

**3-Chloro-5-bromo-2,6-dimethoxy-hydroquinone.**—One g. of the quinone described above was dissolved in 100 cc. of acetone containing a little water. Sulfur dioxide was passed into this solution until it was decolorized. The solution was concentrated to about one-half its original volume from which long, needle-like crystals formed on cooling. These were recrystallized from dil. alcohol, giving a pure product of 3-chloro-5-bromo-2,6-dimethoxy-hydroquinone; m. p., 146°; yield, almost quantitative. It was not analyzed.

**THE DIACETATE.**—The hydroquinone described above was treated with an excess of acetyl chloride and the resulting product crystallized from dilute alcohol; m. p., 85–86°.

*Anal.* Subs., 0.1765: mixed halides, 0.1676. Calcd. for  $C_{12}H_{12}O_6BrCl$ : Cl, 9.64; Br, 21.74. Found: Cl, 9.56; Br, 21.55.

### Summary

It has been shown that the first substituent chlorine atom enters the *meta* position to the hydroxyl group.

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[CONTRIBUTION FROM THE PHYTOCHEMICAL LABORATORY OF THE BUREAU OF CHEMISTRY,  
UNITED STATES DEPARTMENT OF AGRICULTURE]

## NON-VOLATILE CONSTITUENTS OF THE COTTON PLANT

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RECEIVED AUGUST 9, 1926

PUBLISHED OCTOBER 6, 1926

In a previous communication by the present authors<sup>1</sup> an account was given of the odorous constituents of the cotton plant. As those substances were all of a volatile nature, the respective investigation was restricted to such products from the plant as could be obtained by distillation with steam. It seemed to us, however, of importance and interest to extend the investigation by examining also the non-volatile constituents of the plant, as so little had been known regarding them and the work on the subject would thus be rendered more complete. With this purpose in view a quantity of material was collected during the same season and from the same field at Tallulah, Louisiana, as the plants which were previously employed for distillation.

The material used, which was collected during the month of July, 1923, consisted of the branches of the plant which had been stripped from the coarse, woody stems. It therefore comprised chiefly the foliage, "squares" and flowers, all the bolls which had then been developed having been carefully removed. The total weight of fresh material was 169.2 kg., and when thoroughly air-dried and ground it amounted to 34 kg. The loss of weight on drying was thus nearly 80%.

### Experimental Part

**Test for Alkaloid.**—A small portion (25 g.) of the ground, dried material was digested for three days with Prollius' fluid, the liquid then filtered,

<sup>1</sup> Power and Chesnut, *THIS JOURNAL*, **47**, 1751 (1925).

evaporated, and the residue extracted with water acidified with hydrochloric acid. This liquid, when concentrated and filtered, gave distinct precipitates with solutions of potassium mercuric iodide and iodine in potassium iodide, thus indicating the presence of a small amount of a basic substance. These reactions were evidently due to choline, which was subsequently isolated.

**Test for an Enzyme.**—A portion (1 kg.) of the ground, dried material was thoroughly mixed with five liters of distilled water, and a little chloroform was added to prevent bacterial action. After standing for two days, the expressed reddish liquid was filtered, when it amounted to three liters. This was mixed with one and one-half times its volume of alcohol, which produced an abundant, pale brown, curdy precipitate. The latter was collected on a filter, deprived of adhering liquid by means of suction, washed with a little alcohol and finally with ether. It was then spread on a porous plate and dried in a desiccator over sulfuric acid. When dry, it formed a brown mass which could be triturated to a grayish powder, and amounted to 53.6 g. or 5.3% of the material used.

The above-described product did not hydrolyze amygdalin. Its filtered aqueous solution gave none of the characteristic reactions of a protein, but was abundantly precipitated by basic lead acetate. When heated with a caustic alkali, a slight odor of ammonia was developed. The product thus appeared to consist chiefly of gummy and albuminous material, and there was no evidence of the presence of an enzyme that was capable of hydrolyzing  $\beta$ -glucosides.

#### Extraction of the Plant with Alcohol

For the purpose of a complete examination the chief portion (29 kg.) of the ground, dried material was thoroughly extracted by percolation with 95% alcohol. The percolate was first concentrated to a volume of about 10 liters, when it formed a very dark colored liquid. This liquid, which was acid to litmus, was subjected to distillation from a water-bath in order to remove the alcohol as completely as possible.

**Identification of Ammonia and Trimethylamine.**—The alcohol that had been removed by the preceding operation amounted to about 6 l. and was observed to have a decidedly alkaline reaction. This was evidently due to the dissociation by heat of some compounds contained in the original liquid. In order to obtain the volatile alkali the entire distillate was acidified with sulfuric acid, and the greater part of the alcohol then again removed by distillation. The residual acid liquid was made alkaline with sodium hydroxide, distilled, and the distillate collected in dil. hydrochloric acid. This liquid was evaporated to a small volume, and finally in a desiccator over solid potassium hydroxide to dryness. A crystalline salt was thus obtained which amounted to 0.265 g. When examined under the microscope<sup>2</sup> it was found to consist of the same mixture

<sup>2</sup> The authors are indebted to Mr. G. L. Keenan, of the Microchemical Laboratory, Bureau of Chemistry, for assistance during this work in all observations that required the use of a microscope.

of ammonium chloride and trimethylamine hydrochloride as had previously been obtained from the dew and from the emanation of the cotton plant, as also from the products of its distillation with steam.<sup>3</sup> The salt was chiefly in the form of cubical crystals, but also contained some rods which were doubly refractive. Its aqueous solution gave with platinic chloride the characteristic yellow, octahedral crystals of ammonium chloroplatinate, and with lime water it developed the odor of both ammonia and trimethylamine.

### Distillation of the Alcoholic Extract with Steam and Separation of the Essential Oil

After having removed the alcohol as completely as possible from the alcoholic percolate, the dark colored residual extract was distilled in a current of steam. A little alcohol first passed over and subsequently the liquid became milky with the separation of oily drops. The distillate was distinctly acid to litmus and about 3.5 liters was collected. It was saturated with sodium chloride and extracted with ether, the ethereal solution being washed with water, dried with anhydrous sodium sulfate, and the solvent removed. There was thus obtained 8.9 g. of a pale brownish-yellow essential oil, which had a strong and rather pleasant odor. A drop of the essential oil in about 5 cc. of glacial acetic acid gave, on the addition of a drop of concd. sulfuric acid, a reddish-violet color similar to that of one of the sesquiterpenes previously isolated from a distillate of the plant. It gave a strong reaction for furfural, but no coloration with ferric chloride.

The aqueous distillate, which had been extracted with ether as described above, was distilled in a current of steam and about 1 l. of distillate collected. This liquid contained a relatively small amount of acid which was converted into a barium salt. As the latter was found to contain a considerable proportion of formic acid, this was first removed by treatment with a 4% solution of potassium permanganate, after which the liquid was filtered and concentrated. A perfectly colorless salt was thus obtained from which several fractions of silver salt were prepared and the first two of these were analyzed.

*Anal.* Subs., 0.0990, 0.0673: Ag, 0.0637, 0.0434. Calcd. for  $\text{AgC}_2\text{H}_3\text{O}_2$ : Ag, 64.6. Found: 64.3, 64.5.

The total amount of silver salt obtained, which consisted of practically pure acetate, was about 0.7 g.

After the above-described operation of distillation with steam, there remained in the distillation flask a dark reddish-brown aqueous liquid (A) together with a quantity of a black, oily resin (B). When cold, the aqueous liquid was decanted and the resin washed with three successive portions of hot water, these washings being added to the main portion of aqueous liquid.

### Examination of the Aqueous Liquid (A)

The above-mentioned aqueous liquid was filtered and amounted to about 10 l. It was concentrated under diminished pressure to a volume of 1300 cc., and the water that was thus removed was found to contain an appreciable amount of formic acid.

### Isolation of Quercetin, $\text{C}_{15}\text{H}_{10}\text{O}_7$

The concentrated aqueous liquid, which formed a very dark colored sirup, was extracted with five successive portions of ether. These ethereal extracts were yellowish-brown, and the aqueous washings of them gave a dark green with ferric chloride, but

<sup>3</sup> Ref. 1, p. 1770.

they appeared to contain no tannin. After drying the ethereal extracts with anhydrous sodium sulfate, the greater part of the solvent was removed and the residual liquid brought into a small, tared flask, from which the remainder of the solvent was allowed to evaporate spontaneously. There was thus obtained 9.9 g. of a dark colored, sirupy liquid which contained an appreciable amount of a yellow, crystalline substance. This substance was collected on a filter, washed with a little ether, and dried on a porous tile. It was then dissolved in warm alcohol, in which it was very readily soluble, and a little water added, which caused the separation of a magma of fine, acicular crystals. This was collected, washed with a little water, and dried on a porous tile. The pure substance had a bright lemon-yellow color and weighed 1.4 g. It was analyzed with the following results.<sup>4</sup>

*Anal.* Subs., 0.3665, heated at 110°: 0.0324 g. of H<sub>2</sub>O. H<sub>2</sub>O, 8.84%; 0.2789, heated at 130°: 0.0247 g. of H<sub>2</sub>O. H<sub>2</sub>O, 8.86%. Subs. (anhydrous) 0.1219: CO<sub>2</sub>, 0.2654; H<sub>2</sub>O, 0.0400. Calcd. for C<sub>16</sub>H<sub>10</sub>O<sub>7</sub>: C, 59.58; H, 3.34. Found: C, 59.38; H, 3.68.

The substance was thus found to agree in its composition and characters with quercetin. Although usually stated to crystallize with two molecules of water, which would require 10.65% of H<sub>2</sub>O, in the present instance the substance had probably lost a little water by drying in a desiccator over sulfuric acid before being subjected to heat. Further confirmation of its identity was afforded by the preparation of an acetyl derivative. This was obtained in the form of a felted mass of colorless needles which, when crystallized from alcohol, melted at 189–191° (corr.), and after repeated crystallization from 70% alcohol this melting point remained unchanged.

After the separation of quercetin the residual dark colored, sirupy liquid was diluted with ether and extracted several times successively with a saturated solution of ammonium carbonate, a 10% solution of sodium carbonate, and a 5% solution of sodium hydroxide, the ethereal liquid being then finally washed with water, dried with anhydrous sodium sulfate and the solvent removed. All the alkaline liquids were subsequently acidified with sulfuric acid and extracted several times with ether.

The ammonium carbonate extract yielded a very small amount of a dark colored, sirupy product which was found to be a mixture of acids and was difficult to purify. It gave a violet coloration with ferric chloride, which suggested the presence of salicylic acid. The entire material was consequently esterified with methyl alcohol and sulfuric acid, the mixture extracted with ether, and the ethereal extract distilled with steam. The distillate contained a few oily drops which were heavier than water and possessed the characteristic odor of methyl salicylate together with an odor reminding somewhat of methyl benzoate. The last mentioned odor was apparently due to an acid which was subsequently obtained in small amount from the distillate after hydrolysis. When crystallized twice from hot water it separated in fine, colorless needles which melted at 105–106° and gave no coloration with ferric chloride. Its optical characters indicated it to be neither benzoic acid nor *o*-toluic acid, but the very small amount of material precluded its further investigation.

The sodium carbonate extract of the original ethereal liquid yielded nothing definite, and the sodium hydroxide extract was found to contain only traces of a phenolic substance. This substance formed a dye when coupled in alkaline solution with diazo-*p*-nitrobenzene, but could not be obtained in a pure state. The ethereal liquid which had been successively extracted with the various alkalies was finally washed with water, dried, and the solvent removed, but only a small amount of dark colored, oily material was obtained.

<sup>4</sup> All the ultimate analyses recorded in this paper were made for us by Mr. R. M. Hann, of the Bureau of Chemistry, to whom our best thanks may here be expressed.

### Treatment of the Aqueous Liquid with Basic Lead Acetate

After extracting the original aqueous liquid with ether it was treated with a slight excess of solution of basic lead acetate. This produced an abundant, dense yellow precipitate, which was collected in a large Büchner funnel and the liquid filtered by suction. The precipitate was then again mixed with water, collected as before, and thoroughly washed with water.

**Basic Lead Acetate Precipitate.**—This precipitate was mixed with water, decomposed by hydrogen sulfide, and the mixture filtered. After thoroughly washing the lead sulfide with water the excess of hydrogen sulfide was removed from the filtrate by a current of carbon dioxide. The clear, dark colored aqueous liquid was then concentrated under diminished pressure to a small bulk. On standing for some time a quantity of crystalline material separated. This was collected and found to consist of lead chloride, which had escaped decomposition by the treatment with hydrogen sulfide. The total amount of this salt, when purified, was 20 g. It had evidently been formed from potassium chloride contained in the original aqueous liquid and would correspond to 10.7 g. of the potassium salt. After further treatment of the aqueous liquid with hydrogen sulfide in order to remove the lead completely, it was digested with animal charcoal but still retained a dark color. The liquid gave a deep green with ferric chloride. It also readily reduced Fehling's solution, which was probably due to the presence of some sugar that had been occluded in the original lead precipitate.

The aqueous liquid which had been purified as above described was extracted with five successive portions of ether. This removed only a further small amount (0.35 g.) of the previously identified quercetin. After extraction with ether the aqueous liquid was concentrated under diminished pressure to a small bulk. As it was strongly acid it was first neutralized with pure calcium carbonate, then mixed with purified animal charcoal, and the dried mixture extracted in a Soxhlet apparatus with ethyl acetate. A sirupy product was thus obtained which contained some crystals. For its further purification it was mixed with animal charcoal and the dried mixture extracted in a Soxhlet apparatus with absolute alcohol. On filtering the alcoholic extract a very small amount of a sparingly soluble substance was collected. This was purified by dissolving it in hot alcohol, from which it separated almost completely on cooling. When collected and dried on a porous tile it formed thin laminae and had all the characters of a paraffin hydrocarbon. It melted at 64–66°, and apparently was triacontane,  $C_{30}H_{62}$ .

### Isolation of Succinic Acid

The above-mentioned alcoholic extract from which the hydrocarbon had been separated was allowed to evaporate, when a small amount of a crystalline substance was obtained. This was further purified by crystallization from water with the use of a little animal charcoal. It then formed small, nearly colorless, prismatic crystals, which had a strongly acid reaction, and melted quite sharply at 184–185° (corr.). These characters indicated the substance to be succinic acid, and when mixed with a pure specimen of the latter (m. p., 185–186°) the melting point remained unchanged.

*Anal.* Subs., 0.1195:  $CO_2$ , 0.1786;  $H_2O$ , 0.0541. Calcd. for  $C_4H_6O_4$ : C, 40.66; H, 5.12. Found: C, 40.75; H, 5.07.

From an ammonium salt of the acid a silver salt was prepared, and this, after drying at 100°, was analyzed.

*Anal.* Subs., 0.1239: Ag, 0.0799. Calcd. for  $Ag_2C_4H_4O_4$ : Ag, 65.0. Found: 64.5.

The substance gives with resorcinol and sulfuric acid the color reaction that has

been considered characteristic of succinic acid,<sup>5</sup> but which is generally shown by acids of the fatty series which give internal anhydrides as well as by all the *o*-dicarboxylic acids of the benzene series.

The refractive indices of the acid were determined for us by Mr. G. L. Keenan, of the Microchemical Laboratory, and were found to be in perfect agreement with those of pure succinic acid, which are as follows:<sup>6</sup>  $[\frac{20}{D}]\alpha = 1.453$ ;  $\gamma = 1.610$ , both  $\pm 0.002$ .

The previously described mixture with animal charcoal, which had been extracted with ethyl acetate, was subsequently extracted in a Soxhlet apparatus with alcohol. A quantity of a crystalline substance separated which, when collected and purified, was found to consist of potassium chloride and amounted to 1.9 g. On subsequently evaporating the filtered alcoholic liquid it formed a dark colored sirup. The latter gave a deep green with ferric chloride, but no precipitate with gelatin, and evidently contained no tannin.

**Filtrate from the Basic Lead Acetate Precipitate.**—This liquid was treated with hydrogen sulfide for the removal of the excess of lead, filtered, and the dissolved gas expelled by a current of air. It was then concentrated under diminished pressure, when the dark colored, sirupy product amounted to 865 g.

A very small portion of the sirupy liquid was tested for the possible presence of asparagine and allantoin by means of a solution of mercuric nitrate acidified with nitric acid, but with a negative result. The entire amount of aqueous liquid was then largely diluted with alcohol and allowed to stand for some time, when a quantity (20 g.) of a crystalline substance separated. This was collected by filtration and found to consist of potassium nitrate. On subsequently removing the alcohol by distillation from a water-bath it was observed that the distillate contained ammonia and trimethylamine. The distillation was therefore interrupted, and the liquid allowed to evaporate spontaneously. The portion of alcoholic distillate obtained was acidified with hydrochloric acid and evaporated, when an appreciable quantity of the salts of the above-mentioned bases was obtained.

After the complete removal of the alcohol and somewhat further evaporation, the sirupy aqueous liquid amounted to 713 g. It had a peculiar odor and a strongly acid reaction, and on addition of a solution of potassium mercuric iodide a precipitate was produced. It gave no color with ferric chloride, but strongly reduced Fehling's solution, thus indicating the presence of a considerable proportion of a reducing sugar.

### Isolation of Betaine, $C_5H_{11}O_2N$ , and Choline, $C_5H_{15}O_2N$

A portion (100 g.) of the previously mentioned sirupy, aqueous liquid was acidified with sulfuric acid and a saturated solution of phosphotungstic acid added, when a very abundant precipitate was produced. This precipitate was collected, washed with water, decomposed with a cold, saturated solution of barium hydroxide, and the mixture filtered, after which the excess of barium was removed from the filtrate by carbon dioxide. The liquid was then again filtered, concentrated under diminished pressure to the consistency of a sirup, and the latter repeatedly extracted with alcohol. To this filtered alcoholic liquid a cold saturated alcoholic solution of 50 g. of mercuric chloride was added, and the mixture allowed to stand for a day, when an abundant, nearly colorless, crystalline precipitate was produced. This was collected by filtration with suction and dried on a porous plate, when it amounted to 37 g. It was subsequently dissolved in hot water, and the solution treated with hydrogen sulfide for the removal of the mercury. After

<sup>5</sup> L. Rosenthaler, "Der Nachweis organischer Verbindungen," Ferdinand Enke, Stuttgart, 1914, p. 330.

<sup>6</sup> The refractive indices of succinic acid, as recorded by A. Bolland [*Monatsh.*, 31, 408 (1910)] are apparently not quite correct.

filtration, the nearly colorless liquid was concentrated under diminished pressure to a small volume and transferred to a dish, its further evaporation having then been conducted in a vacuum desiccator over solid potassium hydroxide. After a few days a considerable quantity of large, handsome, colorless crystals had formed. These were collected, washed several times with absolute alcohol, and dried in a desiccator on a porous plate. The first crop of crystals, which evidently consisted of betaine hydrochloride,  $C_5H_{11}O_2N \cdot HCl$ , amounted to 9.05 g. By the further evaporation of the liquid an additional quantity of crystals separated, and the total amount of pure salt obtained was 10.86 g. This would correspond to 0.2% of betaine,  $C_5H_{11}O_2N$ , in the air-dried plant.

The melting point of the substance, as determined by heating quickly in the Mason electrical apparatus,<sup>7</sup> was 244–245°, which was the same as that previously found by Power and Browning<sup>8</sup> for betaine hydrochloride obtained from cotton-root bark. When slowly heated in the usual manner in a capillary tube it melted at 234–235° with evolution of gas.

*Anal.* Subs., 0.1537:  $CO_2$ , 0.2199;  $H_2O$ , 0.1004. Subs., 0.1071, by the Kjeldahl-Gunning method, gave  $NH_3$  equivalent to 6.9 cc. of 0.1 *N*  $HCl$ . Subs., 0.0971:  $AgCl$ , 0.0920. Calcd. for  $C_5H_{12}O_2NCl$ : C, 39.08; H, 7.88; N, 9.12; Cl, 23.1. Found: C, 39.02; H, 7.31; N, 9.03; Cl, 23.4.

A portion of the salt was converted into the chloro-aurate. This separated in bright yellow, glistening laminae, and was analyzed.

*Anal.* Subs., 0.1345: Au, 0.0580. Calcd. for  $C_5H_{12}O_2N \cdot AuCl_4$ : Au, 43.13. Found: 43.12.

The salt formed monoclinic crystals, and the refractive indices, as determined by Mr. G. L. Keenan, of the Microchemical Laboratory, were as follows:  $[\frac{20}{D}]\alpha = 1.514$ ;  $\beta$ , indeterminable;  $\gamma = 1.583$ , both  $\pm 0.002$ .

These optical characters of the substance were found to be in complete agreement with those of an authentic specimen of betaine hydrochloride and, with the exception of the crystalline form, they had not previously been recorded.

After the greater part of the betaine hydrochloride had been separated by crystallization, the residual product was extracted with absolute alcohol, and the alcoholic liquid evaporated. This residue was then extracted with alcohol and the liquid evaporated when a small portion of a deliquescent salt was obtained which was completely soluble in absolute alcohol. This salt had the characters of choline hydrochloride, and all of it was converted into the chloroplatinate, which was obtained in the form of handsome, reddish-yellow plates. The total amount of platinum salt was 2.0 g., which would correspond to 0.78 g. of choline,  $C_5H_{15}O_2N$ , or about 0.02% of the latter in the air-dried plant.

The platinum salt, after drying at 110°, was analyzed.

*Anal.* Subs., 0.1130: Pt, 0.0355. Calcd. for  $(C_5H_{14}ON)_2PtCl_6$ : Pt, 31.67. Found: 31.42.

Although betaine and choline had previously been found in cottonseed,<sup>9</sup> their presence in the herbaceous parts of the plant has now been established.

A second portion (100 g.) of the previously mentioned sirupy, aqueous liquid was mixed with purified sawdust, the mixture dried, and successively extracted in a Soxhlet apparatus with ether, ethyl acetate and alcohol.

<sup>7</sup> Mason, *J. Soc. Chem. Ind.*, **44**, 577 (1925).

<sup>8</sup> Power and Browning, *Pharm. J.*, [4] **39**, 420 (1914).

<sup>9</sup> Ritthausen and Weger, *J. prakt. Chem.*, **30**, 32 (1884). Boehm, *Arch. exptl. Path. Pharmacol.*, **19**, 87 (1885).

The above-mentioned ethereal extract was shaken several times with a saturated solution of ammonium carbonate which, however, removed only a trace of indefinite material. On subsequently washing the ethereal extract with water, drying with anhydrous sodium sulfate and removing the solvent, a small amount of a sirupy product was obtained. This was mixed with animal charcoal, the mixture dried, and extracted with absolute alcohol. The alcoholic extract was then treated with absolute ether, when a small amount of a colorless, handsomely crystalline substance separated. This neutral substance was collected and dried on a porous tile, when it melted at 140–143° and amounted to 0.237 g.

*Anal.* Subs., 0.0980, 0.1126: CO<sub>2</sub>, 0.2295, 0.2631; H<sub>2</sub>O, 0.0528, 0.0643. Calcd. for C<sub>14</sub>H<sub>16</sub>O<sub>5</sub>: C, 63.61; H, 6.10. Found: C, 63.86, 63.72; H, 6.03, 6.39.

This substance does not agree in its characters with any known compound of the calculated formula, and the amount obtained did not permit its further investigation. It gives a brown color with concd. sulfuric acid and no coloration with ferric chloride.

The product obtained by extraction with ethyl acetate was small in amount and yielded nothing of interest. On finally extracting the above-mentioned mixture with alcohol a very dark colored product was obtained. This deposited a quantity (about 3 g.) of crystalline material, which consisted of a mixture of potassium nitrate and chloride. After filtration, the alcoholic liquid was evaporated, diluted with water, treated with animal charcoal and again filtered and evaporated. A sirupy product was thus obtained which readily reduced Fehling's solution and evidently contained a reducing sugar. When heated with a caustic alkali it developed the odor of ammonia and trimethylamine, the latter substance having been produced from the betaine and choline which were known to be present in the original liquid.

### Examination of the Resin (B)

The previously mentioned black, oily resin, which had been thoroughly washed with water, was mixed with purified sawdust, the mixture thoroughly dried, and then extracted in a large Soxhlet apparatus with petroleum ether (b. p., 35–75°). This extract, after the complete removal of the solvent, was a black, viscid oil, and amounted to 909 g. or 3.134% of the dried plant.

The material which had been extracted with petroleum ether was exposed to the air until all of the absorbed solvent had evaporated, and then extracted with ether. After the removal of the greater part of the solvent by distillation, the remainder was allowed to evaporate spontaneously. This ethereal extract was a black, soft solid, which amounted to 91 g. or 0.314% of the dried plant.

On subsequently extracting the material with hot chloroform there was obtained, after the complete removal of the solvent, a black, solid product, which amounted to 9 g. or 0.031% of the dried plant.

The material was finally extracted with hot alcohol. This yielded a black, friable resin, amounting to 125 g. or 0.431% of the dried plant. The total yield of resinous products was thus 1134 g. or 3.91% of the weight of dried plant.

### Petroleum Ether Extract of the Resin

The entire amount of this extract (909 g.) was dissolved in ether and an attempt made to extract the liquid with a 10% solution of sodium carbonate, but no separation could be effected. The ether was therefore removed, and as it seemed necessary to saponify the material directly it was mixed with a solution of 200 g. of potassium hydroxide in 2 l. of alcohol. On the addition of the alkali the odor of both ammonia and trimethylamine was evolved. The mixture was then heated under a reflux condenser for two hours, after which the alcohol was removed, water added and steam passed through the liquid in order to remove the small amount of essential oil that had been



occluded by the resin. To the strongly alkaline mixture a quantity of clean sea sand was added, and the whole heated on a steam-bath for the removal of the water. After having been thoroughly dried, the mixture was extracted by digestion with ether. The ethereal liquids, which were quite black, were shaken with a saturated solution of sodium sulfate in order to remove the soap that was intimately associated with the unsaponifiable material, and the very dark colored aqueous liquid was then repeatedly extracted with ether. All the ethereal liquids were subsequently washed well with water, dried with anhydrous sodium sulfate, and the ether was removed.

After extracting the alkaline mixture containing sand as completely as possible with ether, it was treated with large successive portions of water until all of the soluble material had been removed, and the liquids were then passed through a cloth strainer. These aqueous liquids were also completely extracted with ether, the ethereal liquids being washed with water, dried, and the solvent removed. In this manner the unsaponifiable matter was separated from the large volume of very dark colored, aqueous liquid which contained the combined fatty acids.

**Unsaponifiable Constituents of the Petroleum Ether Extract.**—The above-mentioned ethereal extracts, all of which were united, yielded a quantity of a dark colored, oily product which was observed to contain a considerable amount of a crystalline substance. The entire product was therefore dissolved in hot alcohol and the solution filtered. On cooling, a large proportion of the crystalline substance was deposited, and this was separated by filtration from the very dark colored, oily material that accompanied it. After repeated crystallizations from alcohol with the use of animal charcoal, the substance was obtained in a pure state and amounted to 7.8 g.

### Isolation of a Phytosterol, $C_{27}H_{46}O.H_2O$

The above-mentioned crystalline substance separated from alcohol in handsome, colorless, glistening leaflets, which melted sharply at  $135^\circ$  (corr.). After drying at  $110^\circ$  it was analyzed.

*Anal.* Subs., 0.2094:  $CO_2$ , 0.6439;  $H_2O$ , 0.2249. Calcd. for  $C_{27}H_{46}O$ : C, 83.86; H, 11.99. Found: C, 83.90; H, 12.03.

Subs., 0.5201, on heating at  $110^\circ$ , lost 0.0192 g.  $H_2O$ . Calcd. for  $C_{27}H_{46}O.H_2O$ :  $H_2O$ , 4.5. Found: 3.7.

The substance had probably lost a little water by previous drying in a desiccator over sulfuric acid.

The optical rotation of the substance was determined with the following result: 0.2998 (anhydrous), made up to 25 cc. with chloroform, gave  $\alpha_D = -0.79^\circ$  in a 2dm. tube; whence  $[\alpha]_D^{20} = -32.9^\circ$ .

The above results rendered it evident that the substance was a phytosterol, and it gave the color reactions that are characteristic of this class of compounds. It agrees closely in its characters with the so-called sitosterol.

A small portion of the phytosterol was converted into its acetyl derivative, which, when crystallized from a mixture of ethyl acetate and alcohol, separated in colorless needles, melting sharply at  $121^\circ$  (corr.).

Inasmuch as the refractive indices of the phytosterols appear never to have been recorded, these have now been determined for the above-mentioned substance by Mr. G. L. Keenan, of the Microchemical Laboratory, with the following results:<sup>10</sup>  $[\frac{20}{D}]\alpha = 1.519$ ;  $\beta$ , indeterminate;  $\gamma = 1.549$ ;  $\gamma - \alpha = 0.03$ ; both  $\pm 0.002$ .

<sup>10</sup> Both the original crystallized phytosterol and that which had been rendered anhydrous by heating at  $110^\circ$  gave precisely the same data.

In parallel polarized light, with crossed nicols, the extinction was straight, double refraction was moderately strong and elongation positive. In convergent polarized light, with crossed nicols, interference figures were rare.

It is thought possible that the determination of these optical properties may serve not only for the identification of a phytosterol but also for discriminating between the different groups of this class of substances.

### Isolation of a Paraffin Hydrocarbon, Pentatriacontane, $C_{36}H_{72}$

In the course of purification of the unsaponifiable material there was obtained a small amount of a substance which was very sparingly soluble in cold alcohol and had the appearance of a paraffin hydrocarbon. This substance was separated by the fractional crystallization of the phytosterol, and small portions were also obtained from the previously mentioned, dark colored, oily material after the distillation of the latter in a vacuum, as will subsequently be noted.

All the portions of this sparingly soluble substance were united and purified by repeated solution in hot alcohol and treatment with animal charcoal. The solutions were filtered while hot, with the use of a hot-water funnel, when, on cooling, the substance separated in the form of a soft, gelatinous mass. This was collected on a filter, by suction, washed with a little alcohol and spread on a porous tile. When dried in a desiccator over sulfuric acid it formed a colorless, paraffin-like mass, and amounted to 1.0 g. The substance was not affected by concd. sulfuric acid or nitric acid and melted sharply at  $75^{\circ}$  (corr.), which indicated it to be pentatriacontane,  $C_{36}H_{72}$ .

*Anal.* Subs., 0.1165:  $CO_2$ , 0.3638;  $H_2O$ , 0.1528. Calcd. for  $C_{36}H_{72}$ : C, 85.27; H, 14.73. Found: C, 85.19; H, 14.65.

The identity of the substance as pentatriacontane was thus established.

The previously mentioned, very dark colored oily material from which the phytosterol and pentatriacontane had been separated was found to contain an appreciable amount of a fatty acid. This was evidently due to the partial hydrolysis of the large volumes of aqueous soap solutions, and the consequent removal of the acid with the unsaponifiable material on extracting the alkaline liquids with ether. All the oily material was therefore mixed with an alcoholic solution of 100 g. of potassium hydroxide, the mixture heated, some water added, and the alcohol removed. The oily material was then extracted by ether, the ethereal liquids were thoroughly washed with water, dried and the solvent was removed. The strongly alkaline aqueous liquid obtained by this treatment was reserved for further examination.

After the removal of the fatty acid by the above-described treatment, the oily, unsaponifiable material amounted to 176 g. As no further quantity of crystalline substance could be separated from it directly, the whole of this material was subjected to distillation under a pressure of about 6 mm. The distillate was collected in five fractions, all of which except the one of highest boiling point had a reddish-yellow color and a pleasant, aromatic odor. A small amount of a black resin, which became solid on cooling, remained in the distilling flask. As several of these somewhat viscous liquids contained small amounts of a suspended solid substance, this was separated by filtration and found to consist of the above-described hydrocarbon, pentatriacontane.

An analysis of three of the above fractions indicated them to consist chiefly of hydrocarbons. They were therefore united and fractionally distilled twice over metallic

sodium at a pressure of 5-9 mm. when some resinous material remained in the distilling flask. The liquids were then distilled thrice under ordinary pressure and the following fractions finally collected.

Fraction	I	II	III	IV	V	VI	VII	VIII
B. p., °C.	160-170	170-180	180-200	200-220	220-250	250-280	280-300	300-350
Grams	1.98	4.67	5.89	6.34	3.67	15.20	7.06	18.62
Total 63.43 g.								

It was evident from these results that the original oily liquid consisted of a very complex mixture. In order to ascertain the nature of its constituents, Fractions II, IV, VI and VIII were further investigated.

### Identification of Dipentene, $C_{10}H_{16}$

**Fraction II**, b. p. 170-180°, was a nearly colorless liquid, and had a pleasant, somewhat lemon-like odor. Determinations of its density and refractive index gave the following values:  $d_{15}^{15} = 0.8404$ ;  $n_D^{20} = 1.4693$ . It was optically inactive.

*Anal.* Subs., 0.3115:  $CO_2$ , 1.0076;  $H_2O$ , 0.3250. Calcd. for  $C_{10}H_{16}$ : C, 88.15; H, 11.85. Found: C, 88.24; H, 11.67.

This fraction was thus shown to consist of a terpene, and its general characters were in fairly close agreement with those of dipentene. The presence of the latter was confirmed by the formation of the tetrabromide, which was prepared according to the method of Power and Kleber.<sup>11</sup> This compound was obtained in colorless crystals, which melted with slight decomposition at 122-123° (pure dipentene tetrabromide melts at 124-125°).

**Fraction IV**, b. p. 200-220°, was a pale yellow liquid, having a very pleasant odor;  $d_{15}^{15} = 0.9153$ .

The density of this fraction having been found to be higher than that of either the preceding or succeeding one, its correctness was confirmed by a second determination. It was thus indicated that it contained an oxygenated substance which had not been altered by distillation over sodium, and its analysis showed this to be the case.

*Anal.* Subs., 0.1772, 0.2617:  $CO_2$ , 0.5111, 0.7568;  $H_2O$ , 0.1348, 0.1954. Calcd. for  $C_8H_{10}O$ : C, 78.64; H, 8.25. Found: C, 78.68, 78.89; H, 8.51, 8.35.

The very small amount of this substance available precluded its further investigation.

### Isolation of a Sesquiterpene, $C_{15}H_{24}$

**Fraction VI**, b. p. 250-280°, was a pale yellow liquid having an agreeable odor.

*Anal.* Subs., 0.1504:  $CO_2$ , 0.4858;  $H_2O$ , 0.1601. Calcd. for  $C_{15}H_{24}$ : C, 88.15; H, 11.85. Found: C, 88.12; H, 11.91.

Determinations of the density, optical rotation and refractive index gave the following values:  $d_{15}^{15} = 0.8909$ ;  $\alpha_D$  in a 0.5dc. tube =  $+0.125^\circ$ , whence  $[\alpha]_D^{20} = +0.28^\circ$ ;  $n_D^{20} = 1.4891$ ; molecular refraction, 66.07.

The boiling point, analysis and other characters of the above-described liquid have definitely shown that it consists of a sesquiterpene. The molecular refraction has furthermore indicated that it belongs to the group of dicyclic sesquiterpenes with two ethylenic linkings, the calculated value of which is 66.15. It is not, however, identical with the dicyclic sesquiterpene that had previously been isolated from a distillate of the cotton plant.<sup>12</sup>

<sup>11</sup> Power and Kleber, *Pharm. Rundschau*, N. Y. 12, 160 (1894).

<sup>12</sup> Ref. 1, p. 1763.

A drop of the substance, when dissolved in 5 cc. of glacial acetic acid, gives on the addition of a drop of concd. sulfuric acid a deep purplish-red color, which soon changes to brown.

A portion (12 cc.) of the sesquiterpene, dissolved in twice its volume of absolute ether, and cooled to  $0^{\circ}$ , was saturated with dry hydrogen chloride. The liquid soon acquired a deep purplish color, but no crystalline compound was formed, even after standing for several days.

**Fraction VIII**, b. p.  $300\text{--}350^{\circ}$ . This liquid had a brownish-yellow color and an odor somewhat resembling that of cedar wood. It showed a slight greenish fluorescence;  $d_{15}^{15} = 0.9120$ .

*Anal.* Subs., 0.1407:  $\text{CO}_2$ , 0.4551;  $\text{H}_2\text{O}$ , 0.1469. Calcd. for  $\text{C}_{20}\text{H}_{32}$ : C, 88.15; H, 11.85. Found: C, 88.24; H, 11.68.

This fraction evidently consisted of a mixture of diterpenes, which had probably been formed by polymerization of the lower-boiling constituents of the oil.

**Acid Constituents of the Petroleum Ether Extract.**—After the saponification of the petroleum ether extract of the resin, and the removal of the unsaponifiable constituents by extraction with ether, the large volume of very dark colored, aqueous liquid was acidified with sulfuric acid. This caused the separation of some black, resinous material, and the entire acid liquid was subsequently extracted with several portions of ether in order to remove the higher fatty acids, which will be further considered. A portion of the aqueous acid liquid was then distilled in a current of steam. The slightly acid distillate, which contained a little aldehyde, was made alkaline with sodium hydroxide, concentrated, acidified with sulfuric acid and again distilled. The acid thus obtained was converted into a barium salt, which was found to contain a considerable proportion of formate. After the removal of the formic acid by means of permanganate, a silver salt was prepared and analyzed.

*Anal.* Subs., 0.0338: Ag, 0.0211. Calcd. for  $\text{AgC}_2\text{H}_3\text{O}_2$ : Ag, 64.6. Found: 62.4.

It was evident that this salt consisted chiefly of silver acetate, and the somewhat low percentage of silver was apparently due to the presence of a little butyrate, since a slight odor of butyric acid was developed on moistening the salt with dil. sulfuric acid.

The large volumes of ethereal liquid obtained by extracting the above-mentioned acidified solution with ether had a very dark color. They were washed several times with water, dried with anhydrous sodium sulfate and the ether was removed. To the black, viscous liquid thus obtained some water was added, and steam passed through the mixture until the distillate was only faintly acid. During this operation a small amount of crystalline substance separated in the condenser, which proved to be palmitic acid. It was later obtained in larger amount and completely identified. The distillate, amounting to about 1 l., had a pleasant odor and contained a few oily drops. It was made alkaline with sodium hydroxide and concentrated. From the mixture remaining in the distillation flask, there separated on cooling a quantity of a soft black resin from which nothing further of interest could be obtained.

The above-mentioned distillate, which had been made alkaline with sodium hydroxide and concentrated, deposited on cooling a small quantity of a gelatinous substance. This was collected and purified by solution in hot alcohol, when it was obtained in fine, colorless crystals and found to consist of sodium palmitate. The aqueous alkaline liquid was then acidified with sulfuric acid and distilled in a current of steam, about 2 l. of distillate being collected. The first portion of distillate was cloudy, and a few oily drops separated; it had the odor of the higher fatty acids. The entire distillate was then made alkaline with barium hydroxide, concentrated, filtered and the excess of barium removed by carbon dioxide. On subsequently filtering and evaporating

the liquid a very small amount of a crystalline salt separated. This was collected, washed with a little water, dried at 100°, and analyzed.

*Anal.* Subs., 0.1277: BaSO<sub>4</sub>, 0.0973 = Ba, 0.0573. Calcd. for Ba(C<sub>4</sub>H<sub>7</sub>O<sub>2</sub>)<sub>2</sub>: Ba, 44.1. Found: 44.8.

This salt therefore consisted essentially of barium butyrate, and it developed the odor of butyric acid when moistened with dil. sulfuric acid.

The filtrate from the above-mentioned salt yielded with silver nitrate several fractions of silver salt, which were collected and analyzed.

Fraction	Subs., g.	Ag found, g.	Ag, %
I	0.0707	0.0357	50.5
II	.1085	.0543	50.0
III	.0708	.0367	51.8
IV	.0630	.0336	53.3
V	.0547	.0297	54.3
VI	.0410	.0223	54.4

Calcd. for AgC<sub>4</sub>H<sub>7</sub>O<sub>2</sub>: Ag, 55.4. Calcd. for AgC<sub>5</sub>H<sub>9</sub>O<sub>2</sub>: Ag, 51.7. Calcd. for AgC<sub>6</sub>H<sub>11</sub>O<sub>2</sub>: Ag, 48.4.

The composition and characters of these salts indicated that the respective acids consisted chiefly of butyric and valeric acids, with a small proportion of caproic acid. The total amount of silver salt obtained was 0.73 g.

### Isolation of Palmitic Acid

It has previously been noted that after the separation of the phytosterol and pentacontane from the unsaponifiable constituents of the petroleum extract, a quantity of very dark colored, oily material was obtained which was found still to contain an appreciable amount of fatty acid. This was removed by treating the oily product with potassium hydroxide, when, after separating the unsaponifiable oil, the strongly alkaline aqueous liquid was acidified with sulfuric acid and extracted with ether. The ethereal liquids, when washed, dried, and freed from solvent, yielded a solid, dark colored mass, amounting to 50 g. As no pure substance could be obtained directly from this material it was distilled in a vacuum. A portion then passed over between 135° and 195° (5 mm.) which solidified in the condenser and receiver as a soft, crystalline mass. The larger portion which remained in the distilling flask was resinous and could not be distilled further without decomposition.

The above-mentioned crystalline product was purified by repeated crystallization from alcohol, with the use of a little animal charcoal, when it was obtained in the form of colorless needles, which melted sharply at 61°. <sup>13</sup>

*Anal.* Subs., 0.1324: CO<sub>2</sub>, 0.3633; H<sub>2</sub>O, 0.1525. Calcd. for C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>: C, 74.93; H, 12.58. Found: C, 74.86; H, 12.89.

The substance was thus identified as palmitic acid, and the amount obtained was 1.05 g.

### Ether Extract of the Resin

The entire amount of this extract (91 g.) was dissolved in a large volume of ether and the ethereal solution shaken successively with a saturated solution of ammonium carbonate, a 10% solution of sodium carbonate and a 5% solution of sodium hydroxide. These alkaline liquids, after being washed with a little ether, were acidified with sulfuric acid and extracted with ether.

<sup>13</sup> Although palmitic acid is usually stated to melt at 62°, it has been observed by Reissert [*Ber.*, 23, 2243 (1890)] to melt at 60.75° in a capillary tube.

The ammonium carbonate extract yielded nothing definite, but from the sodium carbonate extract there was obtained a little of the previously described quercetin and a phytosterolin. A much larger quantity of the latter substance was obtained from the chloroform extract and will subsequently be described. In addition to these substances a very small quantity of an acid was separated which formed a nearly colorless, felted mass of rods and needles. It melted at 188–189° and in aqueous solution gave a dark bluish-green color with ferric chloride, which indicated that it contains a phenolic group. When coupled in a dilute alkaline solution with diazo-*p*-nitrobenzene a dye was obtained, but the amount of material was too small for its further investigation.

The material obtained by extraction with a 5% solution of sodium hydroxide was very dark in color and completely amorphous. It had a phenolic odor, and for the purpose of purification was brought into a flask with a little water and the mixture distilled in a current of steam. The first portion of the distillate (25 cc.) was clear and colorless, and possessed a phenolic odor, but gave no coloration with ferric chloride. It was examined for us spectrophotometrically by Dr. H. Wales, of the Bureau of Chemistry, with the following results. When coupled in a dilute alkaline solution with diazo-*p*-nitrobenzene it yielded a dye which dissolved with the development of a yellowish-red color in water, a purplish-red in alcohol, and an indigo-blue in acetone. These solutions gave the following absorption spectrum maxima: in water, 4950 Å.; in alcohol, 5250 Å.; and in acetone 5950 Å.

The phenolic substance was thus observed to differ from that obtained by the authors from a distillate of the cotton plant,<sup>14</sup> and it also did not agree with any of the known phenols which have thus far been examined spectrophotometrically.<sup>15</sup>

After extracting the ether extract of the resin with the above-mentioned alkalis the ethereal liquid was washed with water, dried and the solvent removed. A very dark colored product was thus obtained from which, after purification, some colorless, crystalline material was separated. This consisted chiefly of the previously isolated paraffin hydrocarbon with a little phytosterolin.

### Chloroform Extract of the Resin

The entire amount of this extract (9 g.) was dissolved in warm alcohol, the solution treated with animal charcoal and filtered while hot. On cooling, a very sparingly soluble, grayish substance separated. This was collected, washed with a little alcohol, and dried. In order to purify the substance, it was mixed with animal charcoal and extracted in a Soxhlet apparatus with hot alcohol. It was thus obtained in the form of a perfectly white, microcrystalline powder, and weighed 1.3 g.

### Isolation of a Phytosterolin (Phytosterol Glucoside), $C_{33}H_{56}O_6$

The above-described substance melted at 218–223°, and had the general characters of a phytosterol glucoside.<sup>16</sup> When a minute quantity of the substance was dissolved in chloroform with a little acetic anhydride, and a drop of concd. sulfuric acid subsequently added, it gave the color reactions that are characteristic of the phytosterols. It was analyzed with the following result.

*Anal.* Subs., 0.1193:  $CO_2$ , 0.3162;  $H_2O$ , 0.1099. Calcd. for  $C_{33}H_{56}O_6$ : C, 72.2; H, 10.3. Found: C, 72.3; H, 10.3.

A portion of the substance was acetylated by heating with acetic anhydride and a little anhydrous sodium acetate. The mixture was then poured into water and allowed to stand for some time, when the solid product was collected, washed with water and

<sup>14</sup> Ref. 1, p. 1766.

<sup>15</sup> Compare Wales and Palkin, *THIS JOURNAL*, **48**, 810 (1926).

<sup>16</sup> Power and Salway, *J. Chem. Soc.*, **103**, 399 (1913).

dried. This was dissolved in hot alcohol and the solution filtered when, on cooling, the acetylated substance separated in colorless plates, having a silky luster. It melted sharply at 165–166°.

In order to ascertain the character of the phytosterol contained in this glucoside, a small portion (0.2 g.) of the original substance was hydrolyzed.<sup>17</sup> For this purpose it was first dissolved in 20 cc. of hot amyl alcohol and 5 cc. of an aqueous 15% solution of hydrogen chloride added, together with 10 cc. of ethyl alcohol in order to form a homogeneous liquid. After heating for two hours in a flask with a reflux condenser the mixture was distilled in a current of steam in order to remove the alcohol. The substance that separated from the aqueous acid liquid on cooling was collected, washed with water, dried, and finally crystallized from alcohol, with the use of a little animal charcoal. It was thus obtained in colorless, glistening laminae, which melted at 135°, and was evidently identical with the phytosterol which previously had been isolated in considerable quantity from the petroleum ether extract of the resin.

The aqueous acid liquid from which the phytosterol had been separated was concentrated, when a small amount of a sirup was obtained which readily reduced Fehling's solution on heating and evidently contained a reducing sugar. The amount of substance was not sufficient for the preparation of the osazone.

The foregoing results had completely established the identity of the above-described substance as a phytosterol glucoside. It is apparently identical with the substance obtained from jalap tubers, which was originally considered to be a dihydric alcohol and designated as "ipurganol."<sup>18</sup>

### Alcohol Extract of the Resin

The portion of the original resin which had been finally obtained by extraction with alcohol amounted to 125 g. and was a black, amorphous solid. In order to ascertain whether it contained any substance of a glucosidic nature it was dissolved in alcohol, a little dil. sulfuric acid added and the mixture heated for two hours in a flask with a reflux condenser. The greater part of the alcohol was then removed by distillation, and the contents of the flask were diluted with water, which caused the separation of a quantity of dark colored resin. The aqueous acid liquid was subsequently extracted several times with ether, when a small amount of a yellow, crystalline substance was obtained. This substance in alcoholic solution gave a greenish color with ferric chloride, and apparently consisted of quercetin.

After extracting the above-mentioned aqueous liquid with ether, the sulfuric acid was removed by barium hydroxide and, after filtration, the excess of barium removed by carbon dioxide. The liquid was again filtered, treated with animal charcoal and evaporated, when a small amount of a sirupy product was obtained which readily reduced Fehling's solution on heating. It was thus indicated that a very small proportion of the alcohol extract of the resin was glucosidic.

### Summary

The material employed for an investigation of the non-volatile constituents of the cotton plant consisted of the branches that had been stripped from the coarse woody stems and from which the bolls had been removed. It therefore comprised chiefly the foliage, together with the "squares" and flowers. On drying the fresh material it lost nearly 80% of its weight.

<sup>17</sup> Ref. 16, p. 404.

<sup>18</sup> Power and Rogerson, *THIS JOURNAL*, **32**, 89 (1910).

For the purpose of its investigation a quantity (29 kg.) of the dried and ground material was extracted with alcohol. Although the extract was acid to litmus, the alcohol recovered by distillation from a water-bath was alkaline and contained an appreciable amount of ammonia and trimethylamine. On subsequently distilling the concentrated alcoholic extract with steam, a small amount of an essential oil was obtained, together with formic and acetic acids. The essential oil gave a strong reaction for furfural. As it could not be considered a normal product of distillation, and as the volatile constituents of the plant had previously been fully investigated, it was not further examined.

After the distillation of the alcoholic extract with steam there was obtained a dark colored aqueous liquid and a quantity of a black, oily resin. These products were separately examined with respect to their constituents.

From the above-mentioned, dark colored aqueous liquid the following substances were isolated: (1) potassium nitrate and potassium chloride, both in considerable amounts; (2) quercetin,  $C_{15}H_{10}O_7$ ; (3) betaine,  $C_5H_{11}O_2N$ ; (4) choline,  $C_5H_{15}O_2N$ ; (5) succinic acid. The presence of a very small amount of salicylic acid was also indicated, together with a crystalline acid that melted at  $105-106^\circ$  and gave no coloration with ferric chloride. A small amount of a neutral crystalline substance which melted at  $140-143^\circ$ , was likewise obtained, but its identity could not be determined.

The aqueous liquid from which the above-mentioned substances had been isolated strongly reduced Fehling's solution on heating, and evidently contained a considerable proportion of a reducing sugar.

The above-mentioned black, oily resin amounted to 909 g., or 3.134% of the dry material employed.

From the crude resinous product, after its extraction with various solvents and subsequent hydrolysis of some of the extracts, the following substances were obtained: (6) a phytosterol,  $C_{27}H_{46}O \cdot H_2O$  (m. p.,  $135^\circ$ ); (7) a phytosterolin (phytosterol glucoside),  $C_{33}H_{56}O_6$  (m. p.,  $218-223^\circ$ ), which yielded on hydrolysis a phytosterol (m. p.,  $135^\circ$ ) and a sugar which undoubtedly was glucose; (8) pentatriacontane,  $C_{35}H_{72}$  (m. p.,  $75^\circ$ ); (9) palmitic acid; (10) an acid of phenolic character (m. p.,  $188-189^\circ$ ); (11) a mixture of volatile acids, consisting apparently of butyric, valeric and caproic acids, the first two predominating; (12) a small amount of a phenolic substance, which could not be identified with any of the phenols which have thus far been examined spectrophotometrically. By the distillation in a vacuum of a portion of the unsaponifiable material a mixture of terpenes was obtained from which the following substances were isolated: (13) dipentene,  $C_{10}H_{16}$  (tetrabromide, m. p.  $122-123^\circ$ ); (14) an oxygenated compound (b. p.,  $200-220$ ) which appears to possess the formula  $C_8H_{10}O$ ; (15) an optically active, dicyclic sesquiterpene,  $C_{15}H_{24}$ ; (16) a product (b. p.,  $300-350^\circ$ ) which evidently consisted of diterpenes,  $C_{20}H_{32}$ .



Inasmuch as we had previously determined the presence of trimethylamine, together with ammonia, in a distillate from the cotton plant, and also in the emanations from the living plant, it was presumed that the primary source of the first-mentioned alkali was choline. The correctness of this view has now been rendered evident by the isolation of both choline and betaine from the plant.

WASHINGTON, D. C.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF  
SASKATCHEWAN]

## ACTION OF ANILINE ON GLUCOSE IN ACETIC ACID SOLUTION. II

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RECEIVED AUGUST 17, 1926

PUBLISHED OCTOBER 6, 1926

Recently<sup>1</sup> it has been pointed out that alcoholic solutions of glucose, aniline and acetic acid become brown at room temperature more rapidly than comparable solutions of any of the components. It was shown that the first step in the production of the colored material consisted in the formation of glucose-anilide, a reaction which is catalyzed by the acid. The appearance of the colored material is, however, by no means confined to aniline, as *o*- and *p*-toluidine undergo similar changes in the presence of glucose and acetic acid. The effect of the acid, again, is to speed up the reaction between the amine and the carbohydrate. Further, acetic acid is not essential, as potassium dihydrogen phosphate functions as well, not only in catalyzing the reaction resulting in the formation of the anilide but also in the further changes leading to the production of the brown material.

The first step in the reaction is the formation of glucose-anilide and this substance undergoes further changes to produce the colored material. Now it has been shown<sup>2</sup> that this anilide has the butylene oxide structure and yet apparently the compound is somewhat unstable. True, when quite pure and dry, the anilide can be kept in contact with air for a year at least<sup>1</sup> without change but solutions, especially in the presence of acid, soon become colored. Likewise, it is rather rapidly hydrolyzed and reduces Fehling's solution. In addition, it is found that solutions of glucose, aniline and acetic acid, which contain glucose-anilide, show a condition of reactivity greater than that of each of the components. In general such acid solutions reduce oxidizing agents more readily than corresponding blanks. Thus, in such solutions of glucose, aniline and acetic acid, three days old, the aniline is more readily oxidized to quinone by potassium dichromate than is aniline in the presence of acetic acid. Again, these acid

<sup>1</sup> Cameron, *THIS JOURNAL*, **48**, 2233 (1926).

<sup>2</sup> Irvine and Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908).