able proportions of 1 → 6 linkages, such as B1375, B1385, B1383, etc. Assuming that the 19% of 1 → 4 like units present in this dextran were mostly at branch points and that chains of 1 → 4 like units did not occur, as appears likely from the failure of this dextran to precipitate more than about 12 μg. of antibody N from antiserum $9D_3$ (*cf.* Fig. 1), a branching ratio of 4.3 can be calculated for B742 LR and for B1385 and B1375. Despite the finding that such a ratio allows the latter two dextrans to be effective in precipitating antibody and permits the existence of substantial numbers of terminal sequences of four 1 → 6 glucopyranose units, B742 LR was only $^1/_6$ to $^1/_7$ as effective per unit weight in precipitating anti-dextran. Accordingly one would predict that the structure of B742 LR would differ from those of the other dextrans in that many of the 1 → 4 branches are so distributed as to reduce disproportionately the number of terminal non-reducing sequences of four or more glucose units and increase the length of the inner chains of 1 → 6 units. A 1 → 4 branch occurring on the second or third glucose unit from a non-reducing end group would substantially reduce the capacity of that group to combine with 1 → 6 anti-dextran, since it has been shown that the capacity of isomaltose to bind anti-dextran was only about $^1/_{60}$ that of isomaltotriose.

Recent inhibition studies (E. A. K. in preparation)[26] have shown that the antibodies are heterogeneous with combining sites ranging in size from units complementary to four to six (1 → 6) anhydroglucopyranose units. The capacities of the tetrasaccharide and hexasaccharide to inhibit precipitation of anti-dextran by dextran are sufficiently close so that for the present discussion branching would probably have to occur nearer to the non-reducing end than the fourth or third ring to cause the striking decrease in reactivity noted. The correctness of this prediction almost must necessarily await elucidation of the structure of this dextran. It has been shown previously[27] that a preparation of B742 highly comparable with B742 LR[8b] but having only 75% 1 → 6 linkages was more resistant to the action of dextranase from *Penicillium funiculosum* NRRL strain 1768 than was another dextran having the same content of 1 → 6 linkages. When treated with a comparable preparation of 1768 dextranase in this Laboratory, dextran B742 LR likewise showed a lower conversion to reducing sugars than did dextrans B1375 and B1385 which, like B742 LR, have 81% 1 → 6 linkages.

The finding that B742 LR differs in its susceptibility to enzymatic degradation from other dextrans with comparable proportions of 1 → 6 linkages is in agreement with the immunochemical findings and provides additional evidence of the unusual structure of this dextran.

Certain minor differences in specificity of the 1 → 6 antibodies remain to be explained, especially the ability of B1299 S3 to precipitate almost all of the antidextran from antiserum $116D_2$ and $1D_7$, whereas it could only precipitate about $^1/_3$ of the antibody from antiserum $36D_2$ and about $^2/_3$ from antiserum $30D_2$. Such variations are quite common to other cross reacting antigen–antibody systems and may be dependent on the heterogeneity in size of the 1 → 6 antibody combining sites.[26]

Acknowledgment.—The authors wish to express their sincere appreciation and thanks to Drs. Allene Jeanes and H. M. Tsuchiya for their numerous suggestions in reviewing the manuscript and to Miss Gloria Petrilli for technical assistance.

(26) E. A. Kabat, in preparation.

(27) H. M. Tsuchiya, A. Jeanes, H. M. Bricker and C. A. Wilham, *J. Bact.*, **64**, 513 (1952).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Effect of Alkali on Carbohydrates. I. Saccharinic Anilides Derived from D-Glucose, L-Arabinose and Cellobiose[1]

BY JOHN W. GREEN

RECEIVED OCTOBER 10, 1955

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 α-metasaccharinic anilides than for the β-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.

In a previous communication[2] 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 R_f values, the anilides of C_3 (lactic), C_4, C_5 and C_6 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,[3] as

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

(2) J. W. Green, THIS JOURNAL, **76**, 5791 (1954).

(3) Leipen, *Monatsh.*, **9**, 45 (1888); C. A. Bischoff and P. Walden, *Ann.*, **279**, 71 (1894).

TABLE I

SACCHARINIC ANILIDES

Substance	M. p., °C.	$[\alpha]^{30}$ In H_2O	$[\alpha]^{30}$ In 95% EtOH	R_f values[c] in Solvent A	R_f values[c] in Solvent B	Ref.	Analyses,[d] % Carbon	Hydrogen	Nitrogen
D,L-Lactic anilide	58–59			0.92	0.80	3, 4			
D,L-2,4-Dihydroxybutyric anilide	115–116			.86	.62		61.54	6.74	7.25
C_5-Anilide	119.5–120.5	...	+59°	.75	.35				
D-α-Glucometasaccharinic anilide	89–90	...	+40°	.60	.22		56.28	6.66	5.59
D-β-Glucometasaccharinic anilide	123–124	...	−63°	.60	.18		56.44	6.70	5.58
D-α-Isosaccharinic anilide	169–171	+13°		..	.24	9			
D-α-Glucosaccharinic anilide	193–195	+58°[a]	+55°[b]	..	.32		56.68	6.78	5.66

NOTE: All rotations were determined on a Bausch and Lomb wedge saccharimeter. [a] 20°; [b] 25°; [c] solvent A is 9:1:2 v./v. acetone–water–benzene. Solvent B is 30:1:20 v./v. acetone–water–benzene. [d] Calcd. for C_4 anilide: C, 61.50; H, 6.60; N, 7.18. Calcd. for C_6 anilide: C, 56.66; H, 6.66; N, 5.50.

shown by comparison of the R_f value with that of an authentic sample, prepared from lactic acid,[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.[5]

The third and fourth spots, for mixtures derived from L-arabinose and D-glucose, were shown to be the anilides of C_5 and C_6 saccharinic acids, respectively.[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_3 and C_4 anilides are washed from the sheet, has allowed development of the C_5 and C_6 regions into two spots in each case. Nef[5] has shown the formation of isomeric pairs of C_5 and C_6 acids from pentoses and hexoses, respectively.

The C_6 saccharinic anilide, originally reported[2] with a specific rotation of −20°, has thus been shown to be a mixture of roughly 60% D-β-glucometasaccharinic anilide (I) and 40% D-α-glucometasaccharinic anilide. The structure of these anilides was shown by an independent preparation from known samples of calcium α- and β-glucometasaccharinates.[6]

One of the two C_5-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_5-metasaccharinic anilide." The other anilide, of higher R_f value, is a sirup of negative rotation and probably the L-*erythro* isomer.

It is interesting to note, for both the C_6 and C_5 metasaccharinic anilides, that the α-isomer moves more rapidly on paper than does the β-isomer.

Anilides were also prepared from D-α-glucosaccharin[7] and from D-α-isosaccharin.[8] The latter anilide has been reported by Utkin.[9] Both of these anilides move much more rapidly on paper than do the glucometasaccharinic 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_5-metasaccharinic anilides.

Some small scale periodate oxidations were performed, according to the method of Lemieux and Bauer.[10] Oxidation of milligram quantities of the various anilides was followed by paper chromatography of the resulting oxidation products and observation under ultraviolet light. The D-α-glucosaccharinic anilide gave only a blank sheet, and the D-α-isosaccharinic anilide a diffuse pattern. However, the C_6 and C_5 metasaccharinic anilides (I and III), obtained from either D-glucose or L-arabinose, gave well defined spots in the C_4 region. These probably represent a C_4 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–CONHPh grouping 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 C_4 fragments (II and IV), the anilides of 2-deoxytetruronic acids, should be enantiomorphs when formed from I and III, or the α- and β-isomers, and would thus interrelate the structures of the C_5 and C_6 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 *N* NaOH, as studied by Nef.[5] 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.

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

(4) M. L. Fein and E. M. Filachione, THIS JOURNAL, **75**, 297 (1953).

(5) J. U. Nef, *Ann.*, **376**, 1 (1910).

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

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

(8) W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 2245 (1953).

(9) L. M. Utkin and G. O. Grabilina, *Doklady Akad. Nauk. S.S.S.R.*, **93**, 301 (1953); *C. A.*, **48**, 12676 (1954).

(10) R. U. Lemieux and H. F. Bauer, *Can. J. Chem.*, **31**, 814 (1953).