Notes

[Chem. Pharm. Bull. 24(11)2869—2870(1976)]

UDC 547.633.4.04 : 547.457.1.04

New Synthesis of Phenolphthalein β-Glucuronide

Toshio Nambara, Norimasa Takizawa, Junichi Goto, and Kazutake Shimada

Pharmaceutical Institute, Tohoku University[1]

(Received January 26, 1976)

Convenient synthesis of phenolphthalein β-glucuronide (IV), which is widely used for the assay of β-glucuronidase activity as a substrate, has been described. Koenigs-Knorr reaction of phenolphthalin methyl ester (I) with methyl acetobromoglucuronate in the presence of cadmium carbonate as a catalyst provided the monoglucuronide acetate-methyl ester (II) which on oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone was led to the phenolphthalein derivative (III). Elimination of protecting groups with methanolic sodium hydroxide afforded the desired compound (IV) in a satisfactory yield.

Phenolphthalein mono-β-D-glucopyranosiduronic acid (phenolphthalein β-glucuronide) was first isolated by DiSomma from the rabbit urine following administration of phenolphthalein.[2] This glucuronide is widely used as a substrate for the assay of β-glucuronidase activity since the proposal by Talalay, *et al.*[3] In order to avoid the tedious work for separation and purification of this urinary metabolite, the synthesis of phenolphthalein β-glucuronide by Koenigs-Knorr reaction from phenolphthalein employing quinoline as the solvent has previously been reported.[4] However, this synthetic method is not always satisfactory in respect with the yield. We now wish to report a more convenient way for preparation of phenolphthalein β-glucuronide.

First, phenolphthalin methyl ester (I) was chosen as a starting material to attain the facile condensation of acetobromosugar with aglycone. Recently Bernstein and his coworker recommended the use of cadmium carbonate as a suitable catalyst for preparation of the aryl

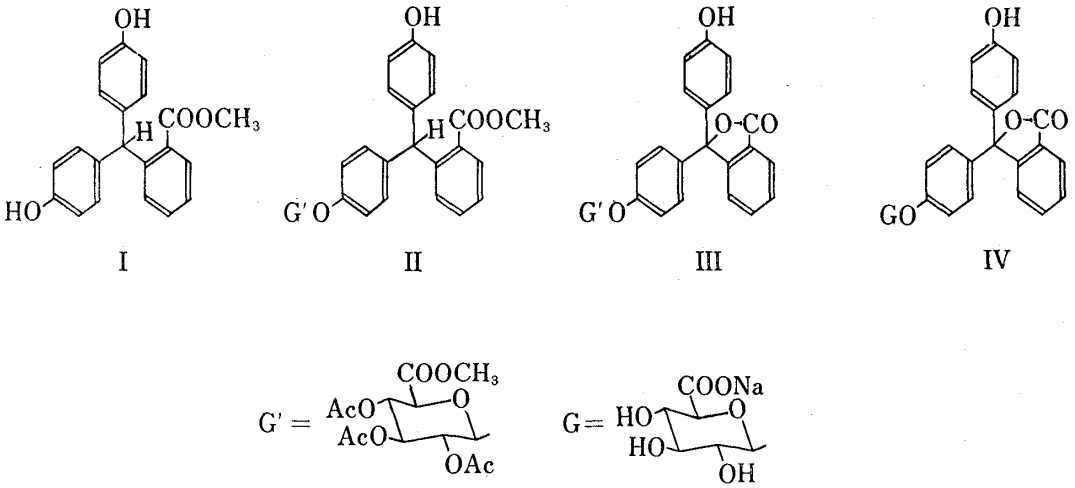

Chart 1

1) Location: *Aobayama, Sendai.*
2) A.A. DiSomma, *J. Biol. Chem.*, **133**, 277 (1940).
3) P. Talalay, W.H. Fishman, and C. Huggins, *J. Biol. Chem.*, **166**, 757 (1946).
4) C.A. Marsh and L.M. Reid, *Biochim. Biophys. Acta*, **97**, 597 (1965).

glucuronide by Koenigs-Knorr reaction.[5] Indeed condensation of I with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate in the presence of cadmium salt proceeded readily yielding the desired phenolphthalin monoglucuronide acetate-methyl ester (II) in 38% yield. Oxidation of the triphenylmethane system and subsequent lactonization was attained with ease by brief exposure to 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) under the mild conditions. Thus phenolphthalein glucuronide acetate-methyl ester (III) was obtained in a satisfactory yield. Removal of protecting groups was accomplished by treatmnet with methanolic alkali to furnish the desired phenolphthalein glucuronide (IV). The nuclear magnetic resonance (NMR) spectrum of IV was indicative of the formation of the β-glucopyranoside structure. The anomeric proton of the sugar moiety appeared at 5.00 ppm as a doublet (J=7 Hz) indicating a *trans*-diaxial relationship to the vicinal 2'-proton. The evidence for the β-glucuronide linkage was also demonstrated by liberation of phenolphthalein, when IV was incubated with beef-liver β-glucuronidase. In actuality this synthetic sample proved to be entirely identical with the metabolite isolated from the rabbit urine after administration of phenolphthalein.

It is to be noted that the present method is much more convenient and satisfactory in the yield than hitherto known methods. The facile availability of the substrate for the assay of β-glucuronidase will be helpful for biochemical studies.

Experimental[6]

Methyl (2-Methoxycarbonyl-4'-hydroxytriphenylmethane-4''-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (II)——To a solution of phenolphthalin methyl ester (I) (333 mg) in benzene (30 ml) was added $CdCO_3$ (500 mg) and the moisture was azeotropically removed by slow distillation. To this solution was added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (800 mg) in benzene (20 ml) over a period of 1 hr and then refluxed for 1 hr. The resulting solution was passed through a column packed with silica gel and washed with AcOEt. The eluate was redissolved in benzene and chromatographed on silica gel (22 g). Elution with benzene-AcOEt (10: 1) and recrystallization of the eluate from MeOH gave II (246 mg) as colorless needles, mp 234°. *Anal.* Calcd. for $C_{34}H_{34}O_{13}$: C, 62.76; H, 5.27. Found: C, 62.57; H, 5.20. NMR (5% solution in $CDCl_3$) δ: 2.02 (9H, s, $-OCOCH_3$), 3.69 (6H, s, $-COOCH_3$), 4.10 (1H, m, pyranose-C_5-H), 5.25 (4H, m, pyranose-C_1-H, -CH-OAc), 6.38 (1H, s, $(Ar)_3CH$), 6.71—7.88 (12H, m, aromatic H).

Phenolphthalein β-Glucuronide Acetate-Methyl Ester (III)——To a solution of II (200 mg) in MeOH (20 ml) was added DDQ (200 mg) and allowed to stand at room temperature for 1 hr. The resulting solution was evaporated *in vacuo* and extracted with benzene. The benzene extract was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using benzene–AcOEt (7: 1) as the solvent. Upon multiple development elution of the adsorbent corresponding to the spot (^4Rf 0.25) gave III (160 mg) as colorless amorphous substance. mp 112—116°. *Anal.* Calcd. for $C_{33}H_{30}O_{13}$: C, 62.46; H, 4.77. Found: C, 61.85; H, 4.67. NMR (10% solution in $CDCl_3$) δ: 2.05 (9H, s, $-OCOCH_3$), 3.70 (3H, s, $-COOCH_3$), 4.20 (1H, m, pyranose-C_5-H), 5.10—5.50 (4H, m, pyranose-C_1-H, -CH-OAc), 6.70—8.00 (12H, m, aromatic H).

Sodium Phenolphthalein β-D-Glucopyranosiduronate (IV)——A solution of III (100 mg) in MeOH (10 ml) containing NaOH (160 mg) was allowed to stand in a refrigerator overnight. The resulting solution was concentrated *in vacuo* and the residue was redissolved in H_2O. The solution was percolated through a column packed with Amberlite XAD-2 resin and washed with distilled water thoroughly. Elution with MeOH and recrystallization of the eluate from MeOH–iso-PrOH gave IV (46 mg) as colorless amorphous substance. mp 210° (decomp.). $[\alpha]_D^{18}$ −7.2° (c=0.14, MeOH). *Anal.* Calcd. for $C_{26}H_{21}O_{10}Na \cdot H_2O$: C, 58.43; H, 4.34. Found: C, 58.06; H, 4.57. NMR (5% solution in CD_3OD) δ: 3.60 (3H, m, pyranose-CH-OH), 4.00 (1H, m, pyranose-C_5-H), 5.00 (1H, d, J=7 Hz, pyranose-C_1-H), 6.80—8.00 (12H, m, aromatic H). This compound proved to be entirely identical in every respect with the authentic sample isolated from the rabbit urine after administration of phenolphthalein.

Acknowledgement The anthors are indebted to all the staff of central analytical laboratory of this Institute for elemental analyses and spectral measurements.

5) R.B. Conrow and S. Bernstein, *J. Org. Chem.*, **36**, 863 (1971).

6) All melting points were taken on a micro hot-stage apparatus and are uncorrected. NMR spectra were recorded on a Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet.