

The investigation will be pursued and if possible more satisfactory methods devised. Any suggestions from chemists having experience in this line will be thankfully received.

### THE PRINCIPAL AMID OF SUGAR-CANE.

BY EDMUND C. SHOREY.

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IT has been more than forty years since Lawes and Gilbert pointed out that in plants used as feed for stock, part of the nitrogen exists in the amid form. Since then several schemes of analysis have been devised by which to determine the amounts of different forms of nitrogen in plants, and numbers of analyses have been published in accordance with these schemes, so that it has come to be generally accepted by chemists that a part of the nitrogen of all plants, in the growing stage at least, is in the amid form. E. Schulze, who has done more than anyone else to advance our knowledge of the forms in which nitrogen exists in plants, states that the amid compounds vary with different plants, and also with the age and condition of the plant.

In 1892 I determined the total and albuminoid nitrogen content of a number of samples of mature sugar-cane. In the samples then examined I found the albuminoid to be about ninety per cent. of the total nitrogen. Analyses made later of another and less mature variety of cane, showed this non-albuminoid nitrogen to be sometimes as high as twenty-five per cent. of the total nitrogen. The albuminoid nitrogen was determined by precipitating with cupric hydroxide, taking special precautions to prevent any decomposition of the albuminoids by preliminary heating. No attempt was made at that time to determine the character of the non-albuminoid nitrogen.

In January, 1894, a paper was read by W. Maxwell before the Louisiana Sugar Planter's Association on "Organic Solids not Sugar in Cane Juice." The matter of this paper was subsequently issued as a bulletin by the Louisiana Experiment Station. In this paper attention was drawn to the fact already noticed, that all plants contain at some period part of their nitrogen in the amid form: a fact well known to chemists, but apparently overlooked by sugar manufacturers. After giving a number of analyses of cane juice, in which the difference

between the total and albuminoid nitrogen was designated amids, Maxwell stated that the amid of cane juice was found to be asparagin, crystallized preparations of this body having been obtained. He made, however, no statements regarding the physical or chemical properties of the asparagin obtained, nor did he give any information as to how it was ascertained to be asparagin.

Some months after the publication of this paper, there was a discussion in the *Bulletin de l'Association des Chimistes de Sucrierie* between H. Pellet and W. Maxwell as to the possibility of the asparagin of cane juice affecting the polariscopic reading of such solutions. In view of this possibility I determined to make some preparations of the amid compound or compounds in cane juice, and ascertain the rotatory power of the same. This work was begun in January and continued until July of the present year, and covers every variation in age and condition of cane delivered at the mill this year, as well as some samples of very young cane.

As a result of this work I have found that the principal amid compound present in sugar cane is *not optically active*, and is *not asparagin*, but glycocoll or glycocin, an amid not heretofore known to occur in plants.

The method of separating the amid was that commonly used, *viz.*, precipitation with mercuric nitrate. To the juice obtained from the cane sample by pressure, a slight excess of lead subacetate was added, the liquid filtered, and to the filtrate an acid solution of mercuric nitrate was added, and the whole brought to faint acidity if strongly acid, by the cautious addition of caustic soda. The precipitate thrown down, which was white and flocculent, after being well washed, was decomposed by hydrogen sulphide. The filtrate from the mercuric sulphide, after being concentrated to a thin syrup, was allowed to stand, when abundant, well defined crystals were generally formed in thirty-six hours. The filtrate from the mercuric sulphide was always quite strongly acid, and the subsequent crystallization was found to be much more rapid, and the yield larger if the solution were neutralized with ammonia before concentration, and neutrality maintained during concentration by the continued addition of ammonia as the solution became acid again.

This addition of ammonia made no difference in the character of the crystalline body obtained. The crystals after separation from the mother-liquor were purified by recrystallizing twice.

The mercuric nitrate used was prepared as wanted by boiling mercury with an excess of nitric acid until no further reaction for mercurous nitrate was obtained. I have as yet made no analysis of the white insoluble precipitate which mercuric nitrate gives with the amid body in question.

The purified crystals were in the form of plates or four-sided prisms, belonging to the monoclinic system; glassy in appearance, quite hard, grating between the teeth and having a sweetish taste. They were soluble in cold water, but much more readily in hot, soluble in eighty per cent. alcohol, but insoluble in ether. In all, fifteen preparations of these crystals have been obtained, and in all cases the physical properties of the crystals and the chemical properties of the solution have been the same.

The mother-liquor from the crystals was found to contain other nitrogenous bodies not readily obtained in a crystalline form, the nature of which has not yet been ascertained; but they are comparatively small in amount, and, since nearly the whole of the bodies precipitated from cane juice by mercuric nitrate can be obtained in a single crystalline form having the properties of an amid, it is quite justifiable to speak of this body as the principal amid of sugar-cane.

In examining this sugar-cane amid to ascertain if it were optically active, solutions containing from two to four grams of the substance per 100 cc. of water were examined in a 200 mm. tube of a Schmidt and Haensch half-shade polariscope, observations being also made on similar solutions after the addition of caustic soda or nitric acid. In no case was any rotatory power shown. As asparagin is slightly left-handed in water-solution, more strongly so in alkaline solution and strongly right-handed in acid solution it was at once evident that the sugar-cane amid must be an inactive form of asparagin, or some other body not asparagin. A more extended examination soon brought out other points of difference between the sugar-cane amid and asparagin; and on comparison of the properties of this amid with those of the other known bodies, it was shown unmistakably to be glycocoll.

The chief points of difference between asparagin and the sugar-cane amid may be stated as below :

	Sugar-cane amid.	Asparagin.
Optical activity.	Inactive.	Left-handed in water solution.
Water of crystallization.	None.	One molecule lost at 100° C.
Behavior with Fehling's solution.	Does not reduce on boiling.	Reduces on boiling.
Behavior when boiled in alkaline solution.	Gives off NH <sub>3</sub> only if alkali is quite concentrated, leaving HCN in solution.	Gives off NH <sub>3</sub> , leaving aspartic acid in solution.

It will be noted that these properties of the sugar-cane amid are identical with those of glycocoll, and in addition to this correspondence, both produce a gray precipitate of metallic mercury when added to a solution of mercurous nitrate, and both give a red coloration with ferric chloride.

The reaction by which the sugar-cane amid is most readily distinguished from asparagin, and by which also its identity with glycocoll is thoroughly established, is that which results on heating in alkaline solution. As is well known, asparagin gives off ammonia when heated in quite dilute alkaline solution, leaving aspartic acid in solution. On the other hand, glycocoll and the sugar-cane amid do not give off ammonia when heated in alkaline solution unless such solution be strongly alkaline, and after the evolution of ammonia has ceased no aspartic acid is found in the solution, but if hydrochloric acid be added to acidify and the solution heated, hydrocyanic acid is given off and can be detected by the smell, and oxalic acid is left in solution and can be precipitated as calcium oxalate. When operating with very small quantities of the amid, as I often found it necessary to do, the quantity of hydrocyanic acid given off in this reaction is so small that the smell is masked by that of the hydrochloric acid; in this case the presence of hydrocyanic acid can be established in the usual way by placing a drop of yellow ammonium sulphide on a porcelain dish, holding over the boiling solution for a few seconds, removing the excess of sulphide by blowing, and prov-

ing the presence of a thiocyanate by the red coloration produced on addition of ferric chloride.

The sugar-cane amid also gives off hydrocyanic acid when heated with dilute sulphuric acid and manganese dioxide.

When heated in a sealed tube with benzoic acid, the sugar cane amid gives hippuric acid, a condensation characteristic of glycocoll, and one by which it is supposed hippuric acid is formed in the animal body.

The hippuric acid so formed was identified by separating it from any remaining benzoic acid by agitation with petroleum ether, evaporating the insoluble residue to dryness with nitric acid and heating, when the characteristic smell of nitrobenzol was detected.

Glycocoll has been prepared in the usual way from hippuric acid by boiling with dilute sulphuric acid, and the reactions given above as characteristic of that body have been verified throughout.

It having been thus thoroughly established that the principal amid of sugar-cane is glycocoll, it is well to note that there are certain points in which glycocoll resembles asparagin, and the resemblance is such that anyone prone to jump at conclusions would on obtaining a crystallized preparation of the sugar-cane amid immediately pronounce it asparagin. The points of resemblance are these: first, the general appearance, solubility, etc., of the crystals; second, neither gives up any nitrogen when treated with sodium hypobromite in alkaline solution; third, both dissolve cupric hydroxide to a blue solution; and fourth, both contain the same per cent. of nitrogen. Asparagin crystallized with one molecule of water of crystallization and glycocoll each contain theoretically 18.66 per cent. nitrogen, while the average of all samples of the sugar-cane amid was 18.69 per cent. The Gunning method was used in determining the nitrogen, and clean, well-defined crystals were selected for such determinations.

In all, fifteen preparations of the glycocoll have been made from sugar-cane; and the samples of cane from which it has been prepared have included young shoots of cane a few weeks old, the green tops of cane one year old, and mature cane growing at elevations from 400 to 1,200 feet above sea-level. It is fair then

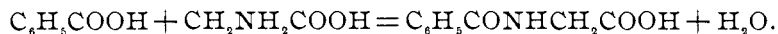
to conclude that glycocoll is not only the principal amid of sugar-cane, but is also a normal constituent of this plant at all periods of its growth.

I have as yet made no attempts to estimate the quantity of glycocoll in sugar-cane or any of its products, but have noticed that larger amounts have been obtained from young than from mature cane.

The average nitrogen content from mature sugar cane is probably about 0.30 per cent., and of this at least 0.22 per cent. is albuminoid nitrogen; so that the amido content cannot be higher than 0.08 per cent., and probably is often less than 0.02 per cent. It is easily seen then that the preparation of any quantity of the sugar-cane amid entails considerable labor, and this coupled with the fact that this work has been done at odd times during a busy grinding season, is my excuse for the incompleteness of this research in some respects.

The identification of glycocoll, glycocine, or amido acetic acid in sugar-cane is of interest from several points of view. Its occurrence has not been noted in a plant before, and it has been considered a body belonging wholly to animal chemistry. It can be prepared from gelatin by heating with acids or alkalis, in fact, was first prepared in this way, and it has been supposed that it was formed in the animal body from gelatin or gelatin-yielding proteids.

It does not seem to occur free in the animal body, and the theory of its formation from proteids seems to have been demanded by the following fact: Benzoic acid, when taken into the stomach, appears in the urine as hippuric acid, and to explain this it has been supposed that the condensation already referred to takes place; *viz.*, benzoic acid and amido acetic acid combine to form hippuric acid with the elimination of water.



This view is strengthened by the fact that when glycocoll is taken with benzoic acid the yield of hippuric acid is increased.

In the urine of herbivorous animals hippuric acid occurs in relatively large quantities, and while the food of such animals has been deemed capable of supplying the benzoic acid part, the

source of the glycocoll to form such large quantities of hippuric acid has been more or less of a puzzle to physiologists.

The identification of glycocoll in sugar-cane and the fact that it has been mistaken for asparagin suggests the probability of its occurrence in other plants, especially the *Graminae*, which form the major part of the food of herbivorous animals; and it is quite likely that the source of the hippuric acid in the urine of such animals will be found in such occurrence.

In vegetable, as in animal physiology, the question of the constitution of proteids is uppermost. Now glycocoll in the physiology of sugar-cane no doubt plays the same part which other amids are known to do in other plants. It is the form in which nitrogen is conveyed to growing parts, and when maturity is reached the amid becomes the albuminoid to become the amid again when a new growth takes place. A number of facts collected during this study of the sugar-cane amid point to the existence in cane of a gelatin or gelatin-yielding proteid which yields glycocoll as one of the products of decomposition. This coupled with the well known and comparatively simple constitution of glycocoll seems a step toward the understanding of at least one proteid of sugar-cane. The further study of this matter promises to be of special interest, both from a physiological and a technical point of view.

From the sugar manufacturer's point of view the presence of glycocoll presents the following points of interest: It is known that at various stages of sugar manufacture ammonia is given off from boiling cane juice, especially if this juice has been rendered alkaline by an excess of lime. This has been stated as due to the decomposition of asparagin into aspartic acid and ammonia. But since we know that the amid is glycocoll and not asparagin, and that glycocoll is not decomposed unless boiled in strongly alkaline solution, it is plain that the ammonia must be derived from the decomposition of albuminoids. When, as is generally the case, cane juice is maintained approximately neutral throughout the course of manufacture, the whole of the glycocoll originally present in the juice should be found in the final molasses.

Whether this theory always carries out in practice, I have not yet ascertained, but I have obtained glycocoll in comparatively

large quantities from several samples of refuse molasses by the same method by which it was obtained from cane juice.

In one sample I determined the amounts of different forms of nitrogen according to a well known scheme of analysis with the following result :

	Per cent.
Nitrogen as free ammonia .....	0.011
Nitrogen as albuminoids.....	0.126
Nitrogen as peptones.....	0.050
Nitrogen as amids.....	0.201
Nitrogen as other forms .....	0.228
Total nitrogen .....	0.616

Free ammonia was determined by distilling with magnesia free from carbonate, and albuminoids by precipitating with cupric hydroxide. The nitrogen designated peptones is that precipitated by phosphotungstic acid after the removal of free ammonia and albuminoids. In the case of cane juice and its products this precipitate does not contain peptone bodies, and its character will be treated in a future paper. After removal of these three forms of nitrogen, amids were determined by boiling for an hour with two per cent. sulphuric acid, neutralizing and distilling with magnesia free from carbonate and doubling the amount of ammoniacal nitrogen obtained for amid nitrogen. This method which is fairly accurate in the case of asparagin which gives up half its nitrogen as ammonia when boiled with dilute acids, is of no value in the case of glycocoll, so that the above analysis should read amids and other forms 0.429 per cent. instead of

	Per cent.
Amids.....	0.201
Other forms.....	0.228

From a number of years' experience in working with cane juice, I am convinced that the formation of molasses is due to mechanical, rather than chemical, conditions, that the presence of viscous non-crystallizable bodies prevents the further crystallization of sugar by rendering the motion of the sugar molecules in the liquid difficult, and the mellassigenic action of crystallizable salts is very slight. A body such as glycocoll would then exert little effect on the crystallization of sugar one way or the other, especially as it exists in cane in such small



amounts, but it is likely that the proteid directly connected with glycocoll will be found to be highly melassigenic.

We have been in the habit of associating the so-called gums or viscous bodies in cane juice with the cellulose or non-nitrogenous constituents of the plant, but we may have to modify this view in the presence of a gelatin-yielding proteid peculiar to sugar-cane and allied plants.

LABORATORY OF KOHALA SUGAR CO.,  
KOHALA, HAWAII, H. I.

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[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE CASE SCHOOL  
OF APPLIED SCIENCE.]

## XXIX.—THE INFLUENCE OF ANTISEPTICS ON THE DIGESTION OF BLOOD FIBRIN BY PEPSIN IN A HYDROCHLORIC ACID SOLUTION.<sup>1</sup>

BY CHARLES F. MABERY AND LEO GOLDSMITH.

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WHILE it may be difficult to perform experiments on artificial digestion under conditions that approximate those of natural digestion in the body, it is possible to determine the influence of substances that exert a retarding influence on the normal chemical reactions which involve the solution of fibrin or albumin by pepsin in an acid solution. The retardation by certain artificial colors, oroline yellow, saffoline, and magenta, was studied by Weber,<sup>2</sup> and the influence of alcohol in different proportions, and other bodies by Chittenden and Mendel.

In a recent number of this Journal,<sup>3</sup> Frank D. Simons gave an account of results which he had obtained in studying the action on the digestive ferments of salicylic acid, formol, several dye-stuffs and essential oils.

In view of the stringent food laws that are everywhere recognized as necessary for the protection of the health and pecuniary interest of the consumer, the influence of adulterations commonly found in foodstuffs and in prepared foods should be well understood.

<sup>1</sup> The experiments described in this paper were made at my suggestion by Mr. Goldsmith, and they formed the subject of his thesis for the degree of Bachelor of Science. The paper was read at the meeting of the Cleveland Chemical Society, June, 1897.

<sup>2</sup> This Journal, 18, 1092.

<sup>3</sup> *Ibid.*, 19, 744.