Synthesis and Optical and Crystallographic Properties of Methyl [Methyl 4-*O*-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate

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Received August 19, 1957

Studies of enzyme specificity required crystalline compounds of known structure as substrates. Amorphous methyl α- and β-D-dimethyl esters of digalacturonic acid have been reported[1] but not crystallized.

The following method of synthesis starting with 4-*O*-α-D-galactopyranosyluronic acid-α-L-galactopyranuronic acid permits a yield of crystalline material to be obtained.

EXPERIMENTAL

Methyl [methyl 4-O-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate. A sample of 0.090 g. (0.00023 mole) of crystalline methyl 4-*O*-α-D-galactopyranosyluronic acid-α-D-galactopyranosiduronic acid[1] was dissolved in 20 ml. of methanol and cooled in an ice-water bath. An ethereal solution of diazomethane prepared from 0.5 g. of *N*-nitrosomethylurea was added at 0° with stirring until a yellow color persisted for 30 min. During the evaporation of excess diazomethane at room temperature, needle-like crystals appeared. The product was recrystallized from ethyl acetate, m.p. 120–121°, 0.071 g. (73%).

Methyl [methyl 4-O-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate. Four grams of brucine 4-*O*-(brucine α-D-galactopyranosyluronate)-α-D-galactopyranuronate monohydrate[2] was refluxed with 150 ml. of 0.2*N* dry hydrochloric acid in methanol for 3 hr. Crystals of brucine hydrochloride formed when the reaction mixture was allowed to stand at 5° overnight. The brucine salt was removed by filtration, washed with dry methanol, and the filtrate diluted with an equal volume of water. Residual brucine was removed from the filtrate with a strongly acidic cation exchange resin (Dowex 50) and the chloride ion removed with a basic anion exchange resin (Permutit S). The solution was evaporated at room temperature to a sirup and then dissolved in methanol. Ethyl acetate was added until the solution was turbid. Seed crystals of methyl [methyl 4-*O*-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate were introduced and, upon evaporation, needle-like crystals were isolated by filtration. A yield of 0.485 (34.6%) of crystalline material, m.p. 118–120°, was obtained. The needle-like crystals were recrystallized from a mixture of methanol and ethyl acetate, and had the following properties; melting point, 120–122°, $[\alpha]^{25}_{D}$ +162.6 (C = 1, H_2O).[3]

Anal. Calcd. for $C_{15}H_{24}O_{13}$: C, 43.67; H, 5.87. Found: C, 43.5; H, 5.78.

Crystals dissolved in water and chromatographed on Whatman No. 1 filter paper with butanol-ethanol-water solvent (40:11:19)[4] revealed no reducing sugar spot with aniline–trichloroacetic acid indicator and only one ester spot appeared with the hydroxamic acid–ferric ion test.[5] The structural formula is presented in Fig. 1.

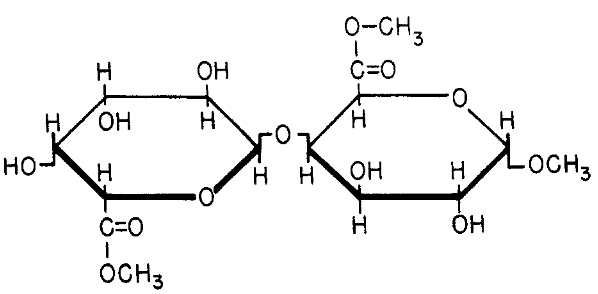

Fig. 1. Structural formula of methyl [methyl 4-*O*-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate

Evaporation of the mother liquor resulted in 0.455 g. of a glassy hygroscopic residue which upon similar chromatographic examination revealed the presence of a moderate amount of methyl [methyl 4-*O*-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate as well as four other esters. One of the esters had the same R_{GA} as methyl (methyl α-D-galactopyranosid)uronate. The other esters were present in small amounts and were not chromatographically identified.

Optical and crystallographic properties. The crystals obtained from methanol–ethyl acetate grew in loose radiating clusters of obliquely terminated prismatic needles. The individual crystals were hollow, being open on the free end, with the cavity tapering toward the base. Crystals suitable for the measurement of silhouettes were grown from a drop of aqueous solution on a slide. The solution must be seeded; otherwise, it will evaporate to a viscous sirup. However, when crystal fragments are crushed in the saturated droplet which is then quickly covered with a cover glass, good

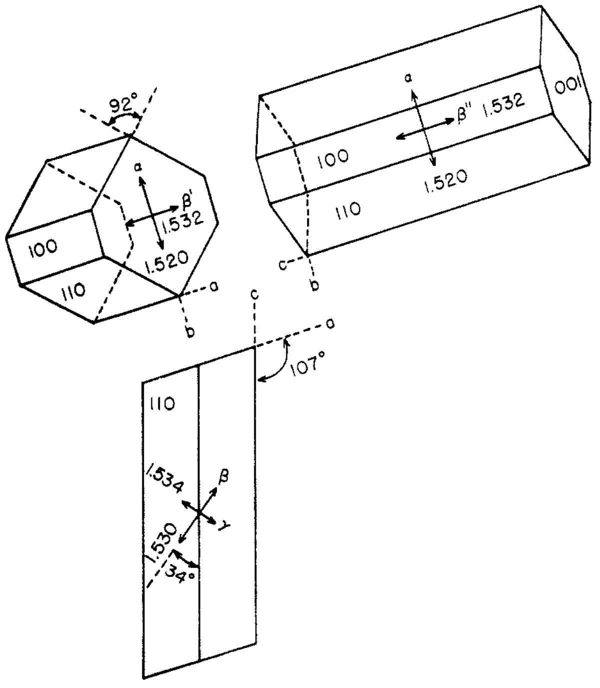

Fig. 2. Orthographic projection of the crystals of methyl [methyl 4-*O*-(methyl α-D-galactopyranosyluronate)]-α-D-galactopyranosiduronate

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[1] R. M. McCready and C. G. Seegmiller, *Arch. Biochem. Biophys.*, **50**, 440 (1954).

[2] R. M. McCready, E. A. McComb, and D. R. Black, *J. Am. Chem. Soc.*, **76**, 3035 (1954).

[3] Rotations were made with a Rudolph Polarimeter with a 0.5 decimeter polarimeter tube.

[4] J. K. N. Jones and W. W. Reid, *J. Chem. Soc.*, 1361 (1954).

[5] M. Abdel-Akher and F. Smith, *J. Am. Chem. Soc.*, **73**, 5859 (1951).

crystals will grow as the solvent evaporates. Fragments of cover glass or of seed crystals should be present under the cover glass in order to obtain crystals thick enough to give distinct interference figures. The immersion oils used for refractive index measurements were removed by flooding repeatedly with petroleum ether or isooctane.

The angular measurements shown in Fig. 2 were made on crystals grown from aqueous solution. Refractive indices were measured on crystals from both sources. The fact that this compound is optically active requires that the crystals be hemimorphic although no forms (that would require this classification) were seen.

Crystal System: Monoclinic, Beta Angle = 107°.

Optical Properties: Refractive Indices (5893A; 27°) $\alpha = 1.520$, $\beta = 1.530$, $\alpha\gamma = 1.534$, β' and $\beta'' = 1.532$ (Fig. 2).

Optic Axial Angle (5893A; 27°) 2E = 65°.

Dispersion: Not noticeable.

Optical Character: Negative.

Acute Bisectrix: α = b.

Extinction: Views having b vertical show oblique extinction with the vibration direction β making an angle of 34° with the length direction (Fig. 2). Such views are rare. They show a hazy acute bisectrix interference figure. The most common view is that of a crystal lying on one of its main faces (110). Such a view which exhibits sweeping extinction, does not show any principal optical direction but exhibits a refractive index for nearly lengthwise vibrations of 1.532. The interference figure for this view shows one optic axis near the edge of the aperture (N.A. = 0.95). Crystals that have the greatest retardation and sharp parallel or symmetrical extinction lie on either (100) or (001). Such views show α crosswise and β'' or β', respectively. The interference figure is symmetrical but centered between the obtuse bisectrix and the optic normal.

Fusion Data: Crystals melt without noticeable decomposition but no crystals grow in the viscous melt which cools to a glass.

Acknowledgment. The authors wish to thank L. M. White and Geraldine Secor for the carbon and hydrogen analyses and N. Floy Bracelin for the drawings.

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Diindazolo[2,3-*a*, 2′,3′-*d*]pyrazine-7,14-dione

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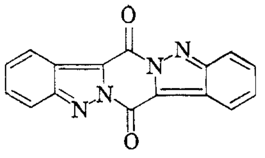
Received August 20, 1957

In the course of preparing some 3-indazolecarboxylic acid derivatives we attempted the preparation of 3-indazolecarbonyl chloride by the action of thionyl chloride on 3-indazolecarboxylic acid. The acid chloride had been reported but not characterized by previous workers.[1] Isolation of the "acid chloride" gave an orange-red compound containing no chlorine. Elemental analyses and saponification equivalent led to the consideration of I as the most likely structure.



I

A search of the literature revealed that von Auwers and Cauer[2] indicated the preparation of I but gave no physical constants or analytical data. However in a later paper von Auwers and Wolter[3] reported the preparation of the 1,2,3,4,8,9,10,11-octahydro derivative of I from the corresponding tetrahydroindazolecarboxylic acid and gave a structure analogous to I.

In common with other workers[4] using diketopiperazine derivatives derived from various 3-pyrazolecarboxylic acids, I was found to be an active acylating agent. On treatment with aqueous dimethylamine, an 85% yield of *N*,*N*-dimethyl-3-indazolecarboxylic acid amide[1] was obtained.

The formation of a polycyclic compound in the benzimidazole group has been reported.[5] The action of thionyl chloride on benzimidazole-2-acetic acid gave a compound whose structure is similar to I and which also readily acylates amines.

EXPERIMENTAL[6]

Diindazolo[2,3-a, 2′,3′-d]pyrazine-7,14-dione (I). A slurry of 53 g. of 3-indazolecarboxylic acid[1] and 100 cc. of thionyl chloride was heated under reflux for 2 hr. A large volume of benzene was added and the product filtered. The reddish-orange solid was suspended in Skelly E and heated on the steam bath to expel thionyl chloride. The product was filtered, washed with pentane, and dried. The yield was 30.0 g. (64%), m.p. >350°. The product was found to be insoluble in common organic solvents and was purified by extraction with boiling acetone for 3 hr. The insoluble orange solid gave the following results.

Anal. Calcd. for $C_{16}H_{18}N_4O_2$: C, 66.66; H, 2.80; N, 19.44; sapon. equiv. 144. Found: C, 66.49; H, 3.24; N, 19.45; sapon. equiv. 144.

N,N-Dimethyl-3-indazolecarboxylic Acid Amide. The diamide I (30 g.) was treated with 200 cc. of 25% aqueous dimethylamine. A mild exothermic reaction resulted and the reaction mixture was allowed to stand overnight at room temperature. The product was isolated by heating on the steam bath to drive off dimethylamine, followed by cooling and filtration. The dried white solid weighed 33.4 g. (85%), m.p. 191–193° (uncorr.). Recrystallization from nitromethane did not alter the melting point. (The reported[1] m.p. is 187–189°).

Anal. Calcd. for $C_{10}H_{11}N_3O$: N, 22.21. Found: N, 21.97.

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(6) Analyses are by Messrs. M. E. Auerbach, K. D. Fleischer, and their staffs.