Anal. Calcd. for $C_{17}H_{18}O_6$: C, 64.20; H, 5.66. Found: C, 64.12; H, 5.72.

Decarboxylation of 8-Isoamyl-7-hydroxy-6-carboxycoumarin.—Five-tenths gram of 8-isoamyl-7-hydroxy-6-carboxycoumarin was decarboxylated with copper bronze in quinoline.[4] Recrystallization from dilute methyl alcohol gave a product which melted at 103–104°; yield 0.06 g. When mixed with an equal quantity of 8-isoamyl-7-hydroxycoumarin, there was no depression of the melting point.

Summary

Condensation of tetrahydrotubanol (2,6-dihydroxyisoamylbenzene) with malic and sulfuric acids yielded 8-isoamyl-7-hydroxycoumarin, which with diazomethane gave 8-isoamyl-7-methoxycoumarin.

Tetrahydrotubaic acid (2,4-dihydroxy-3-isoamylbenzoic acid) with malic and sulfuric acids yielded 8-isoamyl-7-hydroxy-6-carboxycoumarin, which on decarboxylation with copper bronze in quinoline was converted into 8-isoamyl-7-hydroxycoumarin.

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Synthesis of a Lactose Carboxylic Acid (5-*β*-*d*-Galactosido-*α*-*d*-glucoheptonic Acid)[1]

BY RAYMOND M. HANN AND C. S. HUDSON

In a recent note Hudson, Hartley and Purves[2] described a convenient modification of the Kiliani reaction (the condensation of hydrogen cyanide with the carbonyl group of reducing sugars). The present article reports the application of this method to a reducing disaccharide and records the preparation of a pure crystalline thirteen carbon sugar acid from lactose. Reinbrecht[3] prepared sirups containing mixtures of such acids from lactose and from maltose, but no crystalline component was isolated from them. The conditions of formation limit the structure of the acid to 5-*β*-*d*-galactosido-*α*-(or *β*)-*d*-glucoheptonic acid. That it is the substituted *α*-acid follows from its hydrolysis, which yields *d*-galactose and *α*-*d*-glucoheptonic acid. The first sample of the crystalline acid was obtained by way of its pure crystalline quinine salt, but in subsequent experiments it was possible to obtain the acid directly, probably due to the presence of seed crystals in the laboratory. The study of this acid, the lactone of which upon reduction should give 5-*β*-*d*-galactosido-*α*-*d*-glucoheptose, is being continued.

Experimental

5-*β*-*d*-Galactosido-*α*-glucoheptonic Acid.—Solutions containing 200 g. of lactose monohydrate (0.56 mole) in 400 cc. of water, 65 g. of calcium chloride dihydrate in 100 cc. of water (0.88 equivalent) and 40 g. of sodium cyanide in 100 cc. of water (0.82 mole) were poured successively upon 400 g. of crushed ice and the mixture immediately placed in the ice chest. After thirteen days no reducing power remained; the basic calcium salts of the acid product were then thrown down by adding 80 g. of calcium oxide and heating the mixture for four hours on the steam-bath. Eighty grams of filter cel was mixed with the precipitate, which was filtered on a Buchner funnel and washed with lime water until the filtrate was nearly free of chlorides; care to pack the precipitate is necessary to avoid channeling. The basic salts were dissolved in 130 cc. of 1:1 sulfuric acid, the calcium sulfate removed, the amount of calcium in the filtrate measured by an analysis and an equivalent quantity of sulfuric acid added and the calcium sulfate filtered off. Upon concentration to a thick sirup and seeding, a magma formed from the separation of the acid in the course of a few days at room temperature. This was thinned with 60% methyl alcohol, filtered and the crystalline acid washed with absolute methyl alcohol and dried. It was recrystallized from 5 parts of water by adding 10 volumes of hot methyl alcohol; yield 56 g. (26%).

5-*β*-*d*-Galactosido-*α*-*d*-glucoheptonic acid crystallizes in glistening slender prisms which melt at 185–186° (corr.) with decomposition. Its solutions in water give an $[\alpha]^{20}_{D}$ value of +11.2° (0.4315 g. in 25 cc. in a 2-dm. tube rotated 0.388° to the right). The solution showed no mutarotation on standing for several days at room temperature; however, a change was noted when it was heated on the steam-bath, indicating lactone formation. The acid is readily soluble in water but insoluble in methyl or ethyl alcohol.

Anal. Calcd. for $C_{13}H_{24}O_{13}$: C, 40.19; H, 6.23. Found: C, 40.31; H, 6.17. *Titration.* 89.94 mg. consumed 2.32 cc. of 0.1 *N* alkali. Calcd., 2.32 cc. of 0.1 *N* alkali.

Alkaloid Salts of 5-*β*-*d*-Galactosido-*α*-*d*-Glucoheptonic Acid.—The quinine and brucine salts of the acid were obtained by heating aqueous solutions containing about 20% excess of alkaloid for five hours on the steam-bath. The excess was removed by filtration and repeated extraction with chloroform, and the solutions were concentrated

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[1] Publication authorized by the Surgeon General, U. S. Public Health Service.

[2] Hudson, Hartley and Purves, THIS JOURNAL, **56**, 1248 (1934).

[3] Reinbrecht, *Ann.*, **272**, 197 (1892).

in vacuo to a dry sirup and crystallized from suitable solvents.

The quinine salt crystallizes from 1:1 ethyl and methyl alcohol mixtures in colorless opaque nodular crystals melting at 169–170° (corr.) with decomposition. A sample dried *in vacuo* at 110° showed $[\alpha]_D^{20}$ −71.6° and upon recrystallization $[\alpha]_D^{20}$ −71.9° (0.2648 g. in 25 cc. of water in a 2-dm. tube rotated 1.523° to the left).

Anal. Calcd. for $C_{33}H_{48}O_{15}N_2$: C, 55.59; H; 6.79; N, 3.92. Found: C, 55.57; H, 6.67; N, 3.93.

The brucine salt crystallizes from its solution in 5 parts of 85% alcohol in clusters of fine acicular needles, 5 to 6 mm. long, and melts at 172–173° (corr.) with decomposition. A sample dried *in vacuo* at 110° gave $[\alpha]_D^{20}$ −7.6° (0.2286 g. in 25 cc. of water in a 2-dm. tube rotated 0.138° to the left).

Anal. Calcd. for $C_{36}H_{50}O_{17}N_2$: C, 55.21; H, 6.44; N, 3.58. Found: C, 55.19; H, 6.60; N, 3.54.

Proof of the Structure of 5-β-*d*-Galactosido-α-*d*-glucoheptonic Acid.—Twenty grams of quinine salt ($[\alpha]_D^{20}$ −71.5°) was dissolved in 250 cc. of water and the quinine precipitated by addition of 10 cc. of strong ammonia in 50 cc. of water. The quinine was filtered off, the filtrate extracted five times with chloroform, the excess chloroform blown out by air and the ammonium salt decomposed by boiling with 6 g. of barium hydroxide octahydrate until free of ammonia. The barium was balanced out with sulfuric acid and the solution (300 cc.) adjusted to contain 2% of sulfuric acid and refluxed for two hours, when its $[\alpha]_D$ value had become constant. The sulfuric acid was exactly balanced out, the barium sulfate filtered off, and the filtrate containing the heptonic acid formed in the hydrolysis was neutralized with 5% barium hydroxide solution to phenolphthalein end-point. The solution was concentrated *in vacuo* to a dry sirup, which was dissolved in 10 cc. of water and pipetted into 300 cc. of boiling methyl alcohol to precipitate the barium salt. The separated white gummy salt was redissolved in 10 cc. of water and reprecipitated by the same procedure until it did not reduce Fehling's solution. During this process the salt lost its gummy character and became granular. The methyl alcohol extracts were combined, concentrated to a dry sirup and extracted with hot methyl alcohol. From the methyl alcohol extracts 3.1 g. of *d*-galactose was crystallized and identified. The non-reducing solution of the barium salt was decomposed by sulfuric acid and the resulting organic acid solution heated with 7 g. of brucine for five hours on the steam-bath. The excess brucine was removed and the solution concentrated to a dry sirup which was taken up in 25 cc. of 90% alcohol and brought to crystallization by scratching. The yield was 3.5 g. of a material whose $[\alpha]_D^{20}$ value was −18.0°. Recrystallized from 15 cc. of 90% alcohol it gave 1.7 g. rotating −18.2° (0.4217 g. in 25 cc. of water in a 2-dm. tube rotated 0.613° to the left). The salt melted at 155° with decomposition and showed no depression when mixed with an authentic sample of brucine-α-*d*-glucoheptonate. Brucine-β-*d*-glucoheptonate melts at 130° (corr.) and shows $[\alpha]_D^{20}$ −23.8° in water.[4]

Brucine-α-*d*-glucoheptonate has not been described previously. It was prepared from pure α-*d*-glucoheptonic lactone and brucine; it crystallizes from 5 parts of 90% alcohol in nodular clusters of microcrystalline plates and melts at 155° (corr.) with decomposition. In water solution it exhibits $[\alpha]_D^{20}$ −18.4° (0.2356 g. in 25 cc. in a 2-dm. tube rotates 0.346° to the left).

Anal. Calcd. for $C_{30}H_{40}O_{12}N_2$: C, 58.03; H, 6.50; N, 4.52. Found: C, 57.98; H, 6.64; N, 4.45.

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Summary

5-β-*d*-Galactosido-α-*d*-glucoheptonic acid has been isolated in crystalline condition by the action of sodium cyanide and calcium chloride on lactose solutions. Its properties and those of its crystalline quinine and brucine salts are described. Its hydrolysis by acid yields *d*-galactose and α-*d*-glucoheptonic acid.

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[4] Private communication from C. B. Purves.