THE PHYTOCHEMICAL INVESTIGATION OF

Guarea rusbyi (Britton) Rusby

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A B S T R A C T

THE PHYTOCHEMICAL INVESTIGATION OF *Guarea rusbyi* (Britton) Rusby

by E. B. Ritchie

The investigation of *Guarea rusbyi* (Britton) Rusby included the examination of the petroleum ether, benzene, and alcohol extracts of the powdered bark, as well as the application of several screening tests.

The screening tests indicated the presence of small amounts of alkaloidal material. The advantages and disadvantages of several common methods of extraction of alkaloidal material were also investigated. The tests also showed the possible presence of sterols, flavonols, and small quantities of tannins. Anthraquinones were shown to be definitely present, while saponins and glycosides were shown to be absent.

The petroleum ether extract of cocillana bark obtained by cold petroleum represented an average of 1.0% w/w of plant material. Saponification of the extract yielded 59% of unsaponifiable matter, from which β-sitosterol was isolated by column chromatography. Steam distillation of the extract yielded steam-volatile fractions, which were fractionated into several volatile oil fractions by column chromatography.

The benzene extract of cocillana bark (obtained by extraction using a Soxhlet extractor) averaged 1.6% w/w of undefatted bark. Steam distillation of the benzene extracts yielded a steam volatile fraction, which was chromatographed on an alumina column into many fractions, several being volatile oil fractions. From one of these oil fractions, colorless platelets were isolated and characterized as far as the

small quantity permitted. The washings from the condenser after distillation and a steam-volatile fraction (which was not chromatographed) yielded another compound, which was also partially characterized.

The volatile oil fractions obtained by column chromatography of the steam-volatile fractions from the petroleum ether and benzene extracts were combined and fractionally distilled. The distillation fractions were found to be composed of several constituents. Three of the ten fractions formed 2:4-dinitrophenylhydrazones, which were shown to be gross mixtures by the broad melting ranges and by thin layer chromatography. Fractional distillation of one of the larger volatile oil fractions failed to give sharp fractionation of the components.

Investigation of the steam distillation residue indicated the presence of possibly an ammonium salt of an acid and two other fractions, which were not examined in detail.

The defatted bark was then extracted by ethanol only or by ethanol, after treatment of the bark with ammonia, yielding respectively 4.4% w/w and 7.0% w/w of alcohol extracts.

The alcohol extracts deposited a precipitate on concentration. The precipitate was roughly fractionated and each fraction studied. At least one fraction is believed to be triterpenoid in nature, possibly α-amyrin.

Five methods of alkaloidal extraction were tested on a small scale and by process of elimination, three methods

were chosen for large scale extraction. The crude alkaloidal fractions obtained from the alcohol and the alcohol-ammonia extracts represented 0.012% w/w and 0.023% w/w of defatted bark. This material was a dark brown semi-solid which failed to form a picrate derivative. A small amount of reineckate was obtained, but attempts to purify it by crystallization from acetone gradually decomposed this derivative. Thin layer chromatography of the crude alkaloidal fraction revealed three to four spots which would not stain with Dragendorff's reagent. Gas chromatography of the alkaloidal material caused decomposition. The alkaloidal material could not be obtained by steam distillation of the alcohol extract.

Examination of the commercial product, the Fluid Extract of Cocillana, by steam distillation and by an alkaloidal extraction process indicated again that the alkaloidal fraction could not be obtained by steam distillation and that only a small amount of alkaloid was present.

Throughout the examination of the benzene and alcohol extracts of cocillana bark, it was found that the results obtained largely disagreed with the results reported by Dr. Coblentz in 1893. It is believed, therefore, that the species of Guarea sold in commerce today as Guarea rusbyi (Britton) Rusby is not the same species Coblentz examined in 1893.

C O N T E N T S

THE PHYTOCHEMICAL INVESTIGATION OF *Guarea rusbyi* (Britton) Rusby

I N T R O D U C T I O N

The cocillana tree, Guarea rusbyi (Britton) Rusby, was first discovered in 1886 on the slopes of the Andes Mountains in Bolivia, by Dr. H. H. Rusby, while on an exploratory mission for his employers, Messrs. Parke, Davis, and Company. A liquid preparation of the bark of cocillana had been used as an expectorant and to alleviate coughs by the Callahuaya Indians of South America.

Cocillana, in its physiological action, is said to resemble ipecacuanha but is somewhat more stimulating. Excessive doses cause vomiting, purging, and prostration, and are also emmenagogic. It is toxic to humans in 1.3 to 3.3 gram doses.

Cocillana has been administered in various forms such as fluid extract, syrup, drops, lozenges, and pastilles. Until recently, the drug has been used considerably in the United States and Canada in cough syrups and as an expectorant for the treatment of bronchitis, bronchial pneumonia, asthma, and phthisis. Extracts of the bark are also used in several present day proprietary cough remedies (1).

Botanical Description

The cocillana tree is a member of the natural order Meliaceae, a small family of forty genera and three hundred species. The majority of these plants are native to the

warmer regions of Asia and America, with a few in tropical Africa. The only North American representative of this family is the Melia azedarach or China tree, which is extensively cultivated as a fragrant ornamental tree (e.g. the lilac). All members of the Meliaceae are trees or shrubs except for a few resembling herbs.

Economically, the family is noted for its yield of mahogany and similar precious woods. It also includes the "red cedar" of the tropics, the cedrela, which is in fact, a plant totally unlike the juniperus and red cedar of the United States.

From a medicinal standpoint, the Meliaceae family contains numerous plants which tend to display a strong uniformity of properties. These plants are generally placed in the so-called ipecac group. Only azedarach is known in medicine for its distinct emetico-cathartic properties. In India, another member, the Walsura piscidioides, is used as a powerful fish poison. In addition, there are several species of trichilia which resemble the actions of cocillana. Two other species of Guarea, one found in Brazil and the other in Guiana, were independently named Guarea purgans, suggesting a more stimulating effect than the nausea normally associated with cocillana (2).

When Dr. H. H. Rusby was first led to the South American emetico-cathartic drug in 1886, it was known by the natives as cocillana. He learned later, however, that this

name was applied to the tree only in that specific locale. In another region, the same plant was known as Upas and in a more eastern region as Trompillo. The drug is now commonly known as Guapi commercially and the name cocillana should only be used as a synonym.

The first specimens of cocillana were gathered in Guanai, a village on the Mapiri River. Authenticity was ensured (3), since the herbarium specimens used to name and describe the tree and the bark originally used in medicine came from the same tree.

Dr. N. L. Britton completed the original studies on the herbarium specimens. Regarding the plant as a new genus, he named it Sycocarpus rusbyi. In subsequent studies, Dr. Rusby discovered that the tree belonged to the genus Guarea, and gave it its proper name, Guarea rusbyi (Britton) Rusby.

Although there were early misconceptions of the botanical nature of cocillana, in 1893 Dr. Rusby established its place in the Meliaceae family of the genus Guarea. Since the question of its proper species still remained somewhat confusing, Casimir De Candolle monographed the South American species indicating the relative diagnostic values of various structural characteristics (4).

The striking similarity between the species yielding cocillana and Guarea trichiloides became apparent immediately. Since there was no specific difference between the external characteristics of the species, Dr. Rusby dissected both.

He found structural differences in the blossoms and fruits adequate for distinction (5).

Though cocillana could now be differentiated from G. trichiloides, the fact remained that very few of the other genera had been examined thoroughly to prevent confusion with them. No further studies of Guarea species were made, however, until 1922 (6).

In 1893, a full botanical description of Guarea rusbyi was published by Dr. Rusby (7). At the same time, Dr. Rusby published a pharmacognostical description of the bark. Only the thicker bark from the trunk and the larger branches was collected. The macroscopic description of the bark was quoted from the first work of Professor Joseph Schrenk with a few new diagrams added by Dr. Rusby (8).

Similarly, the microscopic description of cocillana bark was quoted directly from Professor Schrenk's publication in "Druggists' Bulletin," 1888, p. 222 (9).

For several years after its discovery, all of the cocillana bark used in America and Europe was known to be of the same kind and quality as the original. In 1908, however, Dr. Rusby received a sample adulterated with a new bark from his Bolivian collector, Mr. Miguel Bang. On examination of the fruit and leaf fragments of the plant, he found it to be another species, which he named Guarea bangii.

During a second expedition to South America in 1922,

Dr. Rusby collected three other plants which closely resembled G. rusbyi. Two of these were other species of Guarea and the third belonged to the Lauraceae family. All were growing in the Guapi-collecting region and were known as Guapi or Trompillo. He collected specimens of each bark for examination by C. W. Ballard (10).

Clinical Studies

The first clinical studies of cocillana were begun in 1889 by Reynold W. Wilcox (11), who experimented mainly with three different dosage forms: a tincture, a fluid extract, and a syrup. The dosage forms were administered in the following manner: the concentrated tincture in one half to two fluid drachm doses every two to eight hours; the fluid extract in five to twenty-five minim doses every three to six hours; and the syrup in one to two fluid drachm doses every four to six hours.

When the drug was given in the crude state (as powdered bark), the following symptoms were noted from one half hour to one and one half hours after administration. Nausea, a desire to vomit, and a metallic taste in the mouth were produced. The nausea was accompanied by an early discharge of mucous and later, by dryness in the throat. These symptoms were not so marked with the use of the fluid extract, tincture, or syrup.

The drug, in any form, sometimes produced a slight headache and on rare occasions, slight dizziness. With large doses, there was a desire to defecate. The latter symptom was scarcely produced by the syrup, suggesting that extracted resinoids contained the purgative substances of the drug. Frequently, a slight increase of perspiration as well as considerable stimulation of the glands of the mucous membranes of both the bronchial tubes and the digestive system resulted.

The primary use of cocillana was indicated in hyperaemia of mucous membrane without any secretion. The drug has been used as a remedy in acute bronchitis, subacute and chronic dry bronchitis, and in chronic diseases of the pulmonary tissues.

In cases of chronic dry bronchitis, cocillana was found to be more capable in removing bronchial mucous than either apomorphine or ipecacuanha. It was more suitable in cases of continued administration because it increased the appetite and acted as a laxative. The increased number of bowel movements were characterized by painless movements which were larger in amount and of greater fluidity.

In cases of chronic diseases of pulmonary tissue, cocillana was beneficial in relieving congestion and for diminishing cough and expectoration.

Cocillana was found to have certain advantages over the use of apomorphine and ipecacuanha as expectorants. One of the most important factors was that the emetic and the

expectorant doses of cocillana were quite distinct. Taken in a single two ounce dose, the syrup caused emesis and a dose of one to two drachms resulted in expectoration. Since cocillana failed to produce nausea so readily, improved the appetite, and promoted regular bowel movements, it was preferable to ipecacuanha. Whereas the use of apomorphine resulted in more profuse, watery secretions, cocillana induced more viscid expectoration, probably by its stimulation of the muciparous glands. Apomorphine required more frequent administration with an onset of action of one-half to one hour and a duration of two to three hours, while cocillana with a slower onset of action of three to six hours had a duration of four to six hours. As a result of their respective onsets of action, apomorphine was used in the first forty-eight hour period of acute bronchitis and cocillana in the latter stages.

Cocillana, like all other expectorants, failed in the treatment of coughs due to pleuritic exudation or of laryngeal or pharyngeal origin. It was inferior to carbonate of ammonia in chronic senile bronchitis and to strychnine, in cases in which stimulation of the respiratory center was desirable.

Histories of specific cases studied by John W. Shoemaker also indicated the advantages of the use of cocillana as an expectorant (12).

Chemical Investigation

The first chemical examinations of the benzene and alcohol extracts of cocillana bark were carried out in 1893 by Virgil Coblentz (13).

Five hundred grams of dry, finely powdered drug were exhausted with hot benzol. After the complete removal of the benzol, 24.95 grams of greenish-gray, syrupy extract were obtained. The extract was mixed with water acidulated with tartaric acid and steam distilled on a water bath until the distillate contained no oily globules. At the end of the distillation, the condenser was incrusted with crystalline masses. These masses were washed out with ether and combined with the portion obtained by ether extraction of the distillate.

The white solid was crystallized several times from alcohol, yielding 0.65 gram of hexagonal plates, which melted sharply at 80°C., after drying over phosphoric oxide. On heating above 80°C., the substance sublimed to white flaky crystals. The compound had a peculiar aromatic taste and was very soluble in ether, chloroform, alcohol, ligroin, acetic ether, and glacial acetic acid but insoluble in alkalies. The compound was classified as a "camphor-like" body, probably a solid hydrocarbon which was peculiar to the phlox species.

The aqueous acid distillate, after removal of the hydrocarbon, indicated the presence of traces of alkaloids on testing with Mayer's and other reagents.

The residue after distillation was freed from water, leaving a pale greenish, brittle, resinous mass (24.3 grams). It was dissolved in a small volume of chloroform and poured into a large excess of alcohol with constant stirring. A white resinous powder precipitated out, leaving a pale green alcoholic solution. The resin was filtered off, washed with alcohol, and again reprecipitated into alcohol. On evaporation, the alcoholic solutions yielded 12.5 grams of a greenish-yellow fixed oil. The oil had a pungent, acrid taste and an earthy odor. It was soluble in ether, chloroform, alcohol, and turpentine and left a permanent greasy stain on paper. It saponified readily with weak soda and yielded elaidin with nitrous acid. The oil was, therefore, a non-drying fixed oil.

The resin obtained (11.8 grams) was a white, impalpable powder, which melted at 122°C. to a clear yellow liquid. On melting and cooling, the resin left a transparent, brittle, pale straw-colored mass which was odorless and tasteless. It was soluble in chloroform, ether, and turpentine and insoluble in alcohol, dilute alcohol, and boiling aqueous caustic potash. The resin was soluble in a large excess of boiling alcoholic potash from which it was reprecipitated on addition of an acid. There was no reaction with any cold acids nor with nitric acid after prolonged boiling. Fusion with caustic

potash gave no reaction. Therefore, the compound was classified as an indifferent resin.

The plant drug was then completely exhausted by 80% alcohol, yielding 9.4 grams of dry extract. On treatment with absolute alcohol, 2.7 grams of insoluble residue consisting of caoutchouc and gummy matter remained.

The alcohol extract was concentrated to a small volume and poured into an excess of ether. After the insoluble portion was deposited, the clear ethereal solution was decanted off and combined with ethereal washings of the precipitated mass.

The brown, ether-insoluble portion was dissolved in water and precipitated with lead subacetate. The precipitate was suspended in water, the lead removed with hydrogen sulphide, and the solution filtered and heated. The solution gave a greenish-black color with ferric chloride, a precipitate with gelatin solutions, and reduced ammoniacal silver nitrate. The presence of a very small amount of a tannin-like principle was thus indicated. The same solution also readily reduced Fehling's solution and the Bismuth test solution on warming. The filtrate from the lead precipitate was freed from excess lead and heated to remove hydrogen sulfide. This showed the presence of glucose by Fehling's and the Bismuth test.

The above solutions were tested for alkaloids and found to be negative.

An alcoholic solution of the ether-soluble extract was poured into water, acidulated with hydrochloric acid. 0.8 gram of a brown resinous precipitate was filtered off. The resin was readily soluble in ether, chloroform, benzol, alcohol, and dilute ammonia but precipitated by acids. It had an intense bitter acrid taste reminiscent of the taste of the crude drug.

The acid filtrate, which was a deep red color with a greenish fluorescence, was shaken with ether, chloroform, and benzol without yielding any appreciable residue. It was then made alkaline with ammonia (intensified the color) and again shaken with several portions of ether. On evaporation, an impure residue was obtained. This was resolved in acidulated water and reprecipitated yielding 0.012 gram of a residue which possessed many characteristics of an alkaloid. A total of 0.014 gram was thus obtained from both the alcoholic and benzol extracts. The usual alkaloidal reagents, such as Mayer's, Lugol's, potassio-cadmic iodide, auric and platinic chlorides, tannic acid, picric acid, all gave distinct reactions. Microscopic crystals were obtained with picric acid but all other salts refused to crystallize. The quantity was too small to test for nitrogen, but the compound was named an alkaloid.

The highly colored alkaline liquid remaining after the extraction of the alkaloid was concentrated on a water bath. During the evaporation, deep crimson drops condensed on the

sides of the beaker. The liquid was distilled in a small flask. The first few milliliters were crimson in color, unaffected by acids, intensified by alkalies, and not abstracted with any volatile solvents. On evaporation in open air, the material was entirely dissipated. The solid extract remaining, on treatment with alcohol to remove salts, consisted of inert extractive.

Coblentz also prepared extracts using distilled water, dilute soda (0.2%), and dilute hydrochloric acid. No extensive studies were made on these extracts as their constituents are common to most plants and are of no interest or value.

Coblentz summarized his results in the following table.

SUMMARY (14)

	%	%
Benzol extract	4.99	
Solid hydrocarbon		0.13
Fixed oil		2.50
Resin		2.36
80% Alcoholic extract	1.88	
Resin 0.16%, alkaloid, tannin and glucose.		
Distilled water extract	5.76	
Starch, mucilage, dextrin, glucose.		
Dilute soda	3.21	
Extractive, albuminoids.		
Dilute hydrochloric acid	1.10	
Extractive, calcium oxalate.		
Lignin	5.84	
Ash	2.65	
Moisture	9.72	
Residue	64.85	

DISCUSSION OF EXPERIMENTAL WORK

I. SCREENING PROCEDURES

The history of cocillana indicates that many years passed after its initial discovery in 1886 by Dr. H. H. Rusby before a complete botanical description of the bark was published. When Dr. Rusby made the discovery (3), he learned that the drug named cocillana was known in other regions as Upas, Guapi, or Trompillo. Eventually, the commercial name Guapi and the synonym cocillana became accepted.

In 1908, the first problem of adulteration of cocillana bark arose. Dr. Rusby was able to draw up a partial description of the adulterating bark (6), from some fruit and leaf fragments and named it Guarea bangii.

While on the Mulford Expedition to Bolivia in 1922, Dr. Rusby found the genuine Guarea rusbyi, Guarea bangii, as well as two other species of Guarea and a tree belonging to the Lauraceae family (a species of Nectandra), all growing in the Guapi-collecting region and known as Guapi or Trompillo. The problem of adulteration of cocillana bark with the barks of any one or all of these species immediately became evident. Dr. Rusby sent bark and herbarium specimens of Guarea rusbyi, Guarea bangii, an unidentified species of Guarea, and the species of Nectandra to Dr. C. W. Ballard for identification (10). Another species of Guarea was supposed to have been sent but the bark specimens were lost

in transit. In addition, Dr. Rusby mentioned the discovery of a fifth species, which he named Guarea alba-rosea, and another species of Guarea planted as a shade tree along a street in eastern Bolivia. Descriptions of the latter two species were not made available.

Modern references (15) (16) for the description of cocillana bark quote standards which are not as stringent as the earlier ones (8) (10), not completely uniform, and rather vague in details. For example, the maximum lengths of the fragments of bark vary from 500 mm. (8), 250 mm. (10), 600 mm. (15), to "variable" lengths (16) and the maximum widths allowed vary from 20-120 mm. (8), 80 mm. (10), 150 mm. (15), to "variable" widths (16). Only two species (Guarea bangii and the Nectandra species) are mentioned as possible adulterants found in cocillana bark.

The cocillana bark (Guarea rusbyi (Britton) Rusby) examined for this paper was purchased from the Penick Company of New York in three lots: powdered bark (mesh number 60), powdered bark (mesh number 100), and whole strips of bark. The latter lot was comminuted to a powder (approximately mesh number 50) and extracted immediately in an attempt to prevent possible losses and changes by drying or oxidation.

On examination of the first two lots of bark (mesh numbers 60 and 100) purchased, the constituents obtained from the benzene and alcohol extracts were different from those reported by Coblentz in his paper. Therefore, the third lot

of bark was purchased as whole bark and was examined by Professor G. Blunden. The sample was reported to be authentic (15) but also worm-eaten, moldy, and containing a larger proportion of wood than specified. The results obtained from the examination of the benzene and alcohol extracts of the third lot were identical to those from the first two lots.

Screening For Alkaloids

Since earlier workers reported the presence of an alkaloid in cocillana bark (13), the bark was first carefully screened for alkaloids. Several methods were used before conclusions were drawn. This served to check the results obtained and furnished valuable information on the advantages and disadvantages of various extraction methods and testing procedures. The value and results of each test are discussed in the following discussion. Each screening test was completed using samples of the two lots of powdered cocillana, one of cinchona bark, an alkaloid-containing drug, and one of liquorice root known to contain no alkaloids. The appropriate extracts were tested with four alkaloidal reagents, Dragendorff's (17), Mayer's (18), silicotungstic acid (19), and Wagner's (20).

Wall's method (21) indicated that a small amount of alkaloidal material was present in the cocillana extracts tested (Table 1, p. 49). The possibility of obtaining false-

negative results by the testing of the dilute aqueous extract (1 ml. extract ≡ 0.2 - 0.4 g. of plant material) is highly probable. Wall et al (22) have modified this method, however, so that one ml. of the test solution is equivalent to four grams of dry plant sample.

After using Wall's confirmatory test (21), the results obtained previously with cocillana bark were altered. After comparison of the results (Table 1, p. 49; Table 2, p. 50), it is evident that alkaloidal reagents containing iodine (Dragendorff's and Wagner's reagents) tend to give false-positive results unless the extract tested is purified previously by an acid-base shakeout. This problem occurred with these reagents in other tests, particularly when partial purification of the extract to be tested was omitted.

The Swanholm Prollius Fluid method (23) is a simple and effective method (Table 3, p. 51). The use of Prollius fluid for the extraction of the alkaloidal material prevents decomposition of plant materials by heat. False-positive tests can be obtained, however, since the acid filtrate is prepared by maceration of the extract with acid for one hour at 80°C. and is not partially purified by an acid-base shakeout before testing.

The Swanholm Hydrochloric Acid method (23) does not seem to be as reliable as the previous methods, since positive tests were obtained even with liquorice with all of the alkaloidal reagents (Table 4, p. 52). Since the plant material

is extracted by maceration with hydrochloric acid for six hours at 80°C., the problem of false-positive results is quite likely to occur. Again, an acid-base shakeout of the extract may overcome this problem.

The Kiang, Douglas, and Morsingh method (24) is simple to carry out and yields reliable results (Table 5, p. 53) except for the common problem with Dragendorff's and Wagner's reagents.

The Kleber method (25) and Arthur's Modification of The Webb method (26), like the Swanholm Prollius Fluid method, both employ Prollius fluid for the extraction of the plant material. Unlike Swanholm's method, Kleber's method and Arthur's method do not apply heat during any stage of their procedures. Therefore, reliable results can be expected without partial purification of the acid test solutions (Table 6, p. 54; Table 7, p. 54).

The Farnsworth and Euler method (27) is the most complicated procedure but it includes a test for quaternary alkaloids in addition to the test for secondary and tertiary alkaloids. Thin layer chromatography of the partially purified extracts indicates the possible number of alkaloids present, a distinct advantage over the precipitation of alkaloids by alkaloidal reagents. However, this procedure requires the use of more elaborate techniques, and prior experience with these techniques is vital.

Under ultraviolet light, the chromatograms of Fraction

1 and Fraction 2 of cocillana extracts A' and B' respectively showed the presence of three blue spots and one blue spot. None of these spots stained with Dragendorff's reagent (Table 8, p. 57). These negative results could possibly be explained on the basis of the use of test solutions which were too dilute. Farnsworth and Euler reported that the detectable concentration of alkaloids in plant samples ranged from as little as 0.5 ug of α-yohimbine to as much as 200 ug of caffeine and ephedrine (still not detectable).

In summary, (Table 9, p. 58), the test results for cocillana bark show evidence for the presence of small amounts of alkaloidal materials.

Screening for Glycosides, Saponins, and Other Substances

A modification of the Stas-Otto process (28) was used to test for the presence of glycosides in cocillana bark. The results of this test, however, should not be regarded as being conclusive evidence for the presence of glycosides only, as positive results are obtained also with carbohydrates. The cocillana bark extract yielded a negative result.

The tests used for the detection of saponins in cocillana bark were Fischer's method (29), using blood agar plates, and Wall's method (21), using a saline red blood cell suspension. Quillaja wood, which was employed as a control in Fischer's method, produced a definite zone of hemolysis

within one hour, while cocillana bark produced no hemolysis even after twelve hours. After refluxing with a methanolic solution of cholesterol, quillaja wood also gave a negative result. This indicated that the hemolysis caused before this treatment was due to the presence of hemolysing saponins, which were then bonded with cholesterol to form a complex, no longer capable of causing hemolysis. The negative result obtained by Fischer's method with cocillana bark was confirmed by the failure of the cocillana extract to hemolyze the red blood cell suspension in Wall's method.

The test for sterols by Wall's method (21) gave an indefinite Liebermann-Burchard reaction (30). The color reaction of this test was obscured by the deep color of the chloroform extract tested.

The Bryant test (31) was used to detect the presence of flavonols in cocillana bark. Since the expected pink color produced by the reduction reaction was difficult to observe in the colored test solution, the reaction mixture was shaken with an equal volume of amyl alcohol. The cocillana reaction mixture developed a pink color in the aqueous layer. This is indicative of the presence of a flavonol in the glycosidic state, since any aglycone present appears as a pink color in the alcohol layer.

Wall's test for tannins (21) indicated that cocillana bark has a lower tannin content than drugs such as cinchona bark and liquorice root (Table 10, p. 62). Hoch's spot

tests (32) are not as reliable as Wall's method, probably due to the method of extraction of the plant material and the concentration of extract tested (Table 11, p. 63).

An alcohol extract of cocillana bark was tested for the presence of anthraquinones by Wasicky's modification of the Borntrager reaction (33). The acidified extract was shaken out with benzene which became bright yellow in color. After shaking out with 10% ammonia, the benzene layer rapidly became bright red in color, indicating the presence of anthraquinones.

In conclusion, the screening tests indicated the absence of glycosides and saponins and the presence of anthraquinones and possibly, sterols and flavonols. These results obtained with the tests for glycosides, saponins, and sterols were substantiated on examination of the petroleum ether, benzene, and alcohol extracts of cocillana bark.

II. THE PETROLEUM ETHER EXTRACTS

Extraction

The cocillana bark (Guarea rusbyi (Britton) Rusby) was defatted by cold percolation with petroleum ether. Each batch of bark (three kilogram batches) was found to be exhaustively extracted after eighteen liters of percolate were collected. Concentration of the combined percolates obtained from the extraction of a total of 17.9 Kg. of bark (mesh number 60) yielded extract A_3 (167.5 g.). Similarly, 16 Kg. of bark (mesh number 100) and 4.5 Kg. of bark (mesh number 50) respectively yielded extracts A_4 (163.6 g.) and A_5 (47.3 g.). The petroleum ether extract represented an average of 1.0% w/w of the plant material.

On cooling in the refrigerator for several weeks, extract A_3 deposited an off-white solid A_{3a}, melting point 69-94°C. The physical appearance of the material, its low solubility in alcohol and high solubility in ether and petroleum ether suggested that A_{3a} was a mixture of esters of long chain fatty acids and/or related substances. This suggestion was substantiated by the infra red spectrum (page 65).

Saponification

Saponification of the petroleum ether extracts yielded

an average of 59.4% w/w of unsaponifiable material and 39.1% w/w of fatty acid fraction.

Two methods of saponification were applied. The British Pharmacopoeia 1958 method (34) is a convenient and standard method giving accurate results. The use of the method of Fitzgerald et al (35) served to check the results and involved an interesting modification, whereby any triterpenoid acids present are precipitated out (p. 68). The petroleum ether extract of cocillana bark, however, failed to precipitate out any solid, indicating the absence of triterpenoid acids in the extract. This indication was substantiated by the fact that triterpenoid acids were not found on further examination of the petroleum ether extract.

The unsaponifiable fraction B_1 (Figure 1) was chromatographed on an alumina column. Over 500 fractions were collected and bulked together as fractions D_1 to D_{10} (Table 12, p. 70). These fractions were examined and in turn combined into larger fractions I to IV (Table 13, p. 71), on the basis of their appearances and weights.

Fraction II (D_3 - D_6) deposited a white solid on standing. After several recrystallizations from ethanol, glistening white needles (IIa) were obtained and identified as β-sitosterol, m.p. 135.0-135.5°C. Compound IIa analyzed correctly for the formula, $C_{29}H_{50}O$ (36); $[\alpha]_D^{24^\circ}$ - 37° (C - 1.3 in chloroform). The infra red spectra of compound IIa and of β-sitosterol were identical. The mixed melting point

of the acetate of compound IIa and of authentic β-sitosterol acetate was not depressed, m.p. 126.5-128°C.

Fraction III (Table 13, p. 71), being a large fraction, was rechromatographed on an alumina column. A large number of fractions were collected together as fractions E_1 to E_{13} (Table 14, p. 73), The infra red spectra of various fractions E_1 to E_{13} indicated the fractions were still quite impure. Only fractions E_4 and E_5 yielded sufficient solid E_{5b} (78 mg.) to be identified as β-sitosterol.

Steam Distillation

Steam distillation of the petroleum ether extract (31.5 g.) yielded a dark brown steam-volatile fraction, F (4.71 g.) (Figure 2). The crude fraction F was chromatographed on an alumina column into several fractions, G_1 to G_9 (Table 15, p. 79). The first four fractions, G_1 to G_4, were pale yellow, mobile oils with pleasant odors. These were combined with other similar oil fractions (H_1 to H_4 and L_1 to L_4) for fractional distillation (p. 80). The remainder of the fractions, G_5 to G_9, were still impure and precipitated only traces of solid material.

A large scale steam distillation of the remainder of extract A_3 and all of extract A_4 yielded a dark brown, steam-volatile fraction F_1 (27.12 g.). Fraction F_1 was chromatographed on an alumina column and several fractions, H_1 to H_7, were collected (Table 16, p. 81). The first fractions, H_1 to

H_4, were yellow, fragrant oils similar to fractions G_1 to G_4 and were combined for fractional distillation (p. 80). The remainder of the fractions, H_5 to H_7, precipitated traces of solids in amounts too small to characterize.

The isolation of the volatile oil fractions and of β-sitosterol from the petroleum ether extract of cocillana bark was not previously reported by Coblentz (13).

III. THE BENZENE EXTRACTS

The benzene extract represented 1.6% w/w of the total weight of undefatted plant material. This percentage is considerably lower than that quoted by Coblentz in his paper (13). He obtained 24.95 grams of benzol extract from 500 grams of undefatted bark (4.99% w/w). Other discrepancies between the results obtained by Coblentz from his benzol extract and those obtained in this paper occurred and are discussed in the appropriate sections.

Steam Distillation

Steam distillation (p. 83) of extract J_1 and extract J_2 respectively yielded fraction K_1 (5.00 g.) and two fractions K_{2a} (9.67 g.) and K_{2b} (1.11 g.) (Figure 3, p. 85).

Each of the steam-volatile fractions K_1, K_{2a}, and K_{2b} precipitated out a white solid on cooling. This solid, however, redissolved in the oils at room temperature and refused to crystallize or precipitate out on the addition of solvents.

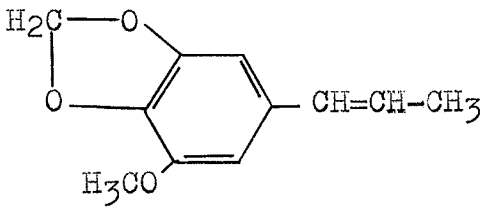
At the completion of the distillation, the condenser was found to be coated with oil droplets containing traces of solid. This material was combined with fraction K_{2b} and allowed to stand. White crystals K_3 (13 mg.) were filtered off and recrystallized (from benzene), m.p. 53.5-55°C. (p. 88).

$[\alpha]_D^{24^\circ}$ -11.5° (C, 1.237 in chloroform); λmax. 257 mu $E^{1\%}_{1\ cm.}$, about 38 (C, 0.01723 in ethanol). The infra red spectra (in carbon disulfide and in chloroform) indicated that compound K_3 was an acid with peaks at: 3,509 cm.$^{-1}$ (weak; hydroxyl), 3,333 to 2,500 cm.$^{-1}$ (broad band with shoulder at 2,667 cm.$^{-1}$, carboxyl group with C-O dimer) (37) and at 1,689 cm.$^{-1}$ (carbonyl). There was little evidence for unsaturation. The absence of other strongly absorbing chromophores was confirmed by the low ultraviolet absorption ($E^{1\%}_{1\ cm.}$, about 38) (38). The failure of K_3 to form a 2:4-dinitrophenylhydrazine derivative verified that the carbonyl group (1,689 cm.$^{-1}$) was not aldehydic or ketonic in nature.

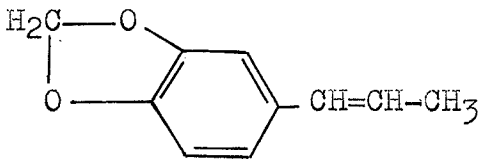
Coblentz, on the other hand, found that the condenser used in the steam distillation of his benzol extract was incrusted with crystalline masses. He combined these crystals with the portion obtained by ether extraction of the distillate and after several crystallizations (from ethanol) obtained hexagonal plates (0.65 g.), m.p. 80°C. (after drying over phosphoric oxide). The compound sublimed above 80°C. and was classified as belonging to "the so-called 'camphor-like' bodies, or more properly, solid hydrocarbons, such being peculiar to the phlox species" (13).

In an attempt to isolate more of compound K_3, fractions K_1 and K_{2a} were combined and chromatographed into several fractions L_1 to L_6 (Table 17, p. 86). Although more of K_3

was not obtained, another compound L_{3a} was isolated. On standing, fraction L_3 yielded colorless crystals L_{3a} (18.9 mg.), subliming at 126-130°C. $[\alpha]_D^{24^\circ}$ +9.5° (C, 0.68 in chloroform); λ max. 255 mu, $E^{1\%}_{1\ cm.}$ 1,004 (C, 0.000675 in ethanol). The infra spectra of compound L_{3a} (in chloroform and in carbon disulfide indicated the presence of a 1:4-disubstituted benzene ring [1,171, 1,110, 1,025, 1,006 cm.$^{-1}$ (all weak) and 832 or 807 cm.$^{-1}$ (strong)(39), and 1,876 cm.$^{-1}$ (medium), 1,757 and 1,721 cm.$^{-1}$ (weak) (40)], with a cis disubstituted double bond [1,653 cm.$^{-1}$ (shoulder), 3,021 cm.$^{-1}$ (medium), 702 cm.$^{-1}$ (strong), the one between 1,420-1,401 cm.$^{-1}$ being hidden (41)], a methyl group (2,941 cm.$^{-1}$), and an acyclic ether grouping 1,087 cm.$^{-1}$ (strong) (41)]. Compounds such as isomyristicin (I) (42) and isosafrole II (43) can be chosen as possible models for the type of compound described, except that a methylene-dioxy grouping is known to be absent in compound L_{3a}.



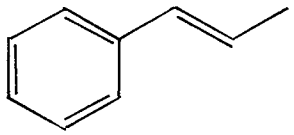
I



II

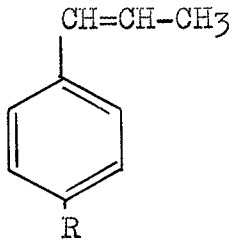
The ultraviolet absorption spectrum of compound L_{3a} indicated the presence of a powerful chromophore such as is found in methyl styrene (III) (44), λ max. 251 mu, $E^{1\%}_{1\ cm.}$ about 1000 (ε, 17,000) (44). It is also known that a methyl sub-

stituent (2,941 cm.$^{-1}$) on a benzene ring produces a bathochromic shift of 5 mu (45).



III

From the evidence given by the infra red and ultraviolet absorption spectra, compound L_{3a} was assigned the partial structure (IV), where R contains an ether linkage.



IV

The amount of compound L_{3a} remaining after attempted purification was too small to obtain a carbon and hydrogen analysis.

Fractional Distillation of The Oil Fractions

The oil fractions G_1 to G_4 (Table 15, p. 79), H_1 to H_4 (Table 16, p. 81) and L_1 to L_4 (Table 17, p. 86) were combined and fractionally distilled to yield fractions N_0 to N_8 (Table 18, p. 90). Although there were some variations in the specific gravities and refractive indices of

the fractions obtained, infra red spectra showed that the oils were all chemically still very similar. The spectra suggested the presence of a saturated substance, possibly of a terpene type, with traces of hydroxyl and carbonyl compounds (weak bands), probably as impurities.

Treatment of the various oil fractions (N_0 to N_8) and of residue N_R with 2:4-dinitrophenylhydrazine reagent produced solid derivatives with fractions N_3, N_5, and N_R (Table 19, p. 91). These derivatives were black in color with wide melting ranges, indicating that they were gross mixtures. Thin layer chromatograms of these derivatives showed five to seven spots in the same regions in each case (Figure 4, p. 94). The presence of several constituents in each oil fraction was further substantiated by thin layer chromatography of the oils (N_3 to N_8), which yielded from five up to eight spots for each fraction in the same regions (Table 21, p. 95). As was to be expected, fraction N_R streaked and failed to resolve into compact spots, under the same conditions.

In an attempt to fractionate the components of one oil fraction, fraction N_6 was redistilled to yield fractions P_0 to P_7 (Table 22, p. 96). The physical constants of fractions P_0 to P_7 showed slight variation but the infra red spectra of fractions P_0 and P_2 were almost identical, suggesting that sharp separation of the components had not been accomplished.

Investigation of Residues J_1R and J_2R

After treatment of residues J_1R and J_2R according to the method of Coblentz (13), an off-white solid M (56 mg.) was obtained, m.p. 239-241°C. with decomposition. The low carbon content of M (31.84%) suggested the presence of a high proportion of other elements and sodium fusion indicated nitrogen. The physical properties of and the analysis results for M suggested a compound such as glycine (46). Although glycine itself has similar physical properties, melts at 233 (completely sintered at 290°C.), and analyses for C (32.00%), the mixed melting point of compound M with glycine was depressed, m.p. 190°C.

The infra red spectrum of compound M (potassium bromide disc) showed weak peaks suggestive of an ammonium salt of an acid (47). The identity of compound M, however, could not be completely established due to the amount available.

Concentration of the alcoholic filtrate remaining precipitated a beige solid M_1, m.p. 65-80°C. Although M_1 was roughly fractionated into three fractions (M_2, M_3, and M_4, p. 98), neither one of fractions M_2 or M_4 could be purified by recrystallization. The infra red spectrum of fraction M_2 suggested the presence of a 1:2-disubstituted benzenoid compound.

A comparison of the results obtained in this section and those obtained in a similar manner by Coblentz (13)

shows total disagreement. Coblentz reported that the residue in the retort after distillation (24.3 grams) was a pale green brittle resinous material (as compared to a dark brown, sticky residue). On precipitation of a chloroform solution of the residue into alcohol, he obtained a white resin (11.8 grams), melting at 122°C. to a clear yellow liquid. This yield is far higher than that obtained in this paper (2.8 g.), even though a larger amount of the benzene extract (89 g.) was steam distilled. Evaporation of the alcoholic filtrates by Coblentz yielded 12.5 grams of a greenish-yellow fixed oil (as compared to a dark brown syrupy residue).

IV. THE ALCOHOL EXTRACTS

Extraction

The major portion of the defatted bark was extracted in a Soxhlet extractor with ethanol 95%. The remainder of the defatted bark was moistened with ammonia prior to extraction with alcohol to determine whether larger amounts of alkaloids were released by this treatment. The first method (ethanol only) yielded dark brown extracts, representing 4.4% w/w of bark defatted by benzene or petroleum ether. The second method, however, yielded dark reddish-brown extracts representing 7.0% w/w of defatted bark.

Coblentz (13) obtained 9.4 grams of dried alcohol extract (1.88% w/w) from 500 grams of powdered bark, previously extracted with benzene. On treatment with absolute ethanol, 2.7 grams of insoluble material remained.

Methods of Extraction of Alkaloidal Material

Several methods of alkaloidal extraction were tested on a small scale to determine which methods were the simplest to use and gave the most successful results.

Method I (48), in which the alcohol extract was macerated with 1% hydrochloric acid for six hours at 80°C.,

yielded the smallest weight of the crude alkaloidal fraction (Table 23, p.102) after acid-base shakeout. The main objection to this method is that the prolonged use of heat with acidic conditions may cause destruction and decomposition of the plant constituents. This method, therefore, was not used on a large scale. It also indicated the presence of only trace amounts of quaternary alkaloids in cocillana bark extracts, since treatment of the acidified aqueous layer with mercuric chloride solution precipitated only a very small amount of solid. Furthermore, the residue recovered, after decomposition of the precipitate, failed to give a positive test with alkaloidal reagents.

Method II (48) involved the addition of dilute acid to the alcohol extract, followed by filtration and acid-base shakeout of the acid filtrate. This method was not only easy to apply but it also yielded a large amount of crude alkaloidal fraction (Table 23, p.102). Quaternary alkaloids remaining in the aqueous layer were precipitated out after acidification and treatment with a saturated aqueous ammonium reineckate solution. Only a small amount of dark brown reineckate was obtained.

Method III (48) was the most complicated process to employ. The alcohol extract was macerated with 1% hydrochloric acid for four hours at 80°C., filtered, and the acid filtrate treated with Mayer's reagent. The dark brown precipitate obtained was then decomposed to recover the

alkaloidal material. The first undesirable feature of this method is the prolonged use of heat with an acid solution (similar to Method I). The second disadvantage is the limited success in the recovery of alkaloidal material from a small amount of precipitate in cases where only small amounts of crude alkaloidal fractions occur.

Method IV, the acid-base shakeout method (p.102), was used to obtain the following information. The first step (extraction of extract Q with ether and then with chloroform) indicated that about 60% of the extract was soluble in ether. The ether-insoluble residue was only very slightly soluble in chloroform and gave negative results with alkaloidal reagents. This indicated that ether was a suitable solvent for the complete extraction of alkaloidal material. Both 20% sodium hydroxide solution and 28% ammonia were found to be equally satisfactory in releasing the alkaloids from the acid layer (Tables 24 and 25, p.106 and108). Ammonia, in particular, produced an intense red color in the aqueous layer, due to the presence of anthraquinones (p. 63). Extraction of the basified aqueous layers with ether completely extracted the freed alkaloids, since extraction with chloroform (after ether extraction) yielded fractions which were negative to alkaloidal reagents.

Method V (13) was used to compare the advantages and disadvantages of Coblentz' procedure with those of the first four methods. Occlusion of part of the alkaloidal material

in the ether-insoluble fraction was almost inevitable due to the tendency of a formation of sticky black material. The proportion of alcohol:ether (1:10), favored the formation of a flocculent tan precipitate rather than the sticky mass. A comparison of the results of Methods IV and V (Tables 24 and 25, p. 106 and 108) indicated that Method IV yielded a larger amount of crude alkaloidal fraction than Method V. The alkaloidal fractions (Q_{1a} and Q_{1b}), however, were shown to simply contain more impurities. Treatment of the acid extracts of the alkaloidal fractions with Mayer's reagent (p. 110) produced only sufficient precipitate in each case to cause a turbidity. The turbidities in each test appeared equal.

Large Scale Extraction of The Alkaloidal Material

Methods II and IV (for reasons already discussed) and Method V (for comparative study of Coblentz' report) were chosen for the large scale extraction of the alkaloidal material.

The organic phase used for the acid-base shakeouts was chloroform. Since the ether-soluble fraction, containing all of the alkaloidal material (p. 104), was completely soluble in chloroform, it was assumed that chloroform was equally appropriate for alkaloidal extraction.

Ammonia (28%) was used to basify the acid extracts to release the alkaloidal material. Although sodium hydroxide

was shown to be as reliable, it was undesirable due to the tendency to cause emulsification with chloroform.

The extracts, prepared by ethanol only, and the extracts, prepared by ethanolic extraction of ammonia-treated bark, respectively yielded 0.012% w/w and 0.023% w/w of crude alkaloidal fractions. A comparison of the yield of crude alkaloidal extract, expressed as percentage of the weight of extract obtained, (0.26 and 0.23% w/w respectively), shows little significant difference between the two types of extraction methods. Ammonia-treated bark, therefore, indirectly releases more alkaloidal material by virtue of producing larger amounts of extract (7.0% w/w as compared to 4.4% w/w, p. 99).

The crude reineckates obtained from the above processes were grossly impure, brown to black precipitates, and small in quantity. Purification by crystallization from acetone gradually caused decomposition. These precipitates, therefore, were not examined further.

The results obtained above by Method V and those obtained by Coblentz are vastly different. The acid-insoluble residue reported by Coblentz was less than 10% of the weight of extract (0.8 g. from 9.4 g. of dry extract) as compared to much more than 10% (p.112). The acid filtrate was described by Coblentz as being of a deep red color with green fluorescence, whereas the acid filtrates obtained were usually yellow to orange in color. The yield of alkaloidal

material obtained by Coblentz (0.003% w/w) was considerably smaller than the yields obtained in this report (0.012% w/w and 0.023% w/w of defatted bark). The significant difference, however, is the fact that Coblentz obtained 15 mg. of amorphous alkaloid (from 500 g. of bark). This was confirmed by Dr. Eccles (49), who isolated an alkaloid, of a light straw-yellow color, in a nearly pure state. In this survey, dark brown, semi-solid alkaloidal material (no solid) was consistently obtained.

The only similarity between the results of the two extraction processes was that ammonia intensified the color of the acid filtrate.

Examination of The Alkaloidal Fraction

Purification of the crude alkaloidal fractions by a second acid-base shakeout was attempted. The purified alkaloidal fraction S still appeared gummy and dark brown in color and the infra red spectra of the crude and purified fraction showed little improvement in the state of purity. The infra red spectra (in chloroform and in carbon disulfide) suggested the presence of a 1:2-disubstituted benzene ring with traces of hydroxyl and carbonyl, possibly as impurities (weak bands). Sodium fusion confirmed the presence of nitrogen.

The infra red spectrum of the alkaloidal fraction bore

a close resemblance to the spectrum of compound M_2 (p. 98). The alkaloidal fraction also possessed similar solubilities, i.e. very soluble in chloroform and only partially soluble in alcohol. An attempt to precipitate out solid by the same method (p.114) yielded only a trace of solid. Sodium fusion of compound M_2 confirmed the absence of nitrogen as well.

A sample of the alkaloidal fraction was subjected to gas vapor chromatography by the Smith, Kline, and French Company of Philadelphia. The Natural Products Section reported (50), however, that the alkaloidal fraction decomposed and could not be identified or purified by this process. They also confirmed the results obtained by thin layer chromatography of the alkaloidal fraction (p.117), i.e. the appearance of three to four spots which would not stain with Dragendorff's reagent.

The preparation of a derivative of the alkaloid fraction was attempted, since Coblentz reported that his alkaloidal compound formed microscopic crystals with picric acid. The preparation of a picrate of alkaloidal fraction S was unsuccessful, but the preparation of a reineckate was slightly more successful (p.115). The acid extract of alkaloidal fraction (0.703 g.) yielded a pink precipitate (0.078 g.), after treatment with saturated aqueous ammonium reineckate solution. Recrystallization of the reineckate (m.p. 128-136°C. with decomposition) from acetone and aqueous acetone gradually caused decomposition. Other attempts to

prepare reineckates produced only small amounts of impure derivative.

Steam Distillation of The Alcohol Extract

The steam distillation of the alcohol extract yielded a light green distillate with greenish-yellow oil droplets and an odor like that of the powdered bark. The steam-volatile fraction T (p.117) was negative to alkaloidal reagents, indicating that the alkaloidal material was not steam-volatile and could not be obtained in this way.

Examination of The Fluid Extract of Cocillana

A commercial product, the Fluid Extract of Cocillana, was purchased from the Penick Co. of New York. According to the information received from the manufacturer (51), cold percolation of undefatted bark (8.3 lbs.) with alcohol, followed by filtration of any sediment, was used to prepare one gallon of extract.

Two liters of the fluid extract were freed from solvent and the residue was processed for the extraction of the alkaloidal material (p.118). The extract (187.7 g., from about 1.7 Kg. of undefatted bark) yielded a brown gummy alkaloidal fraction V (0.025% w/w), which produced slight precipitate on testing with Dragendorff's and Mayer's reagents.

Examination of Solids R and R_1

Solid R (24 g.), obtained from the alcoholic extract of defatted bark (10.5 Kg.), could not be purified by recrystallization only. It was then roughly fractionated into fractions Z_1 - Z_4 (p.120), which could be purified more easily. Recrystallization attempts, however, caused the loss of much of the fractions and it is believed that column chromatography of each fraction would be more suitable for purification purposes.

Solid R_1 (14 g.) obtained from the alcohol extract of defatted bark (4.5 Kg.) treated with ammonia, was roughly subdivided into fractions Y_1 - Y_5 (p.121). The most interesting fraction Y_4 (m.p. 90-150°C.) was eventually purified to a greyish-white compound, Y_{4a}, m.p. 180-184°C.

The difficulty encountered in purifying this fraction and its isolation by concentration of an alcohol extract suggested the presence of a triterpene (52), such as α-amyrin. Sodium fusion indicated the absence of nitrogen, sulphur, and halogen. A mixed melting point of compound Y_{4a} and of authentic α-amyrin (m.p. 169-175°C.) was not depressed but melted over a wide range, m.p. 166-180°C. The infra red spectra of compound Y_{4a} and of α-amyrin (in KBr discs) both showed broad hydroxyl bands respectively at 3,448, 3,268, and 3,390 $cm.^{-1}$ (shoulder, peak, shoulder) and at 3,448 and

3,300 cm.$^{-1}$ (two peaks). The remainder of the spectrum of Y_{4a} indicated that it was still too impure for reliable and complete comparisons. A positive identification of Y_{4a} could not, therefore, be made, due to the amount available and to its still impure state.

CONCLUSION

Screening tests on cocillana bark indicated the presence of small amounts of alkaloids, of anthraquinones, possibly flavonols and sterols, and small quantities of tannins. Tests for glycosides and saponins were negative. The results of these tests were substantiated on examination of the petroleum ether, benzene, and alcohol extracts of cocillana bark.

Several volatile oil fractions were obtained by steam distillation of the petroleum ether extracts. The unsaponifiable fraction of the petroleum ether extract yielded β-sitosterol, a sterol commonly found in plants.

Examination of the benzene extracts yielded results contrary to those reported by Coblentz. The first difference noted was in the amount of benzene extract obtained. Coblentz obtained a benzol extract representing 4.99% w/w of the powdered bark, compared with an average of 1.6% w/w. By steam distillation of the benzene extract, Coblentz obtained and purified a "camphoraceous solid" (0.13% w/w), melting at 80°C. and subliming above this temperature. The same procedure applied to the benzene extracts examined for this paper yielded a few mg. of a white compound, melting at 53.5-55°C. Several volatile oil fractions were also obtained after chromatography of the steam-volatile fraction, and from one of these, another compound, subliming at 126-130°C., was isolated. Neither of

these compounds appears to be the one mentioned by Coblentz. In addition, Coblentz did not report the presence of any volatile oils.

The results from the precipitation of the steam distillation residue into ethanol are also not consistent with Coblentz' findings. For example, Coblentz filtered off 11.8 grams of a white resin (2.36% w/w), melting at 122°C. The alcoholic filtrate, on evaporation, left 12.5 grams (2.50% w/w) of a greenish-yellow fixed oil. In the present examination, a much smaller yield of precipitate was obtained. This precipitate was found to be composed of several small fractions, none melting in the vicinity of 122°C. The alcoholic filtrate left a dark brown, sticky residue after concentration.

Investigation of the alcohol extracts again showed discrepancies between the results. Coblentz obtained a dry alcohol extract (1.88% w/w), from which he isolated 15 mg. of amorphous alkaloid. This alkaloid formed microscopic crystals with picric acid. The present studies indicated that the solvent-free alcohol extracts averaged 4.4% w/w of defatted bark. The alkaloidal fractions obtained were consistently dark brown, semi-solid fractions with a characteristic odor. The alkaloidal material was never isolated in a solid state and failed to form a picrate derivative. A small quantity of a reineckate was obtained, but this derivative slowly decomposed in attempts to crystallize it from acetone.

Steam distillation of the alcohol extract proved that the alkaloidal material was not steam-volatile and could not, therefore, be isolated by this method. Thin layer chromatography of the alkaloidal fraction produced three to four spots, which refused to stain with Dragendorff's reagent.

After concentration and cooling (prior to alkaloidal extraction), the alcohol extracts deposited a precipitate which contained many different components. Partial purification of one fraction yielded a comparatively pure compound, possibly of a triterpenoid nature.

The many discrepancies found on examination of the benzene and alcohol extracts lead to the conclusion that the cocillana bark used in commerce today is probably not the same species of Guarea examined by Dr. V. Coblentz.

It is also possible that seasonal variations may account for the different types and quantities of the plant constituents isolated. The bark examined by Coblentz may have been collected in a season different from the one in which cocillana bark for commercial use is now normally collected.

E X P E R I M E N T A L

Melting points are uncorrected and were determined using the Thomas Hoover Capillary Melting Point Apparatus. In a few cases where only a small amount of solid was available, the melting point was determined on a block.

Rotations were determined in a one decimeter tube with a Bellingham and Stanley polarimeter and a sodium lamp (589 mu). Ultraviolet absorption studies were determined in the stated solvent on the Beckman DU Spectrophotometer and on the Hilger Ultrascan Recording Spectrophotometer. Infra red absorption spectra were recorded by the Beckman IR8 Infrared Spectrophotometer, using an 0.4 mm. path length with carbon disulfide as the solvent, except where otherwise specified. Refractive indices were determined with the Zeiss refractometer at the temperature stated. Carbon and hydrogen analyses were determined by the use of the Coleman Carbon and Hydrogen Analyser.

For thin layer chromatography, Silica Gel G for thin layer chromatography (Fisher Scientific Company) was prepared and then spread on glass plates by the De Saga apparatus. For column chromatography, alumina (Aluminium oxide for chromatographic absorption analysis - B.D.H.) was used. The petroleum ether used boiled over the range 40-60°C.

The author is greatly indebted to the following: Professor G. Blunden, formerly in the Pharmacognosy Department in the School of Pharmacy, for the authentication of cocillana bark; Mr. Walter Kremers and Mr. Gilbert Beaudin

for determination of infrared absorption spectra and carbon and hydrogen analyses; Smith, Kline, and French for gas chromatography of a portion of the alkaloidal fraction.

I. SCREENING PROCEDURES

Cocillana bark was screened for alkaloids, glycosides, saponins, sterols, flavonols, tannins and anthraquinones. In all of the tests for alkaloids, two samples of powdered cocillana bark (Guarea rusbyi (Britton) Rusby), one of powdered cinchona bark (Cinchona succirubra), and one of powdered liquorice root (Glycyrrhiza glabra) were tested at the same time. The results are denoted in the following manner:

Ppte. denotes a precipitate; + denotes turbidity
- denotes no precipitate ; ++ denotes a definite ppte.
± denotes uncertainty ; +++ denotes an abundant ppte.

Screening for Alkaloids

Wall's Method (21)

Thirty grams each of cinchona bark and liquorice root were separately extracted with 600 ml. of ethanol in Soxhlet extractors for twenty-four hours. Five grams of cocillana extract A' and eight grams of cocillana extract B' (both containing some solvent and representing about thirty grams of plant material) were each diluted to 600 ml. with ethanol.

Aliquot portions of the solutions (120 ml.) of the four extracts, equivalent to five to ten grams of plant material,

were evaporated to dryness on a water bath. The residues were each dissolved in twenty-five ml. of hot water and filtered. Each ml. of aqueous solution, therefore, represented 0.2-0.4 gram of plant material.

One ml. of each of the four aqueous extracts were acidified with 0.5 ml. of 1% hydrochloric acid and divided into four portions. The four portions were separately tested with Dragendorff's (17), Mayer's (18), silicotungstic acid (19), and Wagner's (20) reagents. The results are shown in Table 1.

Table 1

Extract	Acidification	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	-	++	+	+	++
Cocillana B'	-	++	±	±	++
Cinchona	-	+++	+++	+++	+++
Liquorice	Yellow ppte.	-	-	-	-

If a precipitate was obtained on acidification or after the addition of an alkaloidal reagent, a confirmatory test was carried out. Five ml. of the aqueous extract were basified with 1% sodium hydroxide solution and extracted with an equal volume of chloroform. The chloroform extract was shaken out with an equal volume of 1% hydrochloric acid and the acid

extract was tested with the reagents. The results for the cocillana extracts A' and B' are shown in Table 2.

Table 2

Extract	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	-	±	±	-
Cocillana B'	-	±	±	-

The Swanholm Prollius Fluid Method (23)

Five grams of each sample of powdered plant material were macerated in thirty ml. of Prollius fluid for fifty-six hours in tightly-stoppered flasks. Prollius fluid is composed of: ether:chloroform:ethanol:ammonia in the ratio 25:8:2.8:1 v/v. After filtration, the marcs were washed with twenty ml. of Prollius fluid. The combined extracts were evaporated to dryness on a water bath and dried in an oven. The residues were macerated with four ml. of 1% hydrochloric acid for one hour at 80°C. After cooling and filtering, the acid filtrates were divided into four portions and tested with alkaloidal reagents. The results are shown in Table 3.

Table 3

Extract	Description	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	Yellow	++	+	+	++
Cocillana B'	Yellow	++	+	+	++
Cinchona	Bright Yellow	+++	+++	+++	+++
Liquorice	Pale Yellow	±	-	-	+

The Swanholm Hydrochloric Acid Method (23)

Five grams of each sample of plant material were mixed with twenty ml. of 1% hydrochloric acid to form slurries. The mixtures were heated at 80°C. for six hours, cooled, and filtered. Sufficient 1% hydrochloric acid was passed through each marc to yield two to three ml. of extracts. Each extract was tested as usual. Table 4 contains the results obtained.

Table 4

Extract	Description	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	Bright Yellow	+++	++	+++	+++
Cocillana B'	Golden Yellow	+++	++	+++	+++
Cinchona	Bright Red	+++	+++	+++	+++
Liquorice	Orange	+++	+++	+++	+++

The Kiang, Douglas, and Morsingh Method (24)

Fifteen grams each of cinchona bark and liquorice root and two separate thirty gram samples of cocillana bark were extracted respectively with 85 ml. each and 170 ml. each of chloroform in Soxhlet extractors. The chloroform was then removed at 40-50°C. by vacuum distillation on a water bath. Each residue was dried in an oven at 65°C. for five to six hours and then rubbed thoroughly with six ml. of 2 N hydrochloric acid. The filtrates were treated as usual. The results are shown in Table 5.

Table 5

Extract	Description	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	Pink	++	+	+	±
Cocillana B'	Orange-pink	++	++	++	+
Cinchona	Dark reddish-brown	+++	+++	+++	+++
Liquorice	Yellow	+	-	-	+

Arthur's Modification of The Webb Method (26)

Small specimen tubes (5 x 1 cm.) were half-filled with plant samples and covered with Prollius fluid. The tubes were stoppered and set aside for two days. The supernatants were drawn off and allowed to evaporate to dryness on watch glasses at room temperature. The residues were rubbed with 1% hydrochloric acid and allowed to stand for one hour. One sample of cocillana (B') was warmed for a few seconds on a water bath with the acid. The results are shown in Table 6.

Table 6

Extract	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	±	±	±	+
Cocillana B'	++	±	±	+
Cinchona	+++	+++	+++	+++
Liquorice	-	-	-	-

The Kleber Method (25)

Fifty grams of each plant sample were mixed with 100-115 ml. of Prollius fluid and shaken mechanically for one hour. The mixtures were then macerated for twenty-four hours and filtered. Each filtrate was extracted with thirty ml. of 2% sulphuric acid. The results of the tests with alkaloidal reagents are shown in Table 7.

Table 7

Extract	Dragendorff's	Mayer's	Silicotungstic Acid	Wagner's
Cocillana A'	++	+	±	++
Cocillana B'	+++	++	+	+++
Cinchona	+++	+++	+++	+++
Liquorice	-	-	-	-

The Farnsworth and Euler Method (27)

Nine grams of cinchona bark, sixteen grams (A') and ten grams (B') of cocillana bark, and six grams of wild cherry bark (Prunus serotina) were tested. Each sample was macerated with 28% ammonia (one ml. per gram) for one hour. The samples were dried on a water bath. Twenty ml. of chloroform were added to each and the mixtures were refluxed for thirty minutes on a water bath under air condensers. The mixtures were filtered and the marcs were again extracted with twenty ml. of fresh chloroform for thirty minutes. The combined filtrates were concentrated on a water bath to about two ml. (Fraction 1).

The marcs were dried overnight and then refluxed with twenty ml. of ethanol containing 0.5% hydrochloric acid for thirty minutes. The mixtures were cooled and filtered. The filtrates were concentrated to yield Fraction 2.

Fraction 1 from each drug sample dissolved in two ml. of chloroform was shaken out once with two ml. of 1% hydrochloric acid. The chloroform layer was discarded and the aqueous layer was basified with 28% ammonia. The basic aqueous layer was extracted twice with one ml. of chloroform. The chloroform extract was spotted on Silica Gel G plates.

The residues of Fraction 2 were each stirred with one ml. of water and filtered through wet filter paper. The

filtrates were spotted on the plates.

The Silica Gel G chromatography plates were prepared in the following manner. Thirty grams of Silica Gel G were mixed with forty ml. of distilled water. Twenty ml. of water were added and the mixture quickly stirred to break up lumps. The mixture was spread over glass plates at a thickness of 50 mu with the De Saga applicator. The plates were air-dried for five minutes and then activated in an oven at 95°C. for three hours. After cooling, the plates were spotted with three drops of the solutions of Fractions 1 and 2. The plates were developed with B.A.W. (n-butanol: acetic acid;water - 4:1:1 v/v) for a distance of fourteen to fifteen cm. and a time of 140 minutes (Fraction 1) and 150 minutes (Fraction 2). After development, the plates were air-dried and viewed under an ultra-violet lamp before spraying with Dragendorff's reagent. The results are shown in Table 8.

Table 8

Fraction and Extract	Ultraviolet Light	Dragendorff's
1-Cocillana A'	Small blue spot at 10 cm.; small blue spot at 13 cm.; streak at the solvent front.	No reaction.
1-Cocillana B'	Similar to cocillana A' but less definite.	No reaction.
1-Cinchona	Blue dot at bottom; turquoise streak up to center.	Reddish-orange color, 4.5 cm. long, in center.
1-Wild cherry	Small blue dot at center; small blue dot at 14 cm.	No reaction.
2-Cocillana A'	Turquoise blue spot at top.	No reaction.
2-Cocillana B'	Same as cocillana A'.	No reaction.
2-Cinchona	Three large spots (blue to turquoise) at center; two small spots between center and base line.	Three large spots stained reddish-orange.
2-Wild cherry	One turquoise spot at top; two turquoise and one green spots between 6-12 cm.; one small spot at about 3 cm.	No reaction.

The results of all of the alkaloidal screening tests applied to cocillana bark are listed in Table 9.

Table 9

Method	Dragen-dorff's	Mayer's	Silico-tungstic Acid	Wagner's
Wall's Method	++	±	±	++
Wall's Confirmatory Test	-	±	±	-
Swanholm Prollius Fluid Method	++	+	+	++
Swanholm Hydrochloric Acid Method	+++	++	+++	+++
Kiang, Douglas and Morsingh Method	++	+	+	±
Arthur's Modification of The Webb Method	+	±	±	+
Kleber Method	++	+	+	++
Farnsworth and Euler Method	-	-	-	-

Screening for Glycosides

A modification of the Stas-Otto process (28) was used to test for glycosides in cocillana bark. Three hundred grams of powdered bark were refluxed for twelve hours on a

water bath with 700 ml. of ethanol containing fourteen grams of tartaric acid. The extract was filtered and the marc washed twice with hot ethanol. The combined alcoholic solutions were evaporated to dryness on a water bath under vacuum. The residue was treated with four fifty ml. portions of hot water. The aqueous extract was shaken out four times with fifty ml. of ether. The combined ethereal solutions were evaporated to dryness. The residue was dissolved in alcohol and tested with Molisch's reagent (53). No violet ring appeared.

Screening for Saponins

In Fischer's method (29), small pieces of quillaja wood (Quillaja saponaris) and of cocillana bark were placed on a blood agar plate and allowed to stand. After one hour, a small zone of hemolysis (a clear zone) surrounded the quillaja sample. After twelve hours, there was a clear zone (about one inch in diameter) around the quillaja sample but none around the cocillana sample.

Samples of both were refluxed for two hours with a saturated solution of cholesterol in methanol. The samples of wood and bark were dried and again placed on blood agar plates. No zones of hemolysis occurred with either of the samples.

In Wall's method (21), an aliquot of cocillana bark

extract (prepared as described on page 48) was evaporated to dryness. The residue was dissolved in ethanol and made up to one liter. One ml. of the aliquot was added to ten ml. of a saline red blood cell suspension. No hemolysis occurred. The test was repeated using one ml. of an aliquot prepared by dissolving the residue in twenty-five ml. of ethanol (as compared to one liter). No hemolysis occurred.

Screening for Sterols

The residue obtained from the evaporation of an aliquot of the alcohol extract of cocillana bark (refer to Wall's method, p. 48) was dissolved in twenty-five ml. of chloroform. One to two ml. of concentrated sulphuric acid were mixed with two to three ml. of the chloroform solution. After one hour, the acid layer was deep red in color and the chloroform layer pale pink. Two to three ml. of fresh chloroform extract were treated with acetic anhydride, followed by the addition of concentrated sulphuric acid. The red color produced by the acetic anhydride changed to a brown color on mixing with the acid (Liebermann-Burchard test) (30).

Screening for Flavonols

The test for flavonols was performed on the original alcoholic aliquots (prepared by Wall's method, p. 48), and

is known as the Bryant test (31). One ml. of each alcohol extract was mixed with one ml. of 10% hydrochloric acid and magnesium turnings. In all cases, it was difficult to note the appearance of a red color produced by the reduction of the magnesium due to the color of the extracts tested. Each reaction mixture was then shaken with five to seven ml. of amyl alcohol and allowed to stand overnight. In the cases of cocillana A' and B', the lower aqueous layer was slightly pink in color. The cinchona test showed a red lower layer and that of liquorice was yellow in color.

Screening for Tannins

The dried aliquots of the alcohol extracts of cocillana A' and B', cinchona, and liquorice prepared by Wall's method were each dissolved in twenty-five ml. of hot water and filtered. A few drops of the aqueous extracts were tested with 1% gelatin solution and ferric chloride, T.S. The results are shown in Table 10.

Table 10

Extract	1% Gelatin	Ferric Chloride, T.S.
Cocillana A'	No reaction	Olive green color
Cocillana B'	No reaction	Olive green color
Cinchona	White precipitate	Dark olive green color
Liquorice	Abundant, light yellow precipitate	Dark brown color and precipitate

In Hoch's spot tests (32), one hundred mg. of cocillana bark and of liquorice root were each macerated in one hundred ml. of distilled water for fifteen minutes at room temperature. The mixtures were filtered and tested (without any dilution) with 1% gelatin solution, 5% ferric sulphate solution, 5% copper acetate solution, and saturated potassium dichromate solution. Each reagent was tested with distilled water as controls. The results are shown in Table 11.

Table 11

Reagent	Cocillana Extract	Liquorice Extract
1% Gelatin solution	-	++
5% Copper acetate solution	-	++
5% Ferric sulphate solution	-	-
Saturated potassium dichromate solution	-	-

Screening for Anthraquinones

An alcohol solution (10 ml.) of cocillana extract prepared from 100 g. of cocillana bark was tested by the Wasicky modification of the Borntrager reaction (33). The solution was boiled with 10% sulphuric acid (4 ml.) for five minutes, cooled, and made up to twelve ml. with water. The acid extract was shaken out with benzene (10 ml.), which in turn was shaken out with 10% ammonia solution (10 ml.). The benzene layer became bright yellow after shaking out the acid extract, but changed to a dark red color after shaking out with 10% ammonia.

II. THE PETROLEUM ETHER EXTRACTS

Extraction

The powdered cocillana bark was defatted by cold percolation with petroleum ether. An attempt to defat the bark in a Soxhlet extractor of twenty liter capacity proved to be unsuccessful due to premature siphoning of the extract.

The percolation was carried out in the following manner. Lots of approximately three kilograms of the powdered bark (mesh number 60) were packed in the customary manner into percolators fitted with stopcocks. After overnight maceration in petroleum ether, the percolation was regulated to flow at the rate of 0.5 to 0.75 l. per hour. The first six liters of percolate collected were bright yellow in color. This fraction was concentrated separately (fraction A_1) from the remaining percolate. As the extraction progressed, the percolate colour gradually diminished. To determine whether the bark had been extracted exhaustively, a residue test was used. One hundred ml. of percolate were evaporated on a water bath to near dryness, dried in the air, and the residue weighed. Percolation was stopped when a maximum of 100 mg. of residue per liter of percolate were obtained. Approximately eighteen liters of percolate were collected from every three kilograms of bark. The

latter twelve liters of percolate were combined and concentrated as a second fraction, A_2.

An electric heating mantle was used to remove the solvent from the percolate until 500 ml. of extract were left. The extract was further concentrated by distillation on a water bath to prevent charring. The extract was then air dried at room temperature until a constant weight was obtained.

The average weight of extract (e.g., A_1) obtained from the first six liters of percolate was 25.5 g. and from the remaining twelve liters (e.g., A_2), 2.6 g. Fractions A_1 and A_2 were found to be similar and were thus combined as one. A total of 167.5 grams of petroleum ether extract (A_3) was, therefore, obtained from 17.9 Kg. of ground bark (mesh number 60). In a similar manner, extract A_4 (163.6 grams) and extract A_5 (47.3 grams) were respectively obtained from 16 Kg. of powdered bark (mesh number 100) and 4.5 Kg. of powdered bark (approximately mesh number 50).

On standing in the refrigerator, extract A_3 deposited a white solid. The syrupy extract was diluted with cold ether and ethanol and the solid (A_{3a}) was filtered off, m.p. 69-94°C., (0.39 g.). An infra red spectrum (C, 1.5 in carbon disulfide) showed peaks at: three between 2,800 and 3,000 $cm.^{-1}$ (methylene linkages), 1,240, 1,163, and 1,110 $cm.^{-1}$ (C-O of triglyceride ester) (54), 1,739 $cm.^{-1}$ (C=O of long chain fatty ester) (55), and 715 $cm.^{-1}$ (long unbranched

paraffin chain, 17-36 carbon atoms) (56). The infra red spectrum of glyceryl monostearate (in carbon disulfide) was very similar. Therefore, solid A_{3a} was not examined further.

Saponification

Two methods of saponification were applied to the petroleum ether extracts.

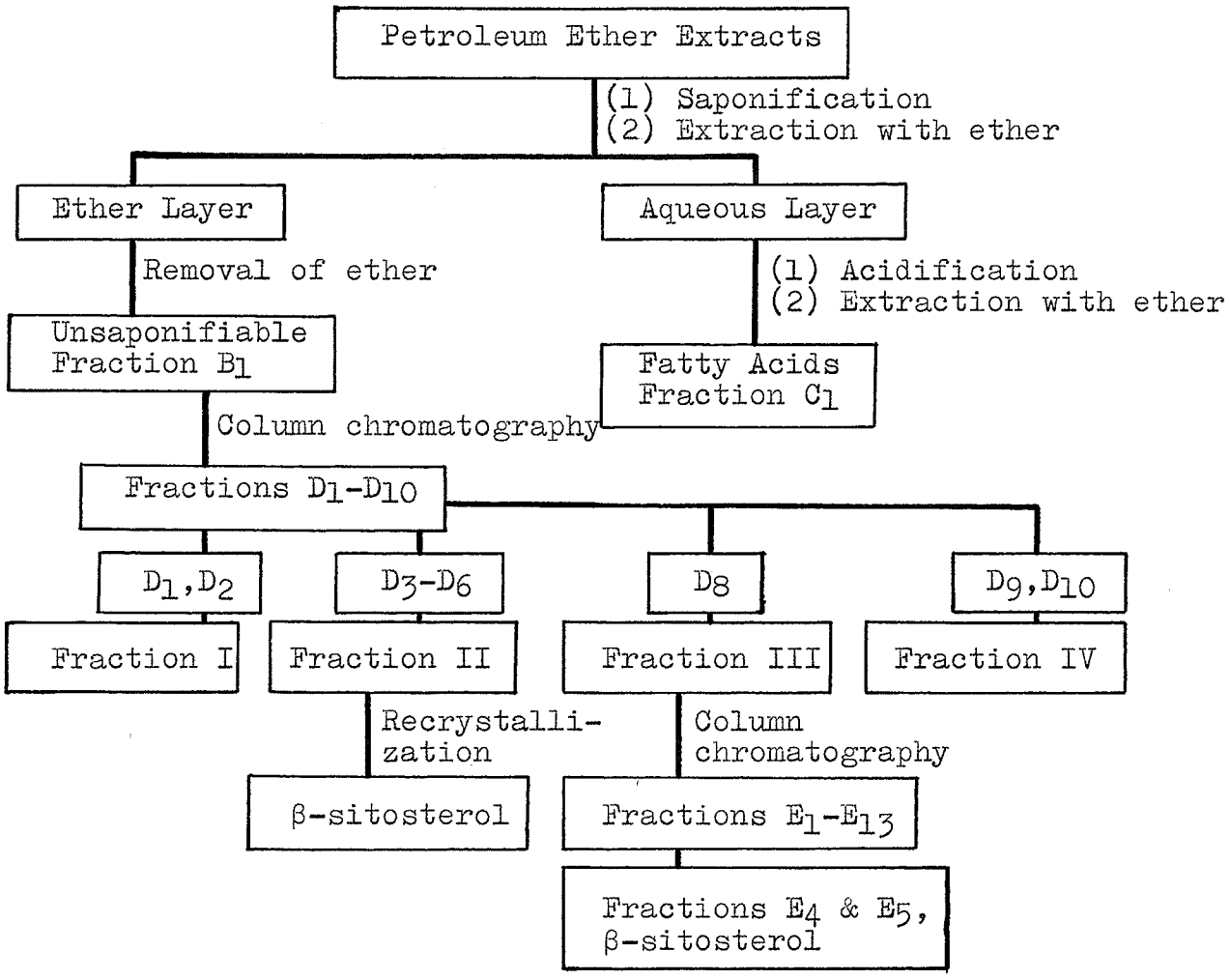
The first method used was the method described in the British Pharmacopoeia 1958 (34). Extract A_3 (31.1 g.) was mixed with N/2 alcoholic potassium hydroxide (375 ml.) and refluxed for one hour on a water bath. The dark, reddish-brown reaction mixture was washed into a separatory funnel with water (600 ml.), forming a turbid yellow solution. This was shaken out several times with ether, until the ether extract was colorless. The combined ether extracts were washed three times with water, filtered, and then washed three times with aqueous N/2 potassium hydroxide. After washing the ether extract free of alkali (to phenolphthalein), the ether was distilled off on a water bath. A small volume of acetone was added and air was blown through to remove the last traces of water. The residue was dried to constant weight in an oven at 65°C. This unsaponifiable fraction B_1 was a reddish colored viscous material (15.77 g.), (Figure 1).

However, an error in the weight of B_1 was later discovered and the experiment was repeated as follows:

extract A_5 (10.0 g.) was treated in the same manner as the above, except that the washed ether extracts were dried over anhydrous sodium sulphate before the ether was distilled off. The unsaponifiable fraction B_2 was dried to constant weight (5.52 g.) in an oven at 70°C.

The second method of saponification used was the method of Fitzgerald et al (35).

Figure 1



Ten grams of extract A_5 were refluxed with ethanol (95%; 250 ml.), containing sodium hydroxide (12 g.), for three hours on a water bath. On cooling, a few mg. of a tan solid were filtered off, m.p. - did not melt at 340°C. The cooled alcoholic reaction mixture was poured into water (300 ml.). Although a turbid solution resulted, no solid precipitated out. The alcohol was removed by warming on a water bath and the cooled aqueous solution was extracted with ether in a separatory funnel until the ether extract was colorless. The washed ether extract was dried and the ether distilled off. The unsaponifiable material B_3 was dried at room temperature to a constant weight (6.36 g.).

The average yield of unsaponifiable material in the petroleum ether extracts was found to be 59.4% w/w of the extract.

The aqueous portions of the saponification reactions were treated to recover the freed fatty acids. The aqueous layer was acidified with 10% or with concentrated sulphuric acid. The reddish-orange aqueous solution changed to a dirty yellow color and a yellow scum settled out. The solution was extracted exhaustively with ether. After washing free from acid (universal indicator paper), the orange ether extract was dried, the ether distilled off, and the residue air-dried to constant weight.

Fatty acid residue C_1 (12.12 g.) and residue C_2 (3.92 g.) were obtained respectively from petroleum ether extracts

A_3 (31.1 g.) and A_5 (10.0 g.). These weights represent an average of 39.1% w/w of the petroleum ether extracts. Residues C_1 and C_2 were not examined further.

Chromatography of The Unsaponifiable Fraction B_1

The total fraction B_1 was dissolved completely in a minimum volume of petroleum ether and placed on a charcoal-cellulose powder (3:1) column, (50.5 cm. x 3.5 cm.). Activated charcoal and Whatman cellulose powder, Standard grade, (W. and R. Balston, Ltd., England) were used. The eluate was collected in 50 ml. fractions and bulked together as shown in Table 12.

Table 12

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Petroleum ether (3 l.)	1-49	D_1	1.5454	Slightly yellow, wax-like residue.
Carbon tetrachloride (6 l.)	50-56	D_2	0.0596	Similar to D_1.
	57-147	D_3	2.6362	Yellow residue with traces of solid.
1% Benzene in carbon tetrachloride (2 l.)	148-183	D_4	0.7422	Similar to D_3.
10% Benzene in carbon tetrachloride (3 l.)	184-245	D_5	1.8350	Similar to D_3.
Benzene (5.5 l.)	246-345	D_6	3.4821	Similar to D_3.
10% Anhydrous ethanol in chloroform (6 l.)	346-352	D_7	0.1330	Yellow residue.
	353-415	D_8	10.1612	Dark brown, mobile residue with a pleasant odor.
	416-468	D_9	1.1642	Dark brown residue.
20% Anhydrous ethanol in chloroform, (3 l.); ethanol 95%, (2 l.)	469-563	D_{10}	1.8195	Similar to D_9.

After examination, the fractions were bulked together into several large fractions (Table 13).

Table 13

Fraction Number	Fractions Bulked	Description
I	D_1 and D_2	Waxy materials when cooled; liquid at 20°C.
II	D_3, D_4, D_5, and D_6	Deep yellow residues, depositing white solid on cooling.
III	D_8	Dark brown, mobile fraction.
IV	D_9 and D_{10}	Dark brown residue.

Investigation of Fraction I

Fraction I solidified to a whitish mass on cooling. However, it melted at room temperature to a clear liquid. It was not examined as more interesting fractions were available.

Investigation of Fraction II

On cooling, fractions D_3 to D_6 (Table 13) each deposited a white crystalline material. Fraction D_5 yielded the largest amount of the solid (IIa). Investigation of fractions D_3,

D_4, and D_6 showed that the same solid (IIa) was found in all of the fractions. Therefore, fractions D_3 to D_6 were bulked together as Fraction II.

Solid IIa was filtered off and the adhering yellow "oil" was washed away with cold petroleum ether. The filtrate was diluted with ethanol and cooled again, yielding more solid. Compound IIa (500 mg., crude, from D_5) was crystallized four times from ethanol yielding glistening white needles, m.p. 135.0-135.5°C. The melting point was undepressed on admixture with authentic β-sitosterol (crystallized from ethanol), m.p. 139.5-140.5°C. Compound IIa gave a pink color with acetic anhydride and when mixed with a drop of concentrated sulphuric acid, a violet color appeared followed by a deep bluish-green (Liebermann-Burchard test), (30). $[\alpha]_D^{24^\circ}$ -37° (C, 1.3 in chloroform).

Found	C, 82.45; H, 12.20.
Calculated for $C_{29}H_{50}O$ (36)	C, 83.99; H, 12.15.

The acetate of the compound IIa and of β-sitosterol were prepared by the Shriner and Fuson method (57). Compound IIa (75 mg.) was refluxed for five minutes with pyridine (8 ml.), and acetic anhydride (5 ml.). The reaction mixture was poured into cold water (30 ml.). The white product was filtered off and recrystallized out of ethanol. The acetate of compound IIa melted at 125.5-126.0°C., and the melting point was undepressed by admixture with authentic β-sitosterol acetate, m.p. 126.5-128°C.

The infra red spectra of compound IIa and of β-sitosterol (C, 2.5 in carbon disulfide in both cases) were identical.

Investigation of Fraction III

Fraction III was dissolved in benzene and placed on an alumina column, (48.5 cm. x 3.5 cm.). 25 ml. fractions of eluate were collected and bulked in the usual manner. The results are given in Table 14.

Table 14

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Benzene (1.5 l.)	1-54	E_1	0.3933	Yellowish, wax-like residue.
20% Ether in benzene (1.5 l.)	55-107	E_2	0.3608	Bright yellow residues with traces of white solid.
Ether-benzene (1:1) (3.5 l.)	108-119	E_3	0.0705	Similar to E_2.
	120-121	E_4	0.2337	Similar to E_2.
	122-141	E_5	1.1164	Dark orange residue.
	142-215	E_6	0.4926	Similar to E_2.
Ether (1.5 l.)	216-269	E_7	0.2449	Reddish-brown residue.

TABLE 14 CONTINUED

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
10% Chloroform in ether, (1.5 l.); Chloroform-ether (1:1); (0.5 l.); Chloroform (0.5 l.)	270-359	E_8	0.3079	Yellow residue with solid.
2% Absolute ethanol in chloroform, (2 l.); 5% Absolute ethanol in chloroform, (1.5 l.); 10% Absolute ethanol in chloroform, (1 l.)	360-411	E_9	0.3090	Brown solid.
	412-491	E_{10}	0.3134	Yellow residue with traces of solid.
95% Ethanol, (2.5 l.); Methanol, (1.0 l.)	492-593	E_{11}	0.4206	Dark reddish-brown residue.
1% Glacial acetic acid in methanol, (0.5 l.); 3% Glacial acetic acid in methanol, (5.5 l.)	594-633	E_{12}	0.0686	Yellow residue.
	634-840	E_{13}	-	

On the basis of quantity and the presence of definite solids, fractions E_1, E_2, E_5, E_6, E_8, and E_{10} were examined

from their infra red spectra. The spectra were used as a guide to the purity of the fractions and to ascertain the existence of any interesting compounds and not as a means of identification. Only fractions E_4 and E_5 were examined further.

Fraction E_4 was dissolved in ether and made cloudy by the addition of ethanol. On cooling, a light yellow solid (E_{4a}) was obtained, m.p. 133-141°C., (8.4 mg.). Fraction E_5, after treatment similar to that of E_4, yielded a yellow solid (E_{5a}), m.p. 128-140°C., (68.7 mg.). A mixed melting point of solids E_{4a} and E_{5a} was undepressed. The solids were combined, crystallized from ethanol and identified as β-sitosterol.

Investigation of Fraction IV

Fraction IV was crystallized from methanol. A dark brown solid was filtered off (91.8 mg.). On warming with methanol, off-white solid particles were precipitated out (did not melt by 315°C.). The fraction was not characterized further.

Steam Distillation

31.5 grams of extract A_3 were mixed with water (100 ml.) and steam distilled. The first portion of distillate

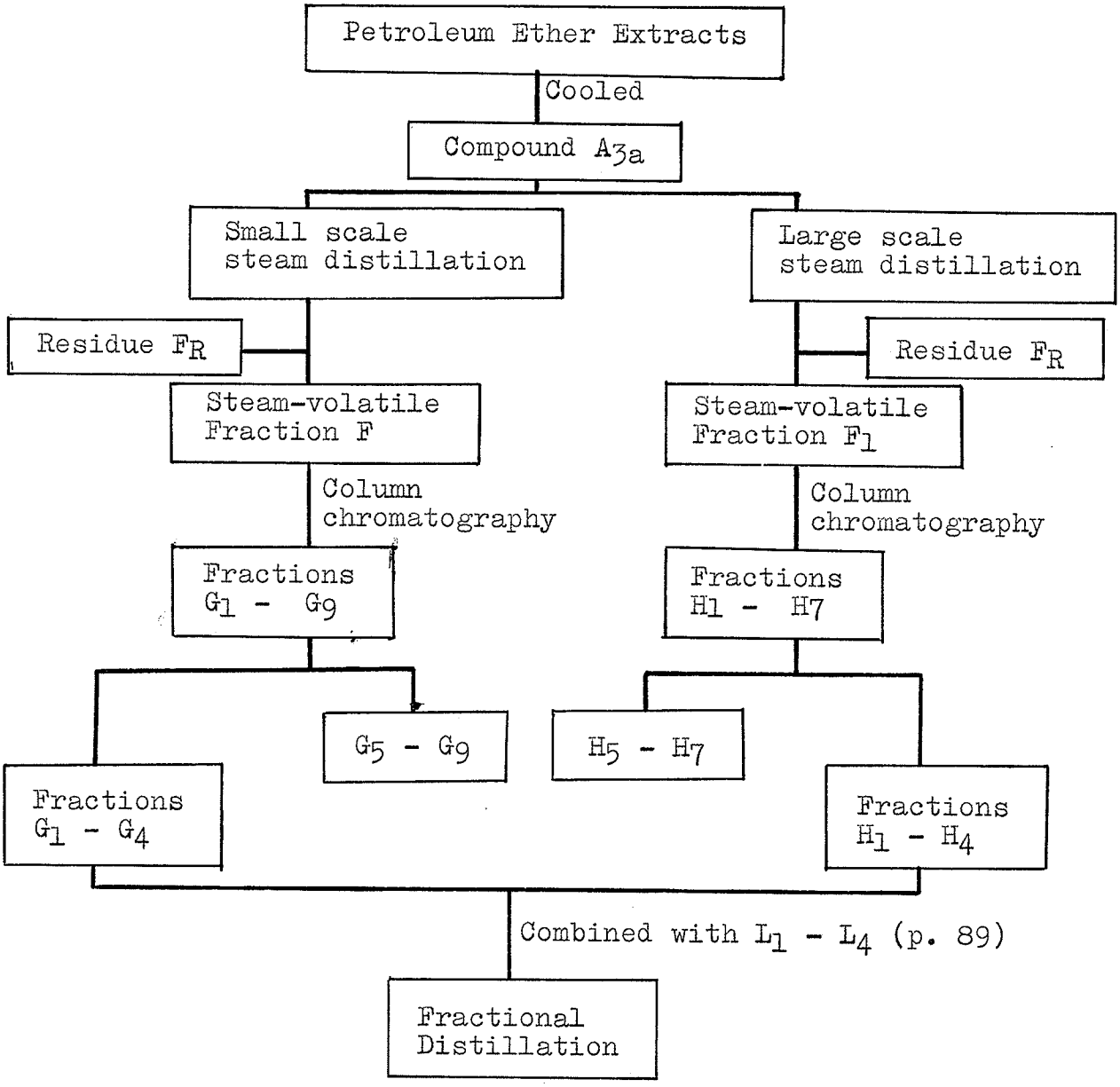
(3.5 l.) contained large droplets of a yellowish-green oil with a peculiar odor and was acidic to litmus. The distillate was extracted with ether until the ether layer was colorless. The aqueous layer was saturated with sodium chloride and extracted several times with ether. Seven liters of neutral distillate were also collected and extracted with ether in one liter portions.

The combined yellow ether extracts were washed, dried over anhydrous sodium sulphate, and the ether removed. A dark brown fraction F was obtained (4.71 g.), (Figure 2).

Similarly, the remainder of extract A_3 and all of extract A_4 were steam distilled to yield a steam-volatile fraction F_1 (27.12 g.).

The residues in the distillation flasks (F_R) were freed from water and not examined further.

Figure 2


Petroleum Ether Extracts
Cooled
Compound A3a
Small scale steam distillation
Large scale steam distillation
Residue FR
Residue FR
Steam-volatile Fraction F
Steam-volatile Fraction F1
Column chromatography
Column chromatography
Fractions G1 - G9
Fractions H1 - H7
G5 - G9
H5 - H7
Fractions G1 - G4
Fractions H1 - H4
Combined with L1 - L4 (p. 89)
Fractional Distillation

Chromatography of Fraction F

The steam-volatile fraction F was taken up in petroleum ether for chromatography on an alumina column, (29.0 cm. x 2 cm.). 25 ml. fractions of eluate were collected and bulked together as shown in Table 15.

Table 15

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Petroleum ether (1.6 l.)	1-4	G_1	1.2000	Yellow, very mobile oil with a pleasant odor.
	5-26	G_2	0.7361	Similar to G_1, but a deeper yellow.
5% Benzene in petroleum ether (0.7 l.)	27-36	G_3	0.2990	More viscous oil.
Benzene (1.5 l.)	37-45	G_4	0.8004	Deep yellow, viscous oils.
	46-66	G_5	0.2861	
10% Ether in benzene (1.0 l.)	67-85	G_6	0.3934	Yellow residue with traces of solid.
Ether (0.5 l.)	86-89	G_7	0.1060	Similar to G_6.
Chloroform (0.4 l.); 10% Ethanol in chloroform, (0.4 l.); 20% Ethanol in chloroform, (0.5 l.)	90-103 104-119	G_8	0.1550	Orange-yellow residue.
95% Ethanol (0.7 l.); Methanol, (1.7 l.)	120-179	G_9	0.4064	Dark brown residue.

Fractions G_1 to G_4 were combined with fractions H_1 to H_4 (Table 16, p. 81) and fractions L_1 to L_4 (Table 17, p. 86) for fractional distillation (p. 89).

Fractions G_5 to G_9 yielded only negligible amounts of solids when warmed with ethanol and were not examined further.

Chromatography of Fraction F_1

A petroleum ether solution of the steam-volatile fraction F_1 was placed on an alumina column, (46 cm. x 3.5 cm.), for chromatography. 25 ml. fractions of eluate were collected and combined as shown in Table 16.

Table 16

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Petroleum ether (1.0 l.)	1-17	H_1	5.9988	Yellow, mobile oil with a fragrant odor.
	18-35	H_2	2.5839	Yellow, less mobile oil.
5% Benzene in petroleum ether (1.0 l.)	36-71	H_2	-	
	72-79	H_3	0.1731	Yellow, thicker oil
Benzene, (1.5 l.); 10% Ether in benzene, (2.0 l.)	80-222	H_4	10.4013	Golden yellow, viscous oil.
Ether, (1.5 l.); Chloroform, (1.0 l.); 5% Ethanol (95) in chloroform, (1.5 l.); 10% Ethanol(95) in chloroform, (0.5 l.)	223-388	H_5	4.3038	Reddish oil with traces of solid.
Ethanol 95% (1.0 l.)	389-434	H_6	0.2357	Dark brown residue.
Methanol (1.0 l.)	435-464	H_7	0.1302	Dark, yellowish-brown residue.

Fractions H_1 to H_4 were combined with fractions G_1 to G_4 (Table 15) and fractionns L_1 to L_4 (Table 17, p. 86) and were fractionally distilled (p. 89).

Fraction H_5 failed to precipitate any solid on warming with ethanol. Fractions H_5 to H_7 were not examined further.

III. THE BENZENE EXTRACTS

Extraction

Batches of approximately three kilograms of powdered bark were extracted exhaustively with benzene in a Soxhlet extractor. Both defatted and undefatted barks were used.

The benzene was removed by vacuum distillation on a water bath. The concentrated extract was air dried at room temperature to constant weight.

Extract J_1 (32.8 g.) was obtained from 3 Kg. of defatted bark (mesh number 60) and extract J_2 (56 g.) was obtained from 7 Kg. of undefatted bark (mesh number 100). Small scale extraction of undefatted bark indicated that the benzene-soluble portion of cocillana bark averaged 1.6% w/w of the plant material.

Steam Distillation

The benzene extracts J_1 and J_2 were independently steam distilled as follows (Figure 3, p. 85).

One hundred ml. of 3% tartaric acid in water were mixed with extract J_1 and the steam distillation carried out. The distillate was cloudy, contained yellowish-green oil globules and was slightly acidic (3.20 ml. of N/10 sodium hydroxide

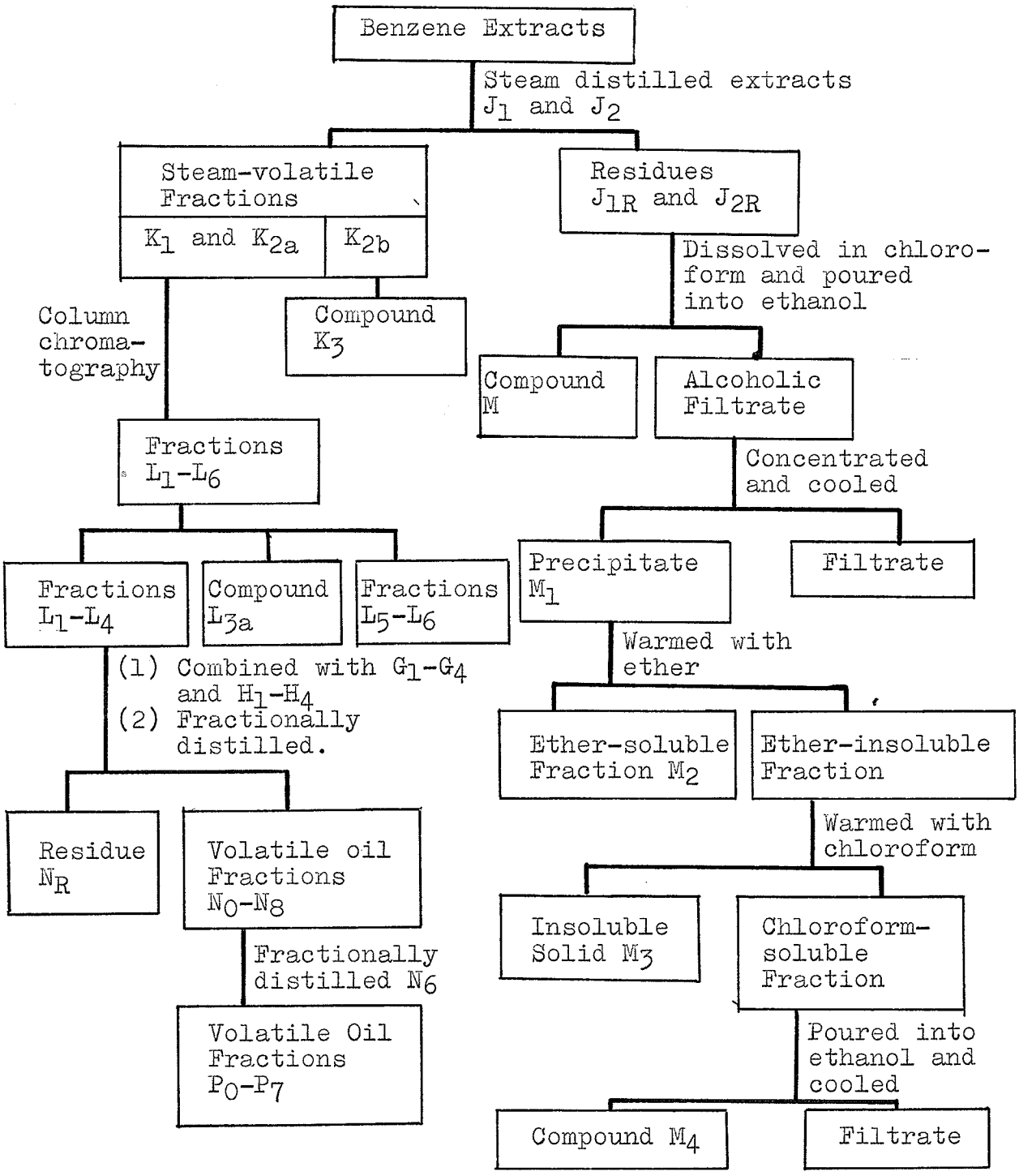
neutralised one liter of the distillate to phenolphthalein, after extraction with ether). A total of 20-21 l. of distillate was collected and extracted in three liter lots with ether (7 x 150 ml.). The aqueous layers were discarded.

The combined ether extracts were washed and dried (anhydrous sodium sulfate), the ether was distilled off and the fraction K_1 was air dried to constant weight (5.00 g.).

Similarly, extract J_2 yielded fraction K_{2a} (9.67 g.) from the first eighteen liters of distillate and fraction K_{2b} (1.11 g.) from the last twelve liters of distillate.

The residues in the distilling flasks (J_{1R} and J_{2R}) were dried and examined (p. 97).

Figure 3


Benzene Extracts
Steam distilled extracts J_1 and J_2
Steam-volatile Fractions
K_1 and K_{2a}
K_{2b}
Residues J_{1R} and J_{2R}
Dissolved in chloroform and poured into ethanol
Column chromatography
Compound K_3
Compound M
Alcoholic Filtrate
Fractions L_1-L_6
Concentrated and cooled
Fractions L_1-L_4
Compound L_{3a}
Fractions L_5-L_6
Precipitate M_1
Filtrate
(1) Combined with G_1-G_4 and H_1-H_4
(2) Fractionally distilled.
Warmed with ether
Ether-soluble Fraction M_2
Ether-insoluble Fraction
Residue N_R
Volatile oil Fractions N_0-N_8
Warmed with chloroform
Insoluble Solid M_3
Chloroform-soluble Fraction
Fractionally distilled N_6
Poured into ethanol and cooled
Volatile Oil Fractions P_0-P_7
Compound M_4
Filtrate

Chromatography of Fractions K1 and K2a

Fractions K_1 and K_{2a} were combined, dissolved in petroleum ether and transferred quantitatively to a column of alumina (44 cm. x 3.5 cm.). 25 ml. fractions of eluate were collected and bulked together as shown in Table 17. Every tenth fraction was checked for optical rotation, using the eluting solvent as a blank.

Table 17

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Petroleum ether (1 l.)	1-42	L_1	2.6906	Pale yellow, mobile oil.
5% Benzene in petroleum ether (0.5 l.)	43-58	L_2	0.7828	Similar to L_1.
Benzene (1.5 l.)	59-72	L_3	0.3723	Pale oil with colorless crystals.
Benzene; 10% ether in benzene, (1 l.); Ether (1.5 l.)	73-210	L_4	3.8653	Yellow to orange viscous oil.

Table 17 Continued

Eluant	Fractions Collected	Fraction Number	Weight (g.)	Description
Chloroform (0.5 l.); 5% Ethanol (95) in chloroform (1.l.); 10% Ethanol (95) in chloroform (0.5 l.)	211-295	L_5	0.3619	Bright yellow residue with a pleasant odor.
Ethanol 95% (1 l.); Methanol (1 l.)	296-365	L_6	0.2693	Dark, reddish-brown residue.

None of the fractions tested showed optical rotation.

Fractions L_1 to L_4 (except for the crystals in L_3) were combined with fractions G_1 to G_4 (Table 15, p. 79) and fractions H_1 to H_4 (Table 16, p. 81) for fractional distillation (p. 89).

The crystals of fraction L_3 were filtered off and washed with cold petroleum ether, yielding colorless platelets, L_{3a} (18.9 mg.), subliming at 126-130°C. $[\alpha]_D^{24^\circ}$ +9.5° (C, 0.68 in chloroform); λ max. 255 mu, $E^{1\%}_{1\ cm.}$ 1,004 (C, 0.000675 in ethanol). The infra red spectra (C, 1.2 in chloroform and C, 2.4 in carbon disulfide) showed peaks at: 3,077 cm.$^{-1}$ (aromatic -CH), 3,021 cm.$^{-1}$ (cis disubstituted double bond), 2,941 cm.$^{-1}$ (shoulder; possibly a methyl),

2,907, 2,841 cm.$^{-1}$ (methylene linkages), 1,876 (weak), 1,757 and 1,721 cm.$^{-1}$ (weak; 1:4-disubstituted benzene ring), 1,653 cm.$^{-1}$ (shoulder; cis disubstituted double bonds), 1,377 cm.$^{-1}$ (C-CH_3 group), 1,235, 1,233, and 1,295 cm.$^{-1}$ (unassigned), 1,171, 1,110, 1,025, 1,006 (all weak) and 832 and 807 cm.$^{-1}$ (strong; 1:4-disubstituted benzene ring), 1,014, 1,000 cm.$^{-1}$ (unassigned), 1,087 cm.$^{-1}$ (acyclic ether), 702 cm.$^{-1}$ (strong; cis disubstituted double bond), 832, 719, 671 cm.$^{-1}$ (unassigned).

Investigation of Fraction K_{2b}

At the completion of the steam distillation of extracts J_1 and J_2, the condenser was washed free from adhering material with ether. These washings combined with fraction K_{2b} deposited a white solid on standing. The solid K_3 was filtered off, washed with cold petroleum ether, and crystallized (from benzene) (13 mg.), m.p. 53.5-55°C. (block). $[\alpha]_D^{24^\circ}$ -11.5° (C, 1.237 in chloroform); λmax. 257 mu, $E^{1\%}_{1\ cm.}$ about 38 (C, 0.01723 in ethanol). The infra red spectra (C, 1.275 in carbon disulfide and C, 2.4 in chloroform) showed peaks at: 3,509 cm.$^{-1}$ (weak; hydroxyl), 3,333 to 2,500, 2,667 cm.$^{-1}$ (broad band with a shoulder; carboxyl), 1,739 and 1,689 cm.$^{-1}$ (shoulder at 1,739 cm.$^{-1}$; C=O), 1,269, 1,250, 1,111, 926 cm.$^{-1}$ (C-O), 1,471 cm.$^{-1}$ (C-CH_3 group), and 714, 671, 645 cm.$^{-1}$ (unassigned).

About three mg. of solid K_3 were dissolved in ethanol (95%; 2 ml.) and 2:4-dinitrophenylhydrazine reagent (referred to as D.N.P.; 2 to 3 ml.) was added. The solution after standing for twelve hours remained clear. Addition of water precipitated out fine dark red needles. The mixed melting point of the needles and of the dinitrophenylhydrazone was undepressed, m.p. 176-187.5°C. Thin layer chromatography (p. 93) also indicated that the needles were identical to the original reagent.

Fractional Distillation of The Volatile Oils

Fractions G_1 to G_4 (Table 15, p. 79), fractions H_1 to H_4 (Table 16, p.81), and fractions L_1 to L_4 (Table 17, p. 86) were combined for fractional distillation. The mixture was subjected to fractional distillation under vacuum on an oil bath. The physical constants of the fractions obtained N_0 to N_8 are summarized in Table 18.

Table 18

Fraction Number	Description	Boiling Point °C./mm. Hg	Weight (g.)	Specific Gravity (H_2O at 26°C.)	Specific Rotation (C, 5 in $CHCl_3$)	Refractive Index t, 24.9°C.
N_0	colorless, mobile oil	73-80/760	0.7947	-	-	-
N_1	cloudy, yellowish oil	80-120/33	0.4869	-	-	-
N_2	yellow oil	120-134/28	0.3758	-	-	-
N_3	yellow oil	154-160/28	3,5900	0.9119 (26°)	+2.18°	1.4880
N_4	yellow oil	160-165/28	3.3840	0.9453 (22°)	+2.19°	1.5036
N_5	yellow oil	90-98/0.12	2.3438	0.9574 (23°)	+0.16°	1.5078
N_6	greenish-yellow oil	94-110/0.09	5.1936	0.9954 (24°)	-1.70°	1.5156
N_7	emerald green, viscous oil	110-118/0.05	2.8495	1.044 (25°)	-0.84°	1.5273
N_8	olive green, viscous oil	127-134/0.10	1.9441	-	-1.52°	-

The residue after the distillation (N_R) was dark brown in color and weighed about 10 g.

Investigation of Oil Fractions N_0 to N_8

Infra red spectra of fractions N_0 to N_8 and N_R were quite similar, indicating that the fractionation of the oils into distinct fractions was not too successful.

Fractions N_0 to N_8 and N_R were each treated with 2:4-dinitrophenylhydrazine reagent (D.N.P. reagent) according to the Shriner and Fuson method (58). The oil (approximately 200 mg.) was dissolved in ethanol (95%; 2 ml.) and freshly-prepared D.N.P. reagent (5-8 ml.) added. The mixtures were allowed to stand for forty-eight hours at room temperature and then examined. The results are shown in Table 19.

Table 19

Oil Fraction	Weight Reacted (mg.)	Results
N_0	202.7	No reaction.
N_2	203.4	Black, gummy mass.
N_3	209.1	Black solid (117.7 mg.; m.p. 53-69°C.)
N_4	235.3	Black, gummy mass.
N_5	222.1	Black solid (169.1 mg.; m.p. 53-71°C.)

Table 19 Continued

Oil Fraction	Weight Reacted (mg.)	Results
N_7	212.4	Black, gummy mass.
N_8	252.0	Black gummy mass.
N_9	316.3	Dark red crystals mainly (353.3 mg.; m.p. 59-96°C.)

Thin layer chromatography of the D.N.P. derivatives of N_5, N_8, N_R, and K_3 (p. 89) was carried out with various solvent systems. The D.N.P. derivatives of camphor and benzaldehyde and D.N.P. reagent itself were run as controls.

The chromatography plates, using Silica Gel G, were prepared as described on page 56. The developing tanks were equilibrated with the developing solvents for at least four hours, depending on the volatility of the solvents used. Five drops of an ethereal solution of each derivative and five drops of D.N.P. reagent were spotted on the plates and developed for a distance of 115 cm.

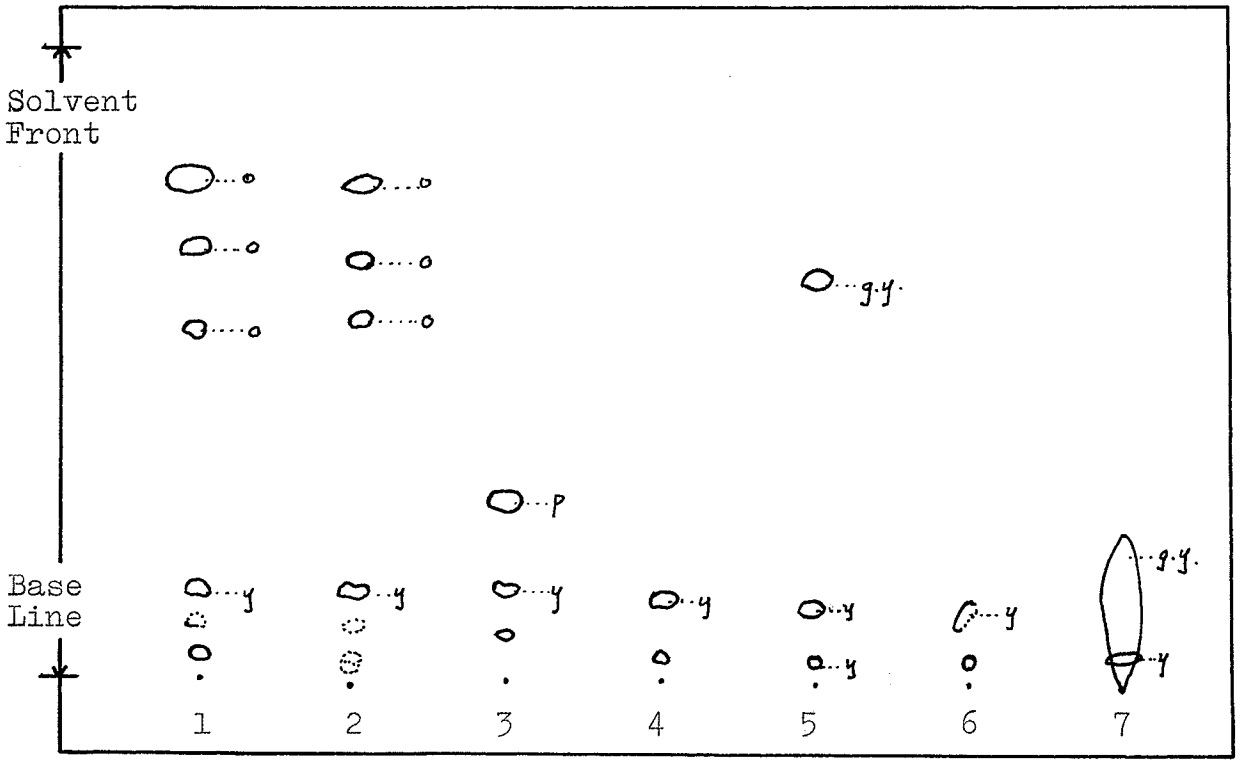
Similar results were obtained by the use of 5% ether in petroleum ether and of 15% benzene in petroleum ether as developing solvents (59). The results are shown in Table 20.

Table 20

D.N.P. Derivative of The Fraction	Results
N_5	Three to four spots.
N_8	Three spots in the same region as N_5.
N_R	One faint pink spot.
K_3	A small faint streak.
Benzaldehyde	One spot.
Camphor	Two spots.
D.N.P. reagent	Similar to K_3.

More complete resolution of the fractions was produced by the use of the solvent system (59), 10% acetone in petroleum ether. The results are shown in Figure 4.

Figure 4



Thin-layer chromatograms of D.N.P. derivatives from samples 1-7. 1, N_5; 2, N_8; 3, N_R; 4, K_3; 5, camphor; 6, benzaldehyde; 7, D.N.P. reagent. Solid lines indicate a well-defined spot; broken lines indicate a faint spot; the following colors are denoted by: O-orange; y-yellow; p-pink; g.y.-golden yellow. Chromatograms were developed with 10% acetone in petroleum ether on a matrix 50 mu) of Silica Gel G.

In an alternative experiment, four drops of an ethereal solution of each oil were spotted on the plates and then developed with 12% ethyl acetate in hexane (60). The dried plates were viewed under ultraviolet and then sprayed with D.N.P. reagent. The results obtained after spraying with D.N.P. reagent are summarized in Table 21.

Table 21

Fractions	Number of Spots
N_3	Five to six spots.
N_4	Up to eight spots.
N_5	Similar to N_4.
N_6	Similar to N_3.
N_7	Similar to N_3.
N_8	Similar to N_4.
N_R	Streaked; no compact spots.

Fractional Distillation of Oil Fraction N_6

Fraction N_6 (5.19 g.; Table 18) was fractionally distilled in the same manner as described on page 89. The data obtained are recorded in Table 22.

Table 22

Fraction Number	Description	Boiling Point °C./mm. Hg	Weight (g.)	Specific Gravity (H_2O at 24°C.)	Refractive Index
P_0	pale yellow, mobile fragrant oil	79.5-84.0/0.02	1.4991	1.005 (25°C.)	1.5099 (24.7°C.)
P_1	similar to P_0	85.0-88.0/0.02	0.8787	1.005 (25°C.)	1.5121 (25.5°C.)
P_2	fragrant, yellow oil	89.0-91.0/0.02	0.8439	1.009 (25°C.)	1.5159 (26°C.)
P_3	yellow oil	98.0-102.5/0.02	0.3709	1.014 (26°C.)	1.5182 (26°C.)
P_4	viscous, yellow oil	103.0-107.0/0.02	0.2608	-	1.5205 (26.5°C.)
P_5	bright yellow, viscous oil	107.5-112.0/0.02	0.1360	-	1.5191 (26.5°C.)
P_6	similar to P_5	112.0-117.0/0.02	0.2991	1.047 (25°C.)	1.5241 (26.5°C.)
P_7	similar to P_5	117.5-119.0/0.02 dropped.	0.2681	1.071 (24°C.)	1.5262 (26.5°C.)

The infra red spectrum of fraction P_0 (C, 1.76 in carbon disulfide) was identical to the spectrum of P_2 (C, 1,5 in carbon disulfide), indicating that the attempted fractionation was not very successful.

Investigation of Residues J_{1R} and J_{2R}

The residues from the steam distillation J_{1R} and J_{2R} were combined, dissolved in a minimum volume of chloroform and poured into an excess of ethanol (95%; 1.5 l.). A fluffy precipitate formed and was filtered off as a light beige solid M (93 mg.), m.p. 235-240°C., with decomposition. After boiling with ethanol 95%, solid M became white (56 mg.), m.p. 239-241°C. with decomposition. Solid M was insoluble in ether, petroleum ether, benzene and chloroform but crystallized out of water as colorless crystals. Sodium fusion showed nitrogen to be present.

Found: C, 31.84; H, 5.51.

Calculated for formula $C_2H_5NO_2$: C, 32.00; H, 6.71.

A mixed melting point with glycine was depressed, m.p. 190°C. The infra red spectrum of compound M (potassium bromide disc) showed peaks at: 3,279 $cm.^{-1}$ (very strong) and 1,408 $cm.^{-1}$ (strong), (ammonium salts, NH_4^+).

The alcoholic filtrate (after precipitation of M) was concentrated and cooled, yielding a beige solid M_1 (1.83 g.), m.p. 65-ca 80°C. Solid M_1 was roughly fractionated into

three fractions in the following manner. The precipitate M_1 was warmed with three portions of ether, producing the ether-soluble fraction M_2 (1.29 g.), m.p. 61-70°C. The ether-insoluble residue was dissolved in chloroform (60-70 ml.), leaving the insoluble fraction M_3 (did not melt at 325°C.). The chloroform solution was poured into ethanol (95%; 175 ml.), thus precipitating fraction M_4 (0.15 g.), m.p. 76-90°C. Repeated attempts to purify fractions M_2 and M_4 by recrystallization from such solvents as ether, benzene, chloroform, methanol, and ethanol failed to reduce their melting ranges. The infra red spectrum of fraction M_2 (C, about 1.5 in carbon disulfide) showed peaks at: 2,950 $cm.^{-1}$ to 2,841 $cm.^{-1}$ (methylene groups), 1,721 and 1,698 $cm.^{-1}$ (carbonyl, possibly of an ester), 1,284, 1,206 $cm.^{-1}$ (possibly $C(CH_3)_3$), and 1,174, 1,098, 1,031, 1,005 $cm.^{-1}$ (weak), 754 $cm.^{-1}$ (strong; 1:2-disubstituted benzene) (61).

The dark brown alcoholic filtrate was not examined further.

IV. THE ALCOHOL EXTRACTS

Extraction and Preparation

The defatted bark was extracted in one of two ways: with ethanol only or with ethanol after moistening thoroughly with 28% ammonia (one ml. per gram of bark).

A typical procedure for the preparation of an extract, with alcohol only, follows. The bark, in three kilogram lots, was extracted exhaustively with ethanol (95%) in a Soxhlet extractor. The extract was filtered hot to remove loose bark particles and then concentrated to about a liter on a water bath under vacuum at 40°C. On cooling, the extract deposited a light tan precipitate (R) which was filtered off and examined later (p.119). The alcoholic filtrate was then concentrated under vacuum until no more solvent distilled, yielding an average of 137 g. of extract (4.4% w/w of defatted bark).

Similarly, defatted bark (3 Kg.) moistened with ammonia and extracted with ethanol (95%) yielded an average of 210 g. of extract (7.0% w/w of defatted bark).

Methods of Extraction of The Alkaloidal Material

Several general methods for the extraction of alkaloids

from alcohol extracts were tested on a small scale basis. The first three methods were carried out on equal amounts of an alcohol extract of cocillana bark (5 grams of extract containing some solvent). The results for these three experiments are shown in Table 23, p.102.

Method I (48)

An alcoholic solution of the cocillana extract (about 5 g.) was macerated with 1% hydrochloric acid on a water bath for six hours at 80°C. After filtration, the acid extract was basified with 28% ammonia and extracted with chloroform. The washed chloroform extracts were dried over anhydrous sodium sulphate and concentrated to constant weight.

The ammoniacal aqueous layer was acidified with dilute hydrochloric acid to pH 2 and treated with mercuric chloride solution (62). The brown precipitate (10 mg.) was suspended in ethanol and hydrogen sulfide was passed through for two minutes. After filtration, the solution was evaporated to dryness. A test of the residue with Dragendorff's reagent was negative.

Method II (48)

The alcoholic extract (5 g.) was mixed with dilute sulphuric acid and filtered. The filtrate was basified with

28% ammonia and extracted with chloroform. The basic fraction was obtained after drying and concentrating the chloroform layer.

The aqueous portion was acidified with dilute sulphuric acid and a saturated aqueous solution of ammonium reineckate was added. A black precipitate (17.5 mg.) was filtered off.

Method III (48)

The alcoholic extract (5 g.) was freed from traces of alcohol and the residue was macerated with 1% hydrochloric acid on a water bath for four hours. The cooled mixture was filtered and the filtrate was treated with Mayer's reagent (18). The dark brown precipitate (70.7 mg.), which was obtained, was decomposed by warming with saturated sodium carbonate solution on a water bath for half an hour. The reaction mixture was filtered and neutralised with dilute hydrochloric acid. After basifying with ammonia, the solution was extracted with chloroform, yielding an alkaloidal fraction of 1.9 mg. A slight turbidity was produced on testing with Dragendorff's reagent.

Table 23

Method	Tertiary Alkaloids		Quaternary Alkaloids		
	Weight (mg.)	Test with Dragendorff's Reagent	Weight of Precipitate (mg.)	Weight Recovered (mg.)	Test
I	14.2	++	Mercuric chloride 10.0	1.8	-
II	87.3	+++	Reinec-kate 17.5	-	-
III	-	-	Mayer's 70.7	1.9	±

Method IV

The simplified acid-base shakeout method of alkaloidal extraction was tested on an alcohol extract (Q; 7.30 g.), prepared from 180 g. of undefatted bark. During concentration of the alcohol extract, about 0.2 g. of solid was filtered off and not examined further, since it was the same type of precipitate (R) which was examined on a large scale (p.119).

Extract Q was warmed with several portions of ether on a water bath until the ether extract was colorless. After concentration, the ether-soluble fraction (Q_1) was obtained (4.57 g.). The ether-insoluble residue was extracted with boiling chloroform until the chloroform extract was colorless.

The chloroform-soluble fraction (Q_2) weighed 0.22 g., leaving 2.43 g. of insoluble material (Figure 5, p.105).

The ether-soluble fraction (Q_1) was extracted with 1% hydrochloric acid (7 x 25 ml.). The ether layer was washed free from acid and the washings were combined with the acid extract.

The acid extract of Q_1 was divided in half, one half being basified with 20% sodium hydroxide solution and the other half with 28% ammonia. Each basified aqueous portion was extracted with ether (5 x 25 ml.), until a test of the ether extract was negative to Mayer's reagent. The ether extracts were washed free from alkali, dried and concentrated as the alkaloidal fractions, Q_{1a} and Q_{1b}. The same basic aqueous portions, after ether extraction, were extracted with chloroform. However, negative tests with Mayer's reagent indicated that the ether had completely extracted the alkaloidal material.

The ethereal solution of fraction Q_1, after acid extraction, was extracted with 20% sodium hydroxide solution (9 x 25 ml.). The reddish-black extract and washings of the ether layer were acidified with concentrated hydrochloric acid. The acidified solution was extracted with ether (2 x 100 ml.; 5 x 50 ml.). The combined ether extracts were washed free from acid and concentrated to yield the acidic fraction (Q_{1c}). The acidified aqueous layer was then extracted with chloroform (1 x 100 ml.; 6 x 60 ml.), which was washed free

from acid and concentrated to yield fraction Q_{1d}.

The ether layer, after extraction with 1% hydrochloric acid and 20% sodium hydroxide solution, was dried and concentrated to yield the neutral fraction (Q_{1e}).

The chloroform-soluble fraction (Q_2) of the original alcohol extract was extracted with 1% hydrochloric acid. The acid extract was basified (half with 20% sodium hydroxide solution and half with 28% ammonia) and each half was extracted with ether. The ether extracts gave negative tests with Mayer's reagent, indicating that fraction Q_2 contained no alkaloids. The results are listed in Table 24.

Figure 5

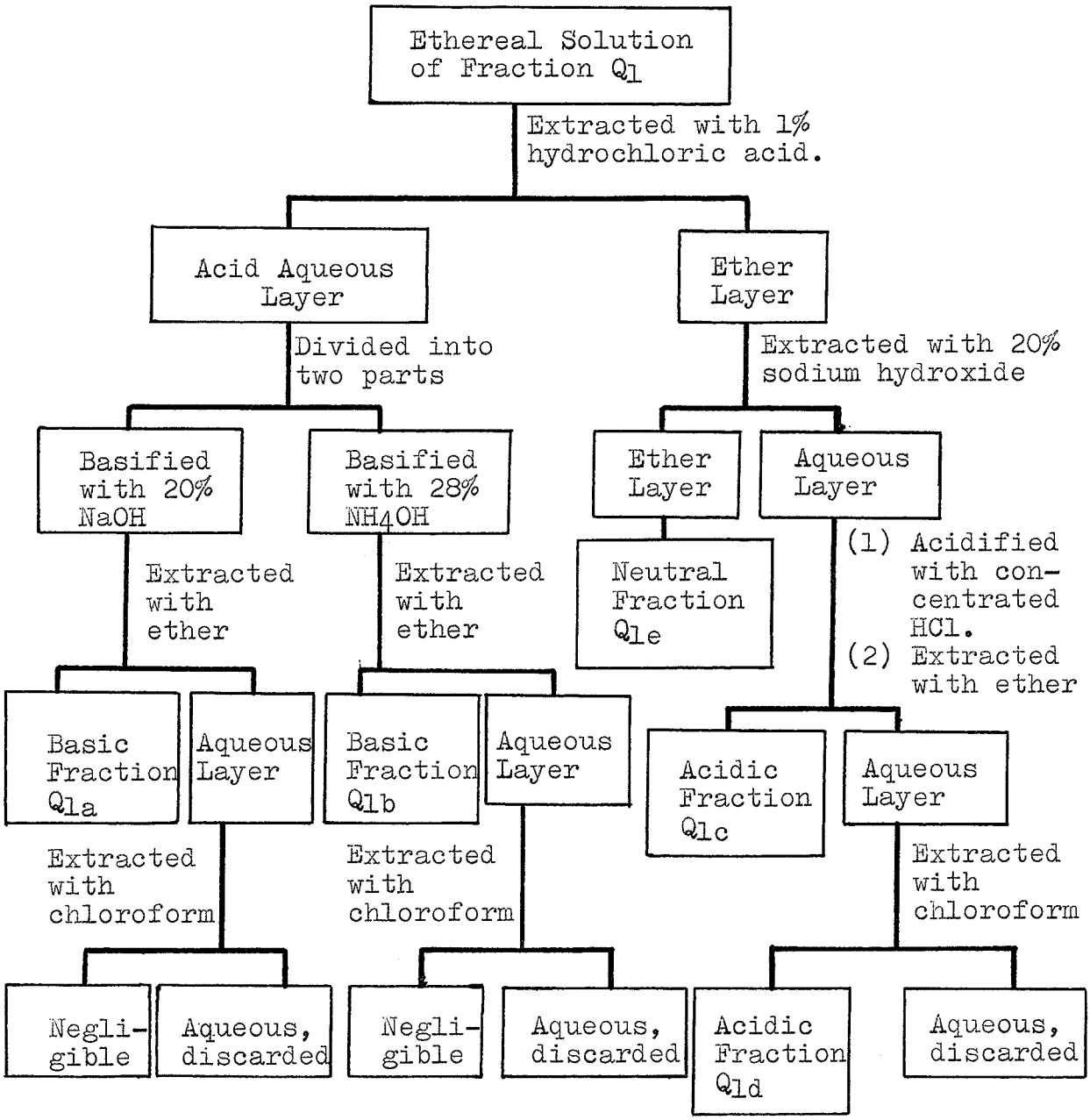

Ethereal Solution of Fraction Q1
Extracted with 1% hydrochloric acid.
Acid Aqueous Layer
Ether Layer
Divided into two parts
Extracted with 20% sodium hydroxide
Basified with 20% NaOH
Basified with 28% NH4OH
Ether Layer
Aqueous Layer
Extracted with ether
Extracted with ether
Neutral Fraction Q1e
(1) Acidified with concentrated HCl.
(2) Extracted with ether
Basic Fraction Q1a
Aqueous Layer
Basic Fraction Q1b
Aqueous Layer
Acidic Fraction Q1c
Aqueous Layer
Extracted with chloroform
Extracted with chloroform
Extracted with chloroform
Negligible
Aqueous, discarded
Negligible
Aqueous, discarded
Acidic Fraction Q1d
Aqueous, discarded

Table 24

Extract	Basic Fraction				Acidic Fraction		Neutral Fraction
	Sodium Hydroxide		Ammonium Hydroxide		Ether-soluble	Chloroform-soluble	
	Ether	Chloroform	Ether	Chloroform			
Ether-soluble, Q_1	45.8 mg. (Q_{1a})	Negligible	63.8 mg. (Q_{1b})	Negligible	1.97 g. (Q_{1c})	0.05 g. (Q_{1d})	1.43 g. (Q_{1e})
Chloroform-soluble, Q_2	Nil	Nil	Nil	Nil	-	-	-

Method V (13)

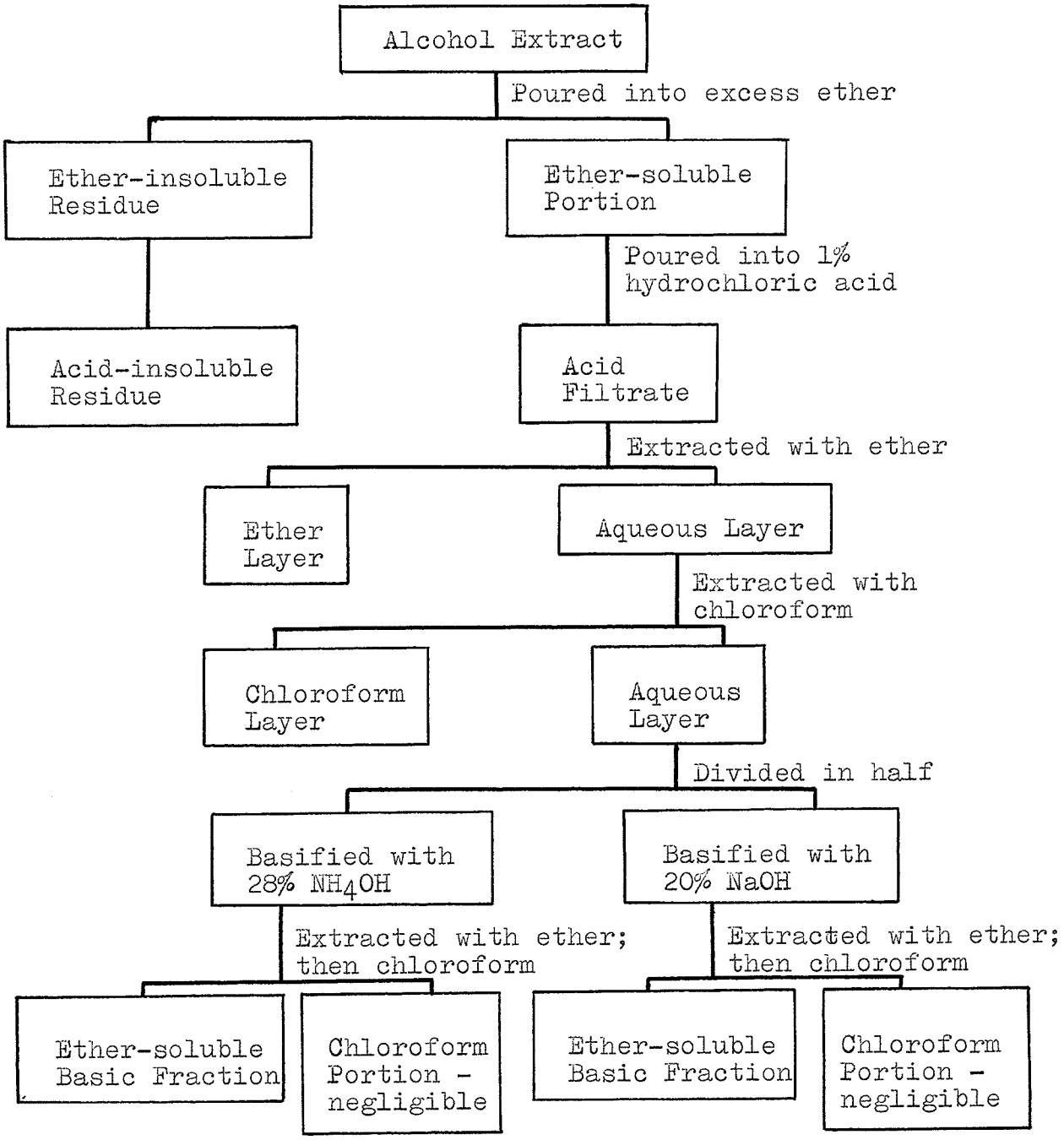
For comparative studies, the method of alkaloidal extraction utilized by Coblentz was carried out. 8.28 g. of an alcohol extract of bark (200 g.) and 13.1 g. of an alcohol extract of bark (180 g.), previously moistened with ammonia (28%), were both tested (Figure 6, p.109).

The extracts were dissolved in small amounts of ethanol (95%) and poured with stirring into an excess of ether. The clear ethereal supernatants were decanted off and combined with ether washings of the insoluble materials precipitated out. Alcoholic solutions of the ether-soluble portions were poured into 1% hydrochloric acid. The acid filtrates were extracted with ether and then with chloroform. The aqueous layers were basified, half with 20% sodium hydroxide and half with 28% ammonia. The basified solutions were each extracted with ether and then with chloroform. The results are shown in Table 25.

Table 25

Extract	Ether-insoluble Residue	Acid-insoluble Residue	Shakeout of Acid Filtrate with:		Shakeout of Bases			
					NH_4OH		NaOH	
			Ether	Chloroform	Ether	Chloroform	Ether	Chloroform
Alcohol Extract, (8.28 g.)	3.66 g.	3.02 g.	1.21 g.	0.24 g.	9.6 mg.	Nil	10.2 mg.	Nil
Alcohol-Ammonia Extract, (13.1 g.)	7.58 g.	2.70 g.	0.64 g.	0.21 g.	11.7 mg.	Nil	12.1 mg.	Nil

Figure 6


Alcohol Extract
Poured into excess ether
Ether-insoluble Residue
Ether-soluble Portion
Poured into 1% hydrochloric acid
Acid-insoluble Residue
Acid Filtrate
Extracted with ether
Ether Layer
Aqueous Layer
Extracted with chloroform
Chloroform Layer
Aqueous Layer
Divided in half
Basified with 28% NH_4OH
Basified with 20% NaOH
Extracted with ether; then chloroform
Extracted with ether; then chloroform
Ether-soluble Basic Fraction
Chloroform Portion - negligible
Ether-soluble Basic Fraction
Chloroform Portion - negligible

The basic fractions (9.6 and 10.2 mg.; Table 25) were combined in chloroform and extracted three times with 1% hydrochloric acid. The acid extracts were shaken out once with chloroform and then Mayer's reagent (25 ml.) was added.

The basic fractions Q_{1a} and Q_{1b} (Table 24, p.106), were similarly extracted and Mayer's reagent was added.

Each of the acid extracts became turbid but the precipitates could not be filtered off.

Large Scale Extraction of The Alkaloidal Material

Three methods (II, IV, and V, pages100,102, and107) were used to extract the alkaloidal material. Typical experiments, using each method, are described.

The extract (202 g.), prepared by alcohol extraction of bark, previously treated with ammonia, was warmed gently on a water bath with 500 ml. of dilute sulphuric acid, (Method II). After cooling and filtering, the reddish-brown filtrate was extracted with chloroform until the chloroform layer was colorless. The chloroform extracts were dried, evaporated, and not examined further, (negative to alkaloidal reagents).

The acid washings and aqueous layer were basified with 28% ammonia (150 ml.) and the cherry red solution was shaken out twenty times with chloroform. After washing, drying (over anhydrous sodium sulphate), and evaporating, the

chloroform extracts yielded 0.675 g. of basic fraction (0.023% w/w of the defatted bark), which gave abundant precipitates with Mayer's and Dragendorff's reagents.

The ammoniacal aqueous portion was acidified with concentrated sulphuric acid (100 ml.), filtered, and mixed with a saturated aqueous ammonium reineckate solution (500 ml.). The dirty red precipitate (0.98 g.), which was filtered off, was dissolved in a large volume of acetone and cooled. Even after concentration to a small volume, the material failed to crystallize out and eventually decomposed on standing.

Method IV of alkaloidal extraction was applied in the following manner. Six liters of a concentrated alcohol extract of defatted bark (10.5 Kg.) were filtered hot to remove bark particles and then concentrated and cooled. A dark brown precipitate was filtered off. The alcoholic filtrate was concentrated to about 0.5 l. and cooled again. More of the dark brown precipitate was obtained and combined with the first one as solid R (examined on p.119). The last traces of alcohol were removed under vacuum on a water bath leaving a dark brown extract (465 g.).

The extract was transferred to a separatory funnel with the aid of chloroform and washed with water. The aqueous extract was acidified and treated with ammonium reineckate solution, producing a brown precipitate. The chloroform layer was extracted with 1% hydrochloric acid until the acid layer was colorless. The combined acid extracts and washings

were basified with 28% ammonia and extracted exhaustively with chloroform. The crude basic fraction obtained weighed 1.235 g., (0.012% w/w of the defatted bark).

Method V (page107) was applied to 537 g. of an alcohol extract of the bark. The extract was, however, first extracted with petroleum ether, giving a cloudy, bright yellow extract (4.48 g.). The petroleum ether extract was not examined further, since attempts to obtain and crystallize the solid present failed. (This modification was used only in this single experiment).

The alcoholic solution (of the original alcohol extract) was poured into ether (5 l.) with stirring. The clear, dark reddish-brown ethereal solution was filtered, leaving blackish-brown ether-insoluble material (242 g.). The ether-insoluble material was tested in the following way. A portion of the material (10 g.) was dissolved in a minimum volume of ethanol, acidified with 10% hydrochloric acid, and filtered. The acid filtrate was shaken out with chloroform, made alkaline with 28% ammonia, and extracted again with chloroform. The latter chloroform extract was extracted with 1% hydrochloric acid. The acid extract was tested with Dragendorff's and Mayer's reagents and found negative in both cases.

The ether-soluble fraction was evaporated, diluted with ethanol, and poured into 3% hydrochloric acid (400 ml.) precipitating out a black insoluble material (171 g.). This

material was negative to Mayer's and Dragendorff's reagents and was not examined further. The acid filtrate was exhaustively extracted with chloroform (in 100 ml. portions), made alkaline with 10% ammonia (175 ml.), and again extracted with chloroform. The chloroform extract yielded a dark brown alkaloidal fraction (0.655 g.).

The ammoniacal aqueous layer was acidified with sulphuric acid and treated with a saturated aqueous ammonium reineckate solution. The dark, reddish-brown precipitate (0.11 g.) was filtered off.

The precipitates obtained with ammonium reineckate solution in all these experiments were grossly impure and eventually decomposed during attempts to purify them by crystallization out of acetone.

Examination of The Alkaloidal Fractions

Purification

An attempt was made to purify a small portion of the crude alkaloidal fractions by a second acid-base shakeout. The crude basic fraction (0.675 g.) was dissolved in chloroform (50 ml.) and extracted ten times with 10% sulphuric acid. The aqueous acid extracts were basified with ammonia (85 ml.) and the turbid yellow layer extracted twelve times with chloroform. The chloroform extracts were washed, dried

(over anhydrous sodium sulphate), and concentrated to a dark brown fraction (0.277 g.).

The remainder of the crude alkaloidal fractions was dissolved in chloroform and shaken out thirteen times with 1% hydrochloric acid. The acid extract was then basified with 28% ammonia and exhaustively extracted with chloroform. The combined chloroform extracts yielded a dark brown fraction, S, (1.046 g.).

The infra red spectra of the alkaloidal fraction, before and after the second acid-base shakeout, (C, about 2.2 in carbon disulfide and C, 21.0 in chloroform, 0.0288 mm. path length), showed peaks at: 3,472 $cm.^{-1}$ (weak; hydroxyl), 2,950, 2,865 $cm.^{-1}$ (methyl groups), 2,924 $cm.^{-1}$ (methylene), 1,727 $cm.^{-1}$ (weak; carbonyl), 1,724, 1,667, 1,585, 1,370 $cm.^{-1}$ (unassigned), 1,206 $cm.^{-1}$ (medium), 1,105 $cm.^{-1}$ (weak), 1,053-1,015 $cm.^{-1}$ (broad band), and 745 $cm.^{-1}$ (strong; 1:2-disubstituted benzene ring) (61).

Sodium fusion indicated the presence of nitrogen.

An attempt was made to precipitate the alkaloid out in the following manner. Fraction S was dissolved in chloroform (5 ml.) and poured into ethanol (95%; 25 ml.). Only a trace of solid appeared.

Preparation of Derivatives

The preparation of a picrate derivative was attempted

by the following procedure (63). A portion of the crude alkaloidal fraction (0.235 g.) was warmed with ethanol (95%; 10 ml.) and filtered. The filtrate was heated with a saturated alcoholic solution of picric acid and allowed to cool for several hours. No solid precipitated or crystallized out.

Another attempt using the purified alkaloidal fraction S (89.7 mg.) and the picric acid reagent (4-5 ml.) failed to yield a derivative.

In an attempt to prepare a reineckate, another portion of the crude fraction (1.95 g.) was dissolved in chloroform (75 ml.) and exhaustively extracted with 1% hydrochloric acid. The yellow acid extract was basified with 1% ammonia and extracted ten times with chloroform. The chloroform extract was washed free from alkali (to Universal indicator paper), dried and concentrated to yield the basic fraction (0.703 g.). This dark brown fraction was dissolved in chloroform (75 ml.) and shaken out five times with 1% hydrochloric acid (25 ml. portions). The acid extract was mixed with freshly prepared ammonium reineckate solution (25-30 ml.) and cooled. A pink precipitate was filtered off (78.1 mg., m.p. 128-136°C., with decomposition).

The reineckate was dissolved in acetone and cooled. The salt refused to crystallize out and decomposed slowly.

Attempts to prepare reineckates using the purified

alkaloidal fraction S yielded only traces of precipitates.

Thin-Layer Chromatography

Silica Gel G chromatography plates were prepared as described on page 56. The alkaloidal fraction S was spotted as a chloroform solution and developed with several solvents for a distance of 6.2 inches. The developed plates were air-dried and viewed under an ultraviolet lamp. The plates were sprayed with Dragendorff's reagent or with 1% iodine in methanol. The results are shown in Table 26.

Table 26

Developing Time (mins.)	Solvent	R_f Values (Examined under Ultraviolet Light)	Reaction with 1% Iodine	Reaction with Dragendorff's Reagent
40	Ethyl	(1) 0.29 (Bright blue) (2) 0.84 (Blue) (3) 0.99 (Green)	A brown spot just above (1) a dark spot at (3).	Slight streak
40	Diethyl ether	(1) 0.57 (Blue) (2) 0.73 (Blue) (3) 0.98 (Green)		No reaction.
40	Benzene	(1) 0.02 (Blue band) (2) 0.04 (Green band) (3) 0.18 (Weak spot blue) (4) - (Blue)	Colored band between (1) and (2); a spot at (3).	-

Table 26 Continued

Developing Time (mins)	Solvent	R_f Values (Examined under Ultraviolet Light)	Reaction with 1% Iodine	Reaction with Dragendorff's Reagent
150	Ethanol (95%)	(1) 0.10 (Blue streak) (2) 0.70 (Bright blue spot) (3) 0.81 (Pale blue spot) (4) 0.95 (Turquoise spot)	Spot above (2) streak from base line	-
150	Isopropanol	(1) 0.28 (Bright blue spot) (2) 0.82 (Blue streak)	-	-

Steam Distillation of The Alcohol Extract

Alcohol-free extract (30 g.) was steam distilled to give eight liters of distillate, which contained oil droplets and was light green in color. It had an odor reminiscent of the odor of the bark. The distillate, in one liter lots, was extracted ten times with ether and the combined ether extracts were concentrated to yield a yellowish-brown fraction T, (0.753 g.). The fraction gave negative results on testing with Mayer's and Dragendorff's reagents.

Examination of The Fluid Extract of Cocillana

One gallon of The Fluid Extract of Cocillana was purchased from the Penick Company of New York. Acidification of a few ml. of the extract with 10% hydrochloric acid, followed by filtration and the addition of a few drops of Mayer's and Dragendorff's reagents, produced only a slight turbidity in each case.

Alkaloidal Extraction

The fluid extract (2 l.) was freed from solvent by vacuum distillation on a water bath, leaving a dark brown residue (187.7 g.). The residue was washed into a separatory funnel with chloroform (300 ml.) and ethanol (75 ml.). Emulsification of the layers prevented an effective acid-base shakeout. The chloroform extract was, therefore, recovered and poured into 1% hydrochloric acid (1.6 l.). The acid filtrate was extracted with chloroform and the aqueous layer was basified with 28% ammonia. The basified layer was extracted with chloroform, which was concentrated (after washing and drying in the usual manner) to yield the crude alkaloidal fraction V, (0.419 g.). This fraction produced slight turbidity with both Mayer's and Dragendorff's reagents.

The basic aqueous layer was acidified with concentrated

sulphuric acid, treated with saturated aqueous ammonium reineckate solution (260 ml.), and allowed to stand. A dark brown precipitate (0.897 g.) was filtered off. It was not examined further due to the tendency of even fairly pure reineckates to decompose gradually.

Steam Distillation

The remainder of the fluid extract was concentrated (367.6 g., not completely dry) and steam distilled after the addition of water (100 ml.). Six liters of distillate containing yellow oil globules were collected. The distillate was divided into two parts and each extracted with ether (six times). On evaporation, the combined ether extracts yielded fraction W (1.62 g.). This fraction gave negative results when tested with Mayer's and Dragendorff's reagents.

Examination of Solids R and R1

Solid R was filtered off as a dark brown precipitate (24.0 g.) from the concentrated alcohol extract of defatted cocillana bark (10.5 Kg.). Attempts to purify portions of this material by crystallization from solvents such as chloroform, benzene, ethanol, methanol, and various mixtures of these solvents scarcely reduced the wide melting range (about 70-150° C.). Chromatography of a portion of the material in

benzene on a column of alumina was unsuccessful as the material clogged the column and would not allow the passage of eluants. The remainder of solid R (16.4 g.) was then fractionated roughly according to its solubilities by warming with various solvents in the following order: petroleum ether (2.1 l.), diethyl ether (0.7 l.), methanol (0.9 l.) and ethanol (0.9 l.). Concentration of each solution respectively yielded fractions Z_1 (4.63 g.), Z_2 (about 6.5 g.), Z_3 (about 1.28 g.) and Z_4 (about 1.9 g.).

Fraction Z_1 (petroleum ether-soluble) was a sticky olive green residue. Addition of cold petroleum ether dissolved a portion of the residue, leaving a solid (tinged green; 0.87 g.) undissolved, m.p. 69-110°C. Attempts to purify the solid by crystallization from petroleum ether, ether, and methanol were limited in success.

Fraction Z_2 (ether-soluble) was dark brown in color. When an ethereal solution of the fraction was poured into methanol, a beige solid (1.92 g.) was obtained, m.p. 73-115°C. This solid could also be obtained by dissolving the fraction in chloroform (15 ml.) and adding ethanol (95 ml.).

Fraction Z_3 (methanol-soluble) deposited a fluffy tan precipitate on concentration, m.p. 95-142°C. Attempts to purify this fraction by crystallization from methanol or by precipitation of a methanolic solution into chloroform indicated the presence of many small fractions of variable melting ranges. Small scale chromatography on an alumina column

yielded fractions, which were still impure, with wide melting ranges.

Fraction Z_4 (ethanol-soluble), on concentration, precipitated out a small portion of tan solid, m.p. 110-150°C. The same problems in purification arose, as with previous fractions.

The insoluble residue Z_5 (3.00 g.) left an ash on ignition and was not examined further.

Solid R_1 was obtained as a blackish-brown precipitate (14 g.), after the concentration of the alcohol extract of defatted bark (4.5 Kg.), moistened with ammonia prior to extraction. Solid R_1 was roughly fractionated into five fractions after warming with solvents in the following order: petroleum ether (1.2 l.), ether (1.7 l.), chloroform (0.9 l.), methanol (0.9 l.), and ethanol (0.8 l.). Concentration of each solution respectively yielded fractions Y_1 (1.73 g.), Y_2 (2.39 g.), Y_3 (2.1 g.), Y_4 (1.84 g.), and Y_5.

Fractions Y_1, Y_2, Y_3, and Y_5 were not examined in great detail. Fraction Y_2, a dark reddish-brown, transparent mass, deposited a tan precipitate (0.6 g.), m.p. 68-93°C., on warming with ether and cooling. Recrystallization of this fraction was unsuccessful. Column chromatography (on alumina) yielded mainly fat-like materials in several fractions. Fraction Y_5, after dilution with ethanol, deposited a small amount of dark brown solid, m.p. 139-164°C.

Fraction Y_4, after several recrystallizations from

methanol with charcoaling, eventually yielded a greyish-white solid, Y_{4a}, m.p. 180-184°C. Sodium fusion indicated the absence of nitrogen, sulphur, and halogen. The infra red spectrum of Y_{4a} (in potassium bromide disc) showed peaks at: 3,448, 3,390, and 3,268 cm.$^{-1}$ (broad band with two shoulders [3,448, 3,268 cm.$^{-1}$] and one peak; hydroxyl). The remainder of the spectrum indicated that Y_{4a} was still impure. The infra red spectrum of authentic α-amyrin (in potassium bromide disc) showed peaks at: 3,448, 3,300 cm.$^{-1}$ (broad band with two peaks; hydroxyl), and several other peaks, characteristic of its structure. The melting point of the α-amyrin purchased (169-175°C.) was not depressed on admixture with compound Y_{4a}, m.p. 166-180°C.

GLOSSARY OF FRACTIONS OBTAINED IN SECTIONS II - IV

II. THE PETROLEUM ETHER EXTRACTS

A_1	p. 64	A typical extraction of 3 Kg. of bark by percolation with petroleum ether.
A_2	p. 65	Similar to A_1.
A_3	p. 65	Extract obtained from 17.9 Kg. of bark (#60).
A_4	p. 65	Extract obtained from 16 Kg. of bark (#100).
A_5	p. 65	Extract obtained from 4.5 Kg. of bark (#50).
A_{3a}	p. 65	White solid obtained by cooling extract A_3.
B_1	p. 66	Unsaponifiable material from 31.1 g. of A_3.
B_2	p. 67	Unsaponifiable material from 10.0 g. of A_5.
B_3	p. 68	Unsaponifiable material from a second attempt on 10.0 g. of A_5.
C_1	p. 68	Free fatty acids from the saponification of 31.1 g. of extract A_3.
C_2	p. 68	Free fatty acids from the saponification of 10.0 g. of extract A_5.
D_1 to D_8 Table,12	p. 70	Chromatography fractions from the unsaponifiable fraction B_1.
I to IV Table 13	p. 71	Fractions D_1-D_8 bulked together.
E_1 to E_{13} Table 14	p. 73	Chromatography fractions of fraction III.
E_{4a}	p. 75	Solid obtained from fraction E_4.
E_{5a}	p. 75	Solid obtained from fraction E_5.

E_{5b}	p. 75	Solids E_{4a} and E_{5a} combined and identified.
F	p. 76	Steam-volatile fraction from 31.5 g. of extract A_3.
F_1	p. 76	Steam-volatile fraction from remainder of A_3 and all of extract A_4.
F_R	p. 76	Combined residues of the two steam distillations.
G_1 to G_9 Table 15	p. 79	Chromatography fractions of F.
H_1 to H_7 Table 16,	p. 81	Chromatography fractions of F_1.

III. THE BENZENE EXTRACTS

J_1	p. 83	Extract from 3 Kg. of defatted bark (#60).
J_2	p. 83	Extract from 7 Kg. of undefatted bark (#100).
J_{1R} and J_{2R}	p. 84	Residues from steam distillation of J_1 and J_2.
K_1	p. 84	Steam-volatile fraction from J_1.
K_{2a} and K_{2b}	p. 84	Steam-volatile fractions from J_2.
L_1 to L_6 Table 17	p. 86	Chromatography fractions of K_1 and K_{2a}.
L_{3a}	p. 87	Crystals isolated from fraction L_3.
M	p. 97	First precipitate obtained from J_{1R} and J_{2R}.
M_1	p. 97	Second precipitate obtained from J_{1R} and J_{2R}.
M_2	p. 98	Ether-soluble fraction of M_1.
M_3	p. 98	Ether- and chloroform-insoluble fraction of M_1.

M_4	p. 98	Chloroform-soluble fraction of M_1.
N_0 to N_8	Table 18 p. 90	Fractions from fractional distillation of G_1-G_4, H_1-H_4, and L_1-L_4.
N_R	p. 91	Residue after fractional distillation.
P_0 to P_7	Table 22 p. 96	Fractions from fractional distillation of fraction N_6.

IV. THE ALCOHOL EXTRACTS

Q	p. 102	Extract from 180 g. of undefatted bark.
Q_1	p. 102	Ether-soluble fraction of Q.
Q_2	p. 103	Chloroform-soluble portion of Q, after ether extraction.
Q_{1a} and Q_{1b}	Table 24 p. 106	Alkaloidal fractions from Q_1.
Q_{1c} and Q_{1d}	Table 24 p. 106	Acidic fractions from Q_1.
R and R_1	p. 111 and p. 121	Precipitates filtered off on concentration of alcohol extracts.
S	p. 114	Purified total alkaloidal fractions.
T	p. 117	Steam-volatile fraction of alcohol extract.
V	p. 118	Alkaloidal fraction obtained from the Fluid Extract of Cocillana.
W	p. 119	Steam-volatile fraction obtained from the Fluid Extract of Cocillana.
Y_1 to Y_5	p. 121	Fractions obtained from solid R_1.
Z_1 to Z_4	p. 120	Fractions obtained from solid R.

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