ORIGINAL CONTRIBUTIONS

RECENT CONTRIBUTIONS TO THE CHEMISTRY OF THE COTTON PLANT AND ITS PRODUCTS[1]

By C. A. Browne

The science of chemistry in its investigation of the various crops which are utilized by man has from necessity confined its attention to only very limited portions of the field. A complete chemical history of an individual species of plants has never as yet been written and we are probably safe in saying that it never will be written. Such a history involves a knowledge of every chemical compound in the roots, stalks, leaves, fruits and other organs of the plant; of the manner in which these various compounds are produced; of the chemical processes which underlie all the various physiological activities such as germination, assimilation and growth; of the chemical reactions involved in the relations between the plant and its surrounding, such as soil, water, air, sunlight, insects, bacteria and other externals; and finally of the chemical utilization of the various parts of the plant for the specific needs of commerce and industry. Even the crops which have been most studied, such as corn or wheat, appear to the chemist only in the resemblance of an unsolved, very complicated cross-word puzzle; with here and there a section filled in, with a few suggestive key words perhaps inserted, but with many blank spaces still remaining to be worked out.

In my brief talk to you this morning upon certain features of the research work of the Bureau of Chemistry I am obliged from lack of time to limit myself to the work which some of our chemists in Washington have been conducting upon the cotton plant. This work has been carried out independently in five of our Bureau laboratories each one of whom had been assigned a separate line of investigation and it is the correlation of some of this work that I wish to bring to your notice.

The attention of mankind had been focused so long upon the valuable fibers or seed-hairs of the cotton plant that chemists overlooked for many centuries other numerous possibilities of utilization. This does not mean, however, that nothing remains to be learned at present about the chemistry of the cotton fiber, which as you know has been employed for making cloth, paper, guncotton, celluloid, artificial silk and many other products. Chemistry is discovering every year new uses for the cotton fiber and there seems to be no limit to the number of ways in which it can be utilized. The cotton fiber, as you know, is pure cellulose and, like sugar, belongs to the carbohydrates; all that is needed for its production is water, air and sunshine. In one respect, therefore, it is an ideal agricultural crop for it

---

[1] Presented before the 16th Annual Convention of the Am. Oil Chem. Soc'y, New Orleans, May, 1925.

removes nothing from the soil. If the seed, leaves and other plant residues were completely restored to the soil after each crop there would be no depletion of fertility. In the early days the cottonseed was occasionally returned to the fields as a fertilizer, but more often it was allowed to decay in piles about the gin houses or disposed of by emptying into streams. It was only about 150 years ago when the London Society for the Encouragement of Arts, Manufactures and Commerce, in 1783, offered a prize for the technical utilization of cottonseed for oil and cattle-food production that chemists began to realize that there was more to the chemistry of the cotton plant than that of its seed hairs. Yet progress was slow and nothing of a serious attempt towards utilization began in the United States until after the Civil War, the annual accumulations of refuse seed about the gins constituting up to this time a great nuisance. In 1873 only 2 per cent of the cottonseed crop was utilized for oil and feed production; in 1890 this had increased to 25 per cent and in 1900 to 50 per cent, the progress thereafter being rapid.

In a recent book published by the Chemical Foundation called "Chemistry in Industry" you will find a very interesting chapter by Mr. Thomas C. Law upon "Cotton and Cotton Products" in which he tells the story of how the cotton plant won the World War by means of the four outstanding contributions which it made in the way of food, clothing, shelter and ammunition. Mr. Law gives a list of thirty or more commercial products which chemistry has manufactured from cottonseed oil and which I will therefore omit discussing in order that I may mention first something about the chemical composition of the oil that is removed from the cottonseed by the commercial process of crushing. Cottonseed oil, which has been a subject of investigation for some time at the Bureau of Chemistry, was found by Jamieson and Baughman to have the following composition:

Glycerides of saturated acids	Per cent
Myristic	0.3
Palmitic	20.0
Stearic	2.0
Arachidic	6.0
Glycerides of unsaturated acids	Per cent
Oleic	35.2
Linolic	41.7

While these figures indicate the relative percentages of each fatty acid in the form of its glyceride, they do not indicate the percentage of the different simple and compound glycerides in the oil. When we consider that three different fatty acids may be united with one glycerol radical, the number of possible glyceride combinations in such a complex mixture as cottonseed oil is seen to be very high. Chemists have not as yet attempted to resolve this complex of mixed glycerides into its various components and this difficult analytical problem must await the future for its solution. The separation of complex fatty substances such as cottonseed oil into their various component simple and compound glycerides is without doubt the most important analytical question which the oil chemist of the future must consider. Various methods of attack have been proposed, such as the carefully-controlled fractional crystallization of large quantities of fat at different temperatures, the fractional extraction of the glycerides of different solubility by means of suitable solvents and the separation of certain components by methods of esterification. The future must determine which method or combination of methods shall be most suitable for the purpose.

An important fact noted by Jamieson and Baughman is that cottonseed oil, freshly expressed in the dark, has no action upon a photographic plate. If the expressed oil, however, be first exposed to the sunlight, it acquires the property of fogging a photographic plate when the latter is placed a few millimeters above the surface of the oil in a dark room. The phenomenon does not take place in an atmosphere deprived of oxygen or of moisture. As Russell has pointed out, it probably depends upon the formation of traces of hydrogen peroxide, the action of which upon a sensitized plate has long been known. The mechanism, by which this hydrogen peroxide is formed, is not as yet thoroughly understood. As the reaction does not take place with saturated glycerides such as stearin it is in all probability connected with the absorption of atmospheric oxygen at the points of unsaturation, one atom of active oxygen being eliminated for every atom of oxygen absorbed. The atom of active oxygen thus eliminated might then unite with atmospheric moisture to form hydrogen peroxide. This active oxygen is no doubt also a potent factor in the spontaneous decomposition of the fat, which is associated with rancidity, as suggested by me in a recent paper upon the spontaneous decomposition of butter fat. The subject of rancidity, however, is too vast a one to be considered in a general paper of this kind.

There is also found in crude cottonseed oil a variety of nonglyceride constituents which Jamieson and Baughman have separated and analyzed. They make up usually from about 0.5 to 1.5 per cent of the crude oil although oils from damaged seed frequently contain larger quantities. These non-glyceride components consist of proteoses, peptones, resin, pentosans, raffinose, inosite phosphates, lecithin phosphatides as well as a small quantity of xanthophyll. These impurities in the oil were found to have emulsifying and lipolytic properties which are no doubt the explanation of the losses occasioned by the retention of the oil in the soap stock and of the decomposition of the oil into free fatty acids.

Certain ingredients of the cottonseed, which are left behind after the expression of the oil, have also been subjected to a careful study by the Bureau of Chemistry. One of the peculiarities of cottonseed, which was early noted in connection with its properties as an animal food, was the poisonous action which it sometimes exercised upon farm animals. The explanation of this toxicity remained a mystery for many years until it was finally attributed by Withers and Carruth about ten years ago to an organic chemical substance of the seed which they called gossypol. A large amount of debate has centered about gossypol. Only within the last year certain English investigators have attributed cottonseed meal poisoning not to gossypol but to a dietary deficiency. The problem, however, has been pretty conclusively settled by Schwartze and Alsberg at the Bureau of Chemistry who have confirmed the findings of Withers and Carruth. They found gossypol to be present in every sample of cottonseed examined and in quantities varying from 0.4 to 1.2 per cent of the raw kernels. The variation in gossypol content was fairly regular in that it tended to vary directly with the oil content, although the reason for this relationship is not as yet understood. Schwartze and Alsberg submit the following proofs that cottonseed poisoning is due to gossypol: (1) gossypol when isolated from cottonseed produces the characteristic symptoms of cottonseed poisoning and (2) the toxicity of various raw cottonseed kernels, when fed to rats, corresponded to their gossypol content. Confirmation of this conclusion is also found in the fact that in the Atlantic Coast States, where the gossypol content of cottonseed averages nearly one per cent, cottonseed meal poisoning is more feared than in the Southwestern States, where gossypol occurs in cottonseed in smaller quantities.

The complete detoxification of cottonseed meal either through removal of this gossypol by solvents or through conversion of the gossypol into harmless conversion products by heating or other means, is one of the most important industrial problems which confronts the food chemist today. Cottonseed will then become a most valuable source of protein in the dietary of man and another claim will be established as to the leadership of King Cotton among agricultural crops.

But there is another reason besides that of toxicity which makes it desirable to eliminate the gossypol from cottonseed before employing it as a food for man or beast. Various studies upon the digestibility of the protein in cottonseed meals have given values which are decidedly below those of meat proteins and casein when fed to rats and other animals. On the other hand the isolated cottonseed globulin after purification has been shown to be capable of supporting growth at the normal rate. This seems to suggest that some substance in the cottonseed diminishes the digestibility of its protein. Jones and Waterman, of the Bureau of Chemistry, were accordingly led to test the effect of gossypol upon the digestibility of cottonseed globulin when treated by artificial digestion with preparations of pepsin and trypsin. The average digestibility of the pure globulin under those conditions was found to be 60 per cent and of the globulin plus one per cent of gossypol only 51 per cent. The depressing effect of the gossypol upon the digestibility of cottonseed proteins is thus clearly indicated.

The function of gossypol in the physiology of the cotton plant is an interesting question upon which to speculate. Poisonous principles are found in the seeds of many plants and they have been supposed by some to act as a means of protection against the attacks of insects and other animals; some natural process of chemical selection, by which cotton plants with seeds of higher gossypol content secure greater immunity against animal enemies, may play a possible part in this connection. Such philosophical inquiries, however, need not detain us. To the practical chemist interested in food technology gossypol is an objectionable cottonseed constituent which it is desirable to eliminate. To the theoretical chemist, interested in purely organic research, gossypol is a substance of phenolic characteristics whose chemical constitution still remains to be determined. A joint attack upon the gossypol problem is now being carried on in America by these two schools and we may look forward with confidence to its ultimate solution.

While the oil has been the ingredient of cottonseed to attract most attention in the past it is probably safe to predict that the interest of chemists in the future will be transferred more and more to the proteins. Jones and Csonka of the Bureau of Chemistry are at present giving considerable attention to the separation and identification of the different proteins of the cottonseed. By extracting with a 10% sodium chloride solution finely-ground cottonseed kernels from which the oil and resins had been previously eliminated by means of benzene, they were able to dissolve over 75 per cent of the total nitrogenous matter. Upon heating this filtered sodium chloride solution at 85°C. they obtained a white flocculent coagulum which after washing and drying was found to contain between five and seven per cent of nitrogen and the unusually high ash content of 68 per cent. This ash upon analysis was found to contain 57.29 per cent of phosphoric acid (P_2O_5), 9.71 per cent of calcium oxide (CaO), 16.62 per cent of magnesium oxide (MgO), and 13.90 per cent of sodium oxide (Na_2O). The identification of this peculiar nitrogenous phosphatic compound, which is perhaps a nucleic acid derivative, is now under way.

The filtrate from the phosphatic coagulum obtained at 85°C. was next treated with ammonium sulfate at 0.23 saturation. The flocculent precipitate thus obtained was filtered off, and, after redissolving and reprecipitating with ammonium sulfate several times, purified by dialysis. The compound thus obtained was designated α-globulin, and it contained 18.22 per cent of nitrogen.

The filtrate from the α-globulin was treated with an additional amount of ammonium sulfate at a final saturation of 0.74, when a second flocculent precipitate settled out which after purification and dialysis was designated β-globulin. This preparation contained 17.77 per cent nitrogen, calculated on an ash-, and moisture-free basis.

The dialysate from the β-globulin was concentrated under reduced pressure and then precipitated with alcohol when a fourth nitrogenous preparation was obtained. This material, calculated on an ash- and moisture-free basis, contained 12.64 per cent of nitrogen and 16.57 per cent of a pentose sugar. The ash content of the air-dried preparation was 4.60 per cent.

Jones and Csonka have determined the distribution of nitrogen, in the proteins which they have isolated from the cottonseed. These and other details of their work will soon be published in a forthcoming paper upon "The Proteins of the Cottonseed."

The examination of the cottonseed meals which are sold in the interstate commerce of the United States has been conducted for many years by the Bureau of Chemistry. A comparison of the analytical data obtained in this work for the cottonseed meals produced in different states over a long period of time has led to some interesting observations. The State of Texas, for example, produces seed of higher protein and less oil content than that of states further east. The complete correlation of these results with climatic conditions, such as rainfall, sunshine and temperature, remains to be worked out.

The cottonseed meal which is sold in interstate commerce has given the Bureau of Chemistry a great deal of trouble in a regulatory way, owing to the fact that mills have failed either to appreciate the importance of delivering meal up to its guaranteed percentage of proteins or to overcome difficulties in manufacture. This has resulted in much low protein meal being placed on the market. Some mills have found it possible to keep their meals up to guarantee, while others think it is impossible. Since the protein content of the finished meal can be lowered to any point the mill desires by the addition of hulls, a great deal of attention has been given to this point by managers of the mills. Of course, the more hulls that are added the more meal there is to sell and the managers of the mills feel that they are giving away value when their meal runs even a few hundredths of a per cent over the guaranteed figure. This has resulted in a large number of cases being brought under the Federal Food and Drugs Act on the charge of low protein. In these days of close business competition manufacturers of every commodity find it necessary to allow themselves the least possible excess in fulfilling their guarantees as to weight or composition. But when they permit themselves no leeway of this kind, the carelessness of workmen, the deterioration of product and other contingencies are very apt to bring their commodities below guarantee. The close cutting of guarantees, like that of corners in automobile driving, will sooner or later bring trouble and is never to be recommended.

Leaving now the seeds of the cotton with their wonderful storehouse of interesting chemical substances, we will turn our attention to the leaves, which constitute the laboratory where the raw materials are manufactured that give rise to the cellulose, oil, protein, gossypol and other organic constituents of the plant. The chemical reactions which take place in the leaf of the cotton plant are of such complexity that they are only imperfectly understood. We know in a general way that in the green chlorophyllaceous cells of the leaf, the water which is absorbed through the roots from the soil and the carbon dioxide which is absorbed through the leaf-pores from the air undergo combination under the influence of sunlight with the formation of sugar which is then transplanted to other parts of the plant where it is converted into starch, cellulose, lignin, oil and other nitrogen-free products. We also know that the nitrogen of the soil water (in the form of nitrates and ammonium salts), enters into organic combination with the products of photosynthesis in order to form various amino acids, which are then transported to the seeds and other organs where they are stored up as protein. This formation of the basic components of carbohydrates, oil and protein we may term the primary chemical functions of the leaf; but in the performance of these major activities there take place numerous complicated side reactions of which we can form only a very imperfect picture. Although the substances formed in these side reactions may be insignificant in actual amount yet they can be of vast importance both to the advantage and to the disadvantage of the plant. The essential oils, which make up the perfume of cotton blossoms, exist only in minute quantities yet they are supposed to perform a most useful function in attracting bees, butterflies and other insects that carry the pollen from flower to flower and thus produce the fertilization which is necessary for seed formation. Odorous volatile substances emanating from other portions of the plant may also act injuriously in attracting insects such as the boll-weevil which do serious harm to the plant. This attracting action of a chemical substance for insects, which is called chemotropism, is so powerful that a single cotton plant growing alone in a large field of corn will give off emanations, unweighable in amount, but yet sufficient to attract weevils from a long distance. The determination of some of these odorous volatile compounds, which are given off by the cotton plant, was considered therefore to be a problem well worth the attention of the Bureau of Chemistry, for if the specific chemotropic substance which attracts the boll-weevil can be discovered and if this substance is one which can be made synthetically there will then be offered a possible means of using this compound as a bait to lure the boll-weevil to its own destruction.

The results which have been obtained by Power and Chesnut of the Bureau of Chemistry in their investigation of the odorous constituents of the cotton plant throw much new light upon the complex chemical reactions which take place in the assimilative organs of vegetable life. The first step in this research was the procuring of a sufficient amount of the volatile constituents of the cotton plant for the purposes of separation and identification. As some of these compounds occur in only minute traces, of a few thousandths or ten-thousandths of a per cent, it was necessary to work upon relatively large quantities of fresh plant material. The total weight of leaves, squares, flowers, etc., of the growing cotton plants subjected to steam distillation was 7255 pounds, or over $3^1/_2$ tons. The total amount of original distillate thus obtained was about 1400 gallons which by redistillation in a small copper still was reduced to 78 gallons. This concentrated distillate was then shipped from the Departmental Laboratory at Tallulah, Louisiana, to the Bureau of Chemistry in Washington, where the final processes of separation and identification were carried out. It would be interesting to describe the methods which Dr. Power and Mr. Chesnut employed in this work, but this would consume too much of our time and I can only refer to their paper which is shortly to be published in the *Journal of the American Chemical Society.* I need only state that by various processes of extraction, fractional distillation, and precipitation they were able to isolate no less than fifteen different volatile chemical substances that were removed from the cotton plant by steam distillation. These include the following:

1. Methyl alcohol, CH_3OH, in large amount.
2. Amyl alcohol, C_4H_9OH, in relatively small amount.
3. Acetaldehyde, C_2H_4O.
4. Acetone, CH_3COCH_3.
5. Vanillin, $C_8H_8O_3$, in very small amount.
6. Formic acid, HCOOH. $\Big\}$ Combined to a greater or less extent with alcohols to form esters.
7. Acetic acid, CH_3COOH.
8. Caproic acid, $C_5H_{11}COOH$.
9. An optically inactive dicyclic sesquiterpene, $C_{15}H_{24}$.
10. A new optically active tricyclic sesquiterpene, $C_{15}H_{24}$.
11. A paraffine hydrocarbon, m. p., 62°, which was apparently nearly pure triacontane, $C_{30}H_{62}$.
12. A deep blue oil, which probably contained the highly unsaturated hydrocarbon azulene, $C_{15}H_{18}$.
13. A phenol in very small amount, which was either a derivative of *m*-cresol, or a similar substance.
14. Ammonia, NH_3.
15. Trimethylamine, $N(CH_3)_3$.

Of this complex mixture of alcohols, aldehydes, esters, hydrocarbons, phenols, and ammonia derivatives which are obtained by distillation of the cotton plant there is one of a particularly powerful and penetrating odor and that is trimethylamine, of which as small a quantity as five ten-millionths of a gram can be distinctly detected by the smell. This substance suggested itself, therefore, as the possible chemotropic agent which attracts the boll-weevil to the cotton plant. It still remained, however, to determine whether trimethylamine, which is obtained by steam distillation of the cotton leaves, is also given off by the plant under natural conditions. This question was answered in the affirmative by Power and Chesnut in two ways; first, by making a chemical examination of the dew gathered from cotton-fields; and, second, by examining the condensed vapor given off by cotton plants when growing under bell-jars. Both dew and condensate were found to contain perceptible amounts of ammonia and trimethylamine.

It had been observed previously by Smith of the Bureau of Chemistry in 1923 that the dew on cotton-leaves was strongly alkaline notwithstanding the fact that the natural juices of the cotton plant have a pronounced acid reaction. An analysis of the dew from cotton leaves, made by Power and Chesnut, indicated the presence not only of the volatile alkalies, ammonia and trimethylamine, but also of a fixed alkali. A consideration of all observations led these investigators to the conclusion that the emanation of ammonia and trimethylamine from the cotton plant is due to their liberation on the surface of the leaves through the action of a fixed alkali carbonate from certain glandular secretions, upon salts of ammonium and salts of some simple organic base such as choline, secreted by other organs of the leaf.

It has been found from preliminary tests by entomologists of the Department of Agriculture that trimethylamine does have an attractive influence upon the boll-weevil, but much more extended experiments must be undertaken before it can be determined whether this substance, either alone or in mixture with other volatile compounds, can be of any practical use in helping to solve the problem of the boll-weevil.

In addition to this study of the odorous volatile constituents of the cotton plant Power and Chesnut are conducting an investigation of the non-volatile organic substances which are removed from cotton leaves by extraction with solvents. A report of this investigation which promises some interesting developments must be postponed, however, to a later date.

Such in brief are a few recent contributions to the chemistry of the cotton plant. The correlation of all that we know concerning it is, however, only a small fragment of what remains to be discovered. I have limited my discussion to a few points only about the chemistry of the seed and leaves of the cotton plant; nothing has been said about the chemistry of its roots. In fact we have very little chemical knowledge about the roots and root processes of our agricultural crops and this is a serious omission. In my opinion some great epoch-making discoveries will be made in this much-neglected field. One of the greatest triumphs of modern industrial chemistry is the fixation of atmospheric nitrogen, yet, for almost numberless ages, atmospheric nitrogen has been quietly fixed and utilized in the root nodules of the clover, pea, bean and other leguminous plants. We can prove that nitrogen is fixed there but of the chemistry of this unobtrusive underground process we have almost no information.

Future advancement in the knowledge of the cotton plant and of other crops will best be accomplished by the most active kind of scientific coöperation. Chemistry is the basis which underlies the existence of soils and crops and animals. Eliminate the elements and processes of chemistry and nothing material is left. Yet, agricultural research demands not only the aid of chemistry but of physics, biology, physiology, meteorology, bacteriology, geology and many other sciences. The chemist who works alone will accomplish but little, but if he collaborates with other investigators in the exploration of the borderland where chemistry and the other sciences overlap he will make discoveries of lasting benefit and importance.

BUREAU OF CHEMISTRY, U. S. DEPT. AGRIC.
WASHINGTON, D. C.

COÖPERATIVE WORK OF THE BUREAU OF STANDARDS[1]

BY N. F. HARRIMAN
ENGINEER-PHYSICIST, U. S. BUREAU OF STANDARDS

Immediately following the World War the growing demands for highly trained technical men to meet the increasing demand for industrial research, threatened the Bureau of Standards with a depletion of its staff and thereby a curtailment of its research activities. This situation led to the establishment by the Bureau of a Research Associate Plan, whereby a manufacturer or association of manufacturers might place a graduate physicist, chemist or engineer, at the Bureau for a period of one or more years to carry on investigation in some particular line.

This plan has proved valuable, both in the training of additional research specialists by association with the Bureau staff and in maintaining the research output of the Bureau in lines which are of special industrial importance.

The Bureau of Standards coöperates, wherever feasible, with the industries in the development and promotion of higher standards and minimum production waste in the commodities of commerce. In effect, therefore, the research associate plan is a plan for coöperative investigation to such ends. The work undertaken under this plan is such that it would be done by the Bureau alone if its funds were sufficient. The co-

---

[1] Presented at the 16th Annual Convention of The Am. Oil Chem. Soc'y, New Orleans, May, 1925.