artemisias, and to state that the plant under cultivation in Holland, which had been referred to A. cina. Berg, by the Bureau of Plant Industry, Washington, was some other species which was unlike any of the named species which they had examined. Their conclusion that A. cina, (Berg), Willkomm is the species from which the genuine commercial drug is obtained, is now accepted and it is stated in the British Pharmacopoeia 1932 that this is the source.

Other Species which contain Santonin

Although A. cina, and A. brevifolia are the only two species which are used for the commercial production of santonin at present, certain other species are known to contain this principle. Viehover and Capen (19) in an examination of 56 American species found santonin to be present in A. mexicana, Willd., A. neo-mexicana, Wooton, and probably A. Wrightii, Asa Gray, which are natives of Mexico and New Mexico. In the other species examined they did not find santonin but were unable to state that it did not occur in them, because they were able to obtain the plants at one stage of growth only. At a different stage of growth santonin might be found in these species. Other species which have been found to contain santonin are A. fragrans, Willd, A. Lercheana, Karel. and Kirel., A. pauciflora, Stechm., and A. gallica, Willd. Probably other species also contain it, as owing to its seasonal occurrence, it is not possible to state that any given species normally does not contain it, unless examinations of plants at various growth stages are made.

C H A P T E R III.

CHEMISTRY

After the discovery of santonin independently by Kahler and by Alms in 1830, Drivon (20) (1865) gave figures showing percentage of constituents in Aleppo santonica. More recently Goodson (21) (1922) gave an analysis of A. afra, Jacq., but he did not find santonin among the constituents of this plant. Aufrecht (loc.cit.) (1923) confirmed Drivon's results by a complete analysis of Aleppo santonica and found also betaine and lecithin in the drug.

Aufrecht's results as percentage of the air dried drug are as follows :- Volatile oil 0.84; Moisture 9.7; Resinous Material (including chlorophyl) 2.16; Santonin 1.6; Lecithin 0.27; Betaine 0.42; gummy, mucilaginous and pectin material precipitated by ethyl alcohol, 14.58; substances reducing Fehling's solution 4.75; free acids (as malic) 1.20; other nitrogen free extractive matter 10.22; crude fibre 35.72; ash 8.44. The ash on analysis yielded the following percentages:- K as K_2O, 11.75, Na as Na_2O 4.36 Ca as CaO 8.30; Mg as MgO 1.75; Fe as Fe_2O_3 0.38, phosphate as P_2O_5 36.44, sulphate as SO_3 11.77, chloride as Cl 5.07, silicate as SiO_3 20.18.

Heckel and Schlagdenhauffen (22) when reporting the presence of santonin in A. gallica, growing in France, reported also the discovery of an alkaloid, not previously noted in any examination. Jahns (23) (1893) investigated A. maritima in a

search for this alkaloid but was able to isolate only two basic substances which he identified as betaine and choline, present to the extent of 0.5 per cent. and 0.1 per cent. respectively. He concluded that Heckel and Schlagdenhauffen had mistaken these substances for an alkaloid. Aufrecht confirmed the presence of betaine.

Later, Merck (loc.cit.) (1894) discovered a new constituent in A. maritima, to which he gave the name "artemisin". Subsequent workers have been unable to confirm the presence of artemisin in this plant although it undoutbedly exists in some species and is probably present in A. maritima also, at some seasons.

Other substances, such as camphors have from time to time been reported to be present, but the only constituents which are now considered to be of any importance and to which the therapeutic action of the drug is attributed are the essential oil, santonin and artemisin. A short description of these three substances follows.

Essential Oil Of Artemisia.

Essential oil of Artemisia was at one time to be found on the drug markets, but it is seldom, if ever, used now, and the only reason for the continuance of the extraction is to facilitate the subsequent extraction of santonin from the herb.

The volatile oils from a number of species of Artemisia have been examined (24) but that of A. maritima or A. gallica does not appear to have received any attention so far. The oils from the other species are all somewhat similar, however, the chief

constituent being cineol (eucalyptol). They are present to the extent of 0.1 to 3 per cent., and as cineol appears to be the only constituent of importance it is not surprising, with the supplies of such as Eucalyptus oil, as a source of this substance, that oil of Artemisia is no longer a commercial article.

An analysis of a typical oil showed the following constituents:- cineol (chief); inactive pinene; terpinene; and traces of α-l- terpineol and a high boiling point sesquiterpene.

The oils are optically active. Some of them contain l-camphor, some d-camphor, and others both enantiomorphs.

Santonin.

Santonin, the lactone of santoninic acid crystallises in flat rhombic plates, practically insoluble in cold water and slightly soluble in hot water. Soluble in 50 parts of cold and 3 parts of hot alcohol, in 2.5 parts of chloroform, and in 140 parts of ether, and in 25 parts of benzene (25); melting point 170 - 174°C., sublimes unchanged. It is insoluble in dilute mineral acids but forms salts of monobasic santonic acid $C_{15}H_{20}O_4$ (isomeric with santoninic acid) with dilute alkalies. On exposure to light santonin turns yellow with the formation of photo- or chromo-santonin which may be reconverted to the colourless form by recrystallisation from alcohol.

Santonin has the empirical formula $C_{15}H_{18}O_3$ and was, until recently, considered to be a derivative of 1 : 4 - dimethyl - 6 - isopropyl naphthalene, having the structural formula shown in Fig.I.

This formula was first proposed by Grassi-Cristaldi and Guzzi (98) in 1892, since when it has been modified in a number of

ways to account for different properties of santonin. None of the proposed new formulae were, however, completely satisfactory, and in 1929 Clemo, Howarth, and Walton (27) began an investigation of the constitution. Having proved the α position of the lactonic oxygen atom, the β position of the propionic group and the five position of the second methyl group, they proposed the formula shown in Fig. II. which they hoped to confirm by synthesis.

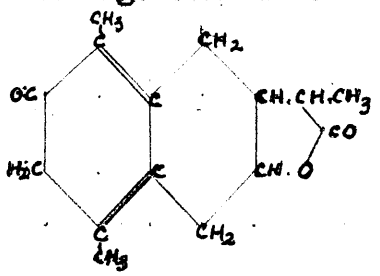

Fig. I.

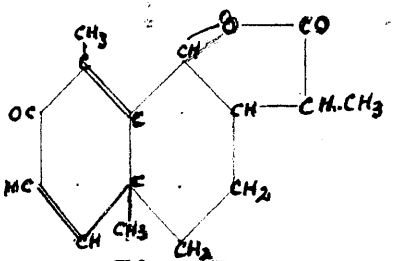

Fig. II.

No synthetical proof has yet appeared, but it seems reasonably certain that the suggested constitution is the correct one for santonin. Ruzicka and Eichenberger (28) have simultaneously been carrying out similar work, and their conclusions and suggested formula are the same as those of Clemo and Howarth.

Santonin is distinguished by a number of colour reactions (29) notably the following :-

Reichards Test; Heated with alcoholic potassium hydroxide solution a carmine, or violet-red, colour is produced.

Wellmann's Test; Warmed with 50 per cent. sulphuric acid and a trace of ferric chloride solution added, santonin gives a yellow coloration, changing to orange, blood-red, violet, and finally lavender.

Theater's Test; Treated with a few drops of 2 per cent. solution of furfuraldehyde in alcohol, and concentrated sulphuric acid added, santonin gives, on warming in a water bath, a series of colours going through purplish-red to carmine-red and then to indigo blue. On standing in the cold a black precipitate is formed.

Jaworowsky's Test; A solution in concentrated sulphuric acid gives on shaking with dilute, acid solution of cerium sulphate, a cherry-red solution which becomes turbid on cooling and on dilution yields a violet precipitate.

Ethyl Nitrite Test; Warmed with a solution of ethyl nitrite and a little potassium hydroxide solution added, a red colour is produced.

Santonin is included in all the pharmacopoeias and for medicinal use it has to a very large extent displaced the herb and galenical preparations. Its chief use is as an anthelmintic, particularly for round worms which it rapidly expels. It has less effect on thread worms and no effect on tape worms. It has been suggested that santonin may be useful in a number of other ways, and there is some clinical evidence to support the statement (30) that it is useful in the treatment of diabetic conditions and certain classes of drug addicts. Its use is often followed by curious disturbances of vision, giving rise to the condition known as xanthopsia. The normal maximum dose has been shown in certain instances to be highly toxic, particularly to children. It would appear that more care should be taken before prescribing this drug

than is commonly thought to be necessary at present, and that its use should not be followed by the administration of oily substances which would increase absorption.

Artemisin.

Artemisin is a white solid forming small needle shaped crystals, not sensitive to light, melting point 200°C., slightly soluble in cold water, more soluble in hot water, slightly soluble in cold alcohol, readily soluble in hot alcohol, insoluble in chloroform (in which santonin is very soluble) and mineral acids, but forms salts with the alkalies.

It has an empirical formula $C_{15}H_{18}O_4$, and is considered to be oxysantonin and the lactone of artemisinic acid, one of the hydrogen atoms of santonin being replaced by an hydroxyl group. The structure usually assigned to it is shown in Fig. III, but this has been based on the assumption that santonin has the structure shown in Fig. I. With the change in the position of the lactonic oxygen atom of santonin, the structure of artemisin will probably be changed accordingly, as in Fig. IV.

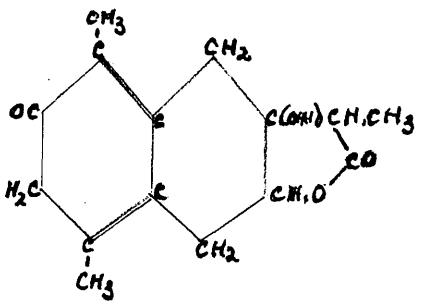

Fig. III.

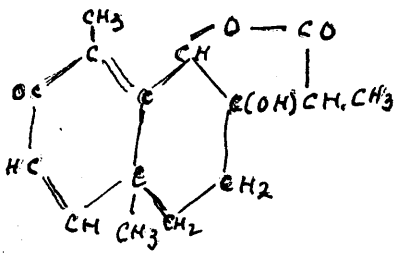

Fig. IV.

until its structure is proved independently by synthesis. Similar work to that done on santonin has been done on artemisin, except for the most recent work, and the two have been shown to be very similar in all their properties, considering the extra hydroxyl group in Artemisin. Hence, the assumption regarding the structure of artemisin is probably more or less justified.

The colour reactions differ from those of santonin. Artemisin with 50 per cent. sulphuric acid and a trace of ferric chloride solution gives a yellowish brown colour; using santonin the test gives a violet or lavender coloration. Artemisin gives a violet coloration when treated with potassium hydroxide solution, while this coloration is given by santonin only if alcoholic solution is used as reagent. On dry distillation dimethylnaphthol and propionic acid are obtained, and in this respect it is identical with santonin.

Artemisin is not much used therapeutically but samples of santonin have on occasion been found to be largely adulterated with it.

PART II.

EXPERIMENTAL

CHAPTER IV.

THE ECOLOGY

The two species which were investigated are Artemisia maritima, Linn., and Artemisia gallica, Willd. The former is a maritime plant growing just within a few inches of average high-tide level so that its roots are moistened or immersed at high-tides, and at high spring tides the whole plant may even be immersed in salt-water. It is found in greatest abundance near where fresh water flows into the sea, but this is not invariable, and the plants have been found in abundance in places where the water is entirely saline, e.g. Blakeney Point and Portsmouth Harbours. More detailed reference is made to the soil requirements in the chapter on soil. A. gallica is a plant which usually grows beside A. maritima and its ecology would appear to be identical with that of the latter.

Both plants grow extensively along the coasts of Europe, and are to be found also in parts of Asia and America. Both are indigenous to Britain. In the local "Floras" of Britain the localities for A. maritima are usually cited but for A. gallica the statement very generally made is that "it may also be found", or that "it is not rare". The writer's observation is that the two species usually grow together.

Geographical Source.

The locality from which the wild plants were collected is at Tynefield, (map, area I.) about two miles east of East Linton in Haddingtonshire. At high tide the area, which is the estuary

of the river Tyne, presents the appearance of an inland lake, there being only a narrow outlet to the sea. At low tide, however, the area is an extensive mud-flat surrounded by a rough-built sea-wall. The circumference of the estuary is between one-and-a-half and two miles, and the plants grow on the sea wall, at high water-mark, along its entire length. They are also to be seen on "Sward" areas which appear as islands at normal high tide, and the selectivity which they display with regard to high water-mark is strikingly obvious on these islands. There are plants only on the top of those islands which are almost submerged, at high tide, while on those which are some inches above high water-mark, the plants grow only round the edges.

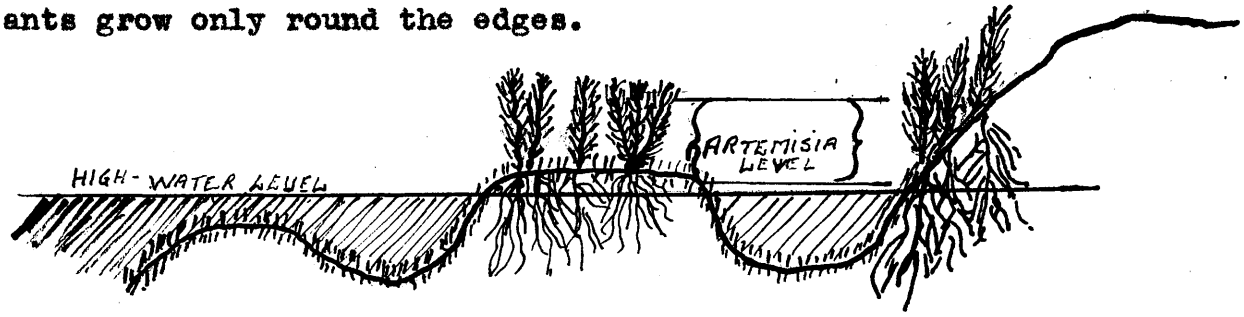

Illustration of Vertical Zonation

For details of other localities see Chapter XI.

SUMMARY OF CHAPTER IV.

The geographical source of the two species and the ecology are described.

CHAPTER V.

THE MORPHOLOGY

Systematic.

A. maritima was first described by Linnaeus (31) in 1753, and A. gallica by Willdenow (32) in 1800. Both species belong to the Seriphidium section of the genus, as do all the species which contain santonin. These two species are the only indigenous members of this section.

A. maritima, Linn.

Synonyms, A. maritima, var. α; var. anglica; var. genuina; Sea Wormwood. Root perennial, long, flexible, tapering, and surmounted by a creeping woody rootstock. Stem herbaceous, branching paniculately from the rootstock and curved, somewhat procumbent at the base, then erect, 9 to 16 inches high; after the primary branching, only floral branchlets occur, pubescent.

Leaves almost or quite sessile, much divided, lower bipinnate with linear segments, upper not so deeply divided; thick, margins thickened, apex rounded, no apiculus, very hairy on both surfaces.

Flowerheads numerous, in spikes on spreading or drooping panicle, ovoid, 3 to 4 mm. long, drooping on a short peduncle and subtended by a linear bract. Composed of 10 to 16 imbricated pubescent bracts, the outer of which are thick with narrow scarious edges and the inner are only two cells thick and with a very broad

membranous margin; enclosed are from 4 to 8 perfect, tubular, yellowish or reddish florets having no pappus; surmounted on a conical naked receptacle. The whole aerial plant is covered with long cottony hairs giving it a hoary or silvery appearance.

Odour distinctive and aromatic.

Flowers August, September.

A. gallica, Willd.

Synonyms, A. maritima, var. β; var. gallica.

Similar to A. maritima except in the following :-

Stem branches arise erect from rootstock and not so long.

Segments of leaves somewhat finer. Branches of panicle erect, and more densely compacted with flowerheads.

Flowerheads sessile and erect, slightly larger; number of bracts 11 to 18; number of florets 4 to 10.

Odour slightly but perceptibly different from that of A. maritima.

Flowers, same time.

Both species show some variation.

Some botanists regard A. gallica as a variety of A. maritima, but the floral characters of the two are quite distinct. Figs. XIII. and XIV. are photographs of the two species which show, clearly, the difference, and this can also be seen on a number of the other photographs.

At the beginning of the investigation the writer's identification of the species was confirmed by sample batches sent to Kew, A.J. Wilmott Esq., of the British Museum, and Miss Vachell

of Cardiff. The specimens sent were identified by them as the two species named.

Additional Morphological Features of the Plants Used.

Wallis and Mowat (loc.cit.) have shown that the microscopic features of the flowerheads of a number of species of Artemisia may be used to distinguish these species. The number and hairiness of the bracts are of particular usefulness for this purpose. When entire plants are available, it is not necessary to refer to these microscopic characters, but they are particularly useful when examining a commercial drug consisting almost entirely of flowerheads.

Other investigators have studied the detailed morphology of some species of Artemisia but Wallis and Mowat appear to be the first to carry out a comparative examination of the species reputed to contain santonin.

In the introduction to their work these authors say that "If characters which may be depended upon for the identification of the drug by an examination of its structural details could be found, a number of samples could be sorted much more rapidly, and, having assured oneself that any given specimen had the correct botanical origin, a chemical assay for the purpose of identification would be unnecessary..... Any samples which did not possess the structure of the genuine drug would need no chemical examination at all".

It is evident from this that the object of the research was to ascertain definite details of the morphological and

anatomical characters by which the different species can be readily identified. It is also to be concluded that Wallis and Mowat considered that only one species of plant, A. cina, (Berg), Willkomm, was to be regarded as the source of santonin, and that the flower-heads of this species invariably contained this principle, which latter point there is no evidence to disprove. O'Connor (33) has pointed out, however, that the name "santonica" is a generic one and that it may be applied to any drug from which santonin may be obtained, and furthermore the pharmacopoeia admits of the use of any species of Artemisia for the extraction of santonin. It may be that Wallis and Mowat intended their work to be extended, and that on the discovery of a new source of santonin, the structural details of the new species should be ascertained. It has not, however, been shown that all species of Artemisia, or even those which are at present known to contain santonin, may be distinguished by such characters. Neither has it been shown that the characters described by these authors are the characters of A. cina, only. Reports regarding the occurrence of santonin in any particular species are conflicting, but in the meantime there appears to be strong reasons for supposing that in the case of A. gallica, at least, the plant growing in one locality may contain santonin and be devoid of it when growing in a different locality.

In this paper it is shown that santonin was found to be present in each one of a large number of batches of A. gallica, and A. maritima, growing in different localities all over Britain. Heckel and Schlagdenhauffen (loc.cit.) reported the presence of

santonin in A. gallica. growing in France in 1885. More recently Mouton (34) (1931) has reported that French A. gallica., probably from a different locality from that examined by the previous authors, contains no santonin, and Viehover and Capen (loc.cit) (1923), and Van de Vyvere (1931) have made similar reports about the American and Belgian plants respectively. The Belgian A. gallica, and A. maritima examined by Van de Vyvere have been examined by the present author also and found to contain no santonin. It is possible that estimations were in some instances made on plants collected at a season when the santonin content was low, or alternatively that the negative results were due to the use of an inferior technique. Until the question is settled one way or another, however, there can be no justification for assuming the invariable occurrence of santonin in any species of plant even at a definite stage of growth.

It is possible that certain morphological features may be developed as a result of the absence or presence of santonin in the plant. Such has been shown (like) for certain other plants, for example, wild Phaseolus lunatus produces black beans, but under cultivation white beans are produced by the same plant, and it is found to contain no poisonous phaseolunatin (stated to be identical with linamarin) which is present in the wild, black beaned plant. It has not so far been found possible to correlate any morphological feature with the presence or absence of santonin.

Critical Morphology of A. maritima and A. gallica.

For the examination, fifty typical sample plants of each species, with only very slightly expanded flowerheads, were selected. These were steeped in chloral hydrate solution in order to render them sufficiently pliable for the bracts to be separated without tearing. Thirty flowerheads were separated from each plant and the number of bracts were counted, but after a few plants had been thus examined it became evident that flowerheads from any one particular plant bore the same number of bracts and florets. When this had been definitely ascertained for both species, the number of flowerheads examined from each plant was reduced to 6.

Bracts and Florets.

The number of bracts and florets were counted on about 600 plants, 50 plants being selected from a batch of each species from six different localities. There was no variation in the average number of these organs, on plants from the different localities. The numbers found to be present on the two species are shown in Table I. From this table it will be seen that, except at the extremes, these characters do not serve to distinguish the two species.

A. maritima. Bracts.

The flowerheads of A. maritima are shortly pedunculate, ovate, or broadly ovate, about 4 mm. in length and covered with a thin woolly felt of long cottony hairs. The bracts are imbricated, the outer being thick, contain chlorophyll and have only a very narrow scarious margin. They are lanceolate ovate,

about 1 mm. broad, and 2.5 mm. long. They bear all over their surface, long cottony hairs having the T shape characteristic of the genus and up to 4 mm. in length, and also shortly-stalked uniserial hairs bearing multicellular glands at the tip. The inner bracts become successively broader until the innermost, which are about 3 to 3.5 mm. in length, are even a little broader than they are long. These inner bracts have a narrow central portion, two cells thick, through which the mid-rib passes, and containing a little chlorophyll and a very broad colourless scarious margin, one cell thick. The parts of these inner bracts which are exposed to the light bear similar hairs also.

TABLE I. BRACT & FLORET NUMBERS

BRACTS:-	A. MARITIMA.	A. GALLICA.
Number Present	10 to 16	11 to 18
Average Number	12.5	14
Most common Numbers	10,11,12,& 14	12,13 & 14
FLORETS:-		
Numbers present	4 to 8	4 to 10*
Average Number	5.8	5.6
Most common Numbers	4,5,6, & 7.	5, & 6.

*(Only two specimens had eight, and only two had ten florets.)

Florets.

The florets are mounted on a naked conical receptacle and usually one or two are not nearly so advanced as the others. There is no pappus but numerous small shining oil glands are

present on the surface of the corolla. The whole lobes of the corolla are very decidedly papillose.

Bract of the Floral Axis.

This bract is linear, about 1 cm. in length, 2 to 3 mm. broad, and very thick. It tapers sharply to a blunt point and is covered with a thin felt of long cottony and glandular hairs similar to those of the bracts of the involucre.

Foliage Leaves.

The segments of these leaves are linear, rounded at the apex and without an apéculus. They are covered with a thick woolly coating of T-shaped hairs. A microscopic examination reveals a continuous line of conducting tissue running parallel to the outer-margin and about halfway between it and the midrib. Other veins branch off from the midrib and join up with this outer conducting strand. Numerous sunken stomata are present.

A. gallica. Bracts.

The flowerheads of A. gallica are sessile, slightly larger than those of A. maritima, being on an average 5.5 mm. in length. They are broadly ovate or rounded in outline and are composed of imbricated bracts, the outer of which are covered with a felt of long cottony hairs. The bracts are also somewhat larger than those of A. maritima, the outer being 1.5 mm. broad and 4 mm. long, while the inner vary in breadth from 2.5 to 6 mm. in breadth, and are about 5 mm. in length. Otherwise the bracts of both plants are similar and no difference could be

detected in the hairs or hairiness or branching of the mid-ribs.

Florets.

These also are indistinguishable from those of A. maritima. The form of the receptacle is the same and the corolla lobes of both species are equally papillose.

Bract of the Floral Axis.

This bract is more curved, and the margins are not thickened to the same extent as in A. maritima, but they are equally hairy and the number of sunken stomata is about equal on the two species. There is no character by which the two may be distinguished.

Foliage Leaves

The segments of the foliage leaves are narrowly linear tapering very sharply to a blunt point on which there is no apiculus. They are not quite so hairy but the form of the hairs, the thickening of the margin and the venation are indistinguishable from that of A. maritima. The transverse sections are also the same. Fig. XV. is a photomicrograph of a transverse section of a segment of a leaf of A. gallica.

Other Features of the two Species.

Transverse sections of the root, rootstock and stem of the two species were also examined but no difference by which they could be distinguished was discovered.

Figs. XVI., XVII., and XVIII. are photomicrographs of transverse sections of the root, rootstock and stem respectively from a specimen of A. gallica.

The flowerheads of these two plants, therefore, show no distinguishing features. On the average there is a similar number of bracts present on each and no great variation in size. None of the other features of the flowerheads are distinctive for only one of the species. Other features which were examined, including those of the bract of the floral axis, the foliage leaves and the anatomy of various organs, show in some instances certain minor differences, but there is no distinctive feature by which the species may be differentiated. The ordinary morphological features by which they may readily be identified, therefore, remain as the only distinguishing features between A. maritima and A. gallica. There are certain differences, however, in the microscopic features of these two species and the species described by Wallis and Mowat, by which they may readily be distinguished from the latter.

SUMMARY OF CHAPTER V.

A description of the two species is given and the characters by which they may be distinguished are pointed out.

An account is given of work done using sample plants, on additional morphological characters which have been shown to be of value in distinguishing between other closely allied species, when only the flowerheads and small portions of the leaves are available for examination, such as in the commercial drug. A study of these characters is not necessary for the identification of the species when entire plants are available, and they were not found to be useful for differentiating between A. maritima and A. gallica, although they would probably serve to distinguish these two species from certain others.

CHAPTER VI.

THE SOIL

A. maritima and A. gallica, both exhibit strongly xerophytic characters and it has been observed that their natural habitat is within the reach of tidal water.

Although the other indigenous Artemisias are inland plants which thrive well even on dry normal soil, it would appear from the literature that most, if not all, of the santonin yielding Artemisias prefer, or may even require, a saline soil.

O'Connor (loc.cit.) in a reference to A. cina says that "all species of Artemisia favour saltish soil, but the species yielding santonin is remarkably exclusive in its selection".

Holmes (35) expresses a similar view and says that "the occurrence of A. maritima and its allies on the Kirghiz Desert in Turkestan may be accounted for by the fact that numerous patches of dried up salt lake occur there, which are watered at intervals by fresh water from the mountains". He further states that he has never seen A. maritima growing wild except near the sea and usually close to where fresh water makes its way to the sea, the soil being on the sea level and frequently inundated at spring tides. He suggests that the degree of salinity of the soil may have some influence on the santonin content of the plants.

The present author agrees entirely with these observations. The plants are so situated that the green

portion is not actually touched by normal high tide, but they have been seen to be almost completely immersed at a high spring tide, and it has been observed that the roots are invariably below the level of average high tide.

TABLE II. COMPARATIVE TABLE OF CONSTITUENTS OF SOILS

	SOIL FROM	
	CORSTORPHINE	TYNEFIELD.
pH. (Water)	6.43	7.02
pH. ($CaCl_2$Soln.)	5.50	6.20
Moisture	3.2	2.9
Loss on Ignition	9.5	12.7
Lime Requirement per cent. as $CaCO_3$	0.120	0.106
Chloride as Cl.	0.35	2.80
Insoluble in HCl.	67.5	65.4
Fe as Fe_2O_3	6.16	3.98
Al as Al_2O_3	5.17	3.68
Ca as CaO	3.67	1.91
Mg as MgO	1.09	0.97
P as P_2O_5	0.0595	0.0563

The soil is often of an alluvial nature and the plants were not encountered on any habitat which was purely sandy. It is not unusual to find the plants growing in the crevices of built sea walls at high water mark and where there is practically no soil round the roots. These remarks apply equally to A. gallica.

An examination of the natural soil in which the Scottish plants under investigation grow, has been carried out, and the results of the examination are compared with those from a sample of the soil from Corstorphine on which the plants were cultivated and which was also examined. The results of the examination of the two soils are shown in Table II.

Any conclusions regarding these soils are self-evident but the difference in chloride content is worthy of remark. The difference in Ca content may be attributed to manuring of the Corstorphine soil.

CHAPTER VII.

METHODS OF EVALUATING THE DRUG

The usual methods of assaying santonica take some time and require a certain technique. Other methods have from time to time been suggested for evaluating a drug for market purposes. These methods only serve to show whether or not a certain minimum of santonin is present. No satisfactory technique whereby they may be applied as quantitative tests has been formulated. They are not always satisfactory for even empirical evaluation. The two most commonly adopted procedures are sublimation and the use of colour tests.

Examination by Sublimation.

This is an empirical method of assay perfected by Van Italie (36) and largely used by him, Viehover and Capen (loc.cit), and Eder and Schneiter (37). These authors did not find it altogether suitable. Other substances present in the plants, such as methyl-aesculetin, sublime at a temperature close to that at which santonin sublimes, and it is therefore necessary to identify the sublimate. Identification is not always possible because of the small amount of sublimate obtained and the necessity of purifying it from oil and resin which distil and are present with it. Finally drugs containing only small percentages of santonin very often yield no crystallisable sublimate.

The method as described by Van Italie and others (38) has

been used by the present author to demonstrate the presence of santonin, and on occasions good sublimates, identifiable as santonin, have been obtained but no attempt was made to get quantitative results.

Estimation by Colour Tests.

Santonin gives a number of distinctive colour reactions, some of which have been generally described in Chapter V. It has from time to time been suggested that advantage might be taken of these colour reactions to formulate a process for the quantitative estimation of santonin present in a drug. According to Feldhoff (39) santonin may be estimated quantitatively by applying the test using potassium hydroxide, and by comparing the colour thus produced with that of a standard coloration. Such tests, however, are generally used empirically only, to determine whether a drug contains more or less than a certain prescribed amount. The observations on their use are summarised in the following paragraphs.

Reichards Test.

This test depends on the reaction of santonin with alcoholic potassium hydroxide solution, producing a red coloration, and is the one on which most attention has been focussed.

Feldhoff made use of this test and stated that it could be used quantitatively. Heyl and Tunmann (40) also used it but stated that working with a crude extract of the drug they

could detect no difference in the colours produced by a good and a bad drug. Eder and Schneiter (loc.cit.), Rosenthaler (41), and Mouton (loc.cit.) state, that with the crude extract of a good drug a yellow-red coloration is produced in the test, but with a drug deficient in santonin a yellow-green colour is obtained.

Opinions differ therefore, and it does not seem that it is very satisfactory to work with a crude extract. The colour of this latter itself may vary from a very dark green using the leaves to quite a pale amber using the commercial flowerheads, and comparison is therefore very difficult, even where possible. The coloration produced is sometimes transient.

It was hoped that this colour test might be developed to give quantitative results and an investigation was carried out using extracts of the drug at various stages of purification, since it had been found that differences in coloration were produced by different drugs. The investigation did not meet with success.

In order to obtain more information about this test a study was made of the reaction using pure santonin with the reagent. The reaction was found to be delicate but the intensity of colour was found, by comparison in a colorimeter, not to be proportional to the amount of santonin present. Attempts were made, by varying the controlling factors such as the concentration and volume of the reagent used, to obtain uniform results, but these were unsuccessful and this test had

to be abandoned as unsuitable for the present requirements.

Ethyl Nitrite Test.

Recently it was stated that the well-known test of treating a warmed solution of santonin in solution of ethyl nitrite with potassium hydroxide to produce a wine-red colour, could be used to differentiate between a good and bad drug, by warming the drug with solution of ethyl nitrite and making alkaline with potassium hydroxide. It was said that a reddish tinge appeared with the genuine article but not with one deficient in santonin. This statement cannot be confirmed.

It may even be remarked that the test on santonin itself is not very successful until technique is acquired as certain controlling factors require a good deal of attention.

Wellmann's Test.

This is carried out by warming santonin with 50 per cent. sulphuric acid (or by adding an equal volume of concentrated sulphuric acid to santonin in water) and adding to the warm mixture a trace of ferric chloride solution. It does not appear to have been applied to the crude drug possibly because of the charring which would result, but it is a very delicate test for santonin itself, giving a series of colours, going from yellow through orange to red and finally violet and lavender.

It was hoped that it might be applicable to a slightly purified extract of the crude drug, and because of its

delicacy and the permanence of the final colours, a thorough investigation of the test was made.

Tests were carried out with pure santonin, and it was found that the change of colour could be stopped, for some hours, at the red or any subsequent stage by simply cooling the mixture. The best procedure for carrying out the test is to have the 50 per cent. sulphuric acid ready prepared, and containing a trace of ferric chloride, and to heat the santonin with it on a water bath until the desired colouration is obtained. Too much ferric chloride prevents the appearance of the colour, and it was found that as little as 0.05 per cent. of B.P. test solution (= 0.0075 per cent. $FeCl_3$) in the sulphuric acid had no limiting effect on the test.

The test was found to be a very delicate one, much less than 0.1 mgm. of santonin giving an intense red colour in 1 c.c. of a reagent of the above strength, or containing any proportion of ferric chloride up to at least 0.2 per cent.

Comparative tests were carried out using weights of santonin (obtained by trituration) of 0.1 mgm., 0.2 mgm., 0.5 mgm., 1 mgm., 2 mgm., and 5 mgm. to find out if the intensity of colour produced was proportional to the weight of santonin present. A difference in intensity was observed but they were not found to be proportional. Keeping the same weights of santonin, the technique of the test was varied as were also controlling factors such as time of heating, temperature, concentration of the reagent and others, but no

discernable advantage was gained. The stage to which the change of coloration was carried was also controlled and examination was made of tests in which the colour had proceeded to the red, violet and lavender stages, but no difference in effect was observed.

Extraction of the colour by immiscible solvents was next tried at various stages of coloration, controlling the concentration of reagent and other factors as already described. After a number of solvents, including chloroform, ether, benzene, acetone, xylol, and toluene, had been found to be unsuitable, as extracting agents of the colour, nitrobenzene and amyl alcohol were found to extract it, but the colour of the nitrobenzene solution changes, after a short time, to a dirty brown, and this was also discarded. The results obtained by examining the amyl alcohol solution of the colouring matter from a large number of tests controlled for all known factors, were no more consistent with the amount of santonin which had been used, than were the previous results.

Finally it was thought that the test might be applied empirically by finding out the minimum amount of santonin, or the minimum concentration of the reagent to limit the test. In this way the minimum amount of drug required to give the coloration would be that amount which contains the minimum of santonin, and so the percentage of santonin could be calculated. Using the reagent as a limiting factor, the amount of santonin could be determined, by ascertaining the maximum amount of drug

which produces the coloration with a particular concentration of reagent, but which does not give an increase in the intensity of colour with an increase in the concentration of ferric chloride. The amount of santonin present would then be proportional to the amount of ferric chloride. An increase in the amount of drug should give no increase in intensity of coloration until a higher concentration of ferric chloride is used in the test.

Although it would appear to be logical to conclude that the test is a quantitative one and that the intensity of colour produced is proportional to the amount of reacting constituents present, this was not found to be the case. It was assumed that the amounts of santonin, ferric chloride and sulphuric acid were the limiting factors, and that temperature, time of heating and state of subdivision of the santonin were other factors which might influence the test. All of these were varied independently and conjointly in a large number of ways, but finally the test had to be discarded, as quantitative results could not be obtained. The reason for the lack of quantitative results cannot be stated. A study of the test gives the impression that quantitative results are possible, but some unrecognised factor appears to influence the results.

Even the minimum amount of santonin required to give the coloration cannot be definitely stated. It was found that a pink coloration was produced by 0.05 mgm. in 5 mil. of a reagent, but in a series of controlled tests no consistent difference could be detected when using $\frac{1}{3}$, 1, 2, 4 and 6 times

this amount. The colours were compared in a colorimeter and no other known factor limited the production of colour.

None of the other colour tests appear likely to be suitable for a quantitative assay, and a number of them are obviously unsuitable. The search for a colorimetric method of assay was accordingly abandoned, such investigation as had been carried out on the colour tests having shown them to be impracticable for quantitative work.

SUMMARY OF CHAPTER VII.

Some account is given of methods which have been proposed for evaluating a drug without carrying out the quantitative assay process.

Work which was done in an attempt to find a procedure, by the use of which these empirical methods might yield quantitative results, is described. The work was, to a large extent, concentrated on colour reactions but no procedure was found whereby satisfactory quantitative results could be obtained.

C H A P T E R VIII.

THE QUANTITATIVE ASSAY OF SANTONIN

Introduction.

Despite the fact that santonin may be isolated in a high state of purity its quantitative separation is a matter of some difficulty. The numerous suggested methods yield results of varying accuracy, and no completely satisfactory method of assay has as yet been formulated. Many methods have been suggested, including gravimetric, polarimetric, and volumetric, but only the first has received any great deal of attention, and it alone has been developed to give consistent results. Gravimetric methods in general attempt the separation of the santonin from resinous matter by a process of crystallisation. A correction for the santonin left in solution in the mother liquor is applied. Most of the methods are at best empirical and are only suitable for the comparative sampling of drugs of similar nature and santonin content. They cannot be applied to a drug with low santonin content because of the magnitude of the correction; neither can they be applied to the analysis of a drug consisting of entire herb or of leaves, alone, or admixed with flowerheads, because of the different nature and larger amount of extractive present.

It has been shown (16) that santonin may be isolated from the leaves as well as the flowerheads of certain species of Artemisia. It is also significant that the commercial

production of santonin is already in operation using the entire Indian herb as source. The material used in the present research consisted of leaves or flowerheads, alone, or both together. In view of these facts it was decided to test all the available methods of assay, to find, if possible, a process generally applicable for the quantitative determination of santonin.

General Principles of the Assay.

The preliminary stages of all the published methods for the determination of santonin are very similar. The general principles for the separation of acids or anhydrides are employed, a variety of solvents being used. In the final stages, however, special technique has been developed by various workers because of the difficulties attending the quantitative separation of the santonin. The choice of solvents is of extreme importance because of the presence of acid resins which react both to solvents and reagents in a manner similar to santonin.

Most of the processes are based on Katz' (42) principle which is as follows:-

Katz' Process.

An ethereal extract of the drug is prepared by continuous extraction and the residue left on evaporation is extracted by heating with barium hydroxide solution. This produces a solution of the barium salt of the santonin leaving some of the inert matter as an insoluble residue, which is

filtered off together with the excess barium, precipitated as carbonate. On acidifying the filtrate with hydrochloric acid the santonin is liberated and is extracted with chloroform by shaking in a separating funnel. The residue left on evaporating the chloroform contains all the santonin and is extracted by heating with 15 per cent. alcohol which dissolves the santonin and some of the resinous matter. The solution is filtered hot, and on cooling and standing the santonin crystallises. Most of the resinous matter remains in solution, but some may remain on the crystalline residue obtained after filtering, especially when the drug under examination has a low santonin content.

A summary of the other processes which were tested in the course of this work is given in Appendix I.

The following are the writer's observations on the various processes.

Observations on the Processes.

(a) Polarimetric.

Favrel's (43) polarimetric assay yields results which approximate closely to those obtained by gravimetric methods. It would appear that some correction should be made for santonin lost in shaking with 15 per cent. sodium carbonate solution as it has been shown (44) that when using pure chemicals, a certain amount of santonin is extracted from the benzene solution by this operation. The figures found for pure chemicals are not applicable for a crude extract, but

it is assumed that there would be some loss to the sodium carbonate solution when it is shaken with a crude chloroformic extract. Dragendorff (45) also gives a correction of 3 mgm. to be added for solubility of santonin in 8 per cent. sodium carbonate solution when 10 c.c. is used to wash crystals. This cannot, however, be confirmed, as it was found that on washing the fairly clean crystals from an assay, with this solution, there was no appreciable diminution in the weight.

The essential oil present in the crude drug is still present in the final solution, and as it is also optically laevo-rotatory, the reading is increased. The oil produces only a small rotation of polarised light, but it is nevertheless sufficient to affect the result quite noticeably in the case of drugs of low santonin content. There is also the possibility of other optically active substances being present and affecting the result, while remaining undetected as interfering agents. Mouton (loc.cit.) gives examples of this and shows that even when santonin was present in the drug, a deviation to the right was observed. No similar phenomenon has been noticed by the present author. This polarimetric method is little used.

(b) Volumetric.

Kariyone and Kimura's Process.

The volumetric assay of Kariyone and Kimura (46) is not satisfactory. The results obtained by using it are always high, evidently due to the saponification of some other

substances. Mouton and Favrel have criticised this method, and their statements that erroneously high results are given by it are in accordance with the findings of the present author. Favrel's statement (47) that the method of the Japanese workers does not extract all the santonin present is, however, unfounded, as the extraction process is the same as that used in Katz' method, which he finds accurate, and which he himself uses in his polarimetric estimation.

Katz' Process.

Katz' (48) volumetric method, probably on account of its length and intricacy, is not much used. Although Katz gives figures for some estimations, which show a close relationship with those obtained by his gravimetric method, the usefulness and accuracy of the method cannot be confirmed as the results obtained were usually much too high. The extra purification by taking up with 15 per cent. alcohol is an advantage over the process of Kariyone and Kimura.

(c) Gravimetric.

With drugs containing at least 1 per cent. of santonin all the gravimetric methods mentioned have been found to give reasonably accurate results, although these are dependent to some extent on individual manipulation, and are very liable to variation with varying conditions. They also give results varying among themselves by reason of the different substances present in the mother liquors from which

the santonin is made to crystallise. Apart from these factors the biggest objection is to the correction required to be made for the solubility of the santonin in the alcohol used in the final stage of all of them, except Palkin's (49). It is difficult to imagine this long, complicated process as a practical method of assay. This correction, usually taken as 6 mgm. per 10 gm. of solution, is necessarily an arbitrary one, since the other substances in solution vary constantly and so influence the solvent action. The correction is usually regarded as small, but in a drug containing 2 per cent. of santonin it corresponds as a rule to about 30 per cent. of the amount of santonin actually recovered. Such a drug is considered good. In an inferior drug containing 1 per cent. of santonin the correction is very nearly, if not over, 100 per cent. of the recovered santonin, a fact which shows how unsatisfactory such a correction is. It also shows why the processes do not give good results or are even inapplicable in the case of drugs of low santonin content. In the case of drugs somewhat low in santonin content, but for which the processes are applicable, the final weight of santonin is inconveniently small, which would not be the case but for the amount left in the alcohol.

None of the processes were found to be entirely suitable for the estimation of a drug consisting of leaves, and containing less than 0.6 per cent. of santonin. The correction, as has been stated, is the chief factor limiting the usefulness

of the processes. Some of the processes gave small quantities of crystals but these were in every case badly contaminated with brown amorphous resinous matter and quite useless as the end product of an assay. Particular mention of only four individual methods of assay need be made; Katz'(loc.cit.), Fromme's (50), Mouton's (51), and Massagetow's (52). The first two in many instances, even when the santonin content was greater than the amount required for the correction, gave no crystals. When crystals were obtained they were so badly contaminated as to be useless. Despite this, by adopting a slight modification, Fromme's process was found to be the most useful of all. The final mother liquors in Mouton's processes, when using the drug described, were found to be strongly coloured green, due to chlorophyll not having been eliminated. No crystals were obtained, and the same remarks as have been made regarding Katz' and Fromme's processes are equally applicable to Mouton's. The process was found to be quite useful, but it is not considered to possess any advantage over Fromme's, and the latter is preferred.

More recently the new process of Massagetow has been found to give accurate results with batches of plant having a low santonin content. The claim by the author that good results can be obtained with a drug containing only 0.2 per cent. of santonin is, however, unjustified. The correction which is required when using 5 gm. of the drug and the quantities of reagents given by the author is equal to 0.012 gm. and it can

be seen that only 0.010 gm. of santonin is present in 5 gm. of drug containing 0.2 per cent of santonin. Although a correction is included in the process it is not inconveniently large and its inclusion cannot be regarded as a serious objection to the process. It is approximately one-third of that required by other processes in which alcoholic mother liquor is used. The number of stages might be objected to if the results obtained by its use were not consistent with the intricacy; the author claims a limit of error of 0.03 per cent., but although the process was found to be reasonably accurate, the smallness of the error cannot be confirmed, doubtless due to insufficient practice with this particular process.

The final stage of the process appears to serve no useful purpose other than the transference of the residue to a flask for weighing purposes. It is suggested that it might be obviated by filtering the alcoholic mother liquor through a tared Gooch crucible, but this is of importance only for shortening the process.

Fromme's process, modified where necessary, was used for the assays during 1930 and 1931. It is as follows :- Thirteen gm. of the power is shaken for one hour with 130 c.c. of chloroform, and 102.5 c.c. (equal to 10 gm. of the drug) is filtered off and evaporated until the residue weighs approximately 8 gm. One hundred c.c. of a fresh saturated solution of barium hydroxide is then added, and the mixture heated on a water-bath until the remainder of the chloroform

has been driven off. The solution is filtered, the filter and flask being washed with hot water, and the filtrate acidified with 5 gm. of 25 per cent. of hydrochloric acid. When lukewarm this acid solution is transferred to a large separator, and the flask rinsed out with 20 c.c. of chloroform, which is then added to the liquid in the separator. After shaking briskly for two minutes, the chloroform is allowed to separate, and is drawn off into a flask, the extraction being repeated twice, with quantities of 20 c.c. of chloroform. The residue left on evaporating these mixed chloroformic solutions is taken up by warming with 7.5 gm. of absolute alcohol, and the solution is poured into 42.5 gm. of hot water. This is filtered immediately, and the filter and flask are washed with two successive 10 gm. lots of a heated mixture of 3 gm. of absolute alcohol and 17 gm. of water. The solution is allowed to cool and stand for twenty-four hours, at the end of which, the crystals which have separated are collected on two counterbalanced, superimposed filter papers. The flask and filter are washed with two successive quantities, each of 10 c.c. of 15 per cent. alcohol. The filters are then dried to constant weight at 110°C., and the crystals obtained are weighed. To the weight of crystals obtained, 0.042 gm. (= 6 mgm. per 10 gm. filtrate without washings) must be added as a correction for the solubility of santonin in the mother liquor. This total weight represents the amount of santonin in 10 gm. of the crude drug.

When the santonin content of the drug is low the

process becomes inoperable, and it is then necessary to adopt some modification in order to obtain crystals. Two methods of modifying the process were tried. In the first attempt to overcome the difficulty, a weight of santonin approximately equal to that which the correction indicated would remain in solution, was added to the hot solution after filtering. This added santonin was taken into account when making the final calculation. The second method adopted was to double the amount of drug and reagents used, up until the stage where the residue is taken up into 15 per cent. alcohol. A single volume of this solvent was used. Some slight error may thus be introduced, by altering the solubility of santonin in the final mother liquor for this has double the normal amount of other dissolved substances. For the purposes of this research, however, the error introduced would be relatively very small, and by comparison with the first method adopted, the result did not appear to be affected. By carrying out this assay with this modification, using a drug which yielded crystals by Fromme's original method without modification, and at the same time carrying out a control using the original unmodified method, the writer could detect no error introduced by the modification.

(d) Preparation of Derivatives.

The processes which take advantage of the ketone group present in santonin were designed primarily for the

estimation of already purified santonin in tablets and such preparations. In these there is no substance having similar ketonic properties, or, if there is, such substances may first of all be eliminated usually quite simply. In dealing with the crude extracts, however, substances not so readily eliminated, such as artemisin and pseudo-santonin, interfere with the result. In addition to these well defined substances, some part of the resins appear also to take part in the reaction with reagents such as hydroxylamine, confusing the result. These also can ultimately be eliminated, but when this is done to a sufficient extent, the preparation of an additive compound is only an additional stage in a straightforward gravimetric estimation.

Attempts were made to estimate the santonin in extracts of varying purity, of the crude drug, by the preparation of oxines, phenylhydrazones and semicarbazones, but consistent results could not be obtained with a reasonable amount of purification. Even after the extracts had been purified up to a stage corresponding to the purity of the final stage in a gravimetric assay, the results were inconsistent. This was attributed to the presence of resinous bodies which remain in colloidal suspension in the final mother liquor of the gravimetric assay while the santonin crystallises out, but which form insoluble compounds with reagents which precipitate ketones.

Fractional crystallisation may be adopted to separate the different additive compounds formed, but this is impracticable for an assay process.

In addition to shortening and simplifying, it is desirable in any projection process that the use of any reagent in which santonin is appreciably soluble, and for which solubility a correction would need to be applied, should be avoided

NEW GRAVIMETRIC METHOD OF ASSAY

Gravimetric processes have been found to be the best for obtaining accurate results. The only one, however, which in its original form, yields consistent results when dealing with a drug of low santonin content, is Massagetow's, and it is rather long. In addition it is very recent and was not available until this research was almost finished. Taking advantage of what appeared to be best in the other processes, a new method of assay has been formulated and after prolonged testing has been found to be eminently suitable for the type of drug used in this research. It has also been used for the estimation of santonin in typical commercial drugs and found to give satisfactory results, and it is believed that it is generally useful for the estimation of all types of crude drugs containing santonin.

No correction is required and good results have been obtained with drugs containing as little as 0.3 per cent. of santonin. With drugs containing less than this amount results may still be obtained, but the accuracy then depends on the type of drug, as in some instances it was found that the crystals were contaminated with resinous matter. This was

found to be particularly so with very poor commercial drugs, and flowerheads alone, which appear to contain a greater amount of resinous matter than do the leaves.

The procedure finally chosen as the result of trial and error is as follows :-

Procedure.

The estimation is carried out by extracting 14 gm. of the dried, coarsely powdered drug, by shaking frequently during six hours with 140 mil. of benzene; 101 mil. of the liquid is filtered off and shaken briskly for five minutes in a separating funnel with 35 mil. of 8 per cent. sodium carbonate solution. Separation is allowed to take place and 80.5 mil. of the benzene solution, corresponding to 8 gm. of the drug, is decanted into a flask and evaporated to dryness on a water-bath. The residue is taken up with 40 c.c. chloroform and the solution is filtered, the flask and filter being washed with two portions each of 20 c.c. of chloroform. The filtered solution is evaporated to dryness on a water-bath. The residue is extracted by heating for ten minutes with 60 mil. of saturated barium hydroxide solution at 95°C. The solution is immediately filtered into a flask, the flask and filter being washed with two portions, each of 10 mil., of saturated barium hydroxide solution at 95°C., and the filtrates united. The flask is then plugged with cotton wool and the solution is allowed to cool, made slightly acid by the addition of 5 mil.

of 25 per cent. hydrochloric acid, and set aside for twenty-four hours to crystallise, being gently agitated occasionally. The crystals are collected in a tared Gooch crucible, any crystals remaining in the crystallising flask being washed into the crucible with small portions of the filtrate. The crucible and crystals are finally washed with two 10 mil. lots of cold water and dried to constant weight at 100°C. After cooling in a desiccator the weight of santonin is found and represents the weight of santonin present in 8 gm. of the crude drug.

Consideration of the New Method.

Benzene was chosen as the extracting agent because it extracts less inert and resinous matter than do the other common organic solvents. It is suggested, however, that commercial crystallisable benzene, completely volatile below 95°C. be used, as was done in the present work. Benzol of commerce cannot be completely removed from the extract at the temperature of the water-bath, at ordinary pressure.

It has been shown (43) that a considerable portion of the resinous matter is extracted from a crude extract of the drug by shaking with 15 per cent. sodium carbonate solution. It has further been shown (44), however, that by shaking a solution of santonin in benzene with 15 per cent. sodium carbonate solution, a proportion of the santonin is taken up by the alkaline layer. Dragendorff uses 8 per cent. sodium carbonate solution to wash crystals from an assay and thus removes any resinous matter which may be adhering to them.

He gives a correction for the solubility of santonin in this solution.

More recent work has shown that santonin is not extracted from benzene solution by shaking with 8 per cent. sodium carbonate solution. As the 15 per cent. solution possesses no advantage over the 8 per cent. for the extraction of resinous matter from a benzene extract, the latter solution was chosen for this assay. That the two solutions are equally effective for the extraction of resinous matter may be demonstrated by first of all extracting two separate volumes of benzene extract of santonica with 8 per cent. sodium carbonate solution. One of the extracted benzene solutions is then extracted with more 8 per cent. sodium carbonate solution and the other with 15 per cent. After separation of the alkali layers, an examination of them shows that the 15 per cent. solution contains very little, if any, more extractive matter than does the 8 per cent., showing that practically no resinous matter remained in the benzene layer after the first extraction, which would have been removed by using 15 per cent. sodium carbonate solution as the <u>first extraction</u> reagent. Mouton has stated that the two layers do not separate readily when a benzene extract of santonica is shaken with 15 per cent. sodium carbonate solution. No difficulty was experienced by the writer in getting the two layers to separate when using 8 per cent. solution. On standing for at most half an hour or with suitable manipulation, separation is complete and the aliquot

part is readily obtained free from the aqueous portion.

Extraction with chloroform serves to eliminate such compounds as artemisin and pseudo-santonin which form insoluble compounds with it. This stage is not considered to be necessary when assaying the plant at present under investigation, as an examination did not reveal the presence of any of these other compounds. In order to make the process generally usable, however, this stage must be included.

By extracting the residue from the chloroformic solution with barium hydroxide solution the santonin is converted to the barium salt, and goes into solution, while some of the resinous matter is left behind on filtering.

It was found that by allowing the solution to cool, before acidifying, better crystals were obtained. By acidifying while hot, small scaly crystals appeared and the colloidal matter which remains in suspension otherwise, showed a tendency to deposit.

The crystals obtained are good clean plates, practically free from contamination of any kind and having a melting-point of 169 to 170°C.

COMPARATIVE RESULTS WITH DIFFERENT METHODS OF ASSAY

The consideration which led to the adoption of the various stages in the new method of assay have already been stated. The general technique having been decided, the various stages had to be experimented with, until the best result was obtained from each, and finally the assay was tested

comparatively with other processes on suitable drugs.

The processes adopted for making the comparison were those of Katz, Fromme and Mouton. It was found, and it has also been shown by others, that when a good drug is being tested, almost identical results can be obtained with these three processes. Eder and Schnieter's (loc.cit.) process was also found to give corresponding results, but for the most part the three previous processes were considered to be sufficient and they alone were used. It had also, however, been observed that satisfactory results can be obtained with any of the processes, using a drug compound of leaves and flowerheads, or leaves alone, only provided an appreciable quantity of santonin is present.

The first three sets of tests were carried out on samples of good commercial santonica which had previously been found to contain 1.48, 2.35 and 1.94 per cent. by Fromme's method. The results of these tests are shown in Table III.

TABLE III. PERCENTAGE OF SANTONIN BY DIFFERENT METHODS OF ASSAY

METHOD	AVERAGE PERCENTAGE OF SANTONIN IN SAMPLE		
	I	II	III
Fromme's	1.48	2.35	1.94
Katz'	1.50	2.36	1.96
Mouton's	1.49	2.36	1.94
New Method	1.48	2.34	1.93

Having found from a number of these assays that comparable results were obtainable, the writer next carried out

tests on batches of plant consisting of leaves, or leaves and flowerheads containing an appreciable quantity of santonin. The results obtained are summarised in the followint table :-

TABLE IV. PERCENTAGE OF SANTONIN BY DIFFERENT METHODS OF ASSAY

METHOD	AVERAGE PERCENTAGE OF SANTONIN IN SAMPLE						
	IV.	V.	VI.	VII.	VIII.	IX.	X.
Fromme's	1.175	1.060	0.805	1.025	1.150	0.960	0.730
Katz'	1.195	1.085	0.840	1.030	1.160	0.990	0.780
Mouton's	1.205	1.100	0.870	1.075	1.165	1.020	0.810
New Method	1.180	1.050	0.795	1.030	1.150	0.95	0.72

NOTE: (The error in the processes is such that results are accurate to the second decimal place at most, but, for purposes of comparison, the actual figures obtained, to the nearest 5 in the third decimal place, are given here.)

From this it will be seen that comparable results are obtained by the use of these four methods, although the variation is, on the average, somewhat greater than that when using the commercial drug. When a high variation is evident the crystals were impure and the extra weight was attributed to the presence of resinous matter. A green colouring matter, probably chlorophyl, is also present on the crystals obtained by using Mouton's process, with a low santonin content drug.

It will also be observed that the results obtained by the new process accord most closely with those obtained by Fromme's process. The reason for this is assumed to be the presence of small quantities of resinous matter adhering to the crystals from the other two methods of assay. The crystals

from Fromme's and the new method are practically free from contamination and were certainly cleaner than the others.

These figures confirmed the usefulness of the new process and it was concluded that accurate results are obtained by using it.

Attention was next turned to drugs containing smaller quantities of santonin. Since there is no correction to be applied it was not considered necessary to prove the accuracy of the process by comparison of the results with those obtained using other methods. The purity of the crystals obtained, as shown by their melting point and freedom from resinous matter was considered to be better evidence of the usefulness of the method than such a comparison, particularly since it had previously been shown that some modification required to be applied to the other processes in order to obtain crystals with a low content drug. As an additional check, however, Fromme's original process, and Fromme's process, modified in two ways as already described, were carried out. Mouton's process similarly modified and without modifications, and Katz' unmodified process were also carried out. The results obtained are shown in Table V. From this it will be seen that while crystals were obtained with all of the processes when drugs with higher santonin content were used, the percentages represented by the weights of these crystals were invariably high, because of the impurities present on them. By a modification of the methods, the results obtained were not improved from the drugs with higher content, due probably to

the extra resinous matter which separates. It has previously been mentioned that the correction also is probably not accurate when any modification is introduced, the amount of solutes in the final mother liquor, for which the correction is required, being doubled. The only advantage of a modification is that an approximate result is obtained from drugs having a lower content.

TABLE V. TABLE OF RESULTS BY DIFFERENT METHODS OF ASSAY

METHOD	PERCENTAGE OF SANTONIN IN SAMPLE							
	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
Fromme's	0.720	0.650	0.645	0.545	0.535	-	-	-
Fromme's M1	0.725	0.665	A	0.570	A	0.460	0.425	-
Fromme's M2	0.725	A	0.655	A	0.545	0.450	0.380	0.150
Mouton's	0.730	0.655	0.660	0.555	0.550	0.505	-	-
Mouton's M1	A	0.660	A	0.565	A	0.525	0.465	-
Mouton's M2	A	A	0.660	A	0.540	0.480	0.385	0.275
Katz'	0.725	0.655	0.650	0.560	0.550	0.520	-	-
New Method	0.715	0.650	0.645	0.540	0.530	0.450	0.370	-
New Method M2	A	A	A	0.550	A	0.450	0.375	0.150

NOTE: M1 indicates that process was modified in first manner described for Fromme's.
M2 indicates that process was modified by the addition of santonin.
A indicates that process was not carried out with this drug.
- indicates that no crystals were obtained.

With drugs of lower santonin content, the three older unmodified processes very often gave no crystals at all. In drugs containing less than about 0.5 per cent. of santonin no

crystals can be obtained as the correction corresponds to only a little less than this percentage. The results obtained by modifying the processes with these drugs were quite unsatisfactory. The crystals obtained, were often very dirty. Table VI. is a comparative table showing the impurity of the residue as evidenced by the approximate melting points, determined as well as was possible with the adhering resinous matter. The melting point of pure santonin is 171 - 174°C.

TABLE VI. COMPARATIVE TABLE OF PERCENTAGE OF SANTONIN BY DIFFERENT METHODS AND ITS APPROXIMATE MELTING POINT (°C.), FROM LOW CONTENT DRUGS.

Method	Sample XIV		Sample XVI		Sample XVII		Sample XVIII	
	% age	M.pt.	% age	M.pt.	% age	M.pt.	% age	M.pt.
Fromme's	0.545	169	-	-	-	-	-	-
Fromme's M1	0.570	164	0.460	167	0.425	162	-	-
Fromme's M2	A	-	0.450	169	0.380	168.5	0.150	167
Mouton's	0.555	167.5	0.505	150?	-	-	-	-
Mouton's M1	0.565	163	0.525	145?	0.465	154?	-	-
Mouton's M2	A	-	0.480	160?	0.385	166	0.175	?
Katz'	0.560	167	0.520	148?	-	-	-	-
New Method	0.540	170	0.450	169.5	0.370	169	-	-
New Method M2	0.550	169	0.450	169	0.375	169	0.150	168.5

These figures show that Fromme's method with some modification is, of all the previous, suggested methods, the most suitable for this type of drug. Even it, however, does not yield crystals as pure as those obtained by the new method and this latter requires no modification with reasonably low

santonin content drugs, there being no correction necessary.

The new method has been found to be quite applicable to air-dry drugs having a santonin content as low as 0.3 per cent; the crystals obtained from drugs containing less than this amount, are somewhat impure. It had been shown to give accurate results, and the extensive use to which it was put in 1932 has confirmed its usefulness.

This process may be modified if necessary by the addition of santonin to the final mother liquor, but it is not advisable to decrease the amount of barium hydroxide solution in proportion to the amount of drug used. Because of the interaction between the reagents with the production of barium carbonate, it is, of course, not permissible in this process to wash the crystals with sodium carbonate solution as may be done in some of the other processes.

Incidental Work.

During the investigation it was necessary to ascertain whether a correction would require to be applied by reason of the extraction of the crude, benzene extract with sodium carbonate solution. The effect of extracting a benzene solution of santonin with 8 per cent. and 15 per cent. sodium carbonate solutions was determined.

The solubility of the benzene used, is not included in any tables, and it was required for the calculation, on which the method adopted for the previous determination, depends. The solubility at 19° C. of santonin in commercial

crystallisable benzene was therefore determined.

The density, at the prevailing laboratory temperature, of the benzene used, was required for calculation of the aliquot part and also, so that the solubility of santonin in the benzene might be stated as a weight in volume percentage, and as a weight in volume of benzene. The density of the benzene at 19°C. was accordingly determined. Details of these three determinations are given in the following chapter.

SUMMARY OF CHAPTER VIII

A large number of assay processes, which were tried in the course of this investigation, in an endeavour to find one suitable for the particular type of drug which was being used, are criticised on the basis of results obtained by their use. Observations as to their usefulness or unsuitability for the present work, are made.

Gravimetric methods having been shown to give the most accurate results, a new method of this type is described. Accurate results may be obtained by this new process which is applicable to the analysis of drugs with a low santonin content for which the other processes are not suitable without the introduction of some modification to increase the final yield of crystals. Comparative results with this new process, and three other accepted processes, on drugs of different character and widely varying santonin content, are shown.

CHAPTER IX.

SOME PHYSICAL PROPERTIES OF SANTONIN

The solubility of Santonin in Benzene

Many of the present methods for assaying santonin in santonica and preparations containing santonin use benzene as the extracting agent, but despite this widespread use, the solubility of santonin in benzene does not appear to have been determined. It is not given in any of the standard reference books, nor is mention made of it in any of the published papers describing the processes in which benzene is used for the extraction. The desire in the present instance was for a figure which, while not necessarily absolute, would be a good working figure for routine laboratory work. Reasonable care was observed and an apparatus giving satisfactory results, used, so that the solubility figure given is close to that which would be obtained using more elaborate methods.

Apparatus and Physical Conditions.

The determinations were carried out at 19°C. (66.2°F.); not a very common temperature for scientific determinations, but a very common one in the laboratory, and therefore in keeping with the exactness of the method. Recrystallised santonin, having a melting point of 171°C., was used, and the benzene was commercial crystallisable benzene, which is usually employed in routine laboratory work. The apparatus used in making the determinations was that described by Campbell (53) for the determination of solubility, and

consists of two weighing bottles connected together by a cork and glass tubing, so that a saturated solution of the substance at any desired temperature can be obtained, without cooling or loss of volatile solvent on filtering. The determinations were made by maintaining the apparatus at 21°C. by means of a thermostat for four days, with frequent shaking, and then at 19°C. for 48 hours, still shaking frequently. The apparatus was then inverted in the thermostat so that filtration took place at the correct temperature. The lower weighing bottle on removal from the cork was immediately stoppered and weighed. The benzene was removed by evaporation at a low temperature (40°C.) and the bottle and contents dried to constant weight at 95°C. It had previously been ascertained that the benzene left no residue when maintained at this temperature for half an hour.

Density of the Solution and of the Benzene.

From the data thus obtained, the weight of santonin soluble in 100 gm. of benzene and the weight in weight percentage solubility, were calculated. To calculate the weight of santonin soluble in 100 mil. of solution, the density of the solution was required, and for the calculation of the weight of santonin soluble in 100 mil. of benzene the density of the benzene was required. These densities were determined with suitable accuracy by means of a density bottle by making a saturated solution at this temperature, using the figures from the data already obtained. The density of saturated solution of santonin in benzene at 19°C., taking the mean of

three determinations, was found to be D. 19° 0.88610 ± 2 X 10^{-5} gm. per mil., and similarly the density of the benzene used was D.19° 0.87915 ± 2 X 10^{-5} gm. per mil.

The figures obtained in the determination of the solubility are set out in the following table :-

TABLE VII. SOLUBILITY OF SANTONIN IN BENZENE

Experiment	Weight of solution	Weight of residue (santonin)	Weight of santonin soluble in		Percentage santonin in solution	
			100 gm. Benzene	100 mil. Benzene	Weight in weight	Weight in volume
1	4.5490	0.1758	4.0199	3.5342	3.8646	3.4244
2	6.5651	0.2543	4.0296	3.5426	3.8735	3.4323
3	5.1944	0.2004	4.0128	3.5279	3.8580	3.4186
4	5.8368	0.2256	4.0205	3.5346	3.8651	3.4249

From this table, taking the mean values, it can be seen that the solubility of santonin in commercial crystallisable benzene, D.19° 0.87915 gm. per mil. at 19°C., is :- 4.0207 gm. in 100 gm. benzene; 3.5348 gm. in 100 mil. of benzene; 3.8653 per cent, weight in weight; 3.4251 per cent. weight in volume. One part of santonin is soluble in 24.871 parts by weight, or 28.292 parts by volume of benzene at 19°C.

These data were required before the determination of the effect of extracting benzene solution of santonin with sodium carbonate solution, could be carried out, by the proposed method.

The Extraction of Santonin from Benzene Solution by Sodium Carbonate Solutions

The final product in the gravimetric assay of santonica consists of crystals of santonin contaminated with a little resinous matter. The crystals may be more or less freed from the contamination by washing on the filter paper with eight per cent. sodium carbonate solution as originally suggested by Dragendorff (loc.cit.), who gives a correction of 0.003 gm. to be added for every 10 c.c. of solution used, to correct for santonin lost by solution. In Favrel's (loc.cit.) assay, an attempt is made to remove the resinous matter at an early stage by shaking the chloroformic solution with 15 per cent. sodium carbonate solution. In this assay no correction is made for the solubility of santonin. As a correction is applied for 8 per cent. solution in Dragendorff's process, and none is made for 15 per cent. solution in Favrel's, it was considered advisable, in view of the importance of such a correction in the assay of santonica, to determine the solubility of santonin in 8 per cent. and 15 per cent. solutions of sodium carbonate (Na_2CO_3, $10H_2O$.).

An acidmetric method is not practicable in view of the large amount of alkali involved, in comparison with the small weight of santonin. The direct gravimetric method also presents a number of difficulties. It was, therefore, decided to make the determinations indirectly by shaking a solution of santonin in benzene with the sodium carbonate solutions. The

solubility of santonin in these latter solutions can then be calculated by determining the amount of santonin left in the benzene layer, using the formula for calculation of partition coefficient, the solubility of santonin being known. Control experiments were carried out at the same time.

Technique.

In making the determinations the following were used :- Recrystallised santonin, melting point 171°C.; commercial crystallisable benzene, and 8 per cent. and 15 per cent. solutions of decahydrated sodium carbonate. Two pipettes, having exactly the same delivery, were used throughout, one for the aqueous, and the other for the benzene and benzene solutions. The santonin in benzene solution was approximately 0.15 per cent. that being a common strength for an extract of santonica.

Sodium carbonate solution in 20 c.c. portions in separating funnels, with 40 c.c. lots of benzene solution were vigorously shaken at frequent intervals for forty-eight hours. Separation was quick and complete. After this treatment 10 c.c. lots of the benzene solution were separated into accurately tared small flasks, the benzene was evaporated at a low temperature and the flasks dried to constant weight at 95°C. In the controls the benzene solution of santonin was replaced by benzene alone. On evaporation of the benzene there was no appreciable residue from any of the controls, showing that any water in solution in the benzene did not carry with it any sodium carbonate. To find the exact amount of santonin present

in the delivery, from the pipette, 10 c.c. lots of the untreated benzene solution were put into tared flasks and similarly dried. The results obtained were as follows :-

Eight Per Cent. W/V Solution of Sodium Carbonate. -

The weight of residue obtained from the extracted benzene solution was in each case the same (within experimental error) as that obtained from an equal volume of untreated benzene solution. Since there was no sodium carbonate in the residue, it follows that no santonin is extracted from the benzene solution, and following the recognised laws of extraction by immiscible solvents, the conclusion is reached that santonin is insoluble at ordinary temperatures in 8 per cent. solution of sodium carbonate.

In Table VIII. a few of the actual figures are shown.

TABLE VIII.

Experiment	Weight in milligrammes from 10 c.c. solution	
	Untreated	After extraction with 8 per cent. sodium carbonate solution
A.	14.4	14.4
B.	14.6	14.3
C.	14.4	14.4
D.	14.4	14.5

Fifteen Per Cent. W/V Solution of Sodium Carbonate.

With this solution the weight of residue from the extracted benzene solution was less than that from an equal

volume of untreated benzene solution. The difference amounted to about one-twelfth of the weight of the latter residue.

The following Table IX. is a table of some of the figures obtained.

TABLE IX.

Experiment	Weight in milligrammes from 10 c.c.solution	
	Untreated	After extraction with 15 per cent. Sodium Carbonate Solution.
A	14.4	13.2
B	14.6	13.2
C	14.4	13.2
D	14.4	13.2

The question arises whether this is a simple solution of santonin in sodium carbonate solution or whether chemical combination takes place. In order to decide this the sodium carbonate solution, which had taken up the santonin, was separated and re-extracted with benzene in a similar manner to the first extraction. No santonin was extracted. This was verified by using larger volumes of the solutions, as the volumes first used gave only comparatively small weights of santonin. From this it is concluded that 8 per cent. weight in volume solution of sodium carbonate has no solvent action on the santonin. A 15 per cent. sodium carbonate solution is strong enough to extract a certain amount of santonin from benzene solution. This is not a simple

solution, and probably involves opening the lactone ring with formation of the sodium salt of the corresponding acid.

It must be observed that these figures were obtained using pure chemicals and that they are not considered to be applicable to solutions in which other substances are present. The solubility of santonin in 8 per cent. sodium carbonate solution may be increased by reason of the presence of much organic matter in that solution, but it was considered that the amount which would be extracted by dissolving in the 8 per cent. sodium carbonate solution would be, relatively, so small as to be negligible for the purposes of the assay.

It was concluded from this investigation that some santonin would be extracted from the benzene extract by shaking with 15 per cent. sodium carbonate solution, and as has been shown, this solution appears to offer no advantage over the 8 per cent. for the purpose for which it is required. The conclusions drawn from this experiment led the author to use 8 per cent. decahydrated sodium carbonate solution in the new gravimetric method of assay.

SUMMARY OF CHAPTER IX.

The solubility at 19°C. of santonin in commercial crystallisable benzene was determined. The procedure adopted and figures obtained in the determination are given.

The density of this benzene at 19°C. has apparently not been determined, and as it was required for stating the solubility as weight in volume of benzene it was determined as an incidental part of the work.

The density of the saturated solution was also determined so that the weight of santonin in a volume of the solution might be stated.

The solubility of santonin in commercial crystallisable benzene was required so that a determination of the effect of extracting a benzene solution of santonin with 8 per cent. and with 15 per cent. sodium carbonate solution might be ascertained, using the partition coefficient method. This latter investigation was carried out and it is shown, that, using pure chemicals, 15 per cent sodium carbonate solution has a definite extractive action on the santonin. Eight per cent. sodium carbonate solution does not extract any santonin from a benzene solution of that substance.

CHAPTER X.

DETERMINATION OF THE SEASONAL VARIATION IN SANTONIN CONTENT

A batch of A. gallica in the vegetative state was collected in July 1929 from Tynefield (Map reference 1) and examined. The estimation for santonin by Fromme's (loc.cit.) method showed 0.81 per cent. of santonin to be present in the leaves of the air-dry herb. The small percentage was attributed to the early stage of development but it was to be expected that it would not be the maximum amount produced by the plant. It was decided then, to collect the plant at successive growth stages during the following seasons, and to trace the variation in the santonin content and its relation to stage of development. Root, stem, leaf and flowerhead of the plant were separated wherever possible and each examined for its santonin content, as this also seemed likely to be of value. Santonin was not found to be present at any time in the root or woody stem.

1930 Material.

Periodic collection of the plant was commenced in May 1930 and carried on at fortnightly intervals until July. From July until September weekly collections were made. Although the time interval was regular, attention was directed more particularly to the stage of development of the plant and such precautions as are possible in dealing with a wild plant distributed over a large area were taken to ensure that increase in development of successive batches of the plant was in

accordance with the time interval which had elapsed from the previous collection. It was not possible to ensure great accuracy in this, but when a collection was made, another batch of plant at, to all appearances, a similar stage of growth, and similarly situated with regard to habitat, to that being collected was marked. By collecting this marked batch at the following collection, reasonable accuracy was ensured. This was done on each occasion.

Early in June a number of plants were transplanted to the ground of the Scottish Society for Research in Plant Breeding at Corstorphine, Midlothian. There, under the supervision of Dr. J.W. Gregor of that Society, the plants were cultivated. These plants also, were collected at successive stages of growth during 1931 and 1932 and examined in order to ascertain the effect on the santonin content, of cultivating the plant in a non-halophytic habitat.

The drying and storing of the plants was controlled and each batch subjected to, as nearly as possible, the same conditions. Drying was carried out by exposing the material to a current of warm air at a temperature of about 45°C. as soon after collection as possible in order to prevent any autoxidation which might otherwise take place.

Thirteen batches of plants at different stages of growth were collected during 1930. The first of those batches consisted almost entirely of fleshy leaves. A shooting stem was present in the fourth and successive batches but flowerheads

were not apparent until the seventh batch, which had however only a very few undeveloped buds. From this, and the succeeding batches, it was possible to separate a small quantity of flowerheads, and these were assayed separately.

TABLE X. TABLE OF PERCENTAGE OF SANTONIN IN 1930, AIR-DRY HERB

BATCH NUMBER	DATE OF COLLECTION	% PART OF PLANT USED	
		Leaves and Finer Stems	Leaves and Flowerheads
1	10th May	0.15	-
2	7th June	0.375	-
3	21st June	0.44	-
4	4th July	0.545	-
5	19th July	0.80	-
6	25th July	0.95	-
7	1st August	1.14	1.14
8	9th August	-	1.99
9	16th August	-	1.66
10	21st August	-	1.095
11	26th August	-	0.76
12	30th August	-	0.805
13	6th September	-	-

(* The roots and stems were also examined, but did not on any occasion yield santonin).

On the tenth batch the leaves surface had started to decrease considerably, and on the eleventh there were comparatively few healthy leaves remaining. This being so, the

assay on the twelfth and thirteenth batches was carried out on flowerheads practically alone. By the time of collection of the twelfth batch, the flowerheads had attained complete development, and in the thirteenth batch they were partially expanded.

The assays were carried out in duplicate, and in some instances in triplicate. Table X. gives the average of the assays, and shows the variation in santonin content in the air-dry leaves, with flowerheads, if present. This variation is shown on Fig. V.

The percentage of santonin present in the flowerheads alone is shown in Table XI., and a graph of these values is given on Fig. V., with that for the air-dry leaves.

TABLE XI. TABLE OF PERCENTAGE OF SANTONIN IN 1930 AIR-DRY FLOWERHEADS

BATCH NUMBER	DATE OF COLLECTION	PERCENTAGE OF SANTONIN IN AIR-DRY FLOWERHEADS
7	1st August	0.48
8	9th August	0.65
9	16th August	0.72
10	21st August	1.18
11	26th August	1.06
12	30th August	0.805
13	6th September	0.190

By comparing these two tables and the graph (Fig.V.) it is seen that the santonin content attained a much higher maximum in the leaves than it did in the flowerheads, and that the maximum amount was present in the leaves when the flowerheads

were just becoming evident. The santonin content of the leaves had dropped considerably before that of the flowerheads had attained its maximum value.

The figures show the variation in santonin content in particular parts of the air-dry herb. Additional figures were taken showing the proportional weight of the different parts present on the fresh plant on collection, and also after drying. After determination of the amount of santonin present in the different parts, the percentage of santonin present in the entire plant in either the fresh or dried condition could then be calculated. The loss in weight on drying of the entire plant and of the separated leaves and stems was also recorded. The amount of santonin in the fresh and dried entire herb could be determined from these data. In addition the amount present in the fresh leaves alone could be calculated.

Considerable time was involved in obtaining these figures, as they are recorded for over 80 batches of plant and a multitude of data may be obtained from them. Other than as a record they are not, however, of much value for this particular research. In Table XII. the data obtained for the plants collected in 1930 are given. They are representative of the figures obtained in successive years and for plants from different localities, details of which are not given in this paper.

From this table it will be seen that the leaves of a plant with almost mature flowerheads and containing 1.66 per cent. of santonin lose approximately 75 per cent. by weight on drying.

TABLE XII. TABLE SHOWING PROPORTIONAL WEIGHTS OF DIFFERENT ORGANS PRESENT ON AERIAL PLANT OF A. GALLICA, IN THE FRESH AND DRY STATE, AND THE LOSS IN WEIGHT ON DRYING OF THE DIFFERENT ORGANS

BATCH NUMBER	Proportional weight of Parts				PERCENTAGE LOSS IN WEIGHT ON DRYING.		
	FRESH		DRIED				
	Leaves and/or Fl'hds.	Stems	Leaves and/or Fl'hds.	Stems	Whole Plant.	Leaves and/or Fl'hds.	Stems
1	100	-	100	-	84	84	-
2	100	-	100	-	82	82	-
3	100	-	100	-	80	80	-
4	88	12	82	18	76.5	78	65
5	85	15	77	23	76	78	64
6	83	17	76	24	74.5	77	64
7	80	20	72	28	74.5	77	64
8	80	20	71	29	74	77	62
9	79	21	64.5	35.5	69	75	48
10	78	22	64	36	65	72	43
11	75	25	65	35	60	65	43
12	68	32	59	41	56.5	62	43
13	65	35	57	43	52.5	58	42

The percentage of santonin shown for the leaves in Table X. must, therefore, be reduced to $\frac{1}{4}$ of that percentage to express the amount of santonin present as a percentage of the fresh leaves, since santonin is included in the weight of the leaves used in the assay and it does not decrease in weight on drying. The percentage of santonin in the fresh leaves and flowerheads of batch nine, 1930, is then $1.66 \times \frac{1}{4} = 0.415$.

Similarly, since the overground plants are composed of 79 per cent. of leaves and flowerheads and 21 per cent. of stem, this percentage must be further reduced by $\frac{79}{100}$, to express the santonin present as a percentage of the fresh aerial plant, i.e. the percentage of santonin present in the living green plant is 0.328.

These figures for the 1930 series of plants, obtained by combining Tables X, and XII, are given in Table XIII, and represented graphically on Fig. VI.

TABLE XIII. TABLE SHOWING CALCULATED PERCENTAGE OF SANTONIN IN THE DRIED AND FRESH ORGANS AND FRESH GREEN PLANT. (TABLES X, AND XII, COMBINED).

BATCH NUMBER.	DRIED LEAVES AND/OR FLOWERHEADS.	FRESH LEAVES AND/OR FLOWERHEADS.	FRESH GEEN PLANT
1	0.15	0.024	0.024
2	0.375	0.068	0.068
3	0.44	0.088	0.088
4	0.545	0.120	0.106
5	0.80	0.176	0.150
6	0.95	0.218	0.181
7	1.14	0.262	0.210
8	1.99	0.457	0.370
9	1.66	0.415	0.330
10	1.09	0.306	0.238
11	0.76	0.266	0.200
12	0.805	0.306	0.210
13	0.190	0.082	0.053

It will be seen from the Fig. VI. that, by taking the figures showing the percentage of santonin in the fresh leaves or plants, the curves are somewhat modified. They are, however, identical in nature as may be demonstrated by increasing the scale of the ordinate and keeping the scale of the abscissa the same. On Fig. VII. the scale of the ordinate has been increased to three times the scale of that used for the graph (Fig. V.) showing the percentage of santonin present in the air-dry herb, and plotted on the same graph for comparison.

The curves are similar but that representing the santonin in the air-dry herb was obtained independently of other data. For that and other reasons the latter has been used in preference to the others in a later part of this work.

1931 Material.

In 1931 only a few batches of the wild plant were obtained. Much of the experimental work connected with the suggested new assay process was done on these during that year. From the appearance of the plants they had not been collected at a time when they might be expected to contain the greatest amount of santonin, as the batch collected in July was only partially developed and on the following batch, collected at the beginning of September, the flowerheads had commenced to expand.

The plants already referred to, which had been transplanted to the ground of the Scottish Society for Research in Plant Breeding, and which will be referred to as the

cultivated plant, were also collected in 1931. The flowerheads on it had just started to expand before it was collected and a bigger yield of santonin would probably have been obtained had it been collected a little earlier. As it was, the santonin content of the leaves had presumably decreased and that of the flowerheads was probably less than the maximum.

The data available are insufficient for plotting a graph, but the figures obtained for the wild and cultivated plants are shown in the following tables, (Tables XIV, and XV.) -

TABLE XIV. WILD

BATCH NUMBER	DATE OF COLLECTION	PART OF PLANT USED		
		LEAVES	LEAVES AND/OR FLOWERHEADS	FLOWERHEADS
1	27th June	0.535	-	-
2	24th July	0.645	-	-
3	7th September	-	0.960	1.025

TABLE XV. CULTIVATED

1	6th August	-	0.730	1.150

It should be noted that the growth of the wild plant was not so far forward as in 1930 and that the cultivated plant was much in advance of the wild. The cultivated plant, collected on August 6th, was at practically the same stage of development as the batch of wild plant collected on September 7th.

1932 Material.

In 1932 batches of the plant were systematically collected

at frequent intervals. On each occasion two batches at different stages of growth were obtained, one batch being taken from the south and more sheltered side and the other, not quite so far advanced, from other parts of the area. The same precautions as were previously taken, to ensure that the plants in each collection were at the appropriate stage of growth in relation to the time which had elapsed from the previous collection, were again taken.

Eleven batches were collected for each of the two series. The first collection was made on the 23rd of June when the plant was at approximately the same stage of growth as the third batch of 1930. There were no flowerheads present on any of the plants in the first three batches but quite a number of buds appeared on the fourth batch collected on the 30th July. Leaves were less evident and flowerheads much more numerous on the seventh batch, although the latter were not fully mature until the tenth batch. By this time there was only a very small proportion of healthy leaves remaining. Some of the flowerheads on the eleventh batch were starting to expand.

Table XVI. shows the variation in santonin content of the aerial parts of the plant without stem.

On examination of these two sets of figures it will be seen that after rising to a maximum just after the flowerheads had appeared, the santonin content decreased for a time and then again rose, but again began to fall before the flowerheads started to expand.

TABLE XVI. TABLE OF PERCENTAGE OF SANTONIN IN 1932 AIR DRY HERB

SERIES 1.

BATCH NUMBER	DATE OF COLLECTION	PART OF PLANT USED		
		LEAVES AND FINE STEMS	LEAVES AND FLOWERHEADS	FLOWERHEADS
1	23rd June	0.59	-	-
2	9th July	0.97	-	-
3	23rd July	1.22	-	-
4	30th July	1.33	1.33	-
5	8th August	-	1.24	-
6	15th August	-	1.04	0.67
7	20th August	-	0.75	0.725
8	27th August	-	0.70	0.985
9	2nd Sept.	-	0.87	1.13
10	9th Sept.	-	1.09	1.09
11	20th Sept.	-	-	0.54

SERIES II.

1	23rd June	0.55	-	-
2	9th July	0.885	-	-
3	23rd July	1.32	-	-
4	30th July	1.60	1.60	-
5	8th August	-	1.54	-
6	15th August	-	1.30	0.695
7	20th August	-	0.90	0.85
8	27th August	-	0.86	1.06
9	2nd Sept.	-	0.975	1.22
10	9th Sept.	-	1.16	1.17
11	20th Sept.	-	-	0.875

With the expansion of the flowerheads the decrease was rapid and in a few days the santonin had practically disappeared.

Fig. VIII, shows the variation more clearly. The variation of the santonin content in the flowerheads alone, is shown on Fig. IX.

The plants comprising Series I. collected from the southern margin of the area appeared, in the earlier batches, to be slightly more advanced in development than those of Series II. With succeeding batches this was, however, not evident, and the plants in both localities reached maturity at the same time. The flowerheads on both series started to expand simultaneously.

In the first two batches of plants, the santonin content of Series I. was slightly higher than that of the other but by the third batch this had been reversed and Series II. had the higher content. Series II. continued to have the higher content right through the successive collections and attained a maximum of about one-fifth more than the maximum in Series I. The flowerheads of Series II. also contained a slightly higher per-centage. No significance is attached to this phenomenon, but the similarity of the curves after the maximum had been reached is worthy of notice. It will be seen that the two curves run almost parallel from this point and show practically the same absolute decrease, increase and again decrease.

On comparison of these two curves with the curve for the 1930 plant on Fig. V, it is evident that they are identical in nature with it. They are somewhat flattened out by

comparison and the primary and secondary maxima occur a little closer together, on the graph for the 1930 plant. The same fluctuations are, however, present, and it is to be noted that while the flowerheads on the 1932 plant occurred a little earlier in the season than they did in 1930, the latter commenced to expand earlier than did those in 1932; that is, the interval between the appearance and expansion of the flowerheads was shorter in 1930 than in 1932. This may afford some explanation for the shorter interval between the appearance of the maxima on the 1930 graph.

1932 A. maritima.

The main part of the work was done on A. gallica, and as soon as it could be identified care was taken to include this species only. This plant covered almost the entire area, there being only a few scattered patches of A. maritima occurring along with it. A few batches of A. maritima were collected in order to ascertain whether this plant contained santonin. Because of the scarcity of the species it was not possible to trace the variation in santonin content as has been done with the A. gallica

On examination, the plants of A. maritima were found to contain quite an appreciable proportion of santonin. On comparison with the yield from plants of A. gallica, at, as far as could be seen, the same stage of growth the percentage from A. maritima would appear to be even slightly higher.

The following table shows the percentage of santonin found to be present in those batches of A. maritima, and batches of A. gallica, at approximately the same stage of development.

TABLE XVII. COMPARATIVE TABLE OF PERCENTAGE OF SANTONIN IN A. GALLICA, AND A. MARITIMA, AT SAME STAGE OF GROWTH

BATCH NUMBER	SANTONIN IN		PERCENTAGE OF DIFFERENCE
	A. GALLICA	A. MARITIMA	
1	1.25	1.39	11.2
2	1.16	1.265	9.05
3	0.875	0.955	9.14

Further reference is made to the comparison in Chapter XI. where the data from the examination of other plants found growing wild in Britain is given. In a number of instances batches of both A. gallica, and A. maritima, were collected separately, when they were found growing together. The results of the examination of these batches generally, showed that A. maritima had a slightly higher content.

1932 Cultivated A. gallica.

The plants cultivated at Corstorphine were collected in four batches at about eleven day intervals in 1932. It was hoped that there would be sufficient material for a larger number of collections, but the plant spread only a little and there was only sufficient for the four collections. Complete data regarding the variation in santonin content in the cultivated

plant is, therefore, not available. It has, however, been shown that the plants continued to produce santonin in their third year of cultivation, although the yield is not so high as that from the wild plant at, as nearly as possible, the same stage of development.

In the first two batches the plants were in the vegetative stage and the stem bore only green leaves. On those of the third batch there was a good number of partly developed flowerheads, and on the fourth the flowerheads were more than half matured.

On the assumption that the santonin varies as it does in the wild plant, the maximum content would be expected to occur at a stage of development intermediate between that of batches 2 and 3.

Table XVIII. shows the results of analyses of these four batches of plant -

TABLE XVIII. TABLE OF PERCENTAGE OF SANTONIN IN 1932, CULTIVATED, AIR-DRY HERB

BATCH NUMBER	DATE OF COLLECTION	PART OF PLANT USED		
		LEAVES AND FINER STEMS.	LEAVES AND FLOWERHEADS	FLOWERHEADS
1	8th July	0.760	-	-
2	21st July	1.045	-	-
3	1st August	-	1.110	-
4	12th August	-	0.980	1.020

It will be seen from the table and Fig. X. that the santonin content shows a variation similar to that of the wild plant, but the evidence is incomplete.

A collective study of the graphs obtained from these assays, shows that they are all similar in nature, and show a rapid rise to a maximum. The fall from the maximum is followed by a subsidiary maximum, after which the santonin rapidly disappears completely. During the fall from the primary maximum, the santonin content of the flowerheads alone is shown to rise but commences to fall again at the same time as the fall, from the secondary maximum in the leaves and flowerheads, commences.

In the following chapter it will be shown that similar curves were obtained from the assay of plants collected in other localities, and in Chapter XIII. an explanation of this variation in the santonin content is put forward.

SUMMARY OF CHAPTER X.

The results of analyses carried out on batches of A. gallica, found growing wild in Scotland and collected periodically over a period of three years, show that the leaves and flowerheads of the plant contain santonin and that there is none in the stem or root.

Santonin reaches a higher maximum in the leaves than in any other part of the plant and that maximum is reached just at the beginning of the formation of flowerheads.

After the maximum the total santonin content does not show a steady decline, but a secondary maximum appears just before the flowerheads commence to expand.

Analysis carried out on cultivated plants show that santonin is still produced by the plant in the third year of cultivation.

A. maritima growing in the same habitat shows a slightly higher percentage of santonin that the A. gallica, at a similar stage of development.

CHAPTER XI.

THE SANTONIN CONTENT OF PLANTS FROM ENGLAND, WALES AND BELGIUM

Introduction.

Despite the large amount of work which has been done on many foreign species of Artemisia, very little attention seems to have been given by plant analysts to the A. maritima, and A. gallica, growing wild in quite a number of localities in Britain. The few investigations on English plants which have been reported, indicated - inconclusively however - that these plants do not contain santonin. No systematic examination for santonin seems to have been made, and those few investigators who have examined isolated batches of the plant appear to have been unfortunate in the material on which they worked.

It has been shown that the plant which gives the best galenical is not necessarily the one containing the most santonin, and the presence or absence of santonin may not even matter. This would appear to be almost probable. It is inconceivable that the merely homoeopathic dose of santonin which can be present in these aqueous preparations could have any remedial effect, firstly because of the insolubility of santonin in water, and secondly because of the minute quantity of santonin represented by the amount of herb used in relation to the amount of water. Galenical preparations in the form of infusions and decoctions of these wild plants are in common use and highly esteemed in many parts of England and to a large extent in the south and south-west of Wales.

Notwithstanding the belief that santonin may have no place in maintaining the popularity of this ancient remedy, it was thought that it would be of interest and perhaps value to make a collection of the plants from as many localities as possible and to examine them for santonin content.

With this object in view serial collections from the coasts of England and Wales were planned for the summer of 1932.

Preliminary Arrangements.

Information regarding the exact localities from which either, or both of the plants, could be collected was somewhat difficult to obtain. Druce (54) in his "Comital Flora of the British Isles" gives a list of 43 vice counties in England and Wales, and 10 in Scotland in which the plants occur, but although the book is recent, much of the data on which the information is based are old and competent naturalists in a number of the vice counties assert that the plants are now extinct in these vice counties. Professor Salisbury, who to a large extent collaborated with Druce in the compilation of this "Comital Flora" confirmed this information. Many of the local "Floras" from which information was sought are also rather old, and on this account too much weight should not be placed in the data in them either. Further, although a sufficiency of plants may occur in any locality for the species to be noted for botanical purpose, there may not be sufficient to make a collection, for the purpose of chemical examination, advisable or even possible. This was

found to be so in a number of localities. These difficulties being apparent, it was decided that the only practical means of obtaining accurate information was by communication with botanists having up-to-date knowledge of the flora of the particular localities. This was done, and a number of people supplied the necessary information regarding the localities with which they were conversant. No up-to-date records could be obtained for some of the vice counties mentioned by Druce, and in these instances the information in local floras was used. Sometimes the plants were found when this was done and sometimes there was no indication of the plant or even of a habitat in which it seemed likely that they would occur, modern industrial development having altered the habitat completely. A striking instance of this was seen in Middlesbrough where the only few plants which now occur are on an unused part of the ground of a modern locomotive works.

On the tour of collection the localities visited were those about which information regarding the presence of the plant had been received. Where no up-to-date information regarding the absence of the plants was available in the other vice counties mentioned by Druce, the plants were assumed to occur and the localities given by the local flors and those which seemed to be typical areas, were visited. The Isle of Man, one of the vice counties, was not visited.

In many of the localities the plants were seen growing wild, and in 28 of them there was sufficient to make collecting

practicable. These 28 localities are in 13 different vice counties. In eleven other vice counties the plant was seen to occur, but in most of these the growth is very sparse. Over sixty separate batches of material were collected from England and Wales and examined.

Druce gives then vice counties in Scotland in which the plant occurs, but in only four of them can the plants now be traced and only from the one area, which has yielded the Scottish plant under investigation, can sufficient material be obtained.

Collection in England and Wales.

Collection was started at the beginning of August 1932 in order that the plant might be at a stage when santonin would be present, if produced by the plant at all.

Particulars such as the proportion of leaves to stems in the fresh and dried conditions, and the loss in weight on drying were taken but are omitted from this work. In a limited number of localities it was possible to arrange for further collections to be made and sent on for examination. Where more than one batch is shown to have been collected, this had been done.

Batches of plant are shown here under the vice county in which they occur, and the order in which the vice counties are arranged is that order in which they were visited in the course of the collection.

South Northumberland.

The most recent "Flora" of this area is Baker & Tate's "A New Flora of Northumberland and Durham" published in 1868, in which the occurrence of A. maritima is described as follows :- "Frequent along the coast line in the salt marshes and by the stream-sides, both the type and gallica." No localities are mentioned. Professor J.W.H. Harrison of Armstrong College, a native of these parts, suggested the areas on either side of Seaton Sluice and Hartley as possible places, but he stated that he had never seen it in any locality in Northumberland. These areas were accordingly visited but no trace of the plant could be found. (A. absinthium is very common).

Durham.

The only locality in this vice county in which the plant was found is on Greatham Creek, (Map - area 2), about a mile north of Port Clarence, and some distance west along the north bank of the creek. This is an area on which a lot of salt marsh work has been done. A. gallica, and A. maritima grow profusely there, within the tidal area. A. maritima is the less common of the two. Fig. XX. is a general view of this area at low tide.

Development was not so far advanced as was that of the Scottish plant and there was no sign of flowerheads on the first collection. Arrangements were made to have additional batches sent on, and four of these were received showing the plant at various stages up to complete development of the flowerheads.

All five batches contained santonin although the amount present was relatively small. The figures obtained from the assays are shown in Table XIX.

TABLE XIX. TABLE OF PERCENTAGE OF SANTONIN IN A. GALLICA FROM GREATHAM CREEK

BATCH NUMBER	DATE OF COLLECTION	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
1	4th August	0.325	Leaf stage before appearance of flower-buds.
2	23rd August	0.74	Buds beginning to appear
3	1st Sept.	0.375	Buds about half developed
4	9th Sept.	0.575	Flowerheads almost completely developed
5	16th Sept.	0.61	Some flowerheads just beginning to expand.

These figures show a variation which is represented on Fig. XI. On comparison of this curve with those obtained from figures for the Scottish plant a similarity in nature will be seen. It will also be noted that the percentage of santonin is appreciably lower than that of the Scottish plant. This was found to be so for all the English and Welsh plants examined; only one showed more than one per cent.

N.E. Yorkshire.

The river Tees marks the northern boundary of Yorkshire and the plants have been reported to occur on the south bank of the river Tees near Middlesbrough. They have not, however, been seen there within recent years, and a search for them proved

fruitless. Neither are the species to be found on the tributary of the Tees about 3 miles west of Middlesbrough, where they were at one time quite profuse. A few shoots were seen growing within one mile of Middlesbrough town hall on the edge of a small marshy patch of ground adjoining the tidal cargo fleet on ground belonging to a locomotive works.

There was, of course, insufficient material for a collection and it is improbable that the species will continue to exist in that locality much longer. Near Whitby a small patch of the plants was encountered on the bank of the river which flows through this town, but here again there was insufficient for a collection.

S.E. Yorkshire.

A number of localities were visited in this vice county but the plants were not found.

The Field Naturalist Society with headquarters in Scarborough, who have catalogues the flora of Yorkshire, have not recorded the presence of A. maritima, or A. gallica, for the county.

S. Lincolnshire.

After Greatham, the first locality in which the species were found to grow in any quantity, was just north of Fosdyke, near Boston. At Fosdyke (Map - area 3) the plants were found in profusion both east and west of the bridge spanning the river Welland, on the north bank of the river. A. gallica formed

by far the major portion of the herb which extended along the bank about a mile west from the bridge and to the east extended right to the sea coast about 2½ miles distant. There were no plants on the south bank of the river.

Fig. XXI. is a view of the area east of the bridge, showing the plant.

A collection of A. gallica was taken from this locality and arrangements made for four additional batches to be sent on for examination. The first batch had only a very few flower-heads beginning to appear. Development was slow as the flower-heads of the fifth batch received some six weeks later were, even then, just commencing to expand.

This series of plant yielded the highest percentage of santonin obtained from any of the plants collected on this tour.

In table XX. the variation in santonin content is shown and the curve representing these percentages is plotted on Fig.XI.

TABLE XX. TABLE OF PERCENTAGE OF SANTONIN IN AIR-DRY A. GALLICA, FROM RIVER WELLAND

BATCH NUMBER	DATE OF COLLECTION	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
1	6th August	0.94	Leaf stage only, no flower-buds.
2	19th August	1.24	Flower-buds just beginning to appear.
3	2nd Sept.	0.625	Buds about half developed.
4	9th Sept.	1.025	Flowerheads fully developed but still unexpanded
5	18th Sept.	0.54	Number of flowerheads expanded.

It will be noted that here again a seasonal variation in santonin content occurs.

The plants were also seen on a number of localities north and south of Fosdyke in Lincoln, and they may be said to grow more or less profusely all round the Wash. No other collection was made from this vice county.

W. Norfolk & N.E. Norfolk.

A. maritima and A. gallica are common along the whole sea coast of Norfolk, except on the most south-easternly part. Localities on which they are profuse are, Belton's Marsh near Terrington; Holme Marsh; Brancaster Staithe; Scolt Head; Burnham Overy; Thornham Marsh and Blakeney, East of the Watch House.

A batch of A. gallica was collected from Belton's Marsh (Map - area 4), and other two batches were obtained at later dates. A batch was collected from Holme Marsh and another from Blakeney (Map - areas 5 & 6). A second batch was received from the same locality at Blakeney, of which Fig. XXII. is a photograph, about a fortnight later.

The first collections from each locality were at about the same stage of growth. They were not so well developed as had been expected, and the plant bore only very rudimentary flowerheads. The flowerheads were incompletely developed even on the plants collected 3½ weeks later from Belton's Marsh.

The figures obtained in carrying out the assays are given in Table XXI.

TABLE XXI. TABLE OF PERCENTAGE OF SANTONIN IN A. gallica.

(a) BELTON'S MARSH.

BATCH NUMBER	DATE OF COLLECTION	PERCENTAGE SANTONIN	STAGE OF DEVELOPMENT
1	8th August	0.69	Very early bud stage
2	23rd August	0.55	Flowerheads about half developed
3	1st Sept.	0.52	Flowerbud not quite mature

(b) HOLME MARSH

1	8th August	0.675	Very early bud stage

(c) BLAKENEY.

1	8th August	0.70	Very early bud stage.
2	20th August	0.64	Flowerheads less than half developed

Suffolk

On the coast of this vice county there are numerous salt marshes and on the majority of these the plants are to be found. Notable localities are the salt marshes west of Aldeburgh and south of Orford (Map - areas 7 & 8) on which the plants grow profusely, and from which a collection of each was made. Other localities on which they were seen, but from which no collection was made, are near Woodbridge on the banks of the River Orwell, near Ipswich, and on the north bank of the river Stour which marks the boundary between Suffolk and Essex.

All the plants from this locality showed numerous but very small and immature flowerheads and they were thus readily distinguishable.

An examination of the four batches obtained showed the percentages given in TableXXII.

TABLE XXII. TABLE OF PERCENTAGE OF SANTONIN IN A. MARITIMA, AND A. GALLICA FROM SUFFOLK

LOCALITY	SPECIES	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
Aldeburgh	A.gallica	0.87	Very early bud stage
"	A.maritima	0.94	"
Orford	A.gallica	0.835	"
"	A.maritima	0.855	"

N. Essex.

Essex, particularly the north, is interesting botanically because of its salt marshes and the number of typical salt marsh plants which grow thereon. A. maritima grows in great abundance in these marshes; A. gallica is also common and grows profusely.

Both plants were first encountered in the northern boundary of the county, the south bank of the river Stour, and were also seen growing freely over the extensive salt marshes in the north-eastern corner of Essex. They also grow abundantly near St. Osyth; near Brightlingsea; round most of Mersea Island, and sparsely on the marsh at Tollesbury. It is not suggested, however, that these are the only localities on which they are

found as the plants apparently occur, sparsely at least, round practically the whole coast of the vice county.

The plants occur together in about equal amounts and a collection of each was made from the river Stour west of Manningtree; north of Walton on the Naze; St. Osyth and Brightlingsea, (Map - areas 9, 10, 11, and 12). They were at a similar stage of growth to those collected in Suffolk, that is, in the very early bud stage; the collections from the river Stour were a little better developed. Results of the analyses of the collections are given in Table XXIII.

TABLE XXIII. TABLE OF PERCENTAGE OF SANTONIN IN A. GALLICA AND A. MARITIMA FROM N. ESSEX.

LOCALITY	SPECIES	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
River Stour	A. gallica	0.92	Early bud stage
"	A. maritima	0.96	Early bud stage
North of Walton on the Naze	A. gallica	0.88	Very early bud stage
"	A. maritima	0.935	Very early bud stage
St. Osyth	A. gallica	0.72	Very early bud stage
"	A. maritima	0.78	Very early bud stage
Brightlingsea	A. gallica	0.69	Very early bud stage
"	A. maritima	0.71	Very early bud stage

S. Essex.

The plants do not occur so frequently in S. Essex as they do in the north, but both of them are to be seen in a number of localities.

No collections were made from this vice county but both plants were seen growing quite abundantly on a number of places on, and opposite, Foulness Island, and also along the banks of the river Crouch towards Burnham.

E. & W. Kent.

Both A. gallica and A. maritima occur frequently in these two vice counties, particularly towards the north. A collection of each plant was made from Pegwell Bay near Ramsgate where they grow profusely, and they were also seen, although not growing so freely, on the estuary of the river Medway, north of Rochester; on a number of localities on Sheppey Island, particularly between Queenborough and Sheerness; on the salt marsh north of Faversham; at Tankerton near Whitstable; and on the estuary of the river Stour. Further south they were not so frequent but were seen growing sparsely south of New Romney near Littlestone-on-Sea.

The two batches collected from Pegwell Bay (Map - area 13) bore young flowerheads, and on examination the A. gallica yielded 0.72 per cent. of santonin, and the A. maritima 0.75 per cent.

E. Sussex.

Both species were growing abundantly near Seaford (Map - area 14), and a collection of each was made from this locality. They do not appear to be very common along this coast.

The plants were at the early bud stage and the

analyses showed 0.54 per cent. in A. gallica and 0.55 per cent in A. maritima.

W. Sussex.

Both species were growing in two localities in west Sussex; near Ferry Station south of Chichester and near Fishbourne. They were well advanced but growth in both places was rather sparse and no collection was made.

S. Hants.

The species were found frequently in this vice county but they were not at all prolific and only small collections of the plant were possible. Townsend's "Flora of Hants" gives Portsmouth and Hayling Island as the localities on which A. maritima is known to occur.

The three places on which there was sufficient for collection are Thornay Harbour, North Hayling Island, and the Portsmouth Harbours towards Farlington Church (Map - areas 15,16 and 17). They were also found on Hurst Point near Lymington, but were not collected.

In these localities A. maritima and A. gallica grow together. Two collections of A. maritima were made, one from Portsmouth Harbours, and one from N. Hayling. Collections of A. gallica were made from these two localities and also from Thornay Harbour.

An additional batch of each species from N. Hayling was received later.

In connection with the examination for santonin content it may be stated here that a few batches of plant gave no crystals by any method, but an amorphous deposit which it was not permissible to filter off, weigh and describe as santonin, was obtained. In such instances no percentage can be stated for santonin, but the presence of a quantity of santonin, less than is required for its gravimetric estimation, was in each of these instances demonstrated by a colour test on the final mother liquor. The test adopted was Wellmann's test, already described, which gave a very deep red and finally lavender solution in each case, showing the presence of santonin.

The percentage of santonin in the plants from this vice county was low. The results of the examination are shown in Table XXIV.

TABLE XXIV. TABLE OF PERCENTAGE OF SANTONIN IN A. MARITIMA AND A. GALLICA FROM S. HANTS

LOCALITY	SPECIES	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
Portsmouth Harbours.	A. gallica	0.56	Early flower-bud stage.
"	A. maritima	0.68	Early flower-bud stage.
N. Hayling	A. gallica	0.645	Early flower-bud stage.
"	A. gallica	0.53	Flowerbuds about half developed
"	A. maritima	0.74	Early flower-bud stage.
"	A. maritima	0.635	Flowerbuds less than half developed.
Thornay Harbour	A. gallica.	Detected by colour test	Flowerbuds more than half developed.

Isle of Wight.

The species were to be seen only in small quantities on the banks of the river Newton. No collection was made.

Dorset

Salt marshes occur near Poole Harbour in this vice county and it was expected that the species would be found in this locality. They were not, however, found there in sufficient quantity to afford a collection, although the plants were encountered in small amounts.

S. Devon.

Information had been received that the species were to be found on Slapton Sands about 12 miles south of Dartmouth. A. absinthium, and A. vulgaris were present in great abundance, but neither of the two required Artemisias were to be found, nor were they encountered in any other locality which was visited in S. Devon.

N. Devon.

The plants which Maplethorpe (55) investigated in 1924, and from which he was unable to isolate any santonin, occur in this vice county. The locality (Map - area 18) from which his material was obtained, and from which collections were made by the present author, is that part of the river Taw known as "The Seven Brethern's Bank". It is situated on the east bank of the river just south of the road bridge into Barnstaple. Both species grow abundantly there. A belt of A. maritima

growing in this locality is shown on Fig. XIII.

The late E.M. Holmes Esq., informed the author that the plants enjoyed a very high local reputation as an anthelmintic. He suggested that further examination was required and expressed the conviction that they contain santonin.

The plants at the time of collection showed quite a variation in stage of development. One batch of A. gallica at the very early bud stage, and another batch at a slightly more advanced stage of growth, were obtained. One batch of A. maritima at the early bud stage was also collected.

On two other localities in N. Devon there was an abundant growth of both species, and a collection of each was made from these places also. One locality is the sea wall south of the Barnstaple-Braunton Road about 2 miles east of Barnstaple, known locally as Bassett's Pitt - (Map - area 19). The other is to the left of the embankment of the toll-road leading from Braunton over Braunton Burrows to the Lighthouse (Map - area 20). Fig. XXIII shows A. maritima, and Fig. XXIV. A. gallica growing in this locality. A fourth locality in this vice county, on which the growth was however very sparse, is on the river wall running parallel with the Bideford-Barnstaple road and about one mile west of Bideford. No collection was made from the last area.

The following table (Table XXV.) shows the results of the examination of these plants. It will be seen that although the percentage present was low, santonin was undoubtedly

present in every batch.

TABLE XXV. TABLE OF PERCENTAGE OF SANTONIN IN A. MARITIMA AND A. GALLICA FROM NORTH DEVON

LOCALITY	SPECIES	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
Barnstaple	A. gallica	0.47	Early flower-bud stage.
"	A. gallica	0.34	Flowerbuds not half developed
"	A. maritima	0.53	Early flower-bud stage.
Bassett's Pit	A. gallica	0.43	Early flower-bud stage.
"	A. maritima	0.475	Early flower-bud stage.
Braunton	A. gallica	Colour Test	Flowerbuds about half developed
"	A. maritima	0.38	Flowerbuds about half developed

N. Somerset

Botanically the species are quite prolific in this vice county, and were seen to occur in six separate localities, but in only two of these was there sufficient to afford a collection. The localities in which they occur are along the Bristol Channel just south of Burnham-on-Sea; on both banks of the tributary of the Axe, which runs through Uphill between Uphill and Bleadon; Woodspring near Weston-Super-Mare; on the bank of the river running beside Kingston-Seymour; on the salt-marsh near Clevedon, and north-east of the Portishead Docks towards Portbury. The two localities (Map - areas 21 and 22) from which collections were made are, the one between Uphill and Bleadon and the Portishead Docks.

A. maritima predominated in these two localities and there was insufficient A. gallica to be of any use for the present purpose. One batch of the former was accordingly taken from each of the two localities. In both batches the plants were at the very early bud stage.

The air-dry leaves and flowerheads of the batch from the Uphill-Bleadon locality yielded 0.60 per cent. of santonin, and using the same parts of the plant from Portishead 0.54 per cent. of santonin was obtained.

W. Gloucester.

The species occur only very sparingly on two localities that were visited in West Gloucester. On the Severn sea-wall at Shepperdine and on the Severn near Berkeley. The older Floras give also the "saltings at Avonmouth on the Gloucestershire side of the river Avon below Bristol", but this area is now completely changed by the erection of docks etc., and the species are no longer to be found there.

Monmouth.

A. gallica was found in only one locality, Marshfield near the Lighthouse (Map - area 23), in Monmouth, and a collection was made from there. Only occasional small patches of A. maritima were to be seen. The plant was further back than most of the others, having only a short shooting stem and very few flowerheads on some plants. The percentage of santonin, 0.35, obtained from the air-dry leaves was low.

Glamorgan.

A. maritima, according to the floras, is common all along the coast of Glamorgan, west of Cardiff, and at the mouths of the tidal rivers. A. gallica is recorded for only one area, on the banks of the river Thaw at Aberthaw (Map - area 25), but Miss Vachell, joint editor of the most recent publication on the flora of Glamorgan, suggested that this species might also be found at the Leys, Gilestone (Map - area 26). It was found in both of these localities and also on the edge of the salt-marsh between Llanmadoc and Whitford Burrows (Map - area 27), North Gower Peninsula. A collection was made from each. One additional batch from Aberthaw was collected about a fortnight later. A clump of this species, growing on the sea wall at the Leys, Gilestone, is shown on the photograph, Fig. XIV.

A. maritima was collected from each of these areas also. It was seen to occur on a number of additional localities and a small collection was made from the bank of the river Ely, at Landough Junction (Map - area 24) about three miles west of Cardiff. Two additional lots were received later at different times from Aberthaw. Figs. XXV. and XXVI. are photographs of the areas at Aberthaw and Llanmadoc respectively from which collections were made.

When the first collection was made from each locality the plants were well advanced and both species were at approximately the same stage of development with regard to

flowerheads. Numerous small flowerheads were present making the differentiation between the two quite distinct, and it was thus assured that the different batches consisted entirely of one type of plant.

In table XXVI. the results of the examination of the various batches of plants collected in this vice county are shown.

TABLE XXVI. PERCENTAGE OF SANTONIN IN AIR-DRY A. GALLICA AND A. MARITIMA, FROM GLAMORGAN.

LOCALITY	SPECIES	SANTONIN IN LEAVES AND FLOWERHEADS	STAGE OF DEVELOPMENT
Gilestone	A. gallica	0.545	Flowerbuds about half developed
"	A. maritima	0.625	Flowerbuds about half developed
Llanmadoc	A. gallica	0.46	Flowerbuds about half developed
"	A. maritima	0.535	Flowerbuds about half developed
River Ely	A. maritima	0.72	Early flowerbud stage
Aberthaw	A. gallica	0.53	Flowerbuds less than half developed
"	A. gallica	0.57	Flowerbuds more than half developed
"	A. maritima	0.63	Flowerbuds less than half developed
"	A. maritima	0.69	Flowerbuds more than half developed
"	A. maritima	0.84	Flowerbuds almost fully developed

It will be seen that the difference in santonin content between the two types was again clearly evident.

Carmarthen

Two localities were visited in this vice county, the estuary of the river Gwendraeth about half a mile south of

Kidwelly (Map - area 28), and the banks of the river Towy near Ferryside. There was no Artemisia on the latter, but both species grow in large quantities on the former locality, of which Fig. XXVII is a photograph, showing the plants. The species were at about the same stage of growth as those collected in Glamorgan, and a collection of each was made. From the air-dry leaves and flowerheads of A. gallica., 0.41 per cent. of santonin was obtained, and the air-dry leaves and flowerheads of A. maritima contained 0.47 per cent.

Pembroke and Cardigan.

The older "Floras" include A. maritima for Cardigan, but according to Dr. J.H. Salter "it certainly does not occur in any of these localities at the present day". He further states that the only specimens he has seen in Cardigan are a few plants growing close to some cottages at Aberayron as if they had been planted, and that "it must certainly be regarded as rare on the West Cardigan Coast".

On enquiry among the older inhabitants of Aberayron it was learned that the plant has not been known to grow wild there during the past four generations at least, and that it has been cultivated continuously during that time. It is known locally as "Pembroke Wormwood" and Pembroke would appear to be the place from which the plant was originally brought, being brought over by the sailors when shipping stone from the Pembrokeshire stone quarries. Information was obtained from

a retired sea-captain who remembered having taken home supplies of the plant while engaged on shipping stones over 70 years ago. This he said had been done even in his grandfather's time as the supply of cultivated plant, obtained by planting the roots brought home by sailors, was not sufficient for the demand, such was its reputation as a medicine. It would appear therefore that this plant should not be regarded as indigenous to Cardigan. Plants seen by the author growing in gardens in Aberayron were typical A. gallica. This species lends itself to cultivation on a normal soil, but A. maritima does not.

Pembroke was not visited but from information received later A. gallica and probably A. maritima would appear to grow abundantly in this vice county on the banks of the river running through Johnstone, near the Pembrokeshire stone quarries.

The large salt marsh near Llannon in Cardigan was also visited but no Artemisia was found. In view of Dr. Salter's remarks, the localities mentioned in the old Floras were not visited.

Merioneth

Localities visited in Merioneth were Dovey (Dyfi) Junction near Glendovey (Glan Dyfi); the salt marsh north of Llanbedr, and the large salt marsh on either side of the Penrhyndeudraeth - Portmadoc Embankment. No Artemisia was found in any of these localities.

Dr. Lloyd Williams stated that A. maritima had been known at one time near Camrhyd on the river Conway, but that it

had not been recorded recently. No plant was found on visiting this locality, nor was there any in the other localities mentioned in the local floras.

It cannot be stated that wild A. maritima or A. gallica require any special type of soil, but it has been remarked that, in all the localities in which they were found, the soil was muddy and not sandy. No area, with the possible exception of Dovey Junction, was seen on the west coast of Wales which appeared a typical locality on which one would expect to find the halophytic Artemisias.

Angelsey.

The older floras give two localities in Angelsey on which A. maritima is to be found. The species has not been recorded recently for these localities, however, and no information about it could be obtained. The two localities, (a) near Llangwyfan Old Church and (b) Trecastell, Aberfraw, were accordingly visited, and so was the salt marsh at Malldraeth, near Newborough, but no Artemisia was seen.

Denbigh and Flint.

In these two vice counties, the estuaries of the Glaslyn and Dwyryd rivers and along the river Clwyd near Rhyl, were visited. The last area seemed to be a likely place and a careful search was made, but no Artemisia was found there, nor in the other two localities, which are sandy.

Cheshire.

The species are said to be common on the muddy inlets of the Mersey, but no more definite information could be obtained and they were seen in only one locality, near Froosham. The specimens were in a backward stage of development, and there was insufficient for a collection.

The plants were not to be seen on such inlets of the River Dee, as were visited. This was the most northernly vice county on the west coast of England in which the species were found as they were not to be seen in Lancashire or Cumberland.

SCOTTISH PLANTS

A. maritima and A. gallica are apparently now known in only four of the ten vice counties given by Druce. In only one of these, Haddingtonshire, is there sufficient plants to afford material for a systematic examination. Such information as is available regarding the plants in these four vice counties is as follows -

Kincardineshire.

A. maritima is not at all common there now, but Professor Craib reports having seen it a few years ago just south of Dunottar Castle, near Stonehaven, and just north of Stonehaven. This locality has not yet been visited by the author.

Fifeshire.

The older floras give quite a number of localities.

There is a specimen of A. maritima in the Royal Botanic Gardens, Edinburgh, collected in 1836 from near Crail and this, and the Kincraig Cliffs west of Elie, seem to be the only localities in Fife on which the plant now occurs. The growth is, however, very sparse and no collection was made from either of these localities.

Haddingtonshire.

Both species appear to have been very common round the coast of Haddingtonshire. Specimens from Tyninghame; near Dunbar; Tynemouth; Luffness and Gullane Links collected prior to 1850 are to be seen in the Herbarium of the Royal Botanic Gardens, Edinburgh.

The plants are now known to occur near North Berwick and at Tynefield (Map - area 1). In the former area growth is not abundant, but in the latter there is a large stretch of tidal water, all round the margin of which the two plants grow profusely. Fig. XIX. is a general view of the area showing the species. Periodic collection was made from this area during three years, and full reference to the results of examination of the plants is made in Chapter X. and elsewhere.

Wigtownshire.

The plants are rare in Wigtownshire and the only locality on which they now appear to grow is on the salt marsh between Cairnhead and Portyerroch, not far from the Isle of Whithorn. Even here, however, they are not plentiful and no

collection of the plants could be made when the locality was visited during the summer of 1932. Both species of the plant are to be seen.

Other Vice Counties.

It is possible that A. maritima and A. gallica still exist in the other counties mentioned by Druce, but up-to-date information regarding the localities in which they occur is not available. It seems very probable, however, from the statements of present-day field botanists, that the species no longer exist in some of these vice counties.

BELGIAN PLANTS

Three batches of A. gallica were received by the author from Mr. R. Van de Vyvere of Brugge. Two of them were collected on the estuary of the river Zwin in Belgium, and the third was from the banks of the river Yser. They were all well advanced and bore well developed but unexpanded flowerheads.

Reports regarding the occurrence of santonin in continental A. gallica vary. Hëckel & Schlagdenhauffen (loc.cit.) reported having found santonin in A. gallica growing in France in 1885, but more recently Mouton (1931) (loc.cit.) reported that specimens examined by him did not contain santonin and similar reports have been made by other workers. Viehover and Capen (loc.cit.) did not find any santonin in the A. gallica growing in America, which they examined.

An examination by the author of the three batches of A. gallica from Belgium did not reveal the presence of santonin in

this plant.

SUMMARY OF CHAPTER XI.

It would appear from published work that environmental conditions affect the presence of santonin in the plant, and the fact that it is to be found in A. gallica and A. maritima from one locality, is no criterion that the same plants growing in other localities will also contain santonin. In order to find out how far this is true for the British plants, it was decided to examine batches collected from as many localities as possible in Britain. Up-to-date information regarding the occurrence of A. gallica and A. maritima in Scotland, England and Wales was accordingly sought and the great majority of the localities in which they are now known to occur, were visited. In many of the localities growth was too sparse to permit of the plant being collected, but separate batches of both were collected from a large number. Examination of these batches revealed the presence of santonin in every instance. The percentage was small in a number of them and none of them showed as high a content as the Scottish plant to which particular attention has been paid, but santonin was nevertheless present. It would further appear that A. maritima produces a slightly higher percentage of santonin than does A. gallica at the same stage of growth and growing in the same locality.

No attempt was made to differentiate between the soils in the various localities as the presence or absence of santonin had first to be determined. There is no evidence that the soil, alone, or in conjunction with other factors, affects the production of santonin, but it has been suggested that the salinity of the soil, may, and it is probable that it does have, a more or less marked effect.

A number of successive batches of the plants were obtained from a few of the localities. These were examined, and the curves obtained by plotting a graph of the percentage of santonin present against time, show a marked similarity in character to the curves obtained for the Scottish plant.

Evidence is not complete but it would appear from the number of localities which are given by older floras, but in which the plants are not now to be found, that they are gradually dying out in this country, particularly in the north. How far this is due to natural factors and how much to modern industrial development, has not been ascertained.

CHAPTER XII.

A. GALLICA UNDER CULTURAL CONDITIONS

A series of experiments was started in 1930 with a view to determining the influence of an increased supply of certain plant nutrients on the santonin content of the plants. For this purpose a number of wild plants were transplanted into separate pots. At the end of the season a single healthy plant was chosen. In order to avoid any variation in santonin content due to genetic influence, this single plant was broken up and each clone planted separately. This was repeated at the end of 1931 and sufficient genetically identical plants were then obtained from the experiment.

The eighteen clones so obtained were potted up and thoroughly established in 6" pots containing a medium loam potting soil. The nutrients selected for treating the plants were the chlorides of sodium, potassium and calcium, the nitrates of sodium, potassium and calcium, and di-potassium hydrogen phosphate. Two lots of each salt, equal to a manurial dressing of 10 cwts. per acre to the top 7" of the soil, were weighed out. One lot of nutrient was added to each pot in early April just when the plants were breaking into growth. Thus there were two plants treated with each salt and 4 pots not supplied with nutrients - these to act as "controls". It was anticipated that these plant nutrients would affect the

metabolism of the plant and through it the santonin content. As will be suggested later, santonin has a definite metabolic function, and if this be accepted there is every reason to expect that its synthesis will have a direct metabolic basis. This underlay the decision to include in the series a soluble phosphate and salts of potassium, and calcium.

It has been shown by Murneek (56), Krauss and Kraybill (57), and other workers, that nitrate nitrogen affects time and amount of flowering, and it has been shown in the present work that there is a relationship between development and santonin content; hence nitrates were included.

The ecological distribution of A. maritima and A. gallica follows the sea shore at a few inches about sea-level and situated so that the soil is kept moist by the tides, hence it was decided to include sodium salts and chlorides.

The treated plants were grown in a cool glass-house in order to prevent damage by wind etcetera, as it had been found during the period when the clones were being built up that potted plants are easily broken and stems blown off.

None of the plants so treated (control or manured) developed past the rosette stage, no attempt to produce elongated stems being made. In view of the behaviour of the controls this result can only be ascribed to the growing of the plants under glass and indicates a peculiar sensitivity of Artemisia to light - the only factor varied from the pre-manuring work when they were found to grow quite normally. Van de Vyvere of

Brugge recently reported a similar sensitivity in A. cina, Berg.

At the time of writing it is impossible to offer results of this manuring programme, but it is hoped to grow the plants in 1933 under more natural environmental conditions, and to obtain plants on which comparative assays of their santonin content can be made.

CHAPTER XIII.

PHYSIOLOGICAL SIGNIFICANCE OF THE OCCURRENCE OF SANTONIN

For many centuries it has been known that Artemisia has the strongest and most efficacious medicinal properties, if collected just before the flowerheads begin to expand. Since its discovery by Kahler and Alms, santonin has been regarded as the active principle. It is a well established fact, however, that santonin does not, by itself, produce the same results, nor is it in certain cases as beneficial, as galenical preparations of the entire plant. It does not, therefore, necessarily follow that the plant yielding the best galenical also contains the highest proportion of santonin.

Commercial santonica consists of the unexpanded flowerheads of the plant, and it is from this that santonin is said to be extracted. Greenish and Pearson (loc.cit.) in 1921 showed, however, that the leaves from plants of A. brevifolia, on which there were only a few undeveloped flowerheads, contained quite an appreciable proportion of santonin, and Goodson (loc.cit.) has asserted that the leaves alone of Artemisia contain santonin. In the present work it is shown that santonin reaches a much higher percentage in the leaves of A. gallica just as the flowerheads are commencing to be formed, and immediately after this the santonin content rapidly decreases. The content of the flowerheads alone traced right through their development does not reach the maximum content found in the leaves.

The flowerheads of the plant from which santonin is extracted commercially may contain the maximum percentage but no

practical evidence of this seems to exist, and it may be that the santonin content of this plant shows similar variation to that of other plants.

It is a well known fact that the synthesis of certain substances, notably alkaloids, such as in Atropa Belladonna, takes place in the leaves, but as the plant becomes older a much higher proportion of these principles is to be found in the younger parts. The function of these substances in the plant metabolism is unknown but they appear to be translocated in the same manner as plastic metabolites in general, from one part of the plant to another, according to the circumstances or conditions under which such transference may prove useful to the plant. For example, it is very general for alkaloids to be translocated to the flowerbuds, prior to gamete formation.

The rôle of santonin also is unknown but it would appear from Goodson's and Greenish's work, and the curves obtained in this work, that the locus of synthesis is in the leaves. From a study of the curves given in this work it is further evident that the santonin is translocated to the developing flowerheads, just as the plastic metabolites are. This, together with the general form of the curves, indicates that the locus of consumption is in the flowerheads.

The curves obtained in this research, pertaining to santonin show close similarity to the curves obtained by Briggs, Kidd and West (58), from Kreusler's (59) data, pertaining to dry matter in the plant. These considerations have led the author

to formulate an hypothesis for the function of santonin in Artemisia.

Dry Matter Curves.

Brigg's, Kidd and West's findings may be summarised as follows :-

The curve of total dry matter over the grand period of growth is a typical "S" curve. The dry matter falls just after germination, rises to a maximum and then commences to fall. As will be seen from Fig. XII, which is copied from Briggs, Kidd and West, and represents their general curve, there are in the secondary, or falling part of the curve, two secondary maxima.

The fall from the primary maximum is attributed by these authors to a diminution in leaf surface whereby the total of the dry matter content is decreased owing to the metabolites being used up quicker than they can be replaced, because of the diminished leaf surface. Santonin is not regarded as a normal plant nutrient, and it will be realised that a decrease in weight of normal plant metabolites due to this cause, would only serve to increase the relative proportion of santonin present. For the present purpose therefore the primary maximum and immediately succeeding falling portion on the curve of Briggs, Kidd and West may be disregarded.

The two secondary maxima of the general dry matter curve coincide with the general curve for santonin. Briggs, Kidd and West ascribe the trough and final fall of the secondary part of their curve to the destruction of dry matter occasioned

by the burst of respiratory activity coincident with gamete production. The first trough coincides with the production of the male gametes, the secondary maximum which follows, with the interval of rest which occurs before the production of the female gametes. The onset of female gamete production produces the later fall in content.

Correlation of Santonin.

The seasonal variation in santonin content may be due to the same influences. The curve of santonin content rises to a maximum just as the flowerheads are being formed and reaches a secondary maximum just before the flowerheads expand. With the expansion of the flowerheads the santonin disappears completely. This variation may be explained by the increased respiratory intensity accompanying the sexual processes, including gamete formation, flower opening, and fertilisation. Bonnier and Mangin (60) have demonstrated an increase in the intensity of respiration coincident with the opening of the flowerheads, and White (61) observed that pollination produces a rapid increase in respiratory activity and showed that the CO_2/O_2 ratio of pollinated carpels of a number of plants is considerably greater than that of the unpollinated gynaecia.

An hypothesis is, therefore, put forward that santonin is formed in the plant as a preparation towards gamete production, and is used as a respiratory substrate coincident with the high respiratory activity, associated with gamete

formation and the other sexual processes of the plant. This hypothesis explains the variation in santonin content and the peculiarly striking coincidence between the author's curves and those of Briggs, Kidd and West.

It was hoped that plants grown under cultural conditions might yield direct confirmation of such an hypothesis, but these plants failed to develop and no results from them are, therefore, available.

SUMMARY OF CHAPTER XIII.

An hypothesis based on the marked similarity between the curves obtained in this work pertaining to santonin content, and those obtained by Briggs, Kidd and West on work pertaining to total dry matter, is suggested in this chapter, to explain the variation in santonin content with relation to the stage of development of the plant. It is suggested that santonin is formed by the plant as a respiratory reserve to be used at times of high respiratory ~~activity~~ intensity associated with the sexual processes in the plant.

C H A P T E R XIV.

CONCLUSIONS.

1). The two species, A. maritima and A. gallica, on which the work was done are morphologically distinct plants often found growing together.. They are xerophytic, growing naturally under the influence of salt-water and therefore usually near the sea. They show a marked sensitivity to the level at which they grow, and are so placed in nature that their roots are continually being moistened by or immersed in salt-water.

2). They show a preference for muddy or alluvial soil, and do not grow well on dry soil. They have not been seen on purely sandy soil and it is concluded that soil of this nature, which drains and dries quickly, is not suitable for their growth. It is probable that habitat, particularly the salinity of it, influences the amount of santonin produced by the plant, but all of the plants collected from a large number of localities in Britain contained at least a small proportion of santonin.

3). The range of variation in certain sub-specific morphological characters is such that, except at the extremes, the two species are not distinguishable by these characters. Neither are they distinguished from each other by the anatomical characters shown in transverse sections of the root, rootstock, stem and leaf.

4). Most of the present methods of assay are unsuitable for

the examination of a drug composed chiefly of dried green leaves, alone, or admixed with flowerheads, and containing small proportions of santonin. Other possible methods of assay, such as by preparation of derivatives and colorimetric reactions, which have been examined are unsuitable for the quantitative estimation of santonin in drugs.

5). A new gravimetric process has been devised for the assay of santonica. It has been found to give accurate results and to be suitable for the assay of drugs of low santonin content. From a comparative study with other processes of assay and different types of drugs, it is concluded that this new process is applicable for the quantitative examination of all types of crude drugs containing santonin.

6). A study of the two species collected systematically from a locality on the east coast of Scotland, over a period of three years, has shown that the santonin content attained a maximum of about 2 per cent. one year, and that in the other years the plants had a maximum of about 1.5 per cent. This study has allowed of the variation in santonin content in relation to stage of growth, being observed, and it has been noted that the maximum content occurs just as the flowerheads are beginning to appear. Using the figures from the assay of leaves and flowerheads, the graphs of the variation during the three seasons plotted against time show a fall from the maximum followed by a rise and the fall from this secondary maximum coincides in time with the

expansion of the flowerheads. The graph, using the figures from the assay of the flowerheads alone, shows a rise and simple fall, and plotted beside the previous graph it is obvious that santonin is transferred from leaf to flowerhead just as plastic metabolites are. It is also obvious that the locus of formation of santonin is in the leaf and the locus of consumption in the flowerhead. The ascending portion of the flowerhead content curve cuts the primary descending portion of the other curve, and descends to meet this other curve, near the point where the latter commences its final descent.

7). Similar variation occurs in the santonin content of plants collected from a number of localities in England, and also in the cultivated Scottish plant.

8). The similarity in nature of the curves obtained, to that of certain curves, chiefly Brigg's, Kidd & West's, relating to Total Dry Matter Content, has provided the basis for advancing an hypothesis that santonin is elaborated as a reserve material for use during the period of increased respiratory activity associated with sexual processes in the plant. While the evidence is not direct, it is believed that considerable support is given to the hypothesis by the marked similarity of the curves and other evidence such as the drift of santonin into the flowerheads as soon as they appear and the rapid disappearance of the santonin with advance in sexual development.

9). Further evidence, in support of this hypothesis has not been obtained owing to the fact that cultivated plants ceased development at the rosette stage.

APPENDIX.

REFERENCES.

FIGURES & MAPS.

APPENDIX,

REFERENCES,

FIGURES & MAP.

APPENDIX I.

METHODS FOR THE ASSAY OF SANTONIN

Observations regarding the processes used for the assay of santonin have already been made in Chapter VIII. In these processes the general principles for the separation of acids or anhydrides is employed, and most of them are based on Katz' (42) method. In the following pages a summary is given of the various processes which were used by the author in an attempt to find one suitable for the assay of low santonin content drugs composed chiefly of dried leaves of Artemisia. Katz' process has been taken as typical and the descriptions of the other processes are confined to pointing out the essential differences between them and Katz' process.

Katz' Process.

In it an ethereal extract of the drug is prepared by continuous extraction and the residue left on evaporation of the ether is extracted by heating with barium hydroxide solution. This produces a solution of the barium salt of the santonin leaving some of the inert matter as an insoluble residue, which is filtered off along with the excess barium, precipitated as carbonate. By acidifying the filtrate with hydrochloric acid the santonin is liberated and is extracted with chloroform by shaking in a separating funnel. The residue left on evaporating the chloroform contains all the

santonin and is extracted by heating with 15 per cent. alcohol which dissolves the santonin and some of the resinous matter. The solution is filtered hot and on cooling and standing the santonin crystallises. Most of the resinous matter remains in solution, although the crystalline residue, obtained on filtering, may be contaminated with it, especially when the drug under examination has a low santonin content.

In the other processes which employ the principles suggested by Katz, the following modifications are made :-

(a) POLARIMETRIC

In Favrel's (43) polarimetric method an additional purification is inserted which consists in shaking the chloroformic solution with 15 per cent. sodium carbonate solution. After separation and evaporation of the chloroformic layer the residue is dissolved in alcohol and the optical rotation is observed.

(b) VOLUMETRIC. Kariyone and Kimura's Method.*

The volumetric process of Kariyone and Kimura (46) is carried out by neutralising in the cold, the alcoholic

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* The abstract of this process in the Yearbook of Pharmacy, 1921, p.128, is misleading, and gives a wrong impression of the process. In the experiment leading to the acid reading, S', there is no drug present; it is in the nature of a blank. If the method described in this abstract is followed the reading S must be greater than S' and the formula cited for the calculation becomes inoperable, a negative result being obtained.

solution of the residue from the chloroformic solution, phenolphthalein being used as indicator. Excess standard potassium hydroxide is then added. After boiling under a reflux for half an hour the excess potassium hydroxide is titrated with hydrochloric acid until again neutral to phenolphthalein. Another portion of equal volume of standard solution of potassium hydroxide is similarly boiled and back-titrated. By difference in the amount of standard acid used in the two experiments the weight of santonin present is calculated.

Katz' Method.

Katz' (48) volumetric method is similar, except that in it there is an additional purification. The residue from the chloroformic solution is taken up with 15 per cent. alcohol as described for his gravimetric assay. After evaporation of this solvent the residue is taken up with absolute alcohol and neutralised in the cold with potassium hydroxide. Excess standard solution of potassium hydroxide is then added and the santonin is saponified by boiling. After dilution and on back-titration with standard hydrochloric acid, the amount of alkali found to have been used in the saponification is an indication of the amount of santonin present.

(c) GRAVIMETRIC. General.

Of the numerous gravimetric processes the best and

most usually employed are, in addition to the original method of Katz, those of Goerlich (62), Fromme (50), and Van den Berg (63), and the method of Eder and Schneiter (37), official in the 1926 German Pharmacopoeia. All of these are modifications of Katz' method. Mouton (51) recently suggested a process based on that of Eder and Schneiter. The processes of Schaap (64) and Palkin (49) are quite distinct from any of the preceding ones, but both are long and somewhat complicated.

Massagetow (52) has recently described a new technique which has also been critically examined by the present author.

Goerlich's Process.

Goerlich's modification consists of precipitating the resins with lead acetate from a weak alcoholic solution of the residue from the ethereal extract. The hot solution is filtered and the filtrate corresponds to the acidified solution of Katz.

Fromme's Process.

The method of Fromme differs from that of Katz in that the preliminary extraction is done in the cold with chloroform and that the excess barium is not precipitated but excess hydrochloric acid is added to the warm solution after filtering. This acid solution is extracted with chloroform and the residue from the chloroformic solution is

dissolved in absolute alcohol, poured into sufficient hot water to give a final strength of alcohol of 15 per cent. and filtered while hot. From this solution the santonin crystallises. This process was adopted at first by the present author and was found to give the most consistent results; later it was replaced by the new process described in Chapter VIII.

Van den Berg's Process.

Van den Berg's process makes use of chloroform as an immiscible solvent. The powdered drug is shaken with water acidified with hydrochloric acid; chloroform, and gum tragacanth as an absorbing agent of the resins, are added and the whole again shaken. The separated chloroformic solution corresponds to the chloroformic solution of Katz.

Eder and Schneiter's Process.

Benzene, which dissolves santonin, dissolves less of the interfering inert matter than do ether and chloroform. Eder and Schneiter make use of it in their preliminary extraction. The residue obtained on evaporating the benzene is taken up by boiling with 15 per cent. alcohol, and this solution, after filtration, is purified by boiling with kaolin and again filtering while hot. The santonin crystallises from the solution on cooling and standing.

Mouton's Process.

Mouton's method is simpler than any of the preceding

processes and is carried out by first treating the dry drug with ammonium hydroxide solution after which it is again dried. By this means some additional constituents of the drug are rendered insoluble in the benzene then used to extract it. The ammonia has no action on the santonin. The residue from the benzene solution is extracted by boiling under a reflux condenser with 15 per cent. alcohol, and on cooling and standing, the santonin crystallises from the solution, filtered while hot.

Schaap's Process.

This process is carried out by extracting the santonin direct from the drug as the calcium salt by treatment with calcium hydroxide and water. To this is added zinc sulphate to precipitate the resins and the solution is filtered. After liberation of the santonin by the addition of acetic acid, the solution is evaporated to dryness, water and calcium carbonate are added, and the solution is again evaporated to dryness. Chloroform is used to extract the residue, and the residue obtained on evaporating the chloroform is taken up with dilute methyl alcohol, from which the santonin crystallises as in the other methods.

Palkin's Process.

The extraction of the drug in Palkin's process is by acetone. After concentration, the santonin is converted

to the salt by the addition of alkali, and the resins are precipitated by calcium chloride. The solution is filtered, the santonin liberated by the addition of acid, extracted by chloroform, and the acid resins removed by washing with alkali of a suitable strength. The chloroform is evaporated, the residue is further purified by converting the santonin to the calcium salt and extracting with acetone, which dissolves the resinous matter but leaves the calcium santonate as a deposit. The residue obtained on filtering is treated with acid to liberate the santonin which is then dissolved in chloroform. On evaporation, the weight of residue represents the amount of santonin present.

Massagetow's Process.

Santonin is extracted as the calcium salt by triturating the drug with slaked lime and is then taken into solution by boiling with water. It is liberated by the addition of hydrochloric acid and the free santonin is extracted from the aqueous portion by shaking with chloroform. The chloroformic solution after filtration is shaken with dilute solution of sodium hydroxide of such a strength that, practically,* no santonin is extracted, but some of the acid resinous matter goes into solution in the alkali. After

* It has been shown that santonin is converted to the respective salts by 15 per cent. sodium carbonate solution, and by cold saturated solutions of barium and calcium hydroxides; i.e. 5.6 per cent. $Ba(OH)_2.8H_2O$ and 0.2 per cent. $Ca(OH)_2$. It would appear, therefore, that, theoretically at least, santonin sufficient to set up equilibrium, would be converted to the salt by the 4 per cent. sodium hydroxide used.

separation the chloroformic solution is shaken with animal charcoal, previously washed with chloroform, filtered and evaporated. The residue is taken up with alcohol as in the other process, but the alcoholic strength of the final solution is not so great. After standing, the solution is filtered, the filter is dried and the residue is dissolved in chloroform and transferred to a tared flask. The solution is evaporated and dried and the weight of residue (santonin) is obtained. A correction for the solubility of santonin in the weak alcoholic filtrate is applied.

Preparation of Derivatives.

From time to time details of processes have appeared for the estimation of santonin by the preparation of deratives, such as are commonly employed for the separation and estimation of ketones. Of these derivatives the most common are oximes, semicarbazones and phenylhydrazones. Such processes as have been suggested have normally been abandoned after testing their accuracy. They have not been found to be successful for the estimation of santonin in the crude drug, however successful they may be for the estimation of this principle in tablets, and similar preparations. A number of attempts based on this principle were made in the course of this investigation but satisfactory results were not obtained in any of them.

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FIG. V. Graph of Seasonal Variation of Santonin Content (1930)

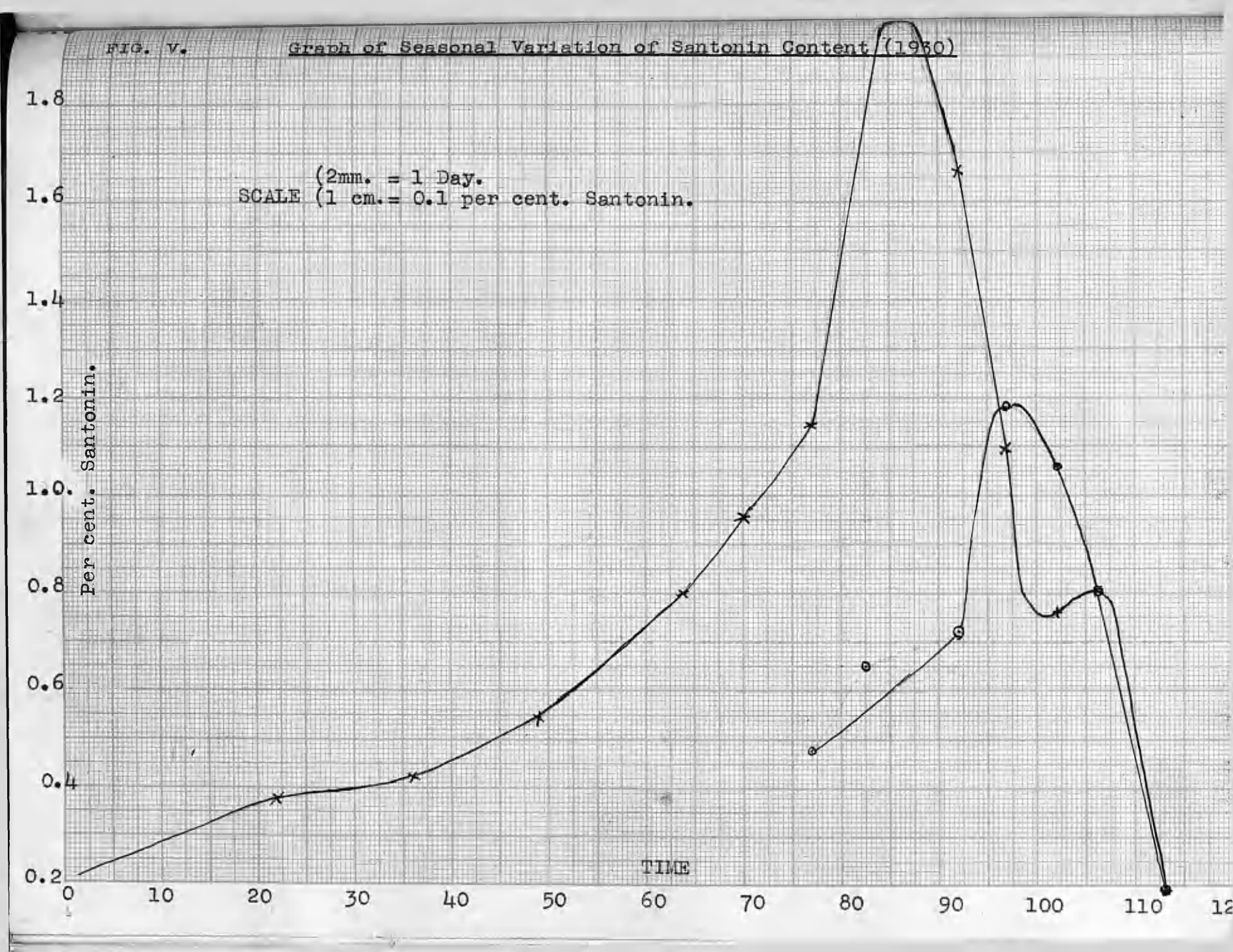


FIG. VI. GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT (1930)

(2 mm. = 1 Day

SCALE (1 cm. = 0.1 per cent. Santonin.

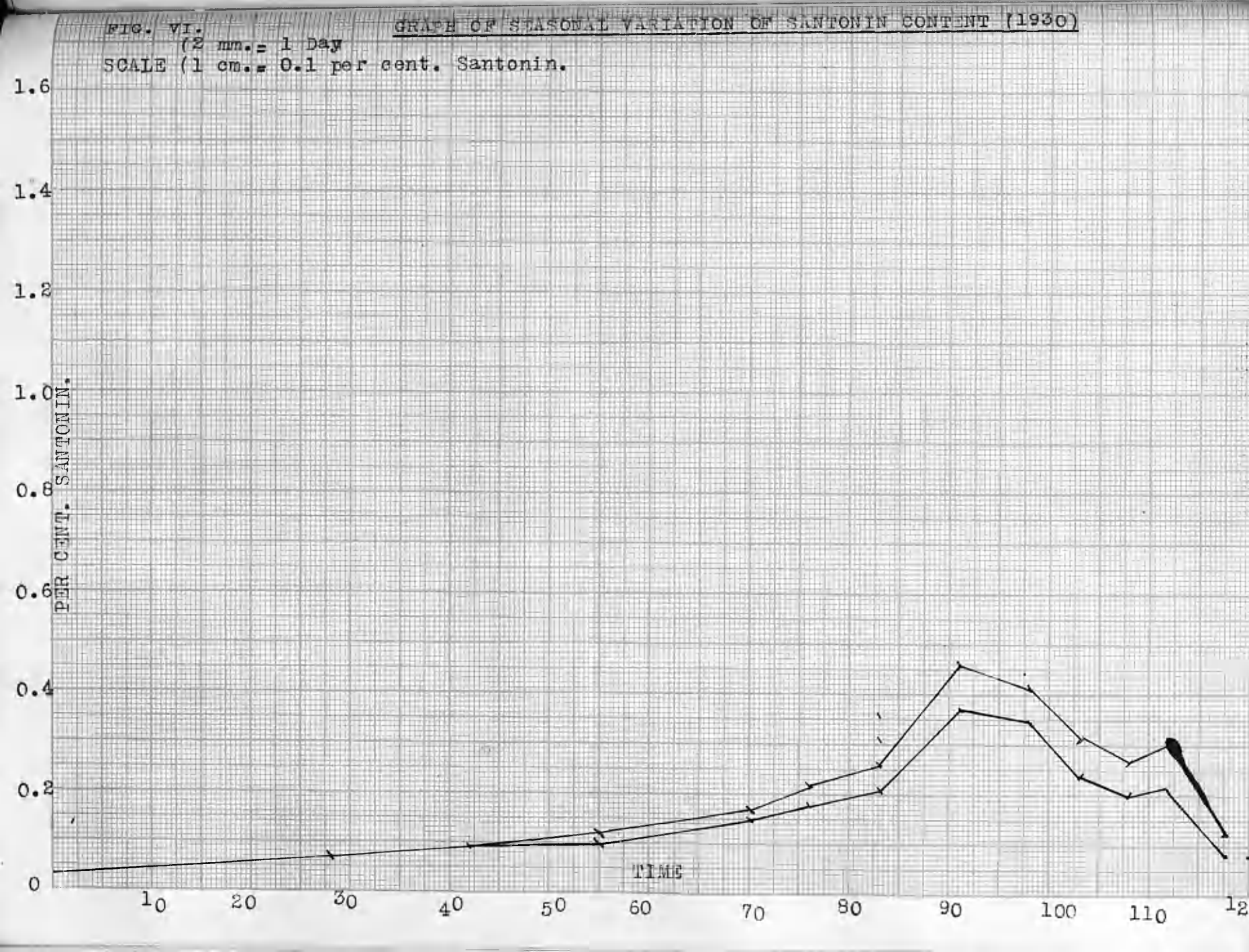


FIG. VII. GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT (1930).

SCALE. (2 mm.= 1 Day.
(1 cm.= 0.1 per cent.Santonin, (DRIED HERB)
(3 cm. = 0·1 " " " (FRESH HERB)

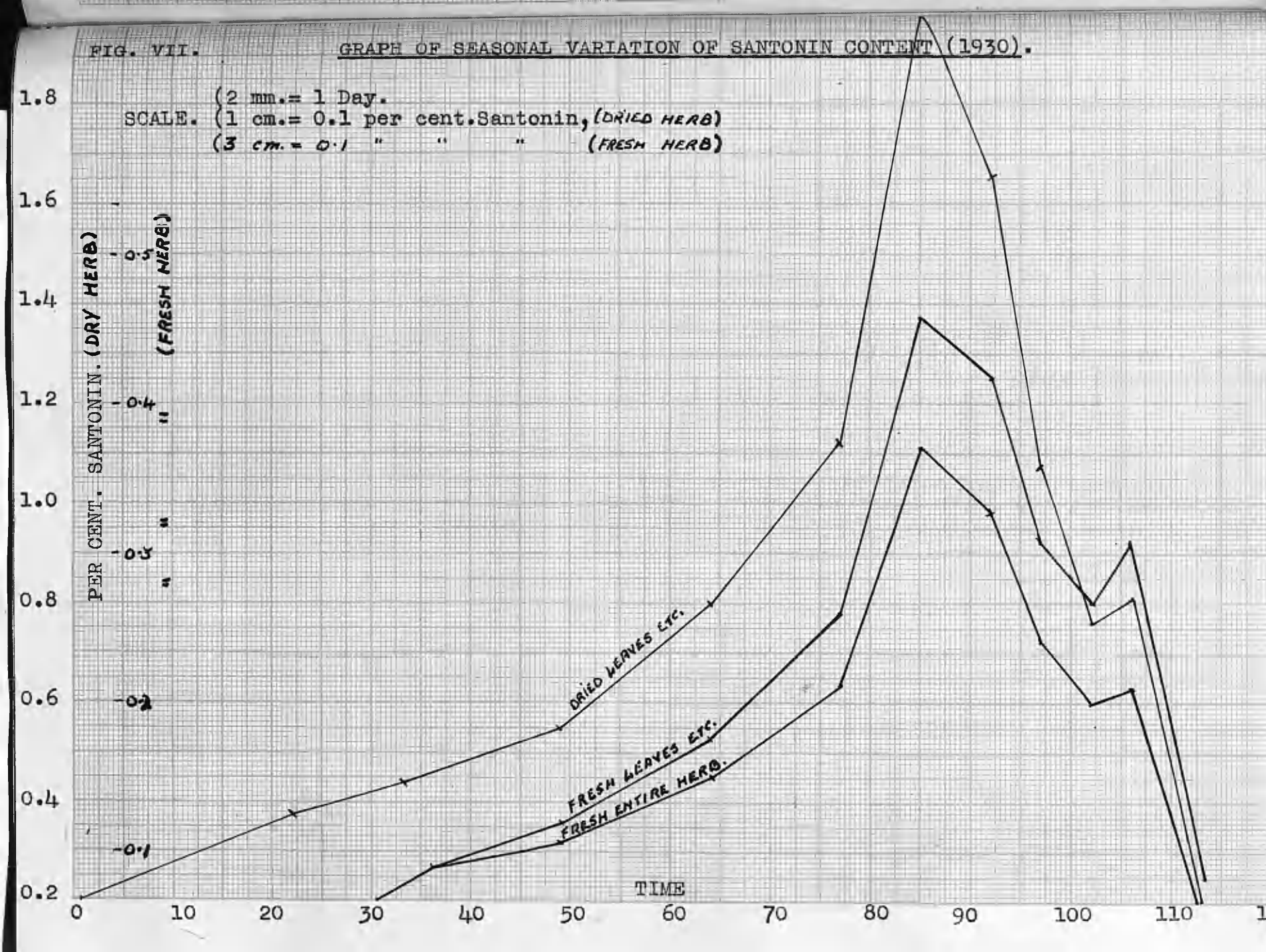


FIG. VIII

GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT (1932)

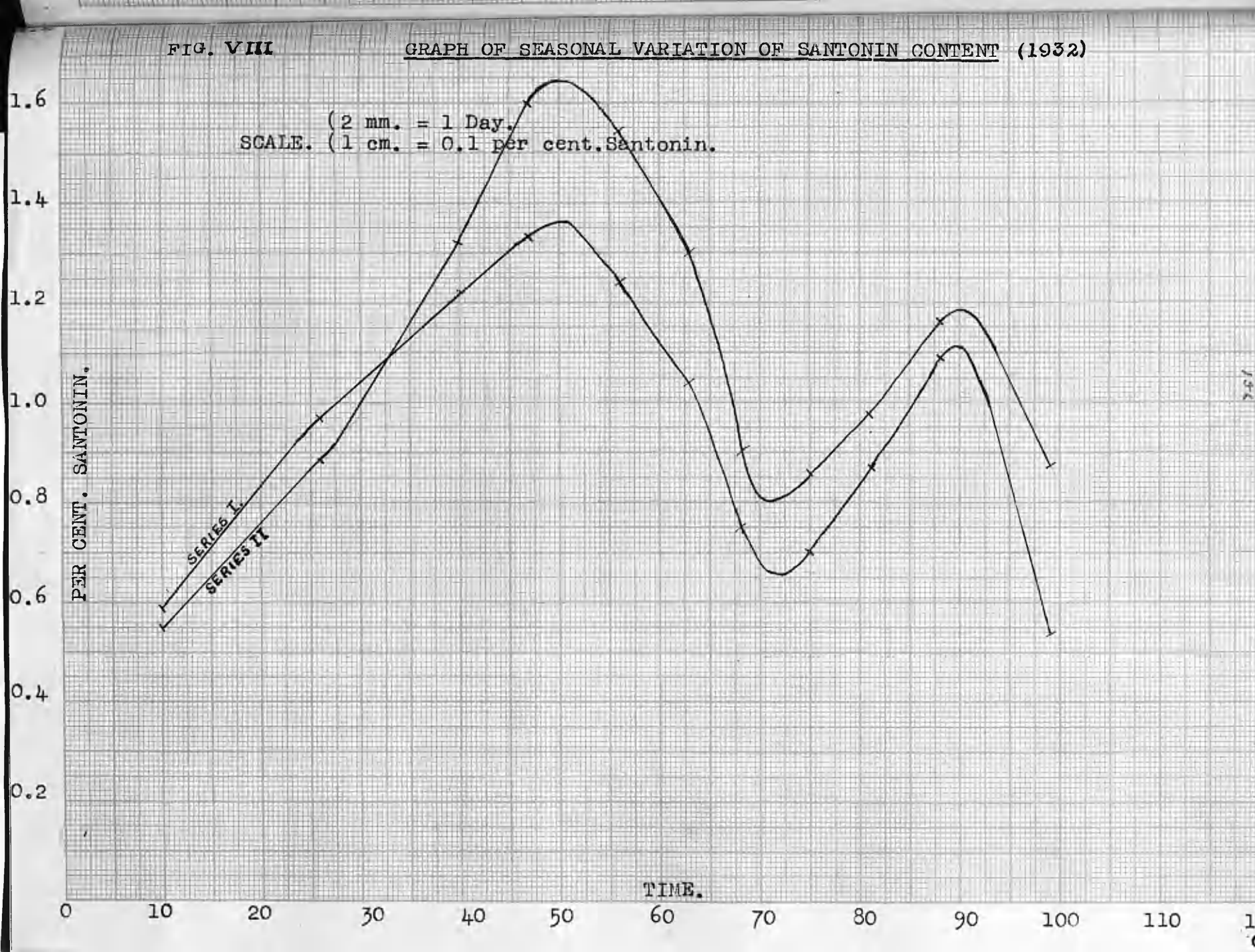


FIG. IX. GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT (1932)

SCALE (2 mm. = 1 Day.
(1 cm. = 0.1 per cent. Santonin.

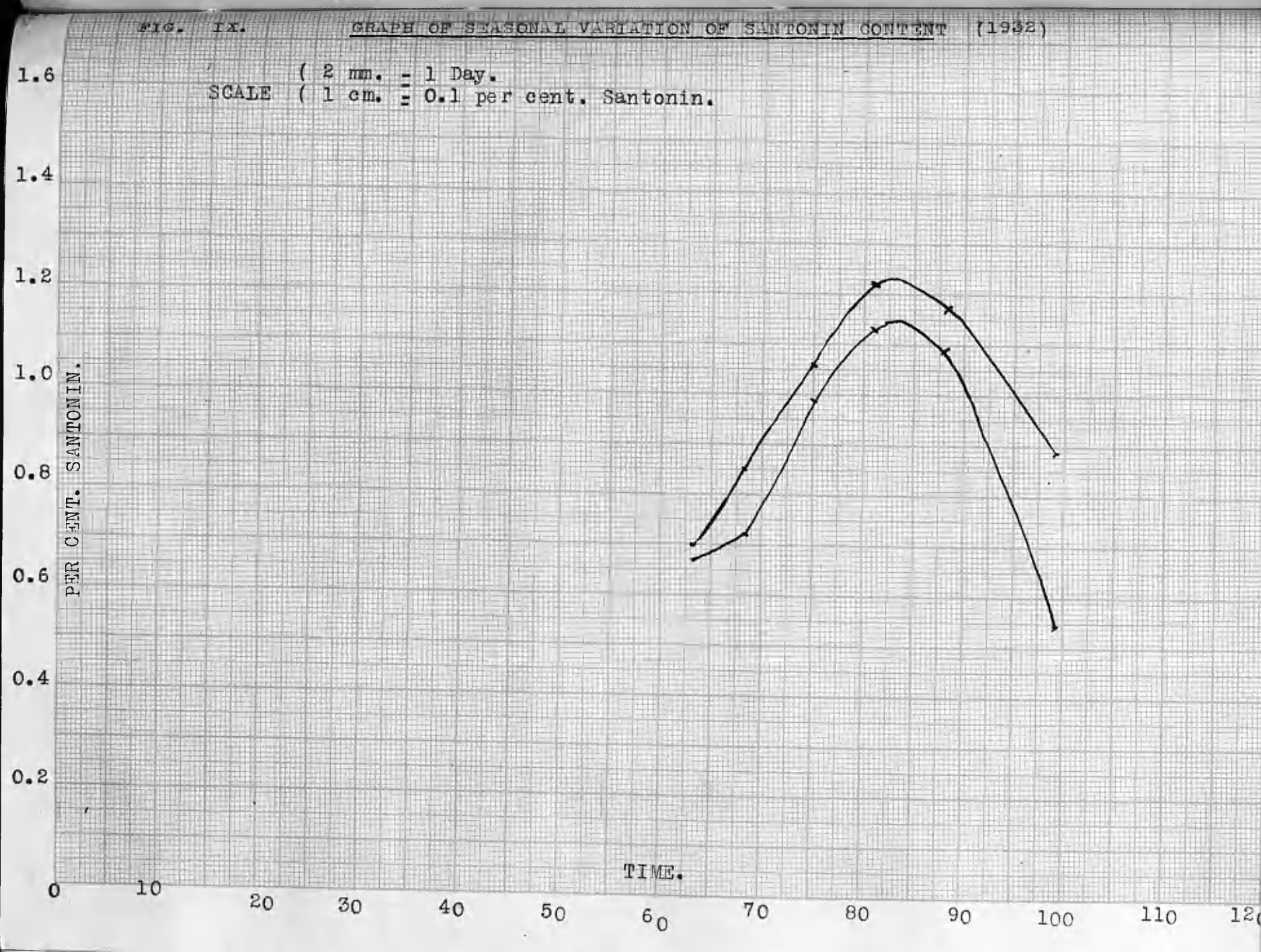
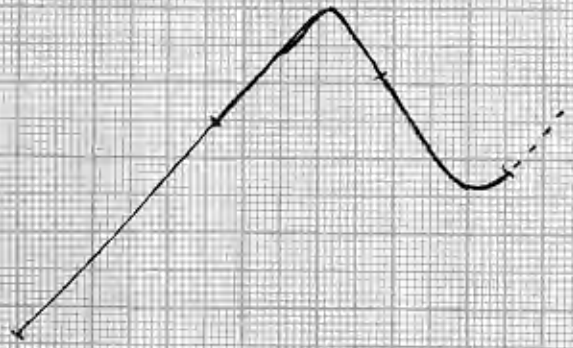


FIG. 8

GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT

SCALE. (2 mm. = 1 Day.
(1 cm. = 0.1 per cent. Santonin.



PER CENT. SANTONIN.

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0

10 20 30 40 50 60 70 80 90 100 110 120

TIME.

FIG. X1 GRAPH OF SEASONAL VARIATION OF SANTONIN CONTENT

SCALE. (2 mm. = 1 Day.
(1 cm. = 0.1 per cent. Santonin.

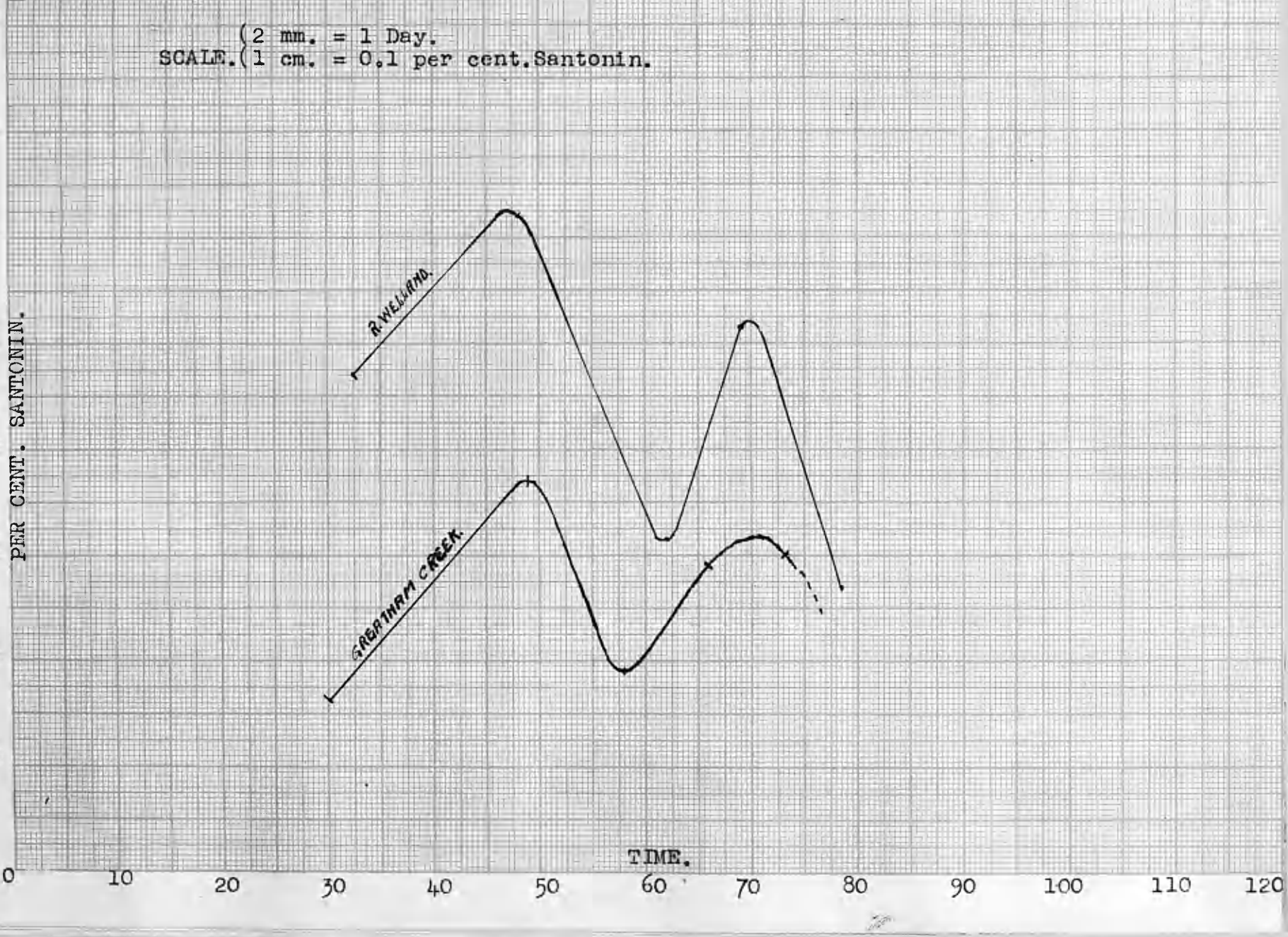


FIG. XII. Brigg's, Kidd's & West's Generalised Form of Relative Growth Rate Curve.

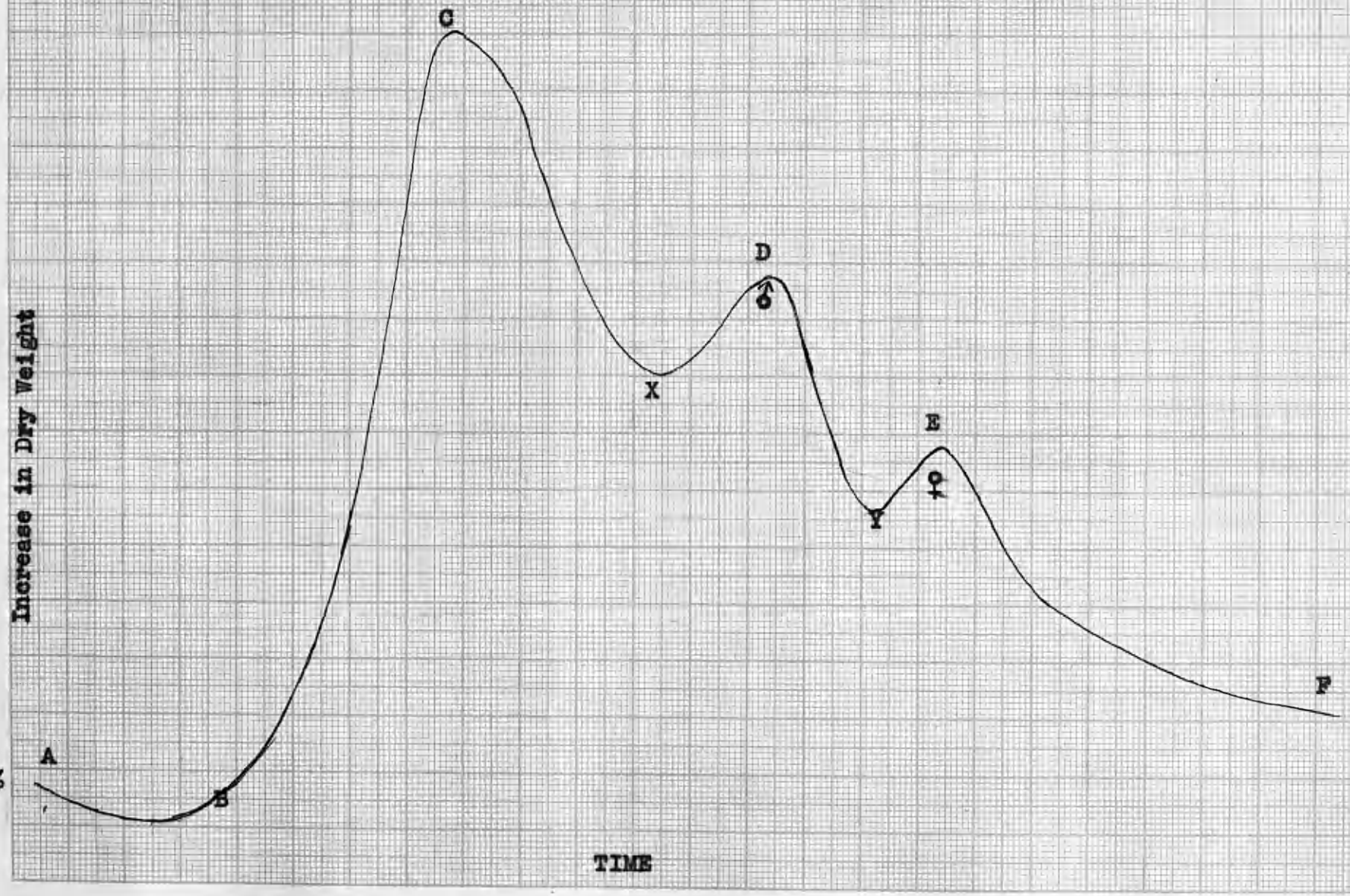


Fig. XIII. Photograph of A. maritima at Barnstaple.

Fig. XIV. Photograph of A. gallica at Gilestone.

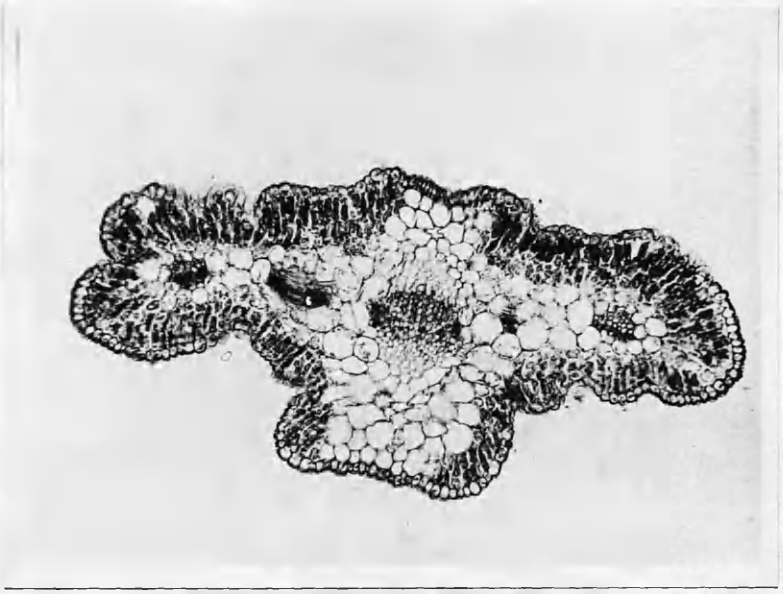


Fig. XV. Photomicrograph of Transverse Section of a segment of leaf of A. gallica. (× 90).

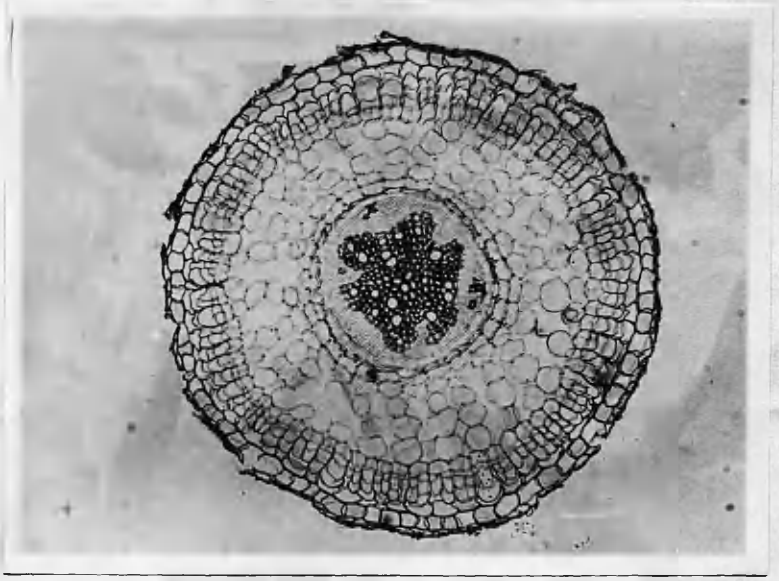


Fig. XVI. Photomicrograph of Transverse Section of Root of A. gallica. (× 90).

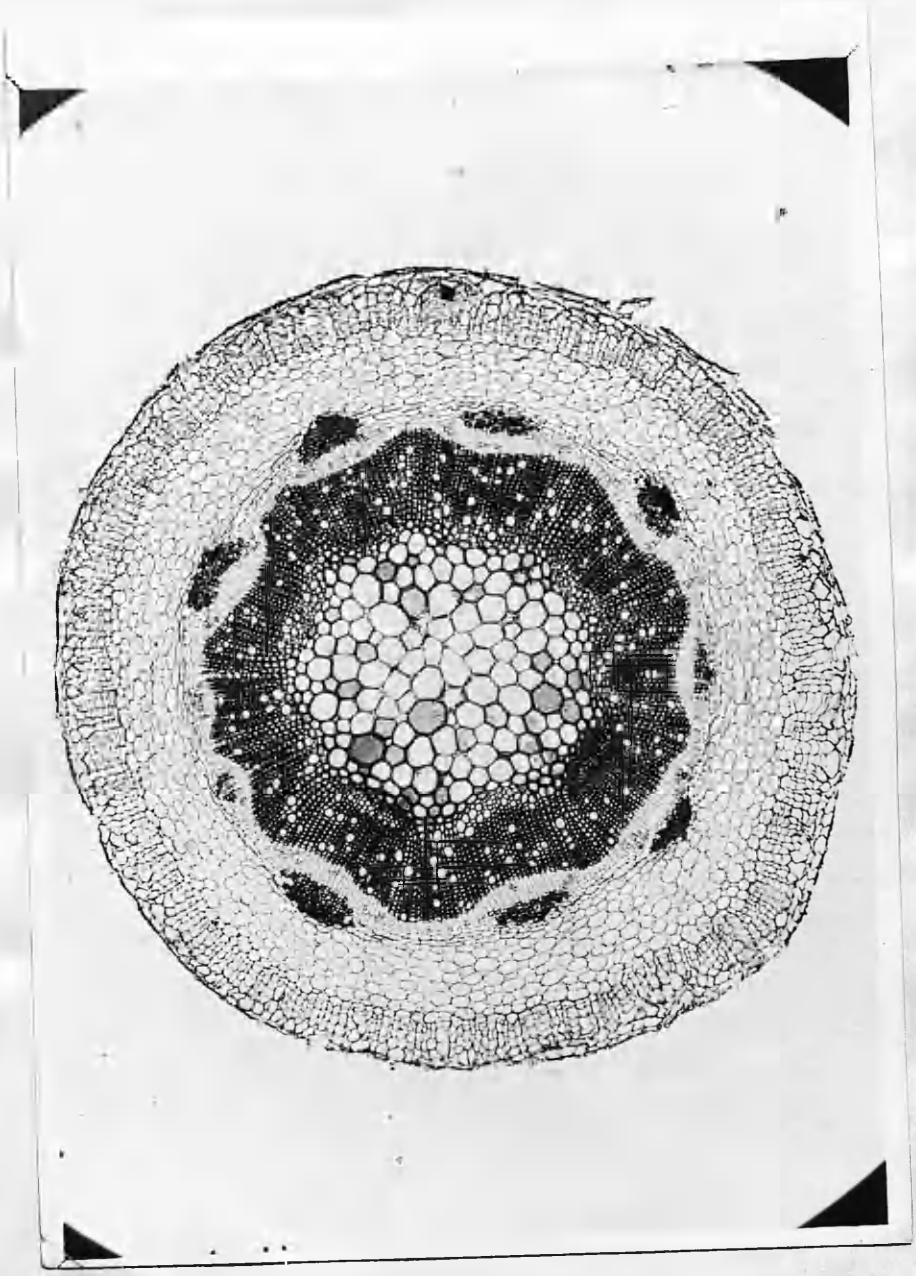


Fig. XVII. Photomicrograph of Transverse Section of Rootstock of A. gallica. (×55).

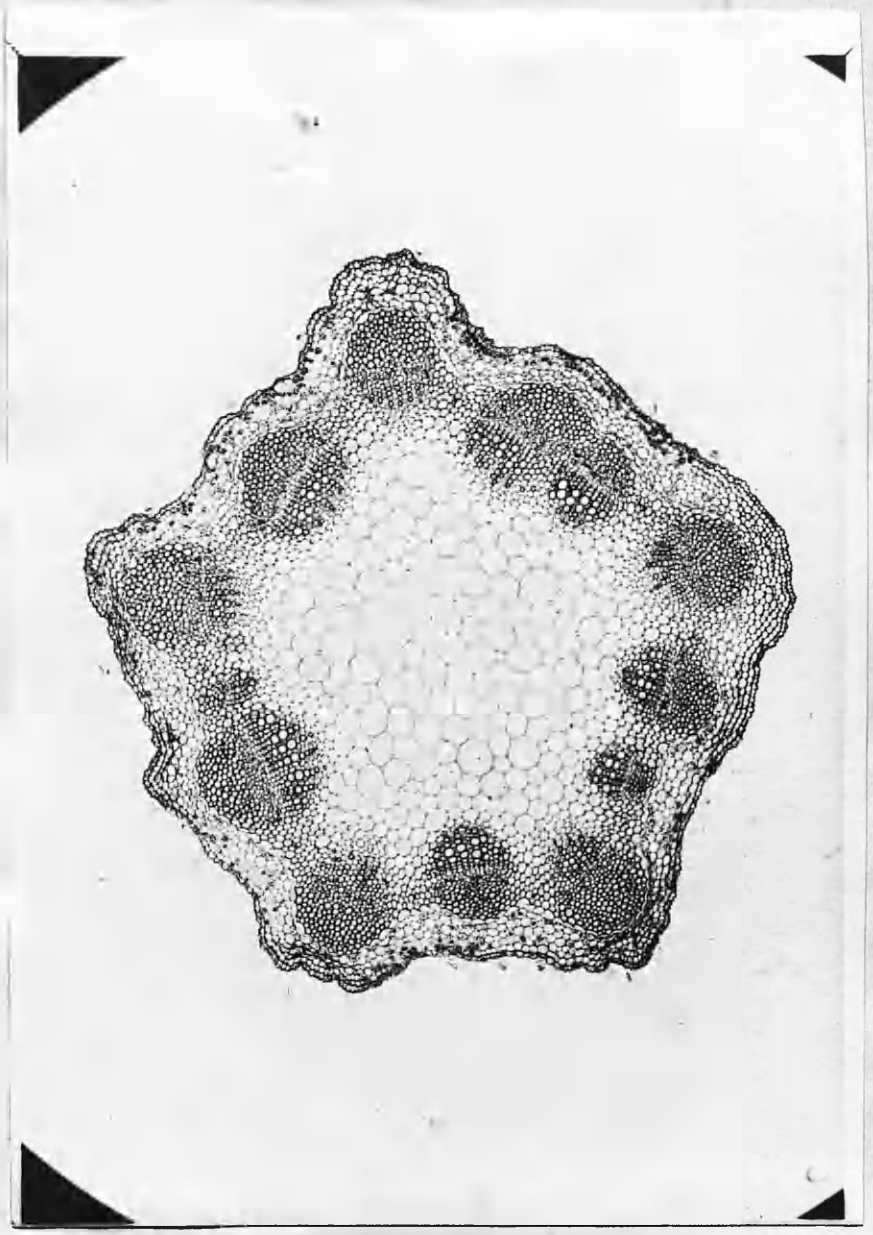


Fig. XVIII. Photomicrograph of Transverse Section of Stem of A. gallica. (× 55).

Fig. XIX. Artemisia at Tynefield.

Fig. XX. Artemisia at Greatham.

Fig. XXI. Artemisia at River Welland.

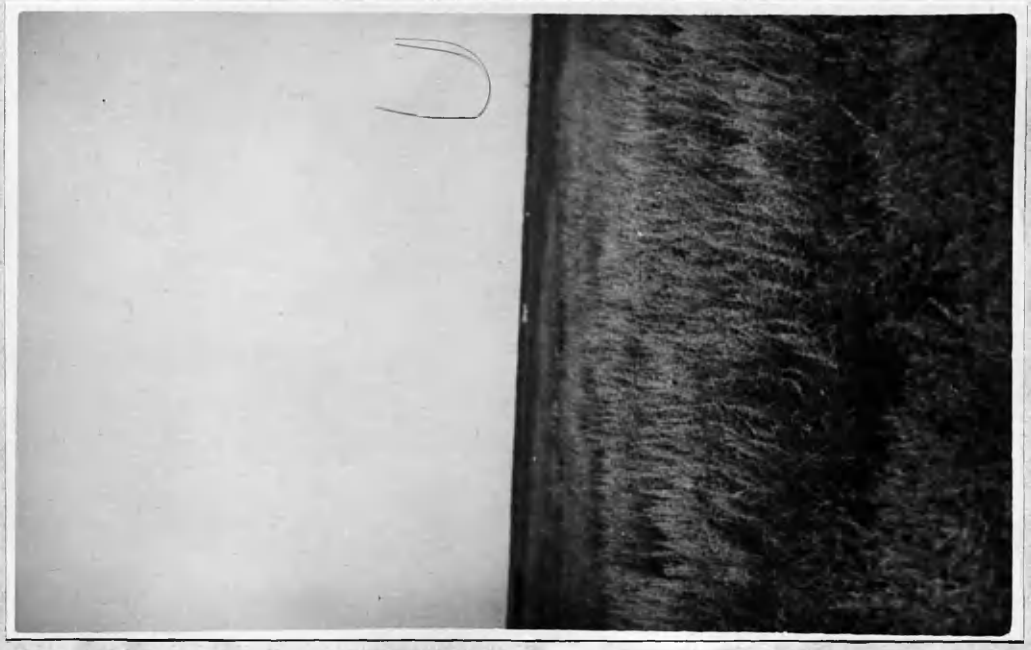


Fig. XXII. Artemisia at Blakeney.

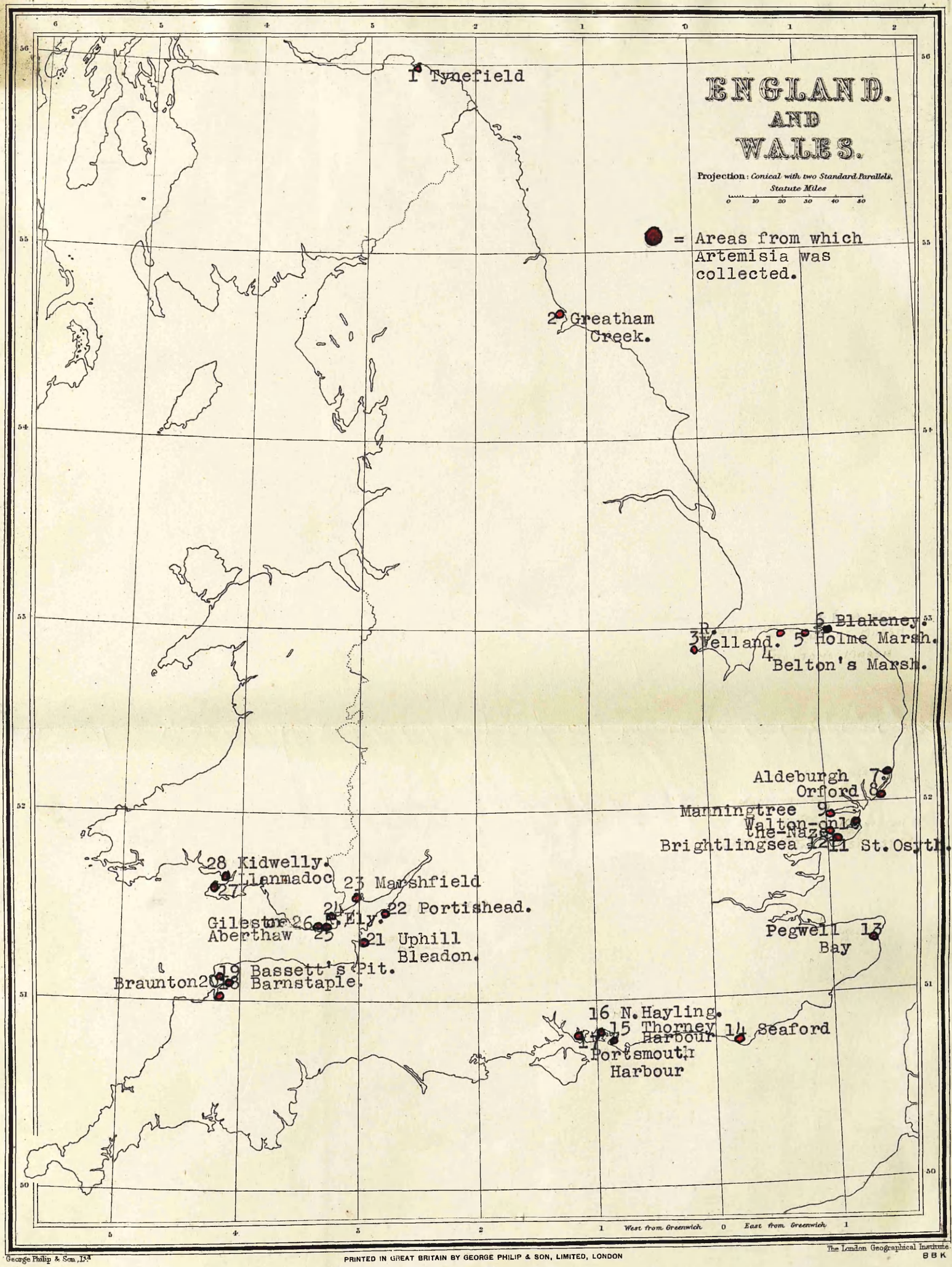
Fig. XXIII. A. maritima at Braunton Burrows.

Fig. XXIV. A. gallica at Braunton Burrows.

Fig. XXV. Artemisia at Aberthaw.

Fig. XXVI. *Artemisia* at Whitford Burrows.

Fig. XXVII. *Artemisia* at Kidwelly.



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Additional papers (two).