[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

Synthesis of 8-Isoamyl-7-methoxycoumarin (Dihydro-osthol)

BY H. L. HALLER AND FRED ACREE, JR.

In view of the recent article by Späth, Takei and Miyajima[1] on the synthesis of dihydro-osthol from tetrahydrotubanol[2] (2,6-dihydroxyisoamylbenzene), a degradation product of rotenone, a report of the work completed in this Laboratory a short time ago seems desirable.

As a result of our work 8-isoamyl-7-methoxycoumarin has been synthesized and found to have a melting point identical with that of dihydro-osthol. A direct comparison of our synthetic product with dihydro-osthol was not possible, however, owing to the fact that osthol occurs in the masterwort only in small quantities, and we have not succeeded in isolating it from any of several lots of commercial masterwort.

In our synthesis tetrahydrotubaic acid[2] (2,4-dihydroxy-3-isoamylbenzoic acid) was taken as the starting material. This compound was decarboxylated to tetrahydrotubanol which, in turn, by condensation with malic acid by the von Pechmann coumarin synthesis, yielded 8-isoamyl-7-hydroxycoumarin. Contrary to the experience of Späth, we had no difficulty in obtaining a crystalline product and on methylation of the 8-isoamyl-7-hydroxycoumarin with diazomethane, 8-isoamyl-7-methoxycoumarin was readily obtained.

The condensation of tetrahydrotubanol and malic acid by sulfuric acid took place readily on the steam-bath, but considerable water-soluble material was formed. The water-soluble compound was not isolated, but it was probably a sulfonated coumarin with the sulfonic acid group in the 6 position. As the temperature is raised the sulfonic acid group is split out. The reaction is comparable to the behavior of phenol which is sulfonated in the ortho position[3] with concentrated sulfuric acid at room temperature. At higher temperatures the sulfonic acid group is split out, and the phenol is sulfonated in the para position. In the substituted coumarin, the para position to the hydroxyl group is occupied. A better yield of the coumarin was obtained when the reaction was carried out at 130°.

(1) Späth, Takei and Miyajima, *Ber.*, **67**, 262 (1934).

(2) Haller and LaForge, THIS JOURNAL, **53**, 4460 (1931); **54**, 1988 (1932); **55**, 3032 (1933).

(3) Richter, "Organische Chemie," Vol. II, 1913, p. 203.

Tetrahydrotubaic acid also condenses readily with malic acid in the presence of sulfuric acid on the steam-bath, with the formation of 8-isoamyl-7-hydroxy-6-carboxycoumarin. In this case the position ortho to the hydroxyl group is blocked by the presence of the carboxyl group, and, therefore, sulfonation does not take place readily.

The 8-isoamyl-7-hydroxy-6-carboxycoumarin was decarboxylated by copper bronze in quinoline[4] with the formation of 8-isoamyl-7-hydroxycoumarin, but the yield was small.

Experimental Part

8-Isoamyl-7-hydroxycoumarin.—Two cc. of concentrated sulfuric acid was added to 1 g. of tetrahydrotubanol (2,6-dihydroxy-isoamylbenzene) intimately mixed with 0.8 g. of thoroughly dried malic acid. Reaction took place with vigorous evolution of gas on heating the mixture to 70–80° on a sand-bath. The temperature was raised to 130–140° and maintained for five minutes. The dark red solution was then carefully poured into ice water. The residue, from evaporation of the ether extract and crystallization from dilute methyl alcohol, yielded 0.5 g. of a product which melted at 104–106°.

Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.41; H, 6.89. Found: C, 72.26; H, 6.97.

8-Isoamyl-7-methoxycoumarin.—An ether solution of diazomethane prepared from 4 cc. of ethyl-N-nitroso-N-methylcarbamate was added to 0.55 g. of 8-isoamyl-7-hydroxycoumarin in 5 cc. of methyl alcohol. The solution was allowed to stand overnight and then filtered, and the solvent was removed. The residual oil readily crystallized. On recrystallization from ligroin (b. p. 57–78°), 0.4 g. of substance melting at 85° was obtained.

Anal. Calcd. for $C_{15}H_{18}O_3$: OCH_3(1), 12.60. Found: OCH_3, 12.55.

8-Isoamyl-7-hydroxy-6-carboxycoumarin.—One gram of tetrahydrotubaic acid, 0.8 g. of malic acid and 3 cc. of concentrated sulfuric acid were heated for one-quarter hour on the steam-bath and poured onto ice. The residue from evaporation of the ether extract and crystallization from methyl alcohol yielded 0.5 g. of a product melting at 224–225°.

Anal. Calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.79. Found: C, 65.34; H, 5.91.

On acetylation with acetic anhydride, 0.1 g. of this product yielded an acetyl derivative, which after recrystallization from methyl alcohol melted at 173–175°; yield 0.9 g.

(4) Shepard, Winslow and Johnson, THIS JOURNAL, **52**, 2083 (1930).

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

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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 $[\alpha]_D^{20}$ 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

(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).