

DEXTRO-ROTARY SUBSTANCE IN BAGASSE.

By E. P. HEDLEY AND F. W. HAYES.

This investigation was undertaken primarily with a view to establishing the fact that some substance, having optical rotatory power, is extracted from apparently sucrose-free bagasse on prolonged boiling; and, if possible, to discover the nature of this substance. The practical importance of the determination is naturally subject to the ability of this substance to remain unprecipitated by the usual clarifying agents.

Samples of 18 months plant cane were taken representing five different varieties of sugar cane: Uba, P.O.J.2725, P.O.J.2878, Co. 290 and Co. 281. The general treatment, and the behaviour of the different samples to various tests are set out in the appended table.

The samples were first finely shredded, as much of the juice as possible expressed in a hydraulic press under 2,500 lbs. pressure, and then lixiviated in changes of cold water for periods of from one week to ten days. When the extract after standing in contact with the bagasse for 12 hours showed no reaction with the molybdate test, and no reading in a 400 mm. tube in the saccharimeter, it was presumed to be free from sucrose. The fibre was then dried at 95-100° C. Sufficient was then weighed out to give a N/4 solution and boiled in the standard apparatus for one hour. The extract, which in all cases gave a pol, ranging from 0.15 to 0.5%, was then divided into two portions, the first being tested without any other treatment, the second being evaporated to half the volume, clarified with neutral lead acetate and de-leaded with a minimum of sodium carbonate. The fibre from this first boiling of one hour was again dried, and a N/4 solution boiled under the same conditions for *three* hours. This extract was then divided into two portions and tested as in the case of the first boiling.

Dealing first with the results from the one hour boiling, it will be noticed that in only two cases is there any drop in pol after clarification, and in these the difference is very small. It can therefore be presumed that the rotary substance is *not* precipitable by lead acetate. The colour reaction to the molybdate test is also very interesting. In all instances the green coloration was most marked and is unlike that given by di-saccharides, hexoses, or pentoses in the *pure* form.

The phloroglucin test ¹ shows the presence of pentoses *or* glucuronic acid and distinguishes from oxy-cellulose. The naphtho-resorcin test ² shows the presence of a pentose and distinguishes from glucuronic acid. These two results therefore, point to the presence of a pentose sugar, either arabinose or xylose.

The furfural yield was determined according to Tollens & Kröber's method ³ and gave comparable

and consistent results on duplication. The results are shown as grams furfural phloroglucide and also calculated as pentose per cent dry fibre. In each case there is a small drop in furfural-yielding substance after clarification. Rather than accounting for the drop in furfural yield between unclarified and clarified solutions by the presence of some non-pentose, it is more probable that the evaporation in presence of the excess of sodium carbonate had a destructive action upon the xylose or arabinose. It should be borne in mind that although substances other than pentoses (furfuroids) may form furfural on distillation with hydrochloric acid, yet the possibility of the presence of glucuronic acid or oxycellulose is discounted by the naphtho-resorcin and phloroglucin tests. Again, sucrose, fructose and other hexoses may form hydroxymethylfurfural on distillation, but Cunningham and Dorée ⁴ show that the furfural condensation takes place rapidly, and if the distillation is stopped when no further pink coloration is shown with aniline acetate paper there is little likelihood of any hydroxymethylfurfural coming over.

To be able to interpret correctly the results of the analyses and tests shown, one must first consider something of the nature of the fibre with which one is dealing, and the changes that may take place in its composition due to prolonged boiling.

In all fibres of this description, whether derived from wood, straw, or any of the higher plants, the carbohydrate portion of the cell wall may be taken as consisting of cellulose, in association with complex poly-saccharides grouped under the name of hemicellulose, as lignin, pectin, gums. Knowing the resistant character of cellulose itself to chemical treatment, the hemicellulose must be looked to as the source of the dextro-rotary substance, which gives every indication of being a sugar, and a *pentose*.

The hydrolysis of pentosans into the corresponding pentose when boiling with a very dilute solution of sodium carbonate (as in bagasse analysis) is extremely likely. Taus ⁵ found that in boiling wood under pressure with water *only*, he obtained as much as 11.19% of sugars. Koch ⁶ carried this further, and boiled the wood after freeing from hemicellulose and pentosans, obtaining a syrup containing:—

Laevulinic acid	7.8%
Mannose	29.2%
Glucose	23.9%
Fructose	trace

Assuming cellulose to consist of glucose residues only, he concludes the hemicelluloses to have been incompletely removed, and the mannose to be derived mainly from the lignin complex.

The analysis of Browne and Blouin ⁷ shows the hydrolytic products of the cane fibre to consist of:—

Cellulose	55%
Xylan	20%
Araban	4%
Lignin	15%
Acetic acid	6%

Again, they show the whole fibre to consist of Cellulose 49 to 51%, Pentosans 27 to 32%, Lignin 15 to 17%.

To account for the optical rotation one must presuppose the hydrolysis of the pentosan to the pentose, as the former is *laevo-rotary*. An indication that this hydrolysis has taken place is the fact that the *laevo-rotary pentosan* is precipitated by lead acetate, whereas the dextro-rotary sugar is not.

Allen ⁸ states that when pentose solutions are treated with basic lead acetate and the excess lead removed with sodium carbonate, the pentoses exercise (quantitatively) a reducing action on Fehling's Solution.

To confirm this a solution of pure xylose polarising 3.6° V. in a 400 mm. tube was treated with Horne's dry basic lead acetate, de-leaded with potassium oxalate and filtered. The saccharimeter reading remained unaffected.

At present it is exceedingly difficult to say in what form the pentosan is originally present in the fibre—whether as lignin, pectin, or gums. Indeed, there exists a great deal of confusion as to the correct classification of, and the distinctions between, these different types of hemicelluloses. Pringsheim ⁹ describes "hemicelluloses" as a poorly defined collective name for polysaccharides having a lesser resistance to hydrolytic agents than cellulose. Onslow ¹⁰ states the cell wall may have, as components, at least six sugars of the pentose and hexose type, and regards it as the non-living record of the metabolic activities of the protoplasm. The cellulose is a complex formed from the condensation of glucose alone; and oxycellulose from glucose and glucuronic acid. Then from the decarboxylation of glucuronic acid a complex of xylose may arise, forming *xylan*. Or by conversion of glucose to galactose, a galactan may be formed. The basal unit of *pectic substances* is galacturonic acid, which in turn may be produced from the oxidation of galactose; the decarboxylation of galacturonic acid forming araban.

So that whether one wishes to regard the source of the hydrolysed pentosans as the lignin, pectins, or gums, matters really little, though they will exist in the form most easily hydrolysed by a very dilute alkali solution, or by boiling water alone.

Sucrose-free fibre from a sample of P.O.J.2878 cane was treated with a 4% solution of sodium hydroxide. The extracted hemicelluloses were then precipitated with alcohol (96% by volume), filtered and dried at a low temperature. The hemicelluloses on solution in boiling water and

hydrolysis with sulphuric acid yielded the dextro-rotary pentose sugar. The fibre which had been extracted was then washed free of alkali and boiled with water; but although this was prolonged for 4 hours no further optically active substance was obtained. Therefore, the dextro-rotation found is due entirely to the hemicelluloses.

The *Pectins* are soluble in water, and Ehrlich ¹¹ states that the so-called insoluble pectin, or protopectin, present in the cell wall of the beet is firmly united to the araban complex, but the combination is split by treatment with hot water.

By some, both gums and pectins are regarded as "degradation products," produced by extensive changes in unligified tissues.

The following quotations, from Onslow ¹² and Pringsheim ¹³ are useful in summing up the position so far as the source of the pentosans is concerned:—

"It appears, looking at the matter in its widest aspect, as if the classification into hemicelluloses and pectic substances might be somewhat artificial. Rather, one would picture the cell wall as consisting, primarily, of cellulose, which may give rise, however, to glucuronic acid and xylose, or, if galactose is incorporated, to galacturonic acid and arabinose. Then alkalis, possibly by destroying certain linkages, will extract some components, the hemicelluloses, from the whole . . ."

"Hemicelluloses are a group of polysaccharides filling the gap between cellulose on the one side and starch, glycogen and inulin on the other. Between these intrude a host of members that are hard to separate, difficult to differentiate and only a few of which occur in the pure form. Closely related to these are gums, mucilages and pectins—all products of sugar condensation. The literature on the subject is a mass of inaccurate and self-contradictory chemical and physiological statements. It is no wonder the nomenclature is unsatisfactory and, for example, compounds characterised as reserve material are classified as hemicelluloses."

As a proof that the optically active substance extracted from the lixiviated bagasse by boiling *was not sucrose*, samples of the extract from the one hour boiling were allowed to stand for one week with the addition of 20 ml. of 4% invertase solution. In no case was there a loss in pol of more than 0.05%.

A further experiment was carried out in which some 18 months Uba plant cane was shredded, the juice expressed with the hydraulic press, and then 250 gms. of the sample covered with water containing 20 ml. of a 4% Invertase solution. Fermentation was vigorous, and was allowed to proceed one week. The water was then changed, the bagasse washed, and kept under water for another week. At the end of that time the extract was tested:—

Pol—Nil.

Molybdate test—Negative.

The fibre was then boiled as in the treatment of samples explained in the table, for one hour and three additional hours. The one hour extract showed 1.4% Pol. and 0.461% Pentose (from furfural distillation). The subsequent three hour extract showed 0.2% Pol. and 0.316% Pentose. This experiment was later repeated giving similar, and quite definite, results.

It is difficult to imagine, then, that the appearance of an optically active substance on boiling may be due to any retention of sucrose in the samples after their prolonged treatment with cold water. Mention has been made by G. C. Dymond and R. M. Bechard (S.A.S.T.A. Conference Proc., 1931, pp. 40-43), who quote E. Haddon (S.A. Sug. Journ., 1930, p. 741) of a sucrose retaining enzyme, but no definite proof of the existence of such an enzyme has been found in the literature on the subject.

Cytase, the enzyme which digests hemicelluloses, is destroyed by heating at 60° C. for 30 minutes¹⁴. So there is no chance of hydrolysis being due to this cause.

Little help is to be looked for from the "barytä" method, which Haddon advocates, in preventing hydrolysis or eliminating the pentose sugars formed. The weak solution of barium hydrate would, as with other dilute alkalis, merely facilitate hydrolysis, and xylose is definitely *not* affected by it.

In an attempt to establish the presence of a pentose in the extract from boiled bagasse the selective fermentation by yeast was resorted to. Samples of Uba, P.O.J. 2725 and 2878 cane were crushed, and the bagasse boiled in the standard apparatus for 45 minutes. The extract was then polarised and the fermentable sugars removed by fermentation with fresh yeast. The tests were done in triplicate. When fermentation was complete a portion of the liquor was clarified with lead subacetate, filtered with kieselguhr, and polarised. The readings were made in a 600 mm. tube and the results reduced to a percentage on fibre. In all cases a dextro-reading was obtained. This varied from 0.05 to 0.15%, the average being 0.10%.

This reading remained constant in the fermented liquor for 3 days, but it then began to drop slowly. A xylose destroying bacteria was suspected. To verify this, portions of a pure xylose solution were left in contact with the same yeast as used for the bagasse extract fermentation, and the effect on polarisation observed.

	Polarisation at start.	After 3 Days.	After 4 days.
1st Yeast	3.6	3.2	3.0
2nd Yeast	3.6	3.0	2.9
Blank	3.6	3.6	3.6

It may therefore be concluded from the action of yeast on the extract, that a non-fermentable sugar

is present, which, however, is slowly utilized by some bacteria or fungi present in the yeast. Had a pure culture been available this latter difficulty might have been obviated.

To sum up, a substance was obtained on boiling sucrose-free bagasse fibre with water, which gave every indication of being a pentose sugar, probably xylose. So in the existing method of bagasse analysis, where the material is boiled with water, some substance, other than sucrose, is formed which affects the polarisation. This result confirms the statements made by Prinsen Geerligs in "Cane Sugar and Its Manufacture," pp. 51-52.

A series of determinations on the varying polarisation figures obtained with different times of boiling was carried out by J. Bruniquel, with one of us, and the results obtained bore out the previous figures of other workers.

His experiments showed a continuous loss in weight of fibre on boiling, and furfural yields roughly proportionate to the time of boiling were obtained from the extracts. An attempt was made to isolate the pentose formed and identify this by means of its osazone, but it would appear that there are slight impurities present which interfere with crystallisation, and yet are difficult of removal without eliminating or destroying the pentose sugar itself.

The conclusions reached by the writers of this paper naturally bring forward the determination, and definition, of what is commonly known as "cane fibre." In this connection both the definition of fibre and the method of bagasse analysis, as recommended by the Committee on Uniformity in Reporting Factory Data of the International Society, would merit further investigation and discussion.

REFERENCES.

- 1 Browne, C. A., "Handbook of Sugar Analysis" (1912), 381.
- 2 Ibid. 383.
- 3 Ibid. 450.
- 4 Biochem. J. (1914), 8, 438.
- 5 Tauss, H. Dinglers polytech. J. (1889), 273, 276-285.
- 6 Koch, W. "Verhalten von Lignocellulose beim Erhitzen mit Wasser unter Druck." Diss. Freiburg (1909), 39.
- 7 Louisiana Agricultural Experiment Station. Bulletin No. 91.
- 8 "Allen's Commercial Organic Analysis" (1924), 502.
- 9 Pringsheim, H. "Chemistry of the Saccharides" (1932), 147.
- 10 Onslow, M. W. "Principles of Plant Biochemistry" (1931), 66.
- 11 Ehrlich, F. Die Pektinstoffe, ihre Konstitution und Bedeutung, Chem. Ztg. (1917), 41. 197-200.
- 12 Onslow, M. W., loc. cit., p. 84.
- 13 Pringsheim, H., loc. cit., p. 307.
- 14 Waksman & Davison "Enzymes" (1926), p. 101.

Experiment Station,
South African Sugar Association,
Mount Edgecombe.

February, 1934.

TREATMENT.	VARIETIES.				
	Uba.	P.O.J. 2725	P.O.J. 2878	Co. 290	Co. 281
<p>All shredded and lixivated with cold H₂O until showing no polarization and negative molybdate test.</p> <p>Fibre dried, weighed and boiled 1 hour.</p> <p>Two portions of the extract:—</p> <p>1. Unclarified: Pol Molybdate test Naphtho-resorcin test Phloroglucinol test Furfural—As phloroglucide As pentose % dry fibre</p> <p>2. (Evaporated to approximately half bulk.) Clarified neutral lead acetate, filtered, de-leaded with a minimum of sodium carbonate and filtered:— Pol Furfural—As phloroglucide As pentose % dry fibre</p>	<p>0.2% Green Positive for pentose Red 0.169 gms. 0.180</p> <p>0.2% 0.0131 gms. 0.0358</p>	<p>0.5% Green Pentose Red 0.0965 gms. 0.255</p> <p>0.5% 0.073 gms. 0.152</p>	<p>0.15% Greenish-blue Faint for pentose Red 0.276 gms. 0.434</p> <p>0.0% 0.141 gms. 0.283</p>	<p>0.4% Greenish-blue Pentose Dark brown 0.1115 gms. 0.2280</p> <p>0.3% 0.0975 gms. 0.201</p>	<p>0.2% Green Faint for pentose Light red 0.1240 gms. 0.200</p> <p>0.2% 0.0908 gms. 0.187</p>
<p>Fibre from the first boiling of 1 hour dried, weighed and boiled for 3½ hours.</p> <p>This extract divided into two portions.</p> <p>1. Unclarified: Pol Molybdate test Naphtho-resorcin test Phloroglucinol test Furfural: As phloroglucide As pentose % dry fibre</p> <p>2. Evaporated and clarified as in first hour's boiling:— Pol Furfural—As phloroglucide As pentose % fibre</p>	<p>0.4% Green Red, and bluish ppt. (pentose or glucuronic acid?) Red 0.0770 gms. 0.071</p>	<p>0.3% Green As for Uba Light red 0.1638 gms. 0.3750</p>	<p>0.3% Green Not positive Light red 0.1415 gms. 0.2850</p>	<p>0.2% Green Not positive Light red 0.1460 gms. 0.2930</p>	<p>0.3% Green Not positive Light red 0.1390 gms. 0.2240</p> <p>0.2% 0.1100 gms. 0.2240</p>

NOTE:—Since reading this paper the authors found that some syrup set aside had crystallised. Only a small weight 0.25 gr. of crystals were obtained which in the crude state melted at 122-128° as against 137-142° of pure xylose in the laboratory stock. 0.15 gr. of these crystals were taken and Bertram's reaction carried out. The resulting double salt of cadmium and cadmium bromide gave 21.2% Br., a theory requiring 21.32% Br. There is therefore no doubt that it is xylose which is formed during the boiling of bagasse which increases the pol reading.

CHAIRMAN: This Association is certainly getting on when we can produce a paper of this description (hear, hear). I think we can be very grateful to Dr. Hedley and Mr. Hayes for this paper. It must have taken a tremendous amount of work.

Mr. MOBERLY: I would like to ask the authors whether they used the tests given by Browne for the determination of xylose. One is done with bromine and cadmium carbonate, and another depends on the action of para-formaldehyde producing certain substances which can be identified.

Mr. HAYES: Neither of these tests were applied to the sugar formed. The colour tests with phloroglucin and naphthoresorcin, although not specific for xylose, show the presence of a pentose.

Mr. DODDS: This is a most interesting and instructive piece of work in a branch of chemistry which is notoriously obscure and difficult,—the chemistry of Sugar—and it is very gratifying to see a paper of this nature presented to the Congress. It has many important practical bearings as well as theoretical ones as the authors have indicated. It seems unfortunate that having gone so far they were unable positively to identify the presence of xylose by crystallisation. There seems to be no doubt that xylose was present, but it would have been more satisfactory perhaps if its existence could have been clinched, so to speak, by crystallising it out. The difficulty of crystallisation of sugars containing impurities is well known. It is often a matter of time, and perhaps if the authors were to give the syrup containing the xylose several months to crystallise out in the desiccator something may be obtained. I have known of several occasions in which crystals have been obtained when the syrup was set on one side and practically almost forgotten.

Mr. HAYES: The great difficulty when dealing with such a dilute solution is to crystallise the xylose in the presence of these slight impurities. The concentration was usually done under reduced pressure, but even so there is a grave risk of decomposition if the solution is allowed to stand for a long time, there is always danger of bacteria affecting the sugar itself and decomposition taking place, unless it is kept under absolutely sterile conditions.

Mr. MOBERLY: There is one point to which attention must be given, especially when we come to consider the actual effect of the presence of these substances on our determinations. There seems to be no way of actually eliminating them in the test, but there is one property of xylose which we should bear in mind, which is that it shows a very strong muta-rotation. It would be interesting to have the times of stability known, so that in doing our bagasse analysis allowance can be made for that to ensure that at any rate the dextro effect is at its maximum at the time polarisation takes place.

Mr. HAYES: The strong muta-rotation of xylose is, of course, well known, and was considered in polarising our xylose solutions. We generally allowed up to an hour so that all signs of it should cease. The same thing was done in the case of the bagasse extracts themselves to make sure that there was no muta-rotation affecting the result.

Mr. MOBERLY: My point was that in doing the actual analysis of bagasse sufficient time should be allowed to elapse for the rotation to reach its maximum.

Mr. DYMOND: Might I ask if all these experiments were carried out with boiling water. Some years ago I prepared a paper on a similar subject, and no increase was shown when water of 50°C. was used.

Mr. HAYES: All the experiments were carried out with boiling water. The sucrose was first extracted in cold water. Although one must agree that there is less possibility of hydrolysis taking place at 50°C., the investigation was undertaken to show that a substance other than sucrose is produced in the standard method of extraction with boiling water.

Dr. HEDLEY: It will be remembered that in Prinsen Geerlig's book the author considers ten minutes is sufficient boiling to extract all sucrose. Nobody appears to adhere to that, most of the other methods recommend an hour's boiling. We have had this argument in private and one or two estates have said they prefer to carry on with a long boiling because they get comparable figures with the past years analyses.

Mr. P. MURRAY: I should like to know how much these dextro-rotary substances increase the pol. of sugar. Have they investigated that at all; can they tell us how much it is throwing up the polarisation to-day? According to this we should not be using too much boiling water. Also, the heavy milling must grind down a lot of this stuff and turn it into some of these substances.

Mr. HAYES: The actual figures obtained are shown at the back of the paper. These are all expressed as a percentage on dry fibre, but allowing approximately 50% moisture the corresponding figure for bagasse itself can easily be computed. It must be borne in mind that there is great difficulty in saying when the sucrose is all extracted and when the hydrolysis actually occurs. It is possible that both take place concurrently. One cannot make any arbitrary deductions from the sucrose percentage shown by one hour or 45 minutes' boiling, because there is no constant content of pentosans or hemicellulose. This varies not only in different canes but in the same variety. In connection with the effect on milling of hot maceration, it has been put forward as an argument that some factories started using hot maceration and obtained increases in extraction and overall recovery. But there is a great difference between sucrose extracted from a milling point of view and a sucrose determination. The latter simply aims at an equal concentration of dissolved substance in the cell and the extracting liquid.

Mr. BECHARD: The authors mention a remark

I made in 1931, regarding the work that had appeared in the reports of the French Association of Chemists. That was in regard to the Naudet process of extraction, which is diffusion following milling. There was quite a lot of interest aroused at the time and yet I notice here that the authors say they can find no definite proof in the literature. If they consulted the bulletins of the French Association of Chemistry they would find some authors claim that it was definitely a dextro-rotary substance which was non-sucrose.

Mr. HAYES: We were not able to find any reference to an enzyme which "retained" sucrose, and if we could obtain that reference we should be very grateful.

CHAIRMAN: Our thanks are due to Dr. Hedley and Mr. Hayes for this interesting paper, and I would ask you to accord them in the usual way. (Loud applause.)

The following paper by Mr. H. H. Dodds was read next.