in the preparation of **12**, gave 1.60 g. of an oil which was chromatographed on Florisil (ligroin-benzene and ether). The early fractions, 0.15 g., were identified as the 3,9(11)-diene **4** (infrared), and were followed by the desired formate, 1 45 g., an oil, $[\alpha]_D$ +116°, $\lambda_{max}^{CS_2}$ 5.70, 5.75, 8.39, 8.48, 9.11 μ.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.40; H, 9.86.

Methyl 12α-chloro-3β-hydroxy-9(11)-cholenate (**16**). Chlorination of 0.40 g. of compound 15 under conditions identical to the preparation of 5, gave a crude product whose infrared curve lacks formate bands. Crystallization of this material from ligroin-benzene gave 0.13 g. of rods, m.p. 121–127.5°, m.p. of mixture with **17** 108–120°, $[\alpha]_D$ +150°. The analytical sample was recrystallized in chloroform-ligroin (35–60°), m.p. 131–135.2°, $[\alpha]_D$ +154°, $\lambda_{max}^{CS_2}$ 5.71, 8.5–8.6, 9.66, 11.42, 14.3–14.4 (w) μ.

Anal. Calcd. for $C_{25}H_{39}O_3Cl$: C, 70.98; H, 9.29; Cl, 8.38. Found: C, 71.28; H, 9.53; Cl, 8.15.

Methyl 12α-chloro-3α-hydroxy-9(11)-cholenate (**17**), prepared from **1** according to Mattox *et al.*,[14] was difficult to obtain in a sharply-melting form. The best sample crystallized from ligroin-ether, m.p. 113–126°, $[\alpha]_D$ +143°, (lit., m.p. 119–124°, $[\alpha]_D$ +149°), $\lambda_{max}^{CS_2}$ 5.70, 8.5–8.6, 9.60, 14.2–14.3 (w) μ.

Methyl 3β,12α-diformoxy-9(11)-cholenate (**18**). A solution of 1.00 g. of **2** in 10 ml. of dimethylformamide was refluxed 48 hr., cooled, diluted with water, and extracted with benzene. The benzene layer was dried over sodium sulfate and evaporated to give 0.60 g. of crude product. Chromatography on Florisil gave 0.21 g. of an unidentified oil (eluted by ligroin) and 0.35 g. of crude product (ligroin-ether 2:1). The latter was rechromatographed on Florisil to give an oil $[\alpha]_D$ +16° (0.77%), $\lambda_{max}^{CS_2}$ 5,70, 5.76, 8.4–8.5 μ.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.24; H, 8.99.

The same product was obtained in an attempt to esterify methyl 12α-chloro-3β-hydroxy-9(11)-cholenate. A solution of 0.26 g. of **16** and 4 drops of concd. sulfuric acid in 20 ml. of 88% formic acid stood at room temperature 4 hr., was poured on ice, and extracted with ether. The ether layer was washed with water, dried over sodium sulfate, and evaporated leaving a chlorine-free (Beilstein) residue. Chromatography on Florisil gave 0.18 g. of an oil with an infrared curve corresponding to that of the diformate prepared from **2**.

Methyl 3α,12α-dimethoxy-9(11)-cholenate (**19**). An excess of potassium was added to a solution of 2.00 g. of **1** in 20 ml. of benzene. After the mixture had refluxed 2 hr. and cooled, methyl iodide (5 ml.) was added, and the mixture again refluxed for 3 hr. Methanol was added to the cooled mixture to destroy the excess potassium. The solution was evaporated to dryness and the residue extracted with ether. Evaporation of the ether left 1.77 g. of an oil which was chromatographed on alumina (ligroin-benzene) and crystallized from acetone, m.p. 190–195° (d), $[\alpha]_D$ +142°, $\lambda_{max}^{CS_2}$ 5.71, 7.92, 8.55, 9.10 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96: H, 10.30. Found: C, 74.81: H, 9.82.

Methyl 3β,12α-dimethoxy-9(11)-cholenate (**20**). A methanolic solution of 2.20 g. of tosylate **2** and 0.40 g. of sodium methoxide was refluxed 60 hr. The methanol was evaporated and the residue extracted with ether; evaporation of the ether left 1.90 g. of crude product. Chromatography on alumina gave 0.94 g. of product **20** (eluted by ligroin-benzene) and 0.8 g. of recovered **2** (eluted by ether). The first fraction was rechromatographed on alumina to give the analytical sample, an oil, $[\alpha]_D$ +113, $\lambda_{max}^{CS_2}$ 5.70, 7.96, 8.52, 9.11, 14.3 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.30. Found: C, 74.52; H, 10.28.

Acknowledgment. The authors are grateful to Mr. George Hayes for conducting the serological tests.

INDIANAPOLIS 7, IND.

(14) V. R. Mattox *et al.*, *J. Biol. Chem.*, **164**, 569 (1946).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Alkaline Degradation of Guaran and Characterization of "β"-D-Isosaccharinic Acid[1]

ROY L. WHISTLER AND J. N. BEMILLER

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Salts of "β"-D-isosaccharinic acid and 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid are obtained in equivalent amounts from oxygen-free alkaline solutions of guaran. An analysis method for mixtures of "α"- and "β"-D-isosaccharinic acids is presented. "β"-D-Isosaccharinic acid is obtained crystalline as its tetrabenzoate, and 5-*O*-α-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone and 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid have been synthesized.

That bases may be useful for the exploration of the chain structures of polysaccharides was first proposed by Corbett, Kenner, and Richards.[2] To show that the procedure is practicable, they treated the linear polysaccharide laminaran with an alkaline solution and isolated the predicted D-glucometasaccharinic acids.[3] Whistler and Corbett[4] have proposed that alkaline degradation can be used to determine branching in a polysaccharide. To test this proposition, the saccharinates from the alkaline treatment of guaran have been identified.

Guaran is a D-galacto-D-mannoglycan (33:67) consisting of a chain of β-(1 → 4)-linked D-mannopyranosyl units with single-unit side chains of D-galactopyranose residues linked α-(1 → 6) to one-half of the D-mannopyranosyl units.[5] The poly-

(1) Journal Paper No. 1690 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

(2) W. M. Corbett, J. Kenner, and G. N. Richards, *Chem. & Ind.* (London), 462 (1953).

(3) W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 1431 (1951).

(4) R. L. Whistler and W. M. Corbett, *J. Am. Chem. Soc.*, **78**, 1003 (1956).

(5) Z. F. Ahmed and R. L. Whistler, *J. Am. Chem. Soc.*, **72**, 2524 (1950).

saccharide is isolated by water extraction and precipitation by ethanol[6] so that no reagents are used which might oxidize the reducing end-unit of the molecule and make it stable to alkali.[4]

Alkaline treatment of 4-*O*-substituted carbohydrates forms isosaccharinates[7] when the substituent group is attached by an etheric or glycosidic linkage. Likewise, the action of alkalies on 4,6-di-*O*-etherified hexoses forms 5-*O*-etherified isosaccharinates.[8] A proposed mechanism is the conversion of the aldose reducing end to a ketose which forms a *β-alkoxy carbonyl* system[9] and ejection of the remainder of the molecule as an alkoxy anion by electron shifts.[10] The former end-unit then rearranges by prototropy to a 2,3-dicarbonyl compound[11] which undergoes a benzilic acid-type rearrangement to isosaccharinate. Therefore, the predicted products from the alkaline treatment of guaran would be D-isosaccharinate and 5-*O*-α-D-galactopyranosyl-D-isosaccharinate in equimolar quantities. Isolated from the reaction mixture are "β"-D-isosaccharinic acid and a modified disaccharide containing "β"-D-isosaccharinic acid and D-galactose. The experimentally determined ratio of "β"-D-isosaccharinic acid to modified disaccharide produced is 1.1:1.0.

"α"-D-Isosaccharinic acid has been used in structural studies of isosaccharinic acids for it is easily isolated as its slightly soluble calcium salt. By oxidations and reductions, "α"-D-isosaccharinic acid was shown to be 3-deoxy-2-*C*-hydroxymethyl-(D-*erythro* or D-*threo*)-pentonic acid.[12] In earlier investigations, a C-2 stereoisomer, "β"-D-isosaccharinic acid was proposed[9,13-15] but never isolated free of "α"-D-isosaccharinic acid and possibly other impurities. In this work, "β"-D-isosaccharinic acid has been isolated as its highly soluble calcium salt and as the stable free acid.

Guaran, the main chain of which is a β-(1 → 4)-linked D-mannoglycan, forms "β"-D-isosaccharinates when treated with alkaline solutions in contrast to the β-(1 → 4)-linked and α-(1 → 4)-linked D-glucoglycans, cellulose and amylose, respectively, both of which give predominantly "α"-D-isosaccharinates.[16,17] "β"-D-Isosaccharinic acid has been obtained by chromatography and/or distillation in a form which is purer than that obtained by differences in solubility of its brucine[13] and calcium[9,14,15] salts and those of "α"-D-isosaccharinic acid. Oxidation of pure "β"-D-isosaccharinic acid with nitric acid produces a compound which is chromatographically identical to the tricarboxylic acid, 2-*C*-carboxy-3-deoxy-D-*glycero*-pentaric acid, formed by nitric acid oxidation of "α"-D-isosaccharinic acid.[18,19] This same tricarboxylic acid was obtained by Kiliani and Eisenlohr[20] by nitric acid oxidation of a residue rich in the "β" isomer obtained after removal of the metasaccharinic acids and most of the "α"-D-isosaccharinic acid from the lactose-alkali reaction mixture. Their findings were used by Nef[13] to support his conclusion that there is a "β"-D-isosaccharinic acid, a C-2 stereoisomer of "α"-D-isosaccharinic acid, although Kiliani and Eisenlohr[20b] had demonstrated that the mixture contained residual "α"-D-isosaccharinic acid.

In oxidation of "β"-D-isosaccharinic acid with periodate, two equivalents of periodate are consumed per mole of acid, and two moles of formaldehyde are formed per mole of acid. Sowden and coworkers[21] have previously reported that two moles of formaldehyde are obtained from each mole of "α"-D-isosaccharinic acid oxidized with periodate. Both findings agree with proposed structures.

Reduction of both "α"- and "β"-D-isosaccharinic acids to 2-methylpentanoic acid affords additional evidence that they are C-2 stereoisomers. A new reduction method[22] has been applied to hydroxy acids, wherein triphenylphosphite and methyl iodide react with the isosaccharinic acids according to the following equation:

$$(PhO)_3P + ROH + CH_3I \longrightarrow RI + CH_3PO(OPh)_2 + PhOH$$

The tetraiodide (RI) is then further reduced to a hydrocarbon with zinc and hydrochloric acid. Racemic 2-methylpentanoic acid is isolated as its

(6) E. Heyne and R. L. Whistler, *J. Am. Chem. Soc.*, **70**, 2249 (1948).

(7) For a general discussion of the alkaline degradation of carbohydrates see R. L. Whistler and J. N. BeMiller, *Advances in Carbohydrate Chem.*, **13**, 289 (1958).

(8) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 2916 (1956).

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

(10) H. S. Isbell, *J. Res. Natl. Bur. Standards*, **32**, 45 (1944).

(11) (a) R. L. Whistler and J. N. BeMiller, *J. Am. Chem. Soc.*, **82**, 3705 (1960); (b) G. Machell and G. N. Richards, *J. Chem. Soc.*, 1932 (1960).

(12) For a review of the structure determination investigations see J. C. Sowden, *Advances in Carbohydrate Chem.*, **12**, 35 (1957).

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

(14) W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 1789 (1954).

(15) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 1810 (1955).

(16) G. N. Richards and H. H. Sephton, *J. Chem. Soc.*, 4492 (1957); G. Machell and G. N. Richards, *J. Chem. Soc.*, 4500 (1957).

(17) (a) J. Kenner and G. N. Richards, *Chem. & Ind.* (London), 1483 (1954); (b) G. Machell and G. N. Richards, *J. Chem. Soc.*, 1199 (1958).

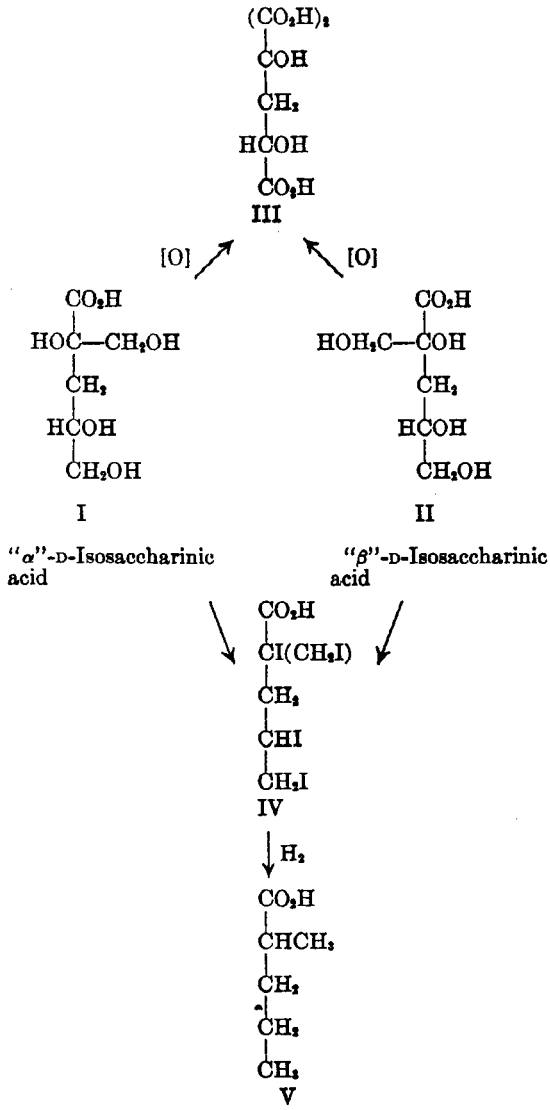
(18) H. Kiliani, *Ber.*, **18**, 631 (1885).

(19) R. L. Whistler and J. N. BeMiller, *J. Am. Chem. Soc.*, **82**, 457 (1960).

(20) (a) H. Kiliani, *Ber.*, **41**, 2650 (1908); (b) H. Kiliani and F. Eisenlohr, *Ber.*, **42**, 2603 (1909).

(21) J. C. Sowden, M. G. Blair, and D. J. Kuenne, *J. Am. Chem. Soc.*, **79**, 6450 (1957).

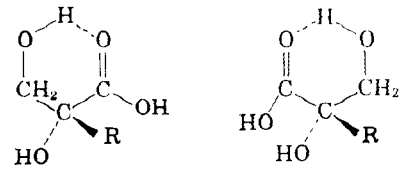
(22) S. R. Landauer and H. N. Rydon, *Chem. & Ind.* (London), 313, (1951); *J. Chem. Soc.*, 2224 (1953); H. N. Rydon and S. R. Landauer, Brit. Patent **695,468** (1953); *Chem. Abstr.*, **48**, 10047 (1954); see also J. B. Lee and M. M. El Sawi, *Chem. & Ind.* (London), 839 (1960).



crystalline *p*-phenylphenacyl ester and amide. The over-all crude yield is about 40%. 2-Methylpentanoic acid has been previously obtained by reduction of "α"-D-isosaccharino-1,4-lactone with hydroiodic acid and red phosphorus.[18] The combined information presented here shows that "β"-D-isosaccharinic acid is indeed a C-2 stereoisomer of "α"-D-isosaccharinic acid, something not heretofore fully demonstrated.

Earlier work in this laboratory[11a] has already shown that both "α"- and "β"-D-isosaccharinic acids are formed exclusively, rapidly, concurrently, and stoichiometrically by the action of alkalies on their dicarbonyl precursor, giving support to the observation that they are C-2 stereoisomers.

Assignment of configuration of "α"- and "β"-D-isosaccharinic acids is tentative, but suggestions are made on the basis of qualitative rules of configuration. Recent observations of Bose and Chatterjee,[23] who have generalized the rule of Klyne and Stokes[24] and applied it to cyclic sugars, are that Hudson's isorotation and lactone rules depend on the relative sizes of the constituents on the asymmetric carbon atom in question. Unfortunately, "β"-D-isosaccharinic acid does not form a lactone. Otherwise configurations of the two acids would be indicated. However, Brewster[25] has extended recent advances in this field to open chain compounds and has given empirical rules for predicting rotatory effects of asymmetric atoms and conformations. His rules cover acids with α-substituents which can hydrogen-bond to the carboxy group to produce cyclic structures. Following these ideas, "α"-D-isosaccharinic acid, having the more negative rotation ($[M]_D$ −64°[26]), would be expected to have the conformation VI (equivalent to structure I). "β"-D-Isosaccharinic acid, having the more positive rotation ($[M]_D$ −2°), should have the conformation VII (equivalent to structure II). However, it must be remembered that other hydrogen-bonding interactions are possible in this case.



Qualitative structural indications of other rules are described below. In these cases, the simple rules of rotation have been modified in accordance with recent observations that the rotation of an asymmetric atom is determined by the polarizability of the attached constituents so that the original rules now indicate if the more polarizable substituent on C-2 is D or L (not necessarily the position of the hydroxyl group as the original rules state). In the case of the isosaccharinic acids, the more polarizable of the two substituents on C-2 is the —CH_2OH group. Therefore, Hudson's "amide rule"[27] as modified by Freudenberg[28] and the phenylhydrazide rule[29,30] might indicate that the —CH_2OH group has the D configuration in "α"-D-isosaccharinic acid due to the positive rotations of the anilide[31–33] and phenylhydrazide.[34]

(23) A. K. Bose and B. G. Chatterjee, *J. Org. Chem.*, 23, 1425 (1958).

(24) W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1979 (1954).

(25) J. H. Brewster, *J. Am. Chem. Soc.*, 81, 5475 (1959).

(26) L. M. Utkin and G. O. Grabilina, *Doklady Akad. Nauk S.S.S.R.*, 93, 301 (1953).

(27) C. S. Hudson, *J. Am. Chem. Soc.*, 40, 813 (1918).

(28) K. Freudenberg and W. Kuhn, *Ber.*, 64, 703 (1931).

(29) P. A. Levene, *J. Biol. Chem.*, 23, 145 (1915); P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, 31, 623 (1917).

(30) C. S. Hudson, *J. Am. Chem. Soc.*, 39, 462 (1917).

(31) Utkin and Grabilina[26] report $[M]_D$ +33° for the anilide.

(32) B. Sorokin, *J. prakt. Chem.*, [2] 37, 318 (1888).

(33) J. W. Green, *J. Am. Chem. Soc.*, 78, 1894 (1956).

(34) $[M]_D$ +50° reported by Nef.[13]

The "acid-salt" rule[29] might also indicate a D configuration for the —CH_2OH group of "α"-D-isosaccharinic acid since the strontium salt is more dextrorotatory[35] than the free acid. On the other hand, since the optical rotation of the calcium salt of "β"-D-isosaccharinic acid is slightly more dextrorotatory than that of the free acid, an L configuration[36] of the —CH_2OH group might be indicated in this acid.

Therefore, it is suggested here that "α"-D-isosaccharinic acid is 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acid (I) and that "β"-D-isosaccharinic acid is 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid (II).

A method has been established for the determination of both "α"- and "β"-D-isosaccharinic acids in mixtures of the two by making use of the fact that "β"-D-isosaccharinic acid is a weaker acid than the "α" form. Titrations are done in 3*M* potassium chloride to enhance ionization of the weak acid (Fig. 1.).

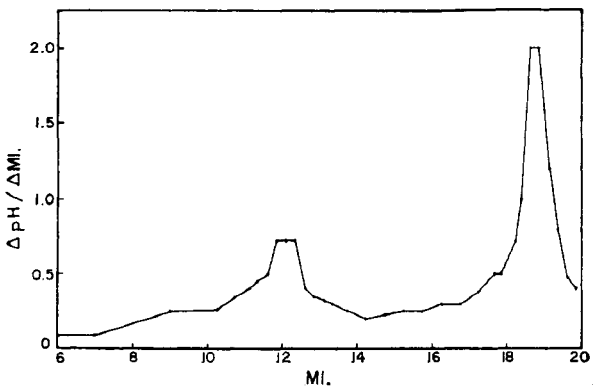


Fig. 1. Differential plot of the back titration (with standard hydrochloric acid in 3*M* potassium chloride) of a mixture of "α"- and "β"-D-isosaccharinic acids in standard sodium hydroxide in 3*M* potassium chloride. The first peak *p*H (10.9–10.4) corresponds to "β"-D-isosaccharinic acid; the second (*p*H 8.5–6.6) to the "α"-form. They were present in approximately equal amounts

The accepted mechanism of alkaline degradation[9] does not explain the preferential formation of "β"-D-isosaccharinic acid, in this case, from a β-(1 → 4)-linked D-mannoglycan. Neither is enough known about the influence of reaction conditions in determining which isomeric D-isosaccharinic acid will be formed. It is known, however, that the reaction conditions can control to some extent the kind of saccharinic acid which is formed[37] and that mannans degrade more slowly than glucans in 0.5–1*N* sodium hydroxide at 100°.[38] It has been shown in this work that "α"- and "β"-D-isosaccharinic acids are stable in alkaline solutions at temperatures up to 125° and are not interconvertible by this treatment. The same observation has been made with the structurally similar hamamelonic acid.[39]

The structure of the modified disaccharide, 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid, is indicated by its synthesis from methyl 2,2′-*O*-isopropylidene-"β"-D-isosaccharinate and 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl bromide by a Koenigs-Knorr reaction. 5-*O*-α-D-Galactopyranosyl-"α"-D-isosaccharino-1,4-lactone has also been synthesized.

Although the products formed are those predicted, complete conversion of the polysaccharide to saccharinic acids was not obtained. However, a new method of alkaline degradation, which used oxygen-free *N* trimethylbenzylammonium hydroxide as the alkaline solution, resulted in 90% of the reaction products being soluble in 70% ethanol after two weeks at 37°.

Incomplete conversion of the main chain units to saccharinic acids might be due to the formation of D-glucometasaccharinates at the end of the remaining polymer chain which stabilizes it to the further action of alkali. This "stopping reaction" has been demonstrated in the (1 → 4)-linked D-glucoglycans, cellulose[16] and amylose.[17b] Higher temperatures produce a complex mixture of products, possibly due to an alkaline hydrolysis of glycosidic linkages.[40]

EXPERIMENTAL

Chromatography. All chromatographic separations were done at 25° using the following irrigants: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.); B, ethyl acetate–pyridine–water (10:4:3 v./v.); C, 1-butanol–ethanol–water (3:1:1 v./v.); D, ethyl acetate–acetic acid–water (100:13:10 v./v.); E, 1-butanol–ethanol–water (40:11:19 v./v.). The components were detected by spraying the paper chromatograms with silver nitrate and sodium hydroxide.[41]

Degradation. In general, the polysaccharide guaran was degraded by dissolution in an oxygen-free alkaline solution; the reaction was stopped by removing the sodium ions with Amberlite IR-120(H^+) cation-exchange resin, and the non-degraded polysaccharide was removed by the addition of ethanol to a concentration of 70%. The 70% ethanol-soluble, nonvolatile acids were obtained by concentration of the remaining solution under reduced pressure.

When guaran was treated with *N* sodium hydroxide solution saturated with calcium ions at 37° for 119 days, it was 83% degraded. Under conditions that were identical except that the *N* sodium hydroxide solution was replaced by *N* potassium hydroxide, 68% of the polysaccharide reacted. After dissolution in oxygen-free *N* trimethylbenzylammonium hydroxide[42] and standing at 37° for 141 days, only 10% of polysaccharidic material could be recovered. In another experiment, 1 g. of guaran was dissolved in 500 ml. of oxygen-free water and a 100% excess of calcium hy-

(35) $[M]_D$ −13° reported by Nef.[13]

(36) W. W. Pigman and R. M. Goepp, Jr., *Chemistry of the Carbohydrates*, Academic, New York, p. 297 (1948).

(37) G. Machell and G. N. Richards, *Tappi*, **41**, 12 (1958).

(38) H. Richtzenhain and B. Abrahamsson, *Svensk Papperstidn.*, **57**, 538 (1954).

(39) O. T. Schmidt and C. C. Weber-Molster, *Ann.*, **515**, 43 (1934).

(40) B. Lindberg, *Svensk Papperstidn.*, **59**, 531 (1956).

(41) W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, **166**, 444 (1950).

(42) "Triton B," see J. Stuchík, M. Tichý, and V. Procházka, *Chem. Listy*, **50**, 662 (1956); *Chem. Abstr.*, **50**, 8497 (1956).

droxide (0.304 g.) was added. The mixture was heated 65 hr. in an autoclave at about 120°, cooled, decationized with Amberlite IR-120(H^+) ion-exchange resin, and concentrated; 0.65 g. or 65% of the polysaccharide was insoluble in 70% ethanol. A complex mixture of products including volatile acids was formed so that high-temperature treatment is unsuitable for the alkaline degradation of this polysaccharide.

To prepare large amounts of the two degradation products, 150 g. of guaran was dispersed in 6750 ml. of water and stirred 12 hr. at 80°. To this dispersion was added 300 ml. of concd. hydrochloric acid in 600 ml. of water, and stirring was continued 3 hr. The solution was then neutralized with sodium hydrogen carbonate and cooled to room temperature while a stream of nitrogen was passed through it. Fifteen grams of calcium hydroxide was added to the cooled, oxygen-free solution which was stirred 9 days at room temperature, heated to 80° for 15 hr., and filtered. The filtrate, which was neutral, was concentrated to about 1.5 l. and held at 0° overnight; no precipitate or crystals formed. It was then decationized and treated with ethanol as described before.

The 70% ethanol-soluble material from each reaction was concentrated under reduced pressure to a sirup, dissolved in water, and passed through an Amberlite IR-120(H^+) cation-exchange resin column. The eluate was concentrated under reduced pressure, and the resulting sirup was fractionated by cellulose column chromatography using irrigant D. Fractions were rechromatographed until each isolated component was chromatographically homogeneous when examined by paper chromatography with irrigants A, B, and E.

To prepare large amounts of calcium "β"-D-isosaccharinate alone, 50 g. of guaran was dissolved in 5 l. of oxygen-free, saturated lime water and kept 70 days at room temperature with stirring. This turbid solution was then concentrated under reduced pressure to about 2 l. and stored 7 days at 0°. The mixture was centrifuged, and two volumes of ethanol were added to the centrifugate. The alcohol-soluble fraction was concentrated under reduced pressure as far as possible; yield about 18 g. of clear highly soluble sirup. Repeated attempts to remove water by azeotropic distillation did not cause crystallization. This material had a small positive rotation and was soluble in absolute ethanol.

"β"-D-Isosaccharinic acid was also produced from lactose in low yields. A solution of lactose (2 kg. in 18 l. of water) was treated with 528 g. of calcium hydroxide, and the resulting mixture was layered with toluene and stirred slowly 3 days at room temperature. The mixture was then heated 10 hr. in a boiling water bath and filtered. The filtrate was concentrated under reduced pressure to a volume of 6 l. and stored 18 hr. at 0°. Calcium "α"-D-isosaccharinate (310 g.) was removed by filtration. Three volumes of ethanol were added to the filtrate with rapid stirring, and the mixture was again filtered. The filtrate was concentrated under reduced pressure to a thick sirup. This sirup was dissolved in a liter of water, and 9 l. of ethanol were added to the solution. The mixture was filtered once more, and the filtrate was passed through a column of Amberlite IR-120(H^+) cation-exchange resin. After concentration of the eluate under reduced pressure (35–40°), "β"-D-isosaccharinic acid was purified by paper chromatography with irrigant A; yield 0.4–0.6 g. of a sirup. The chromatographic flow rates of this sirup in an acidic, a basic, and a neutral irrigant were identical to those of the acid isolated from the alkaline degradation of guaran; $[\alpha]_D^{25}$ −1° (*c* 5.9, water).

"β"-D-Isosaccharinic acid. This acid had R_g,R_{ga},[43] and R_l[44] values of 4.9, 2.8–3.1, and 1.2, respectively, with irrigant A, a R_g value of 2.0 with irrigant B, a R_α[45] value of 0.75 in irrigant B, and a R_α[45] value of 0.85 in irrigant D. Attempts to "lactonize" this material by heating it with cation-exchange resins and by autoclaving a solution of it in 0.1*N* hydrochloric acid at 20 p.s.i. failed to change the flow rates in either acidic or basic irrigants. Refluxing the material with aqueous or ethanolic sodium hydroxide changed its flow rate slightly in the basic irrigant but not in the acidic ones. This indication that it is not a lactone is substantiated by the fact that it does not give the hydroxamic acid ester test as described by Lein.[46] "α"-D-Isosaccharino-1,4-lactone gives a typical test under the same conditions.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.0. Found: C, 39.7[47]

Neither crystallization nor lactonization was obtained by repeatedly distilling benzene-ethanol (2:1 w./w.) or toluene from the sirup, by drying it over phosphorus pentoxide, or lyophilization. Instead it was found that sirupy "β"-D-isosaccharinic acid can be distilled slowly at about 0.1 μ pressure and a bath temperature of 85–110°. Pure, distilled "β"-D-isosaccharinic acid has a rotation, α_D^{25} + 0.49°; $[\alpha]_D^{25}$ −1° (*c* 2.7, water).

A crude brucine salt was obtained; m.p. 185–220° dec., $[\alpha]_D^{25}$ −11° (*c* 0.8, water).[48] The tetrabenzoate was prepared as follows: 0.5 g. of "β"-D-isosaccharinic acid was dissolved in 3.7 ml. of dry pyridine at 0°. To this cold solution was added 1.6 ml. of benzoyl chloride. The crystal slurry was kept one hr. at 0° and then one hr. at 25°. The crystals were washed twice by mixing with petroleum ether (b.p. 35–37°) and decantation. The partially crystalline mass was then recrystallized from 75% ethanol and air-dried; yield 0.78 g. of fine needles, m.p. 68°. Recrystallization from 75% ethanol gives 0.62 g. of needles; m.p. 73–73.5°, $[\alpha]_D^{25}$ +1° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{34}H_{32}O_{10}$: benzoyl, 8.4 ml. of 0.1*N* sodium hydroxide per 100 mg. Found: benzoyl (Kunz method), 8.2 ml.

"α"-D-Isosaccharino-1,4-lactone tribenzoate, prepared in the same way, gave 0.84 g. of fine needles; m.p. 105°. After a recrystallization from 95% ethanol, the yield was 0.76 g.; m.p. 110°, $[\alpha]_D^{25}$ +46° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{27}H_{22}O_8$: C, 68.3; H, 4.6; benzoyl, 8.4 ml. of 0.1*N* sodium hydroxide per 100 mg. Found: C, 68.2; H, 4.6; benzoyl (Kunz method), 8.1 ml.

Attempts to prepare the "β"-D-isosaccharinic acid anilide by the method of Sorokin[26,32] failed. "β"-D-Isosaccharinic acid (0.5 g.) was heated 3 hr. at 150° with 2 g. of freshly distilled aniline. The resulting sirup was then desiccated 4 days over sulfuric acid at <0.001 mm. The remaining sirup was then benzoylated as described above to give "β"-D-isosaccharinic acid tetrabenzoate; yield 0.12 g., m.p. 72–73.5°. This is a further indication that "β"-D-isosaccharinic acid is not lactonized.

Under identical conditions, "α"-D-isosaccharino-1,4-lactone gave a semi-crystalline sirup of "α"-D-isosaccharinic acid anilide[22,33,49] which was benzoylated as described above to give "α"-D-isosaccharinic acid tetrabenzoate anilide; yield, after one recrystallization from 95% ethanol, 0.55 g.,

(43) R_{ga} is the rate of movement relative to D-gluconic acid.

(44) R_l is the rate of movement relative to D-glucono-1,4-lactone.

(45) R_α is the rate of movement relative to "α"-D-isosaccharino-1,4-lactone.

(46) O. G. Lein, Jr., *Anal. Chem.*, 31, 1363 (1956).

(47) R. L. Whistler and J. E. Walsh, unpublished data of chromic acid oxidation.

(48) Melting points of 185–210° dec. and specific rotations at 20° of −20° to −22° have been variously reported for the brucine salt.[9,13,14] However, it is stated that this material could be "lactonized" to give a substance(s) with a positive specific rotation. Therefore, their residual sirups obtained after removal of the slightly soluble calcium "α"-D-isosaccharinate from 4-*O*-substituted-D-hexose-alkali reaction mixtures were probably contaminated with "α"-D-isosaccharinic acid.

(49) Reported by Utkin and Grabilina[26] to have the following constants: m.p. 169–169.5°, $[\alpha]_D^{20}$ +13° (*c* 1.0, water).

m.p. about 165°. After an additional recrystallization, the yield was 0.29 g.; m.p. 181–183.5°, $[\alpha]_D^{25}$ −50° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{40}H_{38}O_9N$: N, 2.1; benzoyl 5.9 ml. of 0.1*N* sodium hydroxide per 100 mg. Found: N, 2.1; benzoyl (Kunz method) 6.2 ml.

When investigated by infrared spectroscopy, neither sirupy "β"-D-isosaccharinic acid nor "β"-D-isosaccharinic acid tetrabenzoate showed any lactone carbonyl absorption. Both crystalline "α"-D-isosaccharino-1,4-lactone and "α"-D-isosaccharino-1,4-lactone tribenzoate had a definite lactone carbonyl absorption at about 5.5 μ.

Oxidation of "β"-D-isosaccharinic acid. Oxidation of 0.0125 g. samples of "β"-D-isosaccharinic acid with periodate for 7 hr. at 25° went with consumption of 2.0 equivalents of periodate per mole of acid, assuming a molecular weight of 180. Oxidation of 0.25 g. samples with periodate produced 1.0 g. of formaldehyde-dimedon (reaction product of formaldehyde and 5,5-dimethyl-1,3-cyclohexanedione) when formaldehyde was determined by the method of Reeves.[50] This is equivalent to 2.0 moles of formaldehyde per mole of acid, again assuming a molecular weight of 180.

A portion (0.47 g.) of the calcium salt of this acid was oxidized with 0.7 ml. of nitric acid (*d*, 1.4) 24 hr. at 35°, 24 hr. at 45°, and then 24 hr. at 50° according to the method of Kiliani.[18] The reaction mixture was diluted with water, and, after standing 12 hr., filtered. The filtrate was concentrated under reduced pressure at 35–40° and purified by paper chromatography with irrigant B. A chromatographically pure sirup was obtained which had R_{ga}[43] and R_i[44] values of 2.9 and 1.2, respectively, with irrigant A; $[\alpha]_D^{25}$ +1° (*c* 4.2, water). This acid had flow rates with irrigants A, B, and E which were identical to those of the corresponding oxidation product from "α"-D-isosaccharinic acid, 2-*C*-carboxy-3-deoxy-D-*glycero*-pentaric acid.[19]

Reduction of D-isosaccharinic acids. A mixture of 31 g. of triphenylphosphite and 21 g. of methyl iodide was refluxed 36 hr. while protected from moisture. To this reaction mixture was then added 3 g. of "α"-D-isosaccharino-1,4-lactone, and the mixture was refluxed another 6 hr. After cooling, 100 ml. of absolute ethanol was carefully added through the condenser, followed immediately by two 10-g. portions of zinc dust. When the reaction had subsided, 5 ml. of concd. hydrochloric acid in 100 ml. of water was added, and the resulting mixture was heated 10–15 min. Following filtration, the filtrate was concentrated under reduced pressure to a sirup. The sirup was dissolved in absolute ethanol which was then evaporated under reduced pressure. This was done three times. The sirup was dissolved in 200 ml. of 50% ethanol, and the solution was adjusted to *p*H 8 with sodium carbonate. The mixture was filtered, and the filtrate was treated with Amberlite IR-120(H^+) ion-exchange resin and extracted with ether. The ether extract was dried over sodium sulfate, evaporated to a small volume, boiled with a little zinc dust, filtered, and evaporated to a sirup which was a mixture of substances; crude yield 0.8 g.

(It was found that the tetraiodo compound could be distilled directly from the reaction mixture or could be distilled over with a high boiling inert solvent, such as ligroin. However, in these cases, the yield was less and the product was contaminated with phenol which was then difficult to remove.)

Part of this sirup was refluxed with thionyl chloride, dissolved in anhydrous ether, and treated with anhydrous ammonia. After filtration and evaporation, 2-methylpentanamide was crystallized from petroleum ether (b.p. 90–100°); m.p. 84–88°.[51] No observable optical rotation in a 1% solution in chloroform. Another portion was converted to its *p*-phenylphenacyl ester; m.p. 64°.[52] 2-Methylpentanoic acid and its derivatives were also produced by the red phosphorus-hydroiodic acid reduction of "α"-D-isosaccharinic acid.

"β"-D-Isosaccharinic acid (6 g.), reduced in an identical manner, gave 2.2 g. of crude 2-methylpentanoic acid, which was converted to its amide, m.p. 84–85°[51] (no observable optical rotation in a 1% solution in chloroform), and its *p*-phenylphenacyl ester; m.p. 64°.[52]

A mixed m.p. of 84–85° was obtained with the 2-methylpentanamide from the reduction of "α"-D-isosaccharinic acid and the 2-methylpentanamide from the reduction of "β"-D-isosaccharinic acid.

Quantitative analyses. Preliminary experiments showed that, although "β"-D-isosaccharinic acid is a weak acid, it can be titrated in a 3*M* potassium chloride solution to enhance its ionization. Titration of the pure acid showed that the inflection point was at *p*H about 10.7 (10.9–10.4) while the inflection point of "α"-D-isosaccharinic acid was at *p*H about 7.4 (8.5–6.6). Titrations were performed by dissolving the acid in standard sodium hydroxide in 3*M* potassium chloride, heating the solution 1 hr. at 95°, and titrating with standard hydrochloric acid in 3*M* potassium chloride. The inflection points were determined by differential plots of Δ *p*H/Δml. against ml. Titrations of this kind showed that "α"-D-isosaccharino-1,4-lactone had an equivalent weight of 162 and that "β"-D-isosaccharinic acid had an equivalent weight of 180.

A mixture of 0.193 g. of "α"-D-isosaccharino-1,4-lactone and 0.206 g. of "β"-D-isosaccharinic acid, titrated in the above manner (Fig. 1), gave 88–90% recovery of each component.

Two and one-half grams of guaran were dissolved in 500 ml. of oxygen-free water and an excess of calcium hydroxide was added to the solution which was kept 21 days at 80° with stirring. Two grams of 70% ethanol-soluble material was isolated by the previously described procedure. The sirup was separated into its components by paper chromatography on Whatman 3*MM* paper with irrigant E. The papers were dried in a forced-draft oven at 60°. Bands were then removed and eluted with water. The eluates were titrated as described before. "β"-D-Isosaccharinic acid and the modified disaccharide containing it were present in an equivalent ratio of 1.1:1.0.

Galactosyl-"β"-D-isosaccharinic acid. This sirupy acid (0.30 g.) had R_g, R_{ga},[43] and R_i[44] values of 0.95, 072, and 0.27, respectively, with irrigant A and an R_g value of 1.0 with irrigant B; $[\alpha]_D^{25}$ + 55° (*c* 1.2, water). An aqueous solution of this modified disaccharide autohydrolyzed at room temperature, with complete hydrolysis occurring in 2–3 days. The final rotation of the hydrolysis mixture was +36.5°. Equimolar amounts of D-galactose and "β"-D-isosaccharinic acid were identified from the resulting solution. This modified disaccharide is believed to be the predicted 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid, and the specific optical rotations agree with those of the synthetic product.

Mannosyl-"β"-D-isosaccharinic acid. When guaran was pretreated by partial acid hydrolysis (1.7% hydrochloric acid at 80° for 3 hr.) prior to alkaline treatment, significant amounts of a second modified disaccharide were obtained. This acid had R_{ga}[43] and R_i[44] values of 1.0 and 0.44, respectively, with irrigant A. Its sirup (0.35 g.) had $[\alpha]_D^{25}$ + 3° (*c* 1.4, water). After autohydrolysis at 5°, the rotation reached a constant value of +6°. D-Mannose and "β"-D-isosaccharinic acid were identified as the hydrolysis products; they were present in equimolar quantities. The origin of this modified disaccharide is unknown.

Synthesis of 5-O-β-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone. 2,2'-Isopropylidene-"α"-D-isosaccharino-1,4-lactone was prepared by the method of Sowden[21]; 3.5 g. of "α"-D-isosaccharino-1,4-lactone was dissolved in 173 ml.

(50) R. E. Reeves, *J. Am. Chem. Soc.*, **63**, 1476 (1941).

(51) S. Ställberg-Stenhagen, *Arkiv Kemi, Mineral. Geol.*, **B24**, No. 15, 14 pp. (1946).

(52) A. Magnani and S. M. McElvain, *J. Am. Chem. Soc.*, **60**, 813 (1938).

of anhydrous acetone and 1.73 ml. of concd. sulfuric acid was added. The solution was kept at room temperature 3 hr., and 100 ml. of dry Amberlite IR-45(OH^-) anion-exchange resin was added. After filtration, the solution was concentrated under reduced pressure to give a partially crystalline sirup. Crystallization from an ether-hexane solution yielded a product with a m.p. of 55–56°.

A mixture of 2.13 g. of this product (0.019 mole) in 50 ml. of anhydrous chloroform, 20 g. of Drierite,[53] 10 g. of silver oxide, 1 g. of iodine, and glass beads was shaken 30 min. in a dark bottle. To this mixture was added 50 ml. of anhydrous chloroform which contained 7.16 g. of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl bromide (0.02 mole), and the shaking was continued. After 17 hr., the test for ionizable bromine was negative, and the mixture was filtered and concentrated to a sirup; yield 9.8 g., $[\alpha]_D^{25}$ + 26° (*c* 1.6, chloroform). Chromatography with irrigant C indicated that there were no unreacted starting materials.

This sirup, 5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) - 2,2′ - *O* - isopropylidene - "α" - D - isosaccharino-1,4-lactone, was dissolved in 80 ml. of 80% glacial acetic acid and heated on a steam plate to remove the isopropylidene group. The rotation became constant after 30 min., and the solution was concentrated under reduced pressure to give a partly acetylated sugar sirup. After addition of 10 ml. of acetic anhydride and 1 g. of fused sodium acetate, the mixture was heated and poured into ice water. The fully acetylated 5-*O*-β-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone was isolated by standard procedures; yield 7.4 g. of thick sirup, $[\alpha]_D^{25}$ + 31° (*c* 10.2, chloroform).

To convert the glycosidic linkage of this disaccharide into the *alpha* configuration, 2.5 g. of the hexaacetate was dissolved in 25 ml. of anhydrous chloroform. Three grams of titanium tetrachloride in 35 ml. of anhydrous chloroform was added, and a yellow precipitate formed immediately. This mixture was refluxed 5 hr. in a water bath (65–70°) and then poured into 250 ml. of ice water. The chloroform layer was separated, washed with water, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to a thick sirup; yield 1.6 g., $[\alpha]_D^{25}$ + 112° (*c* 3.3, chloroform).

This sirup was dissolved in anhydrous methanol at 0° and an excess of barium methoxide was added. After standing 24 hr. at 0°, the mixture was concentrated under reduced pressure to dryness, suspended in water, and treated with an excess of Amberlite IR-120 (H^+) cation-exchange resin. The sirup obtained upon concentration of the resulting solution was purified by removal of trace components by paper chromatography with irrigant A. A sirup was obtained which moved as a single component in irrigant A with R_{ga}[43] and R_i[44] values of 2.9 and 1.1, respectively. It had an R_g value of 3.2 with irrigant B; $[\alpha]_D^{25}$ + 106° (*c* 3.8, water). On the basis of its high positive rotation, this lactone is designated 5-*O*-α-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone. Hydrolysis produced D-galactose, "α"-D-isosaccharinic acid, and "α"-D-isosaccharino-1,4-lactone.

Synthesis of 5-O-α-D-galactopyranosyl-"β"-D-isosaccharinic acid. The above procedure was repeated starting with 2.13 g. of sirupy methyl 2,2′-*O*-isopropylidene-"β"-D-isosaccharinate. The product obtained was a sirup; $[\alpha]_D^{25}$ + 58° (*c* 1.4, water). After autohydrolysis, the solution reached a constant rotation of +37° (based on the original concentration). Investigation of this hydrolyzed solution indicated only D-galactose and "β"-D-isosaccharinic acid. Therefore, this compound has been designated 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid.

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(53) A special form of anhydrous calcium sulfate obtainable from the W. A. Hammond Drierite Co., Xenia, Ohio.

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Simplified Preparation of Sophorose (2-*O*-β-D-glucopyranosyl-D-glucose)

BRUCE COXON AND HEWITT G. FLETCHER, JR.

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Condensation of methyl 4,6-*O*-benzylidene-α-D-glucoside with tetra-*O*-acetyl-α-D-glucopyranosyl bromide gives a sophorose derivative from which the substituting groups may be removed (through successive acetolysis and deacetylation) to give crystalline sophorose in an over-all yield of 30%. This comparatively simple two-step synthesis makes this hitherto rare disaccharide relatively accessible.

Owing to the widespread biological and medical uses to which D-glucose is put, the discovery of sophorose (III), 2-*O*-β-D-glucopyranosyl-D-glucose, in typical "pure" commercial samples of D-glucose[1] focuses new interest on this little-known disaccharide. This interest is enhanced by the discovery of the remarkable activity shown by the sugar in stimulating cellulase production by a strain of *Trichoderma viride*[1] and by the fact that it is a representative of the theoretically interesting but comparatively rare 2-*O*-glycosylaldoses.

The history of sophorose is unusual in that this disaccharide was synthesized prior to its discovery in nature. In 1928 Freudenberg, Toepffer and Anderson[2] described the condensation of methyl 4,6-*O*-benzylidene-α-D-glucoside (I) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Some years later Freudenberg and Soff[3] succeeded in converting the product of this condensation (II) into a disaccharide which they showed to be a 2-*O*-β-D-glucopyranosyl-D-glucose (III); the process involved will

(1) M. Mandels and E. T. Reese, *Biochem. Biophys. Research Communs.*, 1, 338 (1959).

(2) K. Freudenberg, H. Toepffer, and C. C. Andersen, *Ber.*, 61, 1750 (1928).

(3) K. Freudenberg and K. Soff, *Ber.*, 69, 1245 (1936).