CHROMATOGRAPHIC SEPARATION OF SACCHARINIC ACIDS AS THE ANILIDES

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A sample of anilides from kraft black liquor has been fractionated on a cellulose column, but crystallization of the several fractions is very slow, compared with that of anilides obtained from the sugars.

Glycolic acid is apparently not formed by the action of hot alkali on sugars. The spot occupied by glycolic anilide on the paper chromatogram is not identical with any of the four spots (A-D) mentioned above.

QUALITATIVE WORK WITH PAPER CHROMATOGRAMS

Various solvents have been used to develop the chromatograms. They all contain acetone as the principal component, and also 50 mg. Rhodamine B dye per liter. The dye provides a yellow-white fluorescent background in ultraviolet light against which the anilide spots stand out as dark regions.

Originally a 9-1-2 v/v mixture of acetone-water-benzene was used as a developing solvent. Since then a 15-1-2 mixture has been preferred. Also a 30-1-20 mixture has been used as a secondary solvent for the separation of mixtures of C₆ anilides, as the movement of the anilides is very slow (see Fig. 2). Acetone containing 0.5% water has been used for some cases but tends to give streaks on the paper. This last solvent gives better results on the cellulose columns (see below). The $R_f$ values for the anilides in all these solvents are given in Table I.
Qualitatively, by use of paper chromatograms, similar patterns have been found for three hexoses (glucose, mannose and galactose). Two pentoses (arabinose and xylose) also give a similar pattern. The hexoses give three strong spots, for C$_3$, C$_4$ and C$_6$ and only a weak spot for C$_5$. (Nef, in his work on hexoses, found no C$_5$ saccharinic acids at all.) The pentoses give strong spots for C$_3$, C$_4$ and C$_5$, and one or more weak spots in the C$_6$ region. These last spots are probably acids formed by recombination of C$_3$ and C$_4$ fragments.

Further development of the C$_6$ spots obtained from glucose and galactose (see Fig. 1) with 30-1-20 solvent for 15-24 hours (the solvent is allowed to run off the sheet, and also the C$_3$, C$_4$ and C$_5$ spots) has shown the presence of four different C$_6$ isomers, two from each sugar. These are definitely different, as the R$_f$ values shown. Such isomers are expected, as theoretically it is possible to form 10 C$_6$ acids from an aldohexose (1).

Similar development of the C$_5$ region in the case of L-arabinose saccharinic acids has not shown the presence of more than one component. Extended development gave only one long spot; cutting this spot transversely into five sections, elution of each section, and rechromatographing the five solutions gave five spots of identical R$_f$ value. So the original long spot was chromatographically homogeneous (see Fig. 2). Nef has reported only 2 C$_5$ saccharinic acids in his work on the pentoses.
I. Separation of Glucose saccharinic anilides in 15-1-2 solvent.
II. Separation of Glucose C₆ saccharinic anilides in 30-1-20 solvent.
III. Separation of Galactose saccharinic anilides in 15-1-2 solvent.
IV. Separation of Galactose C₆ saccharinic anilides in 30-1-20 solvent.

Note: These are tracings of the spots obtained under ultraviolet light. The II and III separations were run on the same sheet of paper.
Figure 2

Chromatographic Homogeneity of C₅ Fraction

Schematic diagram, showing cutting of elongated spot, run 20 hours in 30-1-20 solvent, into 5 portions, and identical Rₜ values of the 5 solutions, when run again the same time in the same solvent.
Finally a sample of kraft black liquor acids has been fractionated according to the method of Hagglund (Cellulosechemie 5, 81 (1924) with ether and ethanol and the four solutions obtained analyzed as the anilides on paper. These fractions all give very similar patterns, showing C$_3$ to C$_6$ spots, except that the two less soluble fractions (see Fig. 3) also give a very slow spot, which may be the saccharinic acid of an oligosaccharide. Present theory is definitely against this, in favor of fragmentation to small fragments and rebuilding to C$_6$ acids as a maximum.

The mixtures of saccharinic acids obtained from the simple sugars have been examined for glycolic acid, but this compound is apparently not present. Known spots of glycolic anilide (m. 92°) were run on paper (see Fig. 4) and the rate of movement compared with that of the various saccharinic anilides. The glycolic anilide gave a single spot intermediate between the C$_3$ and C$_4$ anilides.

FRACTIONATION ON CELLULOSE COLUMNS

Two columns have been used in this work, a 2 by 20-inch and a 6 by 26-inch column, each filled with Whatman cellulose powder. The maximum capacity of the 2-inch column is 2 grams of material, that of the large column 20 grams. The smaller column operates at just about one-tenth the scale of the larger one, so it can be used for pilot-plant runs.
TABLE I

Rf VALUES OF SACCHARINIC ANILIDES IN VARIOUS SOLVENTS

<table>
<thead>
<tr>
<th>Anilide Fraction</th>
<th>Mixtures of Acetone-water-benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9-1-2</td>
</tr>
<tr>
<td>C3</td>
<td>0.92</td>
</tr>
<tr>
<td>C4</td>
<td>0.86</td>
</tr>
<tr>
<td>C5</td>
<td>0.75</td>
</tr>
<tr>
<td>C6</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each case, the columns, after being packed dry, are washed with 50% ethanol until no more yellow color comes through, then with the desired solvent. Solvent is then drained from the column until the upper level is just even with the top of the packing, and a thick sirup of the given anilide mixture applied. A little powder is sprinkled on top to about 1 cm. height, tamped down gently, and the developing solvent allowed to run through the column at a definite rate. The effluent is collected in successive fractions on a fraction changer. Generally a total of 5000 cc. effluent is collected from the 2-inch column, divided into 25- or 50 cc. fractions. With the larger column, a total volume of 30 liters effluent has been divided, so far, into 100 cc. fractions. In future work, 250 cc. fractions will be taken from the larger column.

Data for the several runs are given in Table II. In the early runs the solvent used was 9-1-2 or 30-1-20 acetone-water-benzene, but in later work it has been found that acetone containing 0.5% water is very effective. This last solvent can be recovered very readily, whereas the composition of the other solvents change on distillation.
Originally the columns were operated at a slow flow rate, as it was felt that a faster rate was objectionable. With acetone, however, it has been found that unrestricted flow (950 and 5-6000 cc. per hour for the two columns) is satisfactory. This fast flow rate is very helpful in that a run can be made with either column during a 9-hour working day.

In all cases, the standard procedure (1) was used to prepare the saccharinic acids and anilides. For the 20-gram runs, the reaction mixture of acids and aniline etc. was refluxed several hours at 80-90°, and then concentrated in vacuo to remove the maximum amount of aniline.

Run 20, made with saccharinic anilides from 20 grams arabinose, is the most satisfactory fractionation made so far, in that the several fractions were obtained with very little overlapping. The gross amounts of these fractions (not recrystallized yields) compare very favorably with the data of Nef (see Table III). The total yield is a little higher, probably because of the presence of a sirupy C₆ anilide.

Below are listed the several crystalline anilides obtained so far in this work. They have been isolated in appreciable amounts on the large column, so they can be crystallized several times, or converted to other derivatives. When they have all been identified, then small runs (1 gram) can be made on the 2-inch column to determine quantitatively how much of each component is present in the various mixtures. The present phase of the work is predominantly qualitative.
**C₄-Anilides**

This fraction has been isolated from several runs and crystallizes readily. The sirup obtained from Run 20 crystallized completely on concentration in vacuo of the solution. The m.p. of a crop, recrystallized several times from ethyl acetate and ligroine (b. 30-60°) was 116.5-18° (total immersion thermometer). The optical rotation of this compound, determined on a 6.4% solution in 95% ethanol, was zero.

This compound is undoubtedly d,l-1,3-dihydroxybutyric acid anilide, and has been converted to the known phenylhydrazide (prepared by Nef). 0.300 g. C₄ anilide and 3 cc. 2 N NaOH were heated in an open dish on the steam bath 90 minutes, the solid residue dissolved in 5 cc. water, 0.6 g. phenylhydrazine hydrochloride added, the solution heated one hour on the steam bath, a little dilute acetic acid added, and the solution again heated to dryness. The residue was stirred with 20 cc. water, the suspension extracted with 20 cc. ethyl acetate, and the latter washed with a little dilute hydrochloric acid and water, then dried over sodium sulfate. The solution was then concentrated on the steam bath to about 10 cc., diluted with ligroine (30-60°) and set aside overnight. The resulting crystals were recrystallized four times from the same solvent combination to a melting point of 129.5-30.5° (corr.). Nef gives 128-30° for the melting point of the phenylhydrazide of 1,3-dihydroxybutyric acid.
An anilide was obtained in earlier work (1,2) in small yield, m. 108-110° (corr.). Now, in Run 20, it has been obtained in good yield (1.5 grams) and m. 119.5-20.5° (corr.). The optical rotation of this compound in 95% ethanol is 59.1°. It is apparently chromatographically pure (see Fig. 3), and may be the anilide of L-threo-1,3,4-trihydroxy-valeric acid (the L-isomer of formula XII, ref. 1). Conversion to the known phenylhydrazide, m. 110° (prepared by Nef) will be needed to confirm this conjecture.

Of the five C₅ acids listed (Table I, ref. 1) only two have been isolated (XI and XII). Nef remarked that while the erythro acid (XI) was formed in greater yield than the threo-isomer, its isolation was extremely difficult. It is possible that the bulk of the C₅ fraction in Run 20 is the erythro acid (6.0 grams out of 7.5 grams total).

C₆-Anilides

Two of these acids have been isolated from glucose, in Run 12. Originally isolated as one fraction, 0.5 gram was fractionated on a cellulose column (2-inch) into two fractions, a fast one (164 mg.) of (a) +39.8° in 95% ethanol, and a slow one (206 mg.) of (a) -52.6°. A very slow fraction, which may only be some of the second fraction in impure state) was obtained in small yield (57 mg.) of (a) -32°. The two
Figure 3

Paper Chromatogram of Saccharinic Anilides
From Kraft Black Liquor

I. From acid fraction soluble in absolute ether
II. From acid fraction soluble in 10-1 ether-ethanol
III. From acid fraction soluble in 1-1 ether-ethanol
IV. From acid fraction soluble in ethanol
main fractions are presumably the alpha and beta-glucometascaccharinic acid anilides (see Formulae I and II, ref. 1). Since the phenyl-hydrazides of these are known (again prepared by Nef), conversion here as for the \( C_4 \) anilide above will show identity.

\( C_3 \)-Anilide

It is very disappointing that the \( C_3 \) fractions obtained so far will not give crystalline lactic anilide. Seeding does not help. The anilide is very soluble in ether, and slightly soluble in ligroine. Impurities present may hinder the crystallization.

SACCHARINIC ACIDS FROM KRAFT BLACK LIQUOR

A sample of 8 liters of kraft black liquor (made from southern pine) was neutralized with 1270 cc. of 7 N sulfuric acid to pH 2.25, the filtrate (a total of 80 liters including washings) concentrated at 50-55° and 25-27 inches vacuum to 2700 cc. of pH 1.4. This was diluted with 2700 cc. 95% ethanol, and the precipitated sodium sulfate removed. The filtrate was concentrated to 1 liter of pH 1.2, diluted with 2500 cc. ethanol and more sodium sulfate removed (573 g. in all).

The filtrate was concentrated to 500 cc., titrated with aq. \( \text{Ba(OH)}_2 \) at pH 6.5-7.0, the solution filtered and run through an IR-120 cation-exchange resin to remove Ba and Na ions. The effluent, of minimum pH 1.7, was concentrated an vacuo to 276 g. dark red sirup. This, by
fractionation with ether and absolute ethanol gave four fractions, listed in Table IV. As shown by Fig. 4, there is not very much fractionation of the saccharinic acids by this method.

A 2-gram sample of this acid (soluble in 1-1-ether-ethanol) was converted to the anilides, and fractionated on the 2-inch column. Several sirupy fractions have been obtained and some are crystallizing slowly. One surprising fact is the reluctance of the C₄ fraction to crystallize. Such fractions, obtained from the sugars, crystallized readily. Perhaps the presence of impurities, as extractives or lignin fragments, have an adverse effect.

FUTURE WORK

The several crystalline anilides will be converted to the phenylhydrazides to determine their structure. The anilides of saccharinic acids derived from D-galactose will be fractionated, especially the C₆ anilides. An attempt will be made to crystallize the C₅ anilides obtained from hexoses, and the C₆ anilides obtained from pentoses.

A few preliminary tests with paper chromatography of the phenylhydrazides and p-nitrophenylhydrazides of the mixed saccharinic acids have been tried. These will be continued with the thought of isolating a crystalline C₃ fraction, and also of a possibly better separation of the mixed C₅ and C₆ fractions.

The crude saccharinic acids obtained from kraft black liquor will be converted to the barium salts first, and then to the anilides, in hope of a better separation of crystalline material.
Figure 4

Paper Chromatogram Showing the Absence of Glycollic Acid in Saccharinic Acid Mixture

This chromatogram was run 4 hours in 30-1-20 solvent, and shows the lower end of the sheet, with \( C_3 \) and \( C_4 \) anilides, and glycollic anilide \( (C_2) \) as an intermediate spot.
TABLE III

YIELDS OF SACCHARINIC ANILIDES FROM ARABINOSE
COMPARSED WITH DATA OF NEF

<table>
<thead>
<tr>
<th>Source</th>
<th>Yields of Saccharinic Anilide Fractions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (20 g. Arabinose originally)</td>
<td>2.0 g.       6.6 g.  7.5 g.  1.0 g.</td>
<td>17.3 g.</td>
</tr>
<tr>
<td>Crystalline crops from Run 20</td>
<td>--           2.0 g.  1.5 g.</td>
<td></td>
</tr>
<tr>
<td>Ids obtained by Nef from 20 g. arabinose, converted to anilide basis</td>
<td>5.5 g. -- - 2.3 g. -- - 1.5 g.</td>
<td>15.9 g.</td>
</tr>
</tbody>
</table>

TABLE IV

YIELDS OF SACCHARINIC ACIDS FROM KRAFT BLACK LIQUOR

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Weight Soluble Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute ether</td>
<td>56.0 g.</td>
</tr>
<tr>
<td>10-1 Ether-ethanol</td>
<td>72.0 g.</td>
</tr>
<tr>
<td>1-1 Ether-ethanol</td>
<td>111.0 g.</td>
</tr>
<tr>
<td>Aqueous ethanol</td>
<td>38.0 g.</td>
</tr>
<tr>
<td>Total</td>
<td>277.0 g.</td>
</tr>
</tbody>
</table>

Note: The amount of black liquor was eight liters.
chromatographic separation of saccharinic anilides from kraft black liquor

In the present report a description is given of the isolation of a sirupy mixture of crude saccharinic acids from kraft black liquor, their conversion to the anilides, the fractionation on a cellulose column, and the isolation of two crystalline C₆-saccharinic acid anilides. One of these is alpha-isosaccharinic acid anilide, the other is probably the beta-isomer.

isolation of the crude saccharinic acids

A sample of kraft black liquor (1000 cc.) was gradually added to 2000 cc. of 3 N sulfuric acid at room temperature. The final pH of the mixture was 1.8. After 3 days the precipitated lignin was filtered and washed several times with distilled water until the pH of the washings had risen to 3.5. At this stage the lignin became very slimy and difficult to filter.

The combined filtrates and washings (8000 cc.) were concentrated in vacuo to 500 cc. (pH 1.0) and added to 1500 cc. of 95% ethanol. The precipitated sodium sulfate was filtered, the filtrate concentrated to 200 cc. and added to 800 cc. ethanol. A smaller precipitate was obtained. The total amount of sodium sulfate was 88 grams.

The filtrate was concentrated, water added, and the solution reconcentrated to remove the ethanol. The sirup was dissolved in 600 cc.
water and this solution extracted portion-wise with a total of 700 cc.
ethyl acetate. In this way quite a bit of red color was removed from
the aqueous solution. The final extraction removed only a little
yellow color. The ethyl acetate extract on concentration gave 13.7 g.
sirup, supposedly noncarbohydrate material.

The aqueous solution still contained a little sulfuric acid.
It was titrated with an aqueous solution of 60-100 grams barium hydroxide.
to pH 8, the barium sulfate removed by filtration and the sulfate-free
filtrate run through an IR-120 cation-exchange resin column. The pH
of the most concentrated portion of the effluent was 1.8, so there seemed
to be no strong acids present.

Concentration of this acidic gave 35.7 grams of a dark red sirup.
This was assumed to contain the saccharinic acids and also possibly some
extractive or degraded lignin material.

CONVERSION OF THE SACCHARINIC ACIDS TO THE ANILIDES

The sirup was dissolved in about 200 cc. of 95% ethanol, 70 cc.
of distilled aniline and about 10 cc. of acetic acid added, and the mix-
ture heated on a boiling water bath for 3-4 hours. The ethanol slowly
distilled, leaving a thick red sirup. This was then concentrated at 20 mm.
and 100°, to remove most of the acetic acid and some of the aniline.

To remove the remaining aniline, the sirup was dissolved in a
minimum of ethanol and this solution added with shaking to 10 volumes
of ethyl acetate. A gummy precipitate formed. The clear red liquid was
shaken with 4 volumes of benzene to give more precipitate. The weight of the combined precipitates (34-1) was 22 grams.

The benzene-ethyl acetate solution presumably contained all the aniline and some saccharinic anilides. It was concentrated to a sirup (34-2) and dissolved in 200 cc. ethyl acetate. This dark red solution was then extracted with several portions of N hydrochloric acid (500 cc in all) until the aqueous extract was neutral. Thus all of the aniline should be removed. Part of the red color was also removed.

The ethyl acetate was then washed with water, which removed only a light yellow color. As this extract seemed to be slightly acidic, the ethyl acetate was extracted with aqueous sodium bicarbonate, which surprisingly removed a large amount of the red color. This extraction was continued until only a light yellow extract was obtained.

The remaining ethyl acetate solution was washed with water, dried over sodium sulfate and concentrated to give 3.9 g. dark red sirup (34-3).

Paper chromatograms were run in 15-1-2 acetone-water-benzene to compare the three fractions. Fraction 34-1, insoluble in ethyl acetate gave a pattern containing strong spots in the C3, C5 and C6 regions, but only a spot of medium intensity in the C4 region. Also a series of very slow-moving spots, well above the C6 region, was noted. Fraction 34-2, the ethyl-acetate-benzene soluble portion before extraction with acid and bicarbonate, gave medium spots for the C6-C5 region, and strong spots in
the C₄ and C₃ region. The 34-3 fraction showed only a strong spot in the C₃ region, hence either the acid or bicarbonate extraction removed all of the C₄, C₅ and C₆ material.

This method of removing excess aniline from the reaction mixture is thus rather costly, much saccharinic acid material being lost. If one assumes that all the originally 35.7 grams sirup were either C₃ or C₆ anilides, the yield of anilides should be 66 and 51 grams respectively. Hence the total amount of anilide obtained (22 + 3.9 = 25.9 grams) is less than half the expected yield.

FRACTIONATION OF THE ANILIDES ON THE CELLULOSE COLUMN

Two fractionations, each with five-gram samples of the ethyl acetate-benzene insoluble sirup (34-1) were made on a 2-inch cellulose column, the solvent being acetone containing 1% water. In experiment 37, the flow of solvent through the column was about 80 cc. per hour, and in experiment 38 the flow rate was 600-800 cc. per hour. The data are given in Table I.

In each case two crystalline C₆ fractions were obtained. The yields were quite small. A larger amount of a C₅ fraction was obtained, but this was a sirup. This fraction should be predominant, being formed from the pentosans present in the hemicelluloses. A lesser amount of mannan fraction should contribute to the C₆ saccharinic acid formation. A smaller amount of C₄ fraction has been obtained, but this is not
crystalline, so cannot be the 1,3-dihydroxybutyric anilide obtained by
the action of alkali on the simple sugars (see Report No. 1, page 7).

One of the C₆ anilides is **alpha-isosaccharinic acid anilide**
(see Figure 1). The melting point is 170°, and the mixed melting
point with an authentic sample (m. 169-70°) is 169.5-70.5°. The
specific rotation of the anilide is +13.4° +1 in water, in comparison
with +14.7° +1° for the authentic sample. The values given in the
literature are m. 169-9.5° and +13.1° (1).

The other C₆ anilide has a melting point of 113-14° and a
specific rotation of -37° in water. This is presumably the anilide
of betaisosaccharinic acid. Further work will be needed to show its
identity, as it has not been reported in the literature.

### TABLE I

PRODUCTS OBTAINED BY FRACTIONATION ON CELLULOSE COLUMN

<table>
<thead>
<tr>
<th>Fraction</th>
<th>R₄ Value</th>
<th>Experiment 37 Effluent cc.</th>
<th>Sirup mg.</th>
<th>Experiment 38 Effluent cc.</th>
<th>Sirup mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₄-anilide</td>
<td>1.0</td>
<td>890-1110</td>
<td>--</td>
<td>1120-1420</td>
<td>--</td>
</tr>
<tr>
<td>C₅-anilide</td>
<td>0.85</td>
<td>1560-2000</td>
<td>630</td>
<td>1840-2300</td>
<td>--</td>
</tr>
<tr>
<td>C₆-anilide</td>
<td>0.75</td>
<td>2170-2400</td>
<td>270</td>
<td>2380-2740</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>2490-3240</td>
<td>205</td>
<td>2810-4260</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>3910-4410</td>
<td>270</td>
<td>4760-5500</td>
<td>210</td>
</tr>
</tbody>
</table>

Note: The R₄ value is a relative value, referring to the cryst. 1,3-di-
hydroxybutyric anilide as 1.0. The above C₄-anilide may not be
the same. The effluents in each case include about 600 cc. of
solvent (forerun) required to displace solvent in the column.
The R₄ 0.75 fraction in each case is the alpha-isosaccharinic
anilide, the 0.73 fraction (m. 142-5°) may be a mixture, and the
0.68 fraction (m. 113-14°) is possibly the beta-isosaccharinic anilide.
Figure 1
The Three Main Types of C₆ Saccharinic Anilides

Figure 2
Various Isosaccharinic anilides expected from 1,4-linked disaccharides
DISCUSSION OF RESULTS

Corbett and Kenner (2) have done extensive work on the effect of lime water at 25° on disaccharides and various monomethyl ethers of glucose and fructose. They have shown that blocking the hydroxyl at C-4, either with a methyl group or by use of a disaccharide, as (maltose or lactose) will give formation of isosaccharinic acids (see Figure 1). Similar blocking at C-3 or use of 1-3 linked disaccharides will cause formation of the metasaccharinic acids. Use of 1-methyl fructose or unsubstituted glucose or fructose gave a mixture of the saccharinic, isosaccharinic and metasaccharinic acids.

Extension of this effect of glycosidic linkages to 1,4-linked polysaccharides found in hemicelluloses (mannans mostly, possibly some galactan) should cause the formation of isosaccharinic acids also. Palmen (3) has reported the formation of 11.4% calcium alpha isosaccharinate by the effect of lime on alpha cellulose.

An assumption should be made that the effect of hot alkali in a kraft cook would be similar to that of saturated lime water at room temperature.

If the effect of alkali on 1,4-linked polysaccharides is to form the isosaccharinic acids, with a branched chain, one would expect that possibly the degradation products, as the C₅ and C₄ acids, would have a similar structure. Hence it is conceivable that the material obtained from the column, with an Rf similar to the 1,3-dihydroxybutyric anilide, may be an alpha-(hydroxymethyl) lactic acid anilide (see Figure 2).
FUTURE WORK

The fractionation of the black liquor saccharinic anilides will be repeated, in an effort to get a maximum yield. The method of removing excess aniline will be altered, to avoid loss of saccharinic anilides. The identity of the second C$_6$ anilide will be attempted, also the crystallization of the C$_4$ and C$_5$ fractions.

Work on kraft black liquor is complicated by the presence of noncarbohydrate material. Thus in this report an extraction of the aqueous acid filtrate with ethyl acetate is described, in an effort to remove noncarbohydrate material. Undoubtedly the aqueous solution contains extraneous compounds which may prevent the crystallization of the anilide sirups obtained on the cellulose column.

Experiments with purer starting materials, as hemicelluloses, holocelluloses, xylan or mannan might give purer products, such as the C$_5$ and C$_4$ anilides shown in Figure 2. I conferred with Dr. Corbett at the American Chemical Society Meeting in Cincinnati last week. He is working with Dr. Whistler at Purdue for a year on the effect of alkali on xylobiose, which should give a C$_5$ isosaccharinic acid, of the type shown in Figure 2. Such compounds are unknown at present, and he reports difficulty in getting crystalline derivatives. Also great interest in this anilide technique.
LITERATURE REFERENCES


Chromatographic Separation of Saccharinic Acids,
As the Anilides, Derived from Glucose,
Arabinose and Celllobiose

The present work deals with the isolation of certain saccharinic acid
anilides, for future use as reference compounds in the analysis of kraft
black liquor. The report is in the form of an article submitted for
publication in the Journal of the American Chemical Society. (This article
is now in the galley proof stage and should appear in print within the next
month.) The content of the article covers both material given in Project
Report No. 1 and new material.
The behavior of seven crystalline saccharinic anilides on the paper chromatogram has been observed. The $R_f$ values vary inversely with the molecular weight, and are greater for the $\alpha$-metasaccharinic anilides than for the $\beta$-isomers. Periodate oxidation of milligram quantities of anilides and subsequent paper chromatography of the fragments identify the "metasaccharinic acid" type of structure. Treatment of cellobiose with hot 8 N NaOH leads to the formation of both iso- and metasaccharinic acids, identified as the anilides.
The Effect of Alkali on Carbohydrates. I. Saccharinic Anilides Derived from D-Glucose, L-Arabinose and Celllobiose

By John W. Green

(1) Presented before the Division of Carbohydrate Chemistry at the Minneapolis Meeting of the American Chemical Society, September 12, 1955.

In a previous communication a method of paper chromatography of saccharinic acids as the anilides was reported. This paper deals with the isolation of five crystalline saccharinic anilides, and a method of structural determination of metasaccharinic anilides by periodate oxidation.

In the original method the anilide mixtures derived from various sugars were resolved on the paper chromatogram into a series of four spots, by use of a 9:1:2 v/v mixture of acetone, water and benzene. Now it has been shown that these spots represent, in order of decreasing Rf values, the anilides of C3 (lactic), C4, C5 and C6 saccharinic acids. Hence, the rate of movement of the various anilides on a paper chromatogram is inversely proportional to the molecular weight (see Table I).

The fastest moving spot is D, L-lactic anilide, as shown by

(3) Leipen, Monatsh., 2, 45 (1888); C. A. Bischoff and P. Walden, Ann., 272, 71 (1894)

comparison of the Rf value with that of an authentic sample, prepared from
### TABLE I

**SACCHARINIC ANILIDES**

<table>
<thead>
<tr>
<th>Substance</th>
<th>M.P. °C</th>
<th>$[\alpha]_{D}^{20}$ in H$_2$O</th>
<th>$95%$ EtOH</th>
<th>Rf in Solvent A</th>
<th>Rf in Solvent B</th>
<th>Reference</th>
<th>Elementary analysis $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D,L-Lactic anilide</td>
<td>58-59</td>
<td>0.92</td>
<td>0.80</td>
<td>3.4</td>
<td></td>
<td></td>
<td>Carbon: 61.54, Hydrogen: 6.74, Nitrogen: 7.25</td>
</tr>
<tr>
<td>D,L-2,4-Dihydroxybutyric anilide</td>
<td>115-116</td>
<td>0.86</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td>Carbon: 61.54, Hydrogen: 6.74, Nitrogen: 7.25</td>
</tr>
<tr>
<td>C$_5$-Anilide</td>
<td>119.5-120.5</td>
<td>--</td>
<td>+59°</td>
<td>0.75</td>
<td>0.35</td>
<td></td>
<td>Carbon: 56.28, Hydrogen: 6.66, Nitrogen: 5.59</td>
</tr>
<tr>
<td>D-$\alpha$-Glucosmesaccharinic anilide</td>
<td>89-90</td>
<td>--</td>
<td>+40°</td>
<td>0.60</td>
<td>0.22</td>
<td></td>
<td>Carbon: 56.44, Hydrogen: 6.70, Nitrogen: 5.58</td>
</tr>
<tr>
<td>D-$\delta$-Glucosmesaccharinic anilide</td>
<td>123-124</td>
<td>--</td>
<td>-63°</td>
<td>0.60</td>
<td>0.18</td>
<td></td>
<td>Carbon: 56.44, Hydrogen: 6.70, Nitrogen: 5.58</td>
</tr>
<tr>
<td>D-$\alpha$-Isosaccharinic anilide</td>
<td>169-171</td>
<td>+13°</td>
<td>--</td>
<td>0.24</td>
<td></td>
<td></td>
<td>Carbon: 56.68, Hydrogen: 6.78, Nitrogen: 5.66</td>
</tr>
<tr>
<td>D-$\alpha$-Glucosaccharinic anilide</td>
<td>193-195</td>
<td>+58°$^1$</td>
<td>+55°$^2$</td>
<td>0.32</td>
<td></td>
<td></td>
<td>Carbon: 56.68, Hydrogen: 6.78, Nitrogen: 5.66</td>
</tr>
</tbody>
</table>

**Notes:** All rotations were determined on a Bausch and Lomb wedge saccharimeter. (1) 20°; (2) 25°; (3) solvent A is 9:1:2 v/v acetone-water-benzene. Solvent B is 30:1:20 v/v acetone-water-benzene.

(4) Calcd. for C$_4$ anilide: C, 61.50; H, 6.60; N, 7.15%. Calcd. for C$_6$ anilide: C, 56.66; H, 6.66; N, 5.56%.
lactic acid\textsuperscript{4}, and purified by distillation. This anilide does not crystallize very readily, and samples isolated by fractionation on a cellulose column have remained sirups, despite seeding.

The second spot is D, L-2,4-dihydroxybutyric anilide. This compound crystallizes very readily. Its structure was shown by conversion to the known phenylhydrazide.\textsuperscript{5}

The third and fourth spots, for mixtures derived from L-arabinose and D-glucose, were shown to be the anilides of C\textsubscript{5} and C\textsubscript{6} saccharinic acids, respectively.\textsuperscript{2} Use of a slower solvent, a 30:1:20 v/v mixture of acetone, water and benzene, and extended development of the chromatogram so that the C\textsubscript{3} and C\textsubscript{4} anilides are washed from the sheet, has allowed development of the C\textsubscript{5} and C\textsubscript{6} regions into two spots in each case. Nef\textsuperscript{5} has shown the formation of isomeric pairs of C\textsubscript{5} and C\textsubscript{6} acids from pentoses and hexoses, respectively.

The C\textsubscript{6} saccharinic anilide, originally reported\textsuperscript{2} with a specific rotation of -20\textdegree, has thus been shown to be a mixture of roughly 60\% D-\beta-glucosetasaccharinic anilide (I) and 40\% D-\alpha-glucosetasaccharinic anilide. The structure of these anilides was shown by an independent preparation from known samples of calcium \alpha- and \beta-glucosetasaccharinates.\textsuperscript{6}
One of the two C<sub>5</sub>-anilides has been isolated in crystalline form. From the data for its rotation (+59°) and the periodate oxidation cited below, it is concluded that this compound is probably the L-threo-2,4,5-trihydroxyvaleric anilide (III) or the "L-β-C<sub>5</sub>-metasaccharinic anilide."

The other anilide, of higher R<sub>f</sub> value, is a sirup of negative rotation and probably the L-erythro isomer.

It is interesting to note, for both the C<sub>6</sub> and C<sub>5</sub> metasaccharinic anilides, that the α-isomer moves more rapidly on paper than does the β-isomer.

Anilides were also prepared from D-α-glucosaccharin and from D-α-isosaccharin. The latter anilide has been reported by Utkin. Both of these anilides move much more rapidly on paper than do the glucosmetasaccharinic anilides, thus showing the effect of the branched chains. The movement of the D-α-glucosaccharinic anilide is almost as fast as that of the C<sub>5</sub>-metasaccharinic anilide.

Some small scale periodate oxidations were performed, according to the method of Lemieux and Bauer. Oxidation of milligram quantities of anilides.

(6) These were kindly supplied by Dr. W. M. Corbett

(7) H. Kiliani, Ber., 15, 2953 (1882)


the various anilides was followed by paper chromatography of the resulting oxidation products and observation under ultraviolet light. The D-α-gluco-
saccharinic anilide gave only a blank sheet, and the D-α-isosaccharinic
anilide a diffuse pattern. However, the C₆ and C₅ metasaccharinic anilides
(I and III), obtained from either D-glucose or L-arabinose, gave well defined
spots in the C₄ region. These probably represent a C₄ fragment (II or IV)
resulting from the scission of the C-4 and C-5 bonds in the original anilides.
This would imply that the -CHOH-CO(NH₂) group is stable to periodate. Such
an assumption is confirmed by experiments with D.L-2,4-dihydroxybutyric
anilide; this compound is unaltered by periodate.

The C₄ fragments (II and IV), the anilides of 2-deoxytetroronic acids,
should be enantiomers when formed from I and III, or the α- and
β-isomers, and would thus interrelate the structures of the C₅ and C₆
metasaccharinic anilides.

With several reference compounds now available, an attempt has
been made to analyze the mixtures of saccharinic acids formed from various
sugars and hot 8 M NaOH, as studied by Nef. This attempt is only qualitative
at present. Two types of impurities are present in the mixtures of saccharinic
anilides obtained, and both undoubtedly interfere with chromatographic separation
on a cellulose column. The first, unconverted acids or lactones, especially
lactic acid, do not show up on the paper chromatogram under ultraviolet light.
Thus, fractions that seem to be chromatographically pure as far as anilide
quality is concerned, may be otherwise impure. The other impurity is the
resin formed by the action of alkali on sugars. Nef reported yields of 5 to
20% of this material, and separated it from the saccharinic acids by a process
of acetylation and extraction with chloroform. In the present work the resin
has been ignored; it has been assumed that it moves very slowly on the chromatograms in relation to the saccharinic anilides.

\[
\begin{align*}
\text{CONHPh} & \quad \text{CONHPh} \\
\text{HO-C-H} & \quad \text{HO-C-H} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{H-C-OH} & \quad \text{NaIO}_4 \quad \rightarrow \quad \text{NaIO}_4 \\
\text{H-C-OH} & \quad \text{CH}_2 + \text{HCO}_2\text{H} + \text{CH}_2\text{O} \\
\text{CH}_2\text{OH} & \quad \text{CHO} \\

\text{I} & \quad \text{II}
\end{align*}
\]

\[
\begin{align*}
\text{CONHPh} & \quad \text{CONHPh} \\
\text{H-C-OH} & \quad \text{H-C-OH} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{HO-C-H} & \quad \text{NaIO}_4 \quad \rightarrow \quad \text{NaIO}_4 \\
\text{CH}_2\text{OH} & \quad \text{CHO} \\

\text{III} & \quad \text{IV}
\end{align*}
\]

Treatment of D-glucose with hot 8 N NaOH gave a mixture of acids as obtained by Nef\(^5\). Separation of the anilides on a cellulose column gave crude yields of 11% C\(_4\) anilide and 20% D-\(\beta\)-glucometasaccharinic anilide (I). The \(\alpha\)-glucometasaccharinic anilide fraction, obtained only as a sirup, did not crystallize on seeding. Two sirupy C\(_5\) anilides were obtained in low yield. These, on periodate oxidation, gave C\(_4\) fragments only, and so are presumably the C\(_5\)-metasaccharinic anilides. The fast C\(_5\) fraction was dextrorotatory, the slower one levorotatory.
Similar fractionation of the saccharinic anilides derived from L-arabinose gave about 6% of the pure C₄ anilide, 5% of the pure 8-C₅-meta-saccharinic anilide (III) and about 4% of a sirupy anilide in the C₆ region. This last fraction is interesting, in that possibly C₃ units are being built up to larger units, as suggested by Sowden¹¹ in the preparation of D-α-

glucosaccharinic acid from D-glucose-1-C⁴.

Treatment of cellobiose with hot 8 N NaOH gave about 15% crude yield of D-α-isosaccharinic acid, (isolated as the anilide) as predicted by the work of Kenner, Corbett and Richards¹² on 1,4-linked disaccharides. Also

D-β-glucosmetasaccharinic anilide (I) and D, L-2,4-dihydroxybutyric anilide were obtained in crude yields of 7 and 8%, respectively. The effect of hot concentrated NaOH is much more drastic than that of saturated lime water at 25°, as used by Kenner et al.¹². With the latter alkali the English workers found, after 200-300 hours, approximately 1 mole each of the isosaccharinic acids and of monose. The monose formed by splitting of the disaccharide linkage in cellobiose is probably converted by the strong caustic to the meta type of acid and products of shorter chain length.

Experimental
Preparation of Saccharinic Anilides.—Each sample of sugar was heated in 8 N NaOH in a steam bath for 8-15 hours; a layer of xylene prevented access of
The cooled solution was diluted up to 10 volumes and run through an IR-120 cation-exchange resin column. The acidic effluent was concentrated to a sirup, then converted to the anilides by heating in ethanol with an excess of aniline and a little acetic acid. For small runs (up to 1 g. of sugar) the heating was effected in a small evaporating dish on the steam bath. For larger runs heating was effected in a distilling flask in a boiling water bath, the ethanol being slowly distilled first at 760 mm., then at 20 mm. In some cases the anilide sirup was finally heated in boiling benzene with removal of water in a water-trap to effect maximum conversion of unaltered acids and lactones to the anilides. No attempt has been made to study the completeness of this reaction, so far.

The resulting dark-red sirup, often containing excess aniline, was decolorized in ethanol with carbon before chromatography.

Paper Chromatography.—Mobile solvent mixtures were made up with 9:1:2 and 30:1:20 v/v acetone-water-benzene, and 40 mg. of Rhodamine B dye added per liter. Chromatograms were run by the descending technique in glass tanks, and the air-dried sheets viewed under short-wave ultraviolet light. Care must be taken that the mobile solvent is not allowed to evaporate in the trough or on the antisiphon rods between runs; any dye residue left on the glass will streak on subsequent paper sheets and give an uneven dye pattern.

Fractionation on Cellulose Columns.—Runs were made on a small dry-packed column (2 by 22 inches) with acetone containing 1% water, at flow rates of 650-800 cc. an hour. Fractionation at a slower rate of 80 cc. an hour gave no improvement in separation. Flow rate on wet-packed columns, as shown by dyes, was more even, and also much faster (3000 cc. an hour). However, most
of the work so far has been done on dry-packed columns. The amount of anilide applied to a column was 1-3 g., with a little Sudan IV dye as a tracer, and about 5 l. of effluent taken, after a fore-run of 750-1000 cc.

Some runs were made with a larger column (6 by 26 inches) containing about 10 pounds of dry-packed Whatman cellulose powder. This was operated at a restricted flow rate of 5 liters an hour, and 55 liters of effluent collected, after a fore-run of 8.5 liters.

Crystallization of Saccharinic Anilides.—The C₄ anilides were crystallized from ether-ligroin or ethyl acetate-ligroin (b.p. 30-60°). Most of the C₅ and C₆ anilides were crystallized from ethyl acetate-ligroin (b.p. 30-60°). Some of the C₆ anilides could be crystallized from ethanol or water. The D-α-glucosaccharinic anilide was not very soluble in most solvents. For rotation measurements it was dissolved in hot ethanol or water at a 1% concentration, but began to crystallize out in the polarimeter tube after one hour at room temperature.

Periodate Oxidation.—One milligram samples of anilides were treated with 20 mg. of sodium metaperiodate in 0.5 cc. of water for 1 hour at 0°. In some cases the anilide was dissolved in 0.1 cc. of ethanol and 0.5 cc. of the aqueous periodate solution added. The excess oxidant was destroyed by addition of 0.1 cc. of 10% aqueous glycol solution, and the solution allowed to evaporate to dryness at room temperature. (Evaporation on a steam bath did not seem to alter the subsequent results.) The residue was extracted with a little acetone, and an aliquot of this extract spotted on paper. The chromatograms were run in 30:1:20 v/v acetonewater-benzene and viewed under ultraviolet light. The R<sub>f</sub> value of the fragments resulting from the C₅ and
and C₆ metasaccharinic anilides was 0.52, and that of the fast spot obtained from the C₆ metasaccharinic anilides only was 0.77.

**Fractionation of Mixed C₆ Anilides.** A 0.500-g. portion of this product, of [α] -20° in 95% EtOH, was fractionated on a 2-inch cellulose column, with 5 liters of acetone at 1020 cc. per hour. The fractions obtained were 0.164 g. of D-a-glucometasaccharinic anilide, 0.206 g. of D-β-glucometasaccharinic anilide (I) and 0.157 g. mixed fractions.

**Conversion of Calcium Gluco-metasaccharinates to the Anilides.** A 0.200-g. portion of calcium D-a-glucometasaccharinate in aqueous solution was passed through an IR-120 cation-exchange resin column to remove the calcium. The acidic effluent was concentrated in vacuo to a sirup, the latter dissolved in a little ethanol, and heated 1 hour on the steam bath with 0.2 cc. of aniline and 0.1 cc. of acetic acid. The product was crystallized from ethyl acetate-ligroin.

The calcium D-β-glucometasaccharinate was treated similarly.

**Fractionation of Saccharinic Anilides Derived from D-Glucose.** A mixture of saccharinic anilides, derived from 10 grams of D-glucose, was fractionated on the 6-inch cellulose column. An initial fore-run of 8.5 liters was followed by 200 fractions of 240 cc. each. The C₄ fraction came through at 12-15 liters effluent volume, the two C₅ fractions at 18-20 liters and 21-27 liters, respectively. The first C₆ fraction was collected at 31-41 liters and the second at 41.5-50.5 liters. Both the C₄ and the a-glucometasaccharinic anilide fractions crystallized spontaneously on concentration in vacuo to sirups.
Fractionation of Saccharinic Anilides Derived from L-Arabinose.—This mixture, derived from 20 g. of L-arabinose, was fractionated on the 6-inch column. A fore-run of 7.5 liters was taken, and then 310 fractions of 100 cc. each. The C₄ fraction was obtained at 6-9.5 liters effluent volume, and the C₅-anilides at 10-21 liters. A slower "C₆-fraction", taken at 21-31 liters, was a sirup.

The C₅-anilide fraction was a mixture, weighing 7.5 g., and crystallized spontaneously on concentration in vacuo. Crystallization from ethyl acetate-ligroin gave 1.5 grams of crystalline product (III), and a levorotatory mother liquor.

Fractionation of Saccharinic Anilides Derived from Cellubios.—Fractionation of saccharinic anilides derived from 1 gram of cellubios was carried out on a 2-inch cellulose column at 650 cc. per hour. After a fore-run of 750 cc., 52 fractions of 43 cc. each and 48 fractions of 86 cc. each were taken. A total of 940 mg. crude yield of anilides was recovered, including: C₃, 170 mg.; C₄, 130 mg.; C₅, 180 mg.; α-isosaccharinic anilide, 215 mg.; and 8-glucometasesaccharinic anilide, 105 mg.

Acknowledgment.—The author gratefully acknowledges the cooperation of Dr. S. F. Darling in furnishing a sample of D-α-isosaccharin, Dr. J. K. N. Jones for advice on the operation of cellulose columns, and H. G. Willemsen for carbon, hydrogen and nitrogen analyses.

The Institute of Paper Chemistry
Appleton, Wisconsin
The present work deals with the isolation of various saccharinic acids in kraft black liquor. This report is in the form of an article submitted for publication to Tappi. (Galley proof has been received and the article should appear in the May issue.) Part of this work was presented in Project Report No. 2. An extensive literature review is also given.
Alkaline Pulping. I. Saccharinic Acids in Kraft Black Liquor

John W. Green

Abstract

Conversion of the crude saccharinic acid mixture present in kraft black liquor to the anilides and subsequent paper chromatography has shown the presence of at least eleven distinct components. Fractionation on a cellulose column has given two crystalline fractions, the anilides of α-D-isosaccharinic acid and of β-D-glucometasaccharinic acid. The possible identity of the other fractions is discussed.

John W. Green, Research Associate, The Institute of Paper Chemistry, Appleton, Wisconsin. This paper was presented before the Symposium on Carbohydrate Constituents of Pulp and Paper Sources, at the Minneapolis Meeting of The American Chemical Society, September 15, 1955.
Alkaline Pulping. I. Saccharinic Acids in Kraft Black Liquor

John W. Green

It has been known for a long time that most of the polysaccharides removed from wood by kraft pulping do not persist in the liquor as such but are converted to acidic material. Thus, Sarnio and Gustafson (1) were able to recover only 2% of the polysaccharides removed from the wood during a kraft cook. While it has been assumed that saccharinic acids are formed from these dissolved polysaccharides, little work has been done on their identity. Klason and Brogerfelt (2) reported the presence of meta, "para" and "sapin" saccharinic acids; the experimental evidence offered was meager. The acidic material in the black liquor has been reported as of the total organic material present. Haglund (3) gave a yield of 18% hydroxy acids and lactones, based on the wood; of this, 5% was lactic acid. Mattson (4) reported a similar yield of nonvolatile acidic material. Read (5) isolated a brucine salt, tentatively identified as that of β-isosaccharinic acid.

Saccharinic Acids from Simple Sugars

Quite extensive work has been done on the formation of saccharinic acids from reducing sugar, especially by Kiliani (6) and Nef (7). The isomerization of hexoses and pentoses to deoxy-aldonic acids with the same elementary formulae, C₆H₁₁₂O₆ and C₅H₁₀O₅, respectively, has been shown in many cases. Thus, D-glucose and D-mannose can be converted to straight-chain metasaccharinic acids (I and II in Table I). Most workers have postulated an isomerization first to the 1,2-enediol (Equation 1), and then to a 3-deoxy osone (A in Equation 2) which is hydrated and undergoes a benzoilic acid rearrangement (interchange of the H and OH groups in B) to the saccharinic acid.
It should be noted that the optical configurations on carbons 4 and 5 in the intermediate A are unchanged, and hence D-glucose or D-mannose will form the same two glucometaccharinic acids. In contrast, D-galactose, with a different configuration on carbon 4, will be converted to two different metaccharinic acids (III and IV).

$$\text{CHO} \quad \text{CHO} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH}$$

$$\text{H-C-OH} \quad \text{HO-C-H} \quad \text{C-OH} \quad \text{C-O} \quad \text{C-OH}$$

$$\text{HO-C-H} \quad \text{HO-C-H} \quad \text{HO-C-H} \quad \text{HO-C-H} \quad \text{C-OH}$$

$$\text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH}$$

$$\text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH}$$

$$\text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH}$$

D-glucose    D-mannose    1,2-enediol    D-glucose    2,3-enediol

$$\text{HO-C-H} \xrightarrow{\text{-H}_2\text{O}} \text{C-H} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2$$

$$\text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH}$$

$$\text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH} \quad \text{H-C-OH}$$

$$\text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH}$$

1,2-enediol    A    B    I    II

$$\text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH}$$

$$\text{C-OH} \quad \text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O}$$

$$\text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O}$$

$$\text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O}$$

$$\text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O} \quad \text{C}=\text{O}$$

$$\text{HOCH}_2\text{-C-OH} \quad \text{HO-C-CH}_2\text{OH}$$

$$\text{2,3-enediol} \quad \text{C} \quad \text{D} \quad \text{V} \quad \text{VI}$$
A second type of acid, isosaccharinic acid (V and VI) is also formed from hexoses (Equation 3). The formation is postulated to go from the 2,3-enediol through a 2,3-diketo intermediate (C) which is hydrated and converted by a benznidic acid rearrangement (exchange of the CH₂OH and CH groupings in D) to the branched-chain acids. Here only the C-5 atom has retained its optical configuration in the intermediate C, and hence all D-hexoses will give the same two isosaccharinic acids.

A third type of acid, alpha-glucosaccharinic acid (VII), with a methyl group as the side chain, was actually the first saccharinic acid prepared by the interaction of alkali and D-glucose. The Beta-isomer has not been isolated, nor either of the two galacto-isomers.

The corresponding C₅-metacaccharinic acids (VIII and IX) can be formed from the pentoses by the action of hot alkali. The mechanism can be postulated as in Equation 2, a 5-carbon desoxy oxone being the intermediate. Here only one asymmetric center, as C-4, is retained from the original sugar; hence, all D-pentoses will form the same type of acid. The corresponding C₅-isosaccharinic acid (X) or C₅-saccharinic acid have not been obtained from any pentoses.

The "parasaccharinic" acid, originally reported by Kiliani, was later shown by Nef (2) to be a mixture.

In addition to the isomorization of aldoses to form acids, a degradation effect has also been observed, with the formation of acids with fewer carbon atoms. Thus, Nef (2) analyzed mixtures of acids obtained from D-glucose and hot 8 M NaOH and obtained not only the C₆-glucometasaccharinic acids (I and II)
but also the C₄ (XI) and the C₃ (X) acids as the optically inactive D,L-mixtures. The C₃ acid is of course the well-known lactic acid. XI and XII were also obtained by alkaline degradation of D-galactose, D-xylose, and L-arabinose. This degradation has been studied only under the above conditions, i.e., where metasaccharinic acids are the main products.

Evans and co-workers (9) made a detailed study of the formation of lactic, acetic and formic acid from sugars and K₂O at various concentrations and temperatures.

**SACCHARINIC ACIDS FROM DISACCHARIDES**

The action of mild alkali (saturated lime water at 25°C) on disaccharides has been studied by Kenner and co-workers (10). This action is rather slow and the glycosidic bond is split, with formation of one mole of saccharinic acid from the reducing portion, and one mole of reducing sugar from the glycosidic portion. In the case of 1,4-linked disaccharides the acid formed is of the isosaccharinic acid type, whereas 1,3-linked disaccharides are split to form the metasaccharinic acids. The formation of "oligosaccharinic acids," where the reducing unit of the disaccharide is converted to a saccharinic acid without splitting the glycosidic bond, is very rare. It has been observed in the case of 1,6-linked melibiose, where 6-α-galactopyranosyl meta- and iso-saccharinic acids were isolated (11).

Whereas with mild alkali (lime water) cellobiose was converted to isosaccharinic acids and glucose (12), the action of hot 8% NaOH gave alpha-isosaccharinic acid, beta-glucosmetasaccharinic acid and degradation products including the C₄-metasaccharinic acid (XI) (13). Under these
conditions the glucose initially formed is undoubtedly converted to the
metasaccharinic acid and degradation products.

Evans and coworkers (14) have studied the effect of KCl of various
strengths on disaccharides at 25° and 50°C, and have shown the presence of
lactic, acetic and formic acid.

SACCHARINIC ACIDS FROM POLYSACCHARIDES

The action of alkali on polysaccharides has received scant atten-
tion, as far as the soluble products are concerned. Sack and Tollens (15)
obtained alpha-isosaccharinic acid from a hydrocellulose by the action of
lime water at 100°C. This product would be expected as arising from the
alkaline cleavage of a 1,4-linkage. Palmen (16) reported 11% of the same
acid from alpha-cellulose and excess of lime in water at 25°C. Corbett
and Kenner (12) showed that laminarin, a 1,3-linked polysaccharide, gave
the glucometasaccharinic acids, whereas the oligosaccharide cellobiose
gave the isosaccharinic acids and the reducing units celotrioses, cellobiose
and glucose. They have discussed the "peeling process" for the alkaline de-
gradation of a polysaccharide (Equation 4) where the reducing end unit is
split off as a saccharinic acid and a new reducing unit is formed on the
new end of the residual polysaccharide. Eventually for a polysaccharide of
n units, n - 1 moles of acid and 1 mole of monose will be formed.

\[
\text{Glu-(Glu)}_n \rightarrow \text{Glu-(Glu)}_{n-1} + I. S. \quad (4)
\]

(Glu = glucosidic unit, Glu\textsuperscript{n} = reducing end unit, I. S. = isosaccharinic acid)

Corbett and Whistler (17) have recently reported on the isolation
of a C\textsubscript{5}-isosaccharinic acid (X), formed by the alkaline degradation of a
partially hydrolyzed xylan with lime water. This type of acid would be expected to be formed by the breaking of a 1,6-linked glycosidic bond.

PAPER CHROMATOGRAPHY OF THE SACCHARINIC ACIDS

In contrast to the extensive work done on paper chromatography of the sugars and oligosaccharides, very little work has been done with the saccharinic acids. One of the difficulties arises from the tendency of the acids to form an equilibrium mixture of acid and lactone; these two components run at different speeds on the paper and streaking may occur. Moilanen and Richtzenhain (12) reported briefly on fractionation on paper, with a 10:1:1:3 mixture of ethyl acetate-water-acetic acid as the mobile solvent. In all cases two spots were obtained from each compound, a fast one for the lactone and a slow one for the acid. Kenner and co-workers (12) have given a few \( R_f \) values for the C₆ saccharinic lactones.

To avoid this overlapping of lactone and acid spots for a mixture of saccharinic acids, a method of converting the mixture to the anilides (Equation 5) has been developed (20). The resulting chromatogram then gives only one spot (of saccharinic anilide) for each saccharinic acid. The chromatogram is treated with a fluorescent

\[
C_9H_{11}O_4\cdot COOH + C_6H_5NH_2 \rightarrow C_9H_{11}O_4\cdot CONHCH_2H_5 + H_2O
\]

(5)
dye [Rhodamine B, incorporated in the mobile solvent] and when the dried sheet is viewed under ultraviolet light, the anilides appear as dark spots against a light background.

In Fig. 1 is shown a chromatogram of the mixture of acids (as the anilides) obtained by the action of hot 2 N NaOH on D-glucose. The
The $R_f$ values are inversely proportional to the molecular weight of the acid, hence lactic acid (C$_3$) moves the most rapidly and the C$_6$ acids the least rapidly.

The branched chain acids (as anilides) move more rapidly than do the straight chain acids. Hence, the alpha-iso-saccharinic anilide has a higher $R_f$ value than the meta-saccharinic acid anilide, and the alpha-glucosaccharinic anilide a still higher value, almost in the region of the C$_5$-acid anilides.

Three methods are available for identification of unknown saccharinic acids by this anilide technique. (1) Comparison of the rate of movement ($R_f$) of the unknown anilides with that of known reference compounds on the paper chromatogram. In all, seven crystalline saccharinic anilides, ranging from C$_3$ to C$_6$, have been prepared as reference compounds (20). (2) Isolation of appreciable amounts of crystalline material by fractionation on a cellulose column. (3) Oxidation of milligram quantities of the unknown anilides with sodium periodate, and paper chromatography of the resulting fragments. In this last method the C$_6$ and C$_5$ meta-saccharinic anilides give definite oxidation products (as new spots on the paper chromatogram) and can thus be distinguished from the iso-saccharinic and saccharinic anilides, which do not give such a pattern (see Fig. 2).

PAPER CHROMATOGRAPHY OF THE SACCHARINIC ACIDS IN KRAFT BLACK LIQUOR

A commercial type of kraft black liquor, made from the cooking of southern pine, was used. From this liquor was isolated a reddish acid.
sirup, soluble in water and ethanol, but not extractible from water with ethyl acetate. The yield was about 4 grams per 100 cc. of black liquor.

This sirup was converted to the anilide and a paper chromatogram run, as shown in Fig. 3. Approximately 10 spots can be detected, ranging from the C3 to the C6 regions. In addition, there is a blurry region above the C6 region which may contain higher molecular weight products. This last region has not been investigated.

Two fractionations of appreciable amounts of the anilide mixture were tried on a cellulose column (21). Partial resolution of the C6 anilides was obtained, and two crystalline anilides isolated. The C3, C4, and C5 anilides, however, moved through the column very rapidly with little or no fractionation (Table II). Improvement in technique for this type of fractionation will undoubtedly lead to better results. The mobile solvent (acetone) has worked very well for mixtures of anilides obtained from simple sugars (13) but the mixture of anilides obtained from black liquor is far more complex.

The first C6 fraction isolated (spot 3 in Fig. 3) is the D-anilide of a-isoascorbic acid. The crude yield was about 4% of the total acidic material. The isolated compound corresponded in Rf value and melting point to the known reference compound, and periodate oxidation gave no recognizable fragments on the paper chromatogram.

The second crystalline compound isolated (spot 1 in Fig. 3) is presumably the anilide of D-glucormetascoronic acid. The crude yield
was about 3-4\% of the total acidic material. The isolated compound corresponded
in $R_f$ value to the known reference compound and gave, on periodate oxidation
and subsequent paper chromatography, two spots similar to those given by
C$_4$-metasaccharinic anilides. Both the melting point and the rotation
of the isolated sample were low. Perhaps with a larger sample a pure product
can be obtained on further crystallisation.

The other components present have not been isolated in crystalline
condition. Some conjecture as to their nature is presented below. This is
based on comparison with reference compounds and periodate oxidation techniques.
Also in Table I mention is given of the possible presence of various acids,
as being derived from the different polysaccharide groupings present in wood.

The compound represented by spot 4 (in Fig. 3) may be the anilide
of a-D-glucosaccharinic acid; it gives no periodate oxidation fragments on a
paper chromatogram, so it cannot be a metasaccharinic acid. The intensity
of this spot on the chromatogram is equal to that of 1 or 4.

Spot 2 is very faint, in comparison with the other 3 spots in
the C$_6$ region, and may be the anilide of $\beta$-D-isosaccharinic acid. (The
reference compound, the anilide of this last acid, is unknown at present.)
Periodate oxidation gave no subsequent spots on the chromatogram.

In the C$_5$ region there are 4 spots. A strong one (5) has the
same rate of movement as the known C$_5$-beta-metaccharinic anilide. Periodate
oxidation of this fraction gave one new spot on the subsequent chromatogram,
diagnostic of a C$_5$-meta type of acid (see Fig. 2). Periodate oxidation of
the three fainter spots (6-8) showed a similar spot for 6 and 8, but not for 7.
Nothing is known of the behavior of the anilide of a C₅-iso acid (X) on periodate oxidation. Further investigation is needed here.

In the C₃-C₄ region, there are three spots. The fastest, (11) is undoubtedly the anilide of lactic acid. Spot 9 may be the anilide of the C₄-meta acid (XI) although a fraction obtained from this area did not crystallize, in contrast to the great tendency of the known reference compound to do so. Spot 10 is unknown.

EXPERIMENTAL

Isolation of the Grude Acids. The alkaline black liquor (100 cc.) was added to a slight excess of 2 N sulphuric acid with stirring at room temperature. After 15 minutes the precipitated lignin was removed by centrifuging, to give a clear yellow liquid of pH 1. The lignin was washed several times (by stirring with 200-cc. portions of water and centrifuging) until a washing with a pH above 3 was obtained. At this stage the lignin became rather colloidal and difficult to centrifuge.

The combined filtrates were treated with a little carbon and filtered through a bed of Celite. The reddish solution was concentrated in vacuo to about 200 cc. and added to 300 cc. 95% ethanol to precipitate the sodium sulphate present. The filtrate, still containing a little sodium sulphate and some sulphuric acid, was concentrated in vacuo again to about 200 cc. to remove the ethanol, and then treated with barium carbonate and barium hydroxide to pH 10. The barium carbonate and sulphate were removed by filtration, and the filtrate passed through a cation-exchange resin to remove soluble barium ion.

The effluent, containing only weak organic acids, was concentrated in vacuo to 200 cc. and extracted thoroughly with ethyl acetate. This extraction
removed about one-third of the soluble material (2 grams) present; this is presumably mostly soluble lignin and extractive material. It was felt desirable to remove this noncarbohydrate material at this stage, even though some of the lower molecular weight saccharinic acids or lactones may be extracted from the water phase. Some of the red color was also removed. The aqueous solution, on concentration in vacuo to dryness, gave an acidic sirup, red in color, and amounting to 4 grams in weight. It is soluble in 95% ethanol.

Conversion of the Acids to the Anilides. The acidic sirup was dissolved in 100 cc. 95% ethanol, 10 cc. aniline added and the solution heated in a distilling flask on a boiling water bath, with slow distillation of the ethanol, during 1-2 hours. The heating was then continued, at 20 mm. (water pump) to remove any water in the system and to complete the formation of the anilides. In this second stage some of the excess aniline was removed. The solution tends to turn a dark red during the heating, and subsequent treatment with carbon will remove part of the color.

Several variations in subsequent heating to form the maximum amount of anilides have been tried. One is to dissolve the sirup in absolute ethanol and distill again to remove water present. Another is to dissolve the sirup in a little ethanol, and then add a larger quantity of benzene (most of the sirup precipitates); the mixture is heated in the water bath with a Dean-Stark water-trap until a perfectly clear benzene distillate is obtained.

The sirup finally obtained consists of anilides, unreacted acids and excess aniline. Attempts to remove the aniline by extraction of the
sirup with benzene (this removed the aniline and \( C_3 \) and \( C_4 \) anilides) and extraction of the benzene solution with aqueous HCl removed not only the aniline but some of the anilides too. Another approach has been to dissolve the sirup in ethanol, add one volume of water, and then pass this solution through a cation-exchange resin to remove the aniline and then through an anion-exchange resin to remove any unreacted acid. All of the sirups fractionated on cellulose columns (see below), however, were prepared by heating with 95\% ethanol and then at 20 mm, as given above.

Paper Chromatography of the Anilides. A 10\% ethanol solution of the mixed anilides was spotted on the starting line of a Whatman No. 1 sheet, 24 inches long. About 100-500 micrograms of material (1-5 microliters of solution) was sufficient to give a satisfactory chromatogram. For some of the fainter spots (2, 6, 7, and 8) elution of the material from the chromatogram and rechromatographing was necessary.

The mobile solvent was a 30:1:20 v/v mixture of acetone-water-benzene containing 40 mg. of Rhodamine B dye per liter; the dye is best dissolved in the acetone and the water and benzene then added.

The chromatograms were run by the descending technique in 12 by 24-inch glass jars. The solvent movement is very rapid, requiring only 5-7 hours to advance to the end of the sheet (21 inches). The dye moves a little slower than the solvent (about 18 inches). The paper is removed from the tank, air dried about 5 minutes and viewed under ultraviolet light. A light of restricted wavelength is needed to view the dark spots of anilide against the light background (1\%).

For further study of the \( C_\text{5} \) and \( C_\text{6} \) anilides, the original spots viewed are outlined with a pencil, and the sheet again run with the same
solvent for 15-24 hours. In this way the C₅ spots will move almost to the
bottom of the sheet and better separation will be achieved.

Periodate Oxidation of Individual Fractions. About 5 milligrams
of material was spotted along one or more sheets at one-inch intervals.
The chromatogram was then run as usual to obtain a good separation of the
desired fraction. The section of paper containing the desired series of
spots was then cut out and eluted with several portions of ethanol. The dye
was removed also but did not seem to interfere with subsequent operations.

The solution was concentrated to dryness, the residue dissolved
in 0.1 cc. ethanol, transferred to a test tube, cooled to 0°C., and 0.4 cc. of
water containing 20 mg. sodium metaperiodate added at 0°C. After one hour
at 0°C., 0.2 cc. of 10% aqueous ethylene glycol was added to react with excess
oxidant. Then 10 cc. of absolute ethanol was added, which precipitated most
of the sodium salts present. The ethanol solution was allowed to concentrate
to dryness, and the residue dissolved in the minimum amount of acetone (about
20 microliters) and this solution spotted on a sheet of paper for chromatography.

In most cases the yield of oxidation fragments, based on the size
of spots obtained on the chromatograms, was 10-20%. In the case of the
crystalline anilides, about 0.2 milligrams of starting material was sufficient.

Fractionation on a Cellulose Column. A concentrated ethanol
solution, containing the anilide sirup prepared from 5% rum of mixed acids
(equivalent to 125 cc. of black liquor) was applied to the top of a cellulose
column, 2 inches in diameter by 22 inches in length (2l). The column had
previously been washed with acetone containing 1% water. Then 5 liters of
solvent were allowed to flow through the column, the first 300 cc. being discarded. The remaining effluent was collected in fractions of approximately 40 cc. each. Approximately 0.5 cc. of each fraction was concentrated to a small volume, spotted on a paper sheet and the spots on the resulting chromato-gram compared with reference compounds. In this way the components in the various fractions were identified.

Two flow rates, of 30 and 300 cc. per hour, were used in the fractionations. The results were similar, except that at the slower rate the various anilides seemed to move through the column in a smaller volume of solvent (see Table II).

Most of the fractions collected contained more than one component. Only in the latter fractions (the C₆ anilides) was a good separation effected, to the extent that crystalline products were isolated. Even here some overlapping intermediate fractions were obtained. These results are in contrast to the better separations obtained with mixtures of saccharinic anilides derived from the simple sugars (12), and may be attributed to (a) the greater number of fractions present in black liquor, (b) the presence of impurities, such as unreacted saccharinic acids, and acidic material derived from lignin and extractives.

The combined fractions containing the α-isosaccharinic anilide (300 and 4000 cc., respectively, for the slow and fast rates) were concentrated to sirups which crystallized readily. The weights of these sirups were 270 and 315 mg., respectively. They were recrystallized repeatedly from a mixture of ethyl acetate and ligroin (b. 30-60°) until a melting point
of 170-171°C. was obtained. The melting point of a mixture of this product and an authentic sample (m. 169-170°C) was 169.5-170.5°C. These products after periodate oxidation did not give any spots on the subsequent chromatogram. The rate of movement of these crystalline products on a chromatogram were similar to that of an authentic sample. The yields of product after the first crystallization were 105 and 95 mg., respectively.

The combined fractions containing the β-glucometasaccharinic anilides were 900 and 800 cc., respectively, (for the two flow rates), and on concentration in vacuo gave 270 and 210 mg., respectively. The first sirup on crystallization from ethyl acetate-ligroin gave 75 mg. of product, m. 113-114°C., with a specific rotation of -77° in water. (An authentic sample of β-glucometasaccharinic anilide, m. 123-124°C, has a specific rotation of -63° in absolute ethanol.) Further recrystallization did not raise the melting point appreciably, so some impurity must be present. This product after periodate oxidation, gave on a chromatogram the two spots typical of a C₆-metasaccharinic anilide (see Fig. 2). The rate of movement of the product on a paper chromatogram was similar to that of an authentic sample.

DISCUSSION OF RESULTS

Saarnio and Gustafsson (1) have determined the amount of the various polysaccharides dissolved in the kraft cooking process and not recovered as such, i.e., probably converted to saccharinic acids (Table III). Thus, it can be seen that half of this material is glucon and mannan and one-fourth is xylan. Based on the Kenner theory of alkaline "peeling" of polysaccharides, the main products should be C- and β-isoclasaccharinic acids (V and VI) derived from the C₆-polysaccharides, and the C₂-isoc acids (X) derived from the xylan.
However, the conditions of a kraft cook (1-2 N NaOH at 160-170°C) are very drastic in contrast to the mild conditions used by Kenner. Hence we would expect, and do find degraded saccharinic acids as well as those of the meta type. It is surprising, however, that the main C₅ saccharinic acid seems to be of the meta type (spot 5, Fig. 3). One would expect at least an equal amount of the iso acids.

The work presented in this paper is very definitely of a preliminary nature. So at present only the possible nature of the various saccharinic acids present in the black liquor can be discussed. It is hoped that, with further refinement of techniques, that this method may serve as a tool in the study of the effect of alkaline pulping on the carbohydrates in the wood.
### TABLE I

**SACCHARINIC ACIDS AND THEIR ORIGINS**

<table>
<thead>
<tr>
<th>Formula of acid</th>
<th>Name</th>
<th>Possible precursor</th>
<th>Presence in black liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH HCOH CH₂</td>
<td>D-α-glucosetasaccharinic acid (I)</td>
<td>Glucan</td>
<td>Probable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannan</td>
<td></td>
</tr>
<tr>
<td>COOH HCOH CH₂</td>
<td>D-β-glucosetasaccharinic acid (II)</td>
<td>Glucan</td>
<td>Isolated as the anilide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannan</td>
<td></td>
</tr>
<tr>
<td>COOH HCOH CH₂</td>
<td>D-α-galactosetasaccharinic acid (III)</td>
<td>Galactan</td>
<td>Possible, only in small amounts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH HCOH CH₂</td>
<td>D-β-galactosetasaccharinic acid (IV)</td>
<td>Galactan</td>
<td>Possible, only in small amounts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH HCOH₂ CH₂</td>
<td>D-α-isosaccharinic acid (V)</td>
<td>Galactan</td>
<td>Isolated as the anilide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannan</td>
<td></td>
</tr>
<tr>
<td>COOH HCOH₂ CH₂</td>
<td>D-β-isosaccharinic acid (VI)</td>
<td>Galactan</td>
<td>Probable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannan</td>
<td></td>
</tr>
<tr>
<td>COOH CH₂ HCOH</td>
<td>D-α-glucosaccharinic acid (VII)</td>
<td>Glucan</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannan</td>
<td></td>
</tr>
</tbody>
</table>
TABLE I (Continued)

SACCHARINIC ACIDS AND THEIR ORIGINS

<table>
<thead>
<tr>
<th>Formula of acid</th>
<th>Name</th>
<th>Possible precursor</th>
<th>Presence in black liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH HCOH CH₂</td>
<td>D-α-C₅-metacarboxylic acid (VIII)</td>
<td>Xylan</td>
<td>Probable</td>
</tr>
<tr>
<td>HCOH CH₂OH</td>
<td>D-β-C₅-metacarboxylic acid (IX)</td>
<td>Xylan</td>
<td>Probable</td>
</tr>
<tr>
<td>HCOH CH₂OH</td>
<td>D,L-C₅-isosaccharinic acid (X)</td>
<td>Xylan</td>
<td>Probable</td>
</tr>
<tr>
<td>COOH C(CH₂)CH₂OH</td>
<td>D,L-2,4-dihydroxybutyric acid (C₄-metacarboxylic acid) (XI)</td>
<td>Glucan, Mannan, Galactan, Xylan, Arabin</td>
<td>Probable</td>
</tr>
<tr>
<td>CH₂OH</td>
<td>D,L-lactic acid (XII)</td>
<td>Glucan, Mannan, Galactan, Xylan, Arabin</td>
<td>Present (3)</td>
</tr>
</tbody>
</table>
# Table II

**Fractionation of Saccharinic Anilides on a Cellulose Column**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Flow rate of 80 cc/hr</th>
<th>Flow rate of 800 cc/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yield, % Effluent, cc</td>
<td>Yield, % Effluent, cc</td>
</tr>
<tr>
<td>C₃-C₆ fraction (mixed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude α-isoaccharinic anilide</td>
<td>5.4</td>
<td>2100-2400</td>
</tr>
<tr>
<td>Crystalline</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Mixture</td>
<td>4.0</td>
<td>2400-3600</td>
</tr>
<tr>
<td>Crude β-glucometaccharinic anilide</td>
<td>5.4</td>
<td>3600-4500</td>
</tr>
<tr>
<td>Crystalline</td>
<td>2.0</td>
<td>--</td>
</tr>
</tbody>
</table>

Note - the anilide mixture originally applied to the column was obtained from 5 g. of acidic sirup. The % yields are weights of anilide obtained based on the original acid mixture. The yields of saccharinic acid would be 70% of the given yields.
### TABLE III

**RECOVERY OF CARBOHYDRATES IN KRAFT PULPING**

<table>
<thead>
<tr>
<th>Polysaccharides</th>
<th>Total Glucan</th>
<th>Mannan</th>
<th>Xylan</th>
<th>Galactan</th>
<th>Ara-</th>
<th>Araban</th>
<th>Uronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present in pine, %</td>
<td>72</td>
<td>46</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Present in kraft pulp, %</td>
<td>46</td>
<td>39</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Removed in liquor, %</td>
<td>26</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Max. amt. in liquor</td>
<td>3.1</td>
<td>0.5</td>
<td>0.6</td>
<td>1.8</td>
<td>0.01</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Min. amt. in liquor</td>
<td>0.45</td>
<td>0.07</td>
<td>trace</td>
<td>0.35</td>
<td>0.02</td>
<td>0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note - The data for polysaccharides in the liquor are from Saarnio and Gustafsson. The data for the wood and pulp are representative only.*
LITERATURE CITED


6. Kiliani, H., et al., Ber. 15:2953 (1882); 16:2625 (1883); 18:631 (1885); 35:3523 (1902); 42:2603 (1909).


9. Evans, W. L., et al., J. Am. Chem. Soc. 48:2665 (1926); 50:1496 (1928); 50:2543 (1928); 52:3680 (1930); 52:4065 (1930); 55:4957 (1933); 60:2847 (1938).

LITERATURE CITED—Continued


Figure 1. Chromatogram of saccharinic anilides formed from glucose.

A. The solvent advanced only to the bottom of the sheet (5-7 hours).
B. The solvent was allowed to drip off the sheet (15-24 hours), thus resolving spots 1 in I and II, and 2 into VIII and IX.
(The Roman numerals refer to the saccharinic acid portion of each anilide.)
Figure 2. Chromatogram of periodate oxidation fragments

A = reference compounds, B = fragments from oxidation of a C₅-nitasaccharinic anilide (II), C = that from a C₅-nitasaccharinic anilide (IX).
(The Roman numerals refer to the saccharinic acid portion of each anilide.)
Figure 3. Chromatogram of saccharinic anilides obtained from black liquor.

A and C are reference compounds, B is the mixture of anilides derived from the black liquor.
(The Roman numerals refer to the saccharinic acid portion of each anilide.)