CCCII. STUDIES ON HEMICELLULOSES.

VI. THE HEMICELLULOSES OF THE FLOWER OF THE HOP (*HUMULUS LUPULUS*).

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THE study of the carbohydrate constituents of the hop flower constitutes an almost entirely neglected chapter in plant biochemistry. On the other hand, the chemistry of the antiseptic principles present in the hop has been the object of intensive research, chiefly owing to the known technical importance of these substances in the brewing processes. Nevertheless, a study of the complex carbohydrates of the hop should be of interest from both academic and technical viewpoints, since on the one hand we believe that this is the first occasion on which the hemicelluloses of floral organs have been described and on the other the colloidal systems formed by such substances in solution may have technical importance. The papers of Fink *et al.* [1935-36] on pectin in hops indicate a growing interest in the nature of this substance and its possible role in brewing technique.

It is proposed to describe studies on the nature of pectin in hops in a subsequent communication, whilst in this paper the preparation and properties of the hemicelluloses will be discussed.

The methods employed in the preparation of the hemicelluloses were similar to those indicated in earlier papers in this series [Norris & Preece, 1930; Preece, 1930], with such modifications as were found necessary in dealing with this particular source and several improvements in technique which have been indicated in the previous communication [Angell & Norris, 1936]. In addition, since the introduction of more exact methods of calculation of analytical results [Norris & Resch, 1935; Angell *et al.* 1936] we believe that we are now in a position to determine the proximate constitution of our products with greater accuracy than hitherto.

A more elaborate treatment of the material prior to extraction of the hemicelluloses was necessary in the case of hops since the amount of substances soluble in organic solvents was greater than in other materials examined by us. Failure to remove such substances from the original material led to considerable difficulty in purifying the final products which were invariably contaminated. The hops in bulk were therefore extracted to exhaustion successively with 95 % ethyl alcohol, methyl alcohol and ether. Extraction with water and hot ammonium oxalate solution followed, whereby all pectin was removed. The removal of lignin presents a considerable difficulty which Norris & Preece [1930] sought to overcome by extraction of the material with a 1 % solution of NaOH in 50 % alcohol. It was considered that while this reagent would remove a large proportion of the lignin, any tendency for the hemicellulose also to pass into solution would be checked by the presence of the alcohol. In a quantitative study Norman [1935] criticized this method on the ground that, in fact, hemicellulose was extracted and lost at this stage. We have carefully examined the material

before and after extraction, and the product obtained by the extraction, in the cases of hops and of maize cobs, and in these two cases at least have found no appreciable loss of furfuraldehyde-yielding substances.

The hemicelluloses were extracted as usual with 4% NaOH and fractionation was carried out with modifications of the method suggested by Norris & Preece [1930]. The first fraction, A, was most completely precipitated by acetic acid at pH 3, a more acid value than that for maize cob hemicelluloses [Angell & Norris, 1936]. The precipitation of fraction B with half a volume of acetone did not occur until the solution was further acidified to pH 2. On the other hand, fraction C was not precipitated at all in very acid solutions—pH lower than 4—but was precipitable by acetone in neutral or slightly acid solutions, with a feeble optimum at pH 5.5. Thus it would seem, as we have indicated in the previous communication, that the optimum pH of precipitation of the hemicellulose is a characteristic of each type.

Further fractionation was effected through the copper gels using cupric sulphate and glycerol for precipitation and acetic acid for decomposition [Angell & Norris, 1936]. In this way four fractions were obtained, namely A_1 , B_1 , B_2 and C_2 , of which A_1 was by far the largest. The yields of B_1 and C_2 were so small as to preclude complete analysis.

Fractions A_1 , B_2 and C_2 showed no striking differences in composition, since they all contained anhydroxylose, anhydroglucose, glycuronic anhydride and methoxyl groups. Quantitative examination revealed, however, that the proportions of each constituent varied very markedly from fraction to fraction. It is still not possible to say that any one fraction represents a single chemical entity, but the widely differing analyses show that some separation has been effected.

The total amount of hemicellulose is small, being approximately 3%, but it will be seen from the above that the hemicelluloses conform to general type, consisting of pentosan, hexosan and uronic anhydride. It would appear therefore that no matter where the site may be, in the floral organs as in this case, or in the seed coats, stems etc. of a number of plants which have already been examined, the general constitution of the hemicelluloses is closely similar.

EXPERIMENTAL.

Analytical methods.

The following is a résumé of the methods employed throughout.

Moisture: by loss in weight on drying to constant weight in an electric oven at 100°.

Ash: by heating to a white ash of constant weight at dull red heat.

Protein: by standard Kjeldahl process; protein calculated as Kjeldahl nitrogen $\times 6.25$.

Alcohol- and ether-soluble: by exhaustive extraction with each solvent in turn in a Soxhlet apparatus.

Cross and Bevan cellulose: by the usual modified procedure. The C. and B. cellulose yielded furfuraldehyde which was calculated to xylan by the equation $X = 0.7673P + 0.0069$ [Angell *et al.* 1936]. "True" cellulose was given by difference between C. and B. cellulose and xylan.

Lignin: by method of Schwalbe [1925] involving treatment with a mixture of 72% sulphuric acid and 18% hydrochloric acid.

Uronic anhydride: by the apparatus of Dickson *et al.* [1930], modified by Norris & Resch [1935].

Furfuraldehydephloroglucide: by the method of Kröber [1901] using a modified apparatus. As an approximate indication of pentosan and urone content in the hops at various stages the "yield of furfuraldehyde" is given by use of the equation $F = 0.5198P + 0.0020$. In mixtures containing xylose, glycuronic anhydride and glucose, anhydroxylose, X , is obtained from the equation

$$X = 0.0060 + 0.7859P - 1.146C,$$

where P = phloroglucide and C = carbon dioxide from urone. Glucose is given approximately by difference [Angell *et al.* 1936].

Pretreatment of hops.

The hemicelluloses were prepared from the fresh unsulphured flowers of the hop, variety Fuggles, grown in Worcestershire and picked in September 1934.

Analysis: The fresh hops contained 76.6 % moisture and the dry hops gave the following analysis:

	%
Ash	8.5
Protein	21.3
Alcohol-ether-soluble	30.6
C. and B. cellulose	15.3
Xylan	2.3
True cellulose	13.0
Lignin	22.8
Furfuraldehyde	6.4

The total hemicellulose present was determined by the method of Preece [1931] and found, on an ash-free basis, to be 3.4 %. An attempt to determine the amounts of each fraction [Buston, 1934] proved unsuccessful owing to the extreme difficulty in separating small amounts of colloidal precipitate.

Extraction with organic solvents. About 6500 g. of hops were dried in air at 50° for 5 days, then ground in a Christie and Norris laboratory mill and immersed immediately in 80 % alcohol in order to minimize enzyme action on rupture of the cells. The net dry weight of hops used was 1510 g.

The ground hops were filtered from the 80 % alcohol, placed in a calico bag and extracted with boiling 95 % alcohol for 10–12 hours on each of 9 days. The ethyl alcohol was then expressed and a similar treatment with methyl alcohol for 8 days followed. After removal of methyl alcohol, the hops were extracted twice with ether under reflux for 3 hours, washed with ether, dried and weighed. The weight after this treatment was 950 g. corresponding to a loss of 37 % on the original dry weight. The furfuraldehyde yield of the extracted hops was 9.8 %, equivalent to 6.2 % on the original dry hops. Since the latter gave a furfuraldehyde yield of 6.4 % it is evident that no appreciable loss of pentosan or uronic anhydride had occurred by reason of the extraction.

Extraction of pectin. Since hop pectin was required for the purpose of another investigation the opportunity was taken to prepare pectin by different treatments and in this way the hops were completely exhausted of pectin. They were extracted successively by water: (i) at room temperature, (ii) at 50°, (iii) at 90°, (iv) under pressure at 125°; and finally three times with hot 0.5 % ammonium oxalate. The residue was thoroughly washed and pressed.

Extraction of lignin. The hops were then extracted twice with boiling 1 % NaOH in 50 % alcohol as suggested by Norris & Preece [1930]. A third extraction with 50 % alcohol only was carried out in order to facilitate removal of the NaOH, after which the hops were filtered off and pressed.

The alcoholic NaOH extract was examined in order to detect any possible loss of hemicellulose at this stage as suggested by Norman [1935]. The solution

was acidified with glacial acetic acid to pH 3.5, a copious brown precipitate being formed. This was reprecipitated from 1% NaOH by acetic acid, washed and dried in graded strengths of alcohol. It was then extracted in a Soxhlet extractor with methyl alcohol, which removed a quantity of brown-coloured material, then with absolute alcohol and ether. After drying *in vacuo* the powder weighed 17.2 g., equivalent to 1.15% of the hops. The product contained 68% of lignin and 70% was unaffected by hydrolysis for 12 hours with 0.5*N* sulphuric acid. From the present viewpoint, however, the most important fact is its failure to yield any furfuraldehyde at all. This shows conclusively that hemicellulose—in particular fraction A, which would be precipitated under the conditions imposed—was not dissolved out by the reagent. A similar result was obtained in the case of maize cobs which were specially re-examined in view of Norman's results.

Extraction of hemicelluloses.

The residue after lignin extraction was allowed to stand overnight with a large volume of cold 4% NaOH, the extract then being filtered off through muslin. This procedure was repeated six times, until the last extracts contained only a negligible quantity of material precipitable by acetone.

Precipitation of hemicellulose A. Preliminary experiments, similar to those described in the previous communication [Angell & Norris, 1936], had shown that the most complete precipitation of fraction A occurred at pH 3. In order to bring the alkaline solution to this acidity it was necessary to use hydrochloric in addition to acetic acid. Accordingly a mixture of these acids was added to the united NaOH extracts until this acidity was reached, the precipitate being allowed to settle out and then separated at the centrifuge or by filtration. The precipitate was redissolved and reprecipitated several times and finally dried in graded strengths of alcohol and *in vacuo*.

For separation through the copper gel the following procedure was adopted, again as the result of preliminary experiments. The fraction is dissolved in 4% NaOH and to the solution is added one-twentieth volume of glycerol, the whole being thoroughly stirred. Stirring is continued whilst one-tenth volume of molar cupric sulphate is added. If the gel formed is bulky it may be filtered through muslin and pressed out; if small in quantity it may be filtered off on a hardened paper. The gel is then stirred into water and decomposed with acetic acid; from the solution it is precipitated by acetone, reprecipitated and dried as usual.

The copper solution after separation of the gel is also treated with acetone for separation of the other fraction if present. In this instance, no A₂ fraction was obtained, the whole of the original precipitate coming down as copper gel of fraction A₁.

Precipitation of hemicellulose B. The filtrate after separation of the first fraction A was brought to pH 2 and one-half volume of acetone added. The precipitate formed was fractionated, as previously described, through the copper gel, and in this case two fractions were obtained, B₁ and B₂.

Precipitation of hemicellulose C. The filtrate from hemicellulose B was adjusted to pH 5.5 and excess acetone added whereby a final precipitate was obtained which on copper treatment was found to consist solely of fraction C₂.

Composition of the hemicelluloses.

The final products varied in colour from white to yellowish brown; they contained varying amounts of ash and lignin, which could not be completely removed. The hemicelluloses were non-reducing and gave no colorations with iodine. Fractions A₁ and B₁ were sparingly soluble in boiling water, but soluble

in alkali; on the other hand B₂ and C₂ were readily soluble in cold water. The yields of each fraction and their analyses are given in Table I.

Table I.

Fraction	Ash %	Lignin %	On a lignin- and ash-free basis				
			Yield		Furfur- aldehyde %	Carbon dioxide %	Methoxyl %
			g.	%			
A ₁	0.85	2.04	24.1	1.6	50.5	3.12	1.43
B ₁	8.9	—	0.6	0.04	24.5*	3.83*	—
B ₂	12.7	12.0	4.0	0.26	39.6	3.53	4.55
C ₂	12.7	10.4	1.5	0.10	19.6	9.70	1.37

* Ash-free basis only.

Hydrolysis of A₁ and B₂ with dilute sulphuric acid and subsequent identification of the sugars formed showed that in each case xylose and glucose were present, whilst the Heidelberg & Goebel [1927] reaction indicated glycuronic acid. Anhydroxylose, X, in the products was calculated from the yields of phloroglucide, P, and carbon dioxide, C, using the equation

$$X = 0.0060 + 0.7859P - 1.146C.$$

Anhydroglucose was then obtained by difference. The limits of accuracy of this procedure have already been discussed [Angell *et al.* 1936].

The yields of B₁ and C₂ were so small that it was not possible to complete a qualitative examination; in the latter case it is possible to calculate the proximate composition from the quantitative results, on the assumption that the hydrolysis products are the same as for A₁ and B₂. The following table shows the percentage composition of the three major fractions.

	Fractions		
	A ₁	B ₂	C ₂
Anhydroxylose	76.1	58.2	20.9
Glycuronic anhydride	12.5	14.1	38.8
Methoxyl	1.4	4.5	1.4
Anhydroglucose (by diff.)	10.0	23.2	38.9

Fraction B₁ was so small in amount as to suggest that it might possibly represent a small proportion of fraction A which had escaped precipitation. In the absence of data on the lignin content of B₁ it is impossible to establish its composition with as much accuracy as in the other cases, but the figures available show that it must consist of 35–39% of anhydroxylose, 15–17% of uronic anhydride and about 47% of anhydroglucose. These values are sufficiently different from any of the others in the above table to show plainly that this fraction has a separate existence.

DISCUSSION.

The composition of the hemicelluloses in every case shows that anhydroxylose is the only pentose present and, as might be anticipated, this is accompanied by glycuronic anhydride and anhydroglucose. It is somewhat surprising that the hemicelluloses present in the floral organs should show such close similarity to those obtained from various woods. Thus O'Dwyer found large amounts of xylan together with glycuronic anhydride and methoxyl groups in hemicelluloses of

beech [1926] and oak [1934], whilst those of mesquite wood were similar [Sands & Gary, 1933]. Nevertheless, it is significant that the dried hops contained some 22 % of lignin, and the products fall into line with Buston's proposed classification [1935]. Thus, according to Buston, non-lignified tissues may be expected to yield pectin and hemicelluloses of the galactose, arabinose and galacturonic acid series, whilst lignified tissues yield little or no pectin and hemicelluloses of the xylose, glucose and glycuronic acid series. It is evident then that hop flower hemicelluloses will fall into the latter class.

We have stressed the impossibility of establishing the individual chemical nature of any one of the hemicellulose fractions and would also point out the danger in attempting, at this stage, any correlation between chemical constitution and physical characteristics. Thus, Buston [1935] has attempted to show that insoluble "A" hemicelluloses consist largely of either pentosan or hexosan units, whilst the soluble "B" and "C" types contain large proportions of uronic anhydride. That this is not universally the case is indicated by fractions A_1 and B_2 in hops: the marked difference in physical properties in this case is to be correlated merely with an alteration in the pentosan/hexosan ratio, the proportion of uronic anhydride remaining substantially unaltered.

Finally, it may be mentioned at this stage that the whole of the work described here was preceded by preliminary operations on hops of the 1933 season. Such work need not be detailed here, since the methods adopted for the 1934 crop were in every way superior. Some evidence, however, was obtained which appears to indicate that the relative amounts and proximate composition of the various fractions of hemicelluloses may be subject to seasonal and other variations.

SUMMARY.

1. The hemicelluloses of the flowers of the hop have been prepared and their nature investigated.
2. The importance of the *pH* of the solutions for precipitating the various fractions is stressed.
3. The fractionation by means of copper solutions is carried out with glycerol-cupric sulphate mixture.
4. The largest fractions are A_1 and B_2 , whilst C_2 and particularly B_1 are small.
5. All fractions consist of anhydroxylose, anhydroglucose and glycuronic anhydride in different proportions and hence the hop hemicelluloses belong to the xylan class usually found in lignified tissues.

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