dilute ammoniacal lead acetate solution. Filtrate and washings were freed from lead with H_2S and concentrated to 16 ml. Phenylhydrazine hydrochloride (1.6 g.) and sodium acetate (2.4 g.) were added and the solution immersed in a boiling water-bath. Crystalline galactose osazone appeared in 17 min. The crystals were separated and recrystallized from 50% ethanol; m.p. 186° (uncor.), mixed m.p., with authentic specimen of galactose phenylosazone 185° (uncor.).

Paper Chromatography.—The hydrolyzed galactinol solution was run in both pyridine and collidine solvents. Reactions to both solvents were similar. α-Naphthol reagent showed no ketoses present. P.D.A. reagent showed a single aldose present, in the galactose location. A.S.N. reagent showed two spots, the second being inositol.

Identification of the Inositol Moiety of Galactinol.—The lead precipitate containing inositol, described above, was freed from lead with H_2S. The filtrate from the lead sulfide was concentrated, and inositol was crystallized from ethanol solution; m.p. 220–222° (uncor.), mixed m.p. with authentic specimen of inositol 220–222° (uncor.); microbiological analysis, 100% inositol.[15] Scherer's[16] test for cyclitols applied to the crystals gave a positive reaction.

Paper Chromatography.—A solution of the crystals run in both pyridine and collidine solvents gave single spots, at the inositol area, which reacted only to ammoniacal silver nitrate.

Anal. Calcd. for $C_6H_{12}O_6$: C, 39.98; H, 6.72. Found: C, 39.85; H, 6.67.[17]

Galactinol Dihydrate.—Galactinol was proved to be a compound containing α-D-galactose and *myo*-inositol. The molecular weight was determined by the freezing point depression of a solution containing 1.890 g. of crystalline galactinol in 10.0 g. of H_2O. The freezing point was depressed 0.906°, corresponding to mol. wt. 388, in the disaccharide range.

Crystalline galactinol (0.2997 g.) heated *in vacuo* at 90° for 16 hr. lost 0.0287 g. equivalent to 9.57% H_2O; theoretical for $C_{12}H_{22}O_{11}\cdot 2H_2O$: 9.52%.

Anal. Calcd. for $C_{12}H_{22}O_{11}\cdot 2H_2O$: C, 38.07; H, 6.93; O, 54.99. Found: C, 38.19; H, 6.99; O (by analysis), 54.85.[17]

These data proved galactinol to be a molecule of 378 mol. wt., containing D-galactose and *myo*-inositol, and crystallizing as a dihydrate. Hydrolysis with melibiase indicates the α-D-galactopyranosyl configuration. Therefore, modern terminology assigns the descriptive name O-α-D-galactopyranosyl-*myo*-inositol.

Some physical properties of galactinol which have been determined are given in Table II.

TABLE II

PHYSICAL PROPERTIES OF GALACTINOL DIHYDRATE

Melting point, open tube, heated slowly:	220–222° (uncor.)
Closed tube:	113–114° (uncor.)
$[\alpha]^{20}D$, estimated by saccharimeter, 2% soln. in H_2O	*ca.* +135.6°
Solubility in H_2O at 20°, by refractometer:	satd. soln. shows 49.9% as anhydride
Mutarotation:	none

***myo*-Inositol.**—Fraction No. 1, from the carbon column was concentrated *in vacuo* and treated with ethanol for crystallization. No extensive tests for identification of the crystals were made, it being considered sufficient to accept the evidence of various qualitative tests. The crystals gave the same reactions in paper chromatography as given by the *myo*-inositol recovered from hydrolyzed galactinol, and by authentic inositol. Scherer's test[16] gave a positive reaction for cyclitol; m.p. 219° (uncor.), mixed m.p. with authentic specimen 219° (uncor.).

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(15) Analysis made by the Laboratory of Vitamin Technology of Chicago, Illinois, by the yeast growth method of L. Atkin, A. S. Schultz, W. L. Williams and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **15**, 141 (1943).

(16) C. A. Browne, "Handbook of Sugar Analysis," ref. 12, p. 758.

(17) Analysis by Huffman Microanalytical Laboratories of Denver, Colorado.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, DEPARTMENT OF SURGERY OF BETH ISRAEL HOSPITAL AND HARVARD MEDICAL SCHOOL]

Synthesis of Phenyl β-D-Glucopyruronoside[1]

BY KWAN-CHUNG TSOU AND ARNOLD M. SELIGMAN

RECEIVED OCTOBER 22, 1952

Phenyl β-D-glucopyruronoside was synthesized by the catalytic oxidation of phenyl β-D-glucopyranoside in the presence of platinum black. Its identity to the natural product was shown by its physical properties and its hydrolysis by β-D-glucuronidase. A previously reported synthesis by Neuberg and Neimann is discussed.

Phenyl β-D-glucopyruronoside (I) was first isolated from urine by Külz[2] in 1890, and although it has since continued to be of biological interest as a detoxification product of phenol,[3] only recently has it been properly characterized (m.p. 161–162°, $[\alpha]^{23}D$ −90.5°).[4] Its synthesis was claimed by Neuberg and Neimann[5] from phenol and acetobromoglucurone in the presence of alkali (m.p. 150–151°, $[\alpha]^{17}_D$ −83.3°, solvent not mentioned). The product was compared with I isolated from the urine of a sheep (m.p. 148–150°),[6] but no mixed melting point with the synthetic product was given. Both Goebel and Babers[7] and we were unable to repeat the Neuberg and Neimann synthesis.

In the course of developing a synthesis for 2-naphthyl β-D-glucopyruronoside[8] from 2-naphthyl β-D-glucopyranoside by the catalytic oxidation first described by Fernandez-Garcia, *et al.*,[9] we were able to prepare I by the same procedure from phenyl β-D-glucopyranoside. Marsh, however, reported[10] that he was unable to prepare I by this

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency and by an Institutional Grant to Harvard University from the American Cancer Society.

(2) E. Külz, *Z. Biol.*, **27**, 248 (1890).

(3) N. E. Artz and E. M. Osman, "Biochemistry of Glucuronic Acid," Academic Press, Inc., New York, N. Y., 1950, p. 39.

(4) G. A. Garton, D. Robinson and R. T. Williams, *Biochem. J.*, **45**, 65 (1949).

(5) C. Neuberg and W. Neimann, *Z. physiol. Chem.*, **44**, 114 (1905).

(6) E. Salkowski and C. Neuberg, *Biochem. Z.*, **2**, 307 (1906).

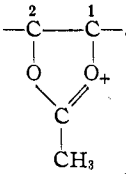
(7) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **100**, 743 (1933).

(8) K.-C. Tsou and A. M. Seligman, THIS JOURNAL, **74**, 5605 (1952); presented before the Organic Division at the 122nd National Meeting of the American Chemical Society, September 14, 1952.

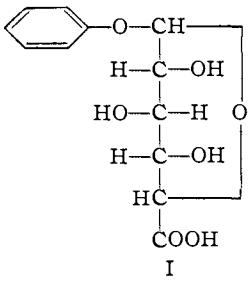
(9) R. Fernandez-Garcia, L. Amores, H. Blay, E. Santiago, H. Soltero-Diaz and A. A. Colon, *El. Crisol*, **4**, 40 (1950).

(10) C. A. Marsh, *J. Chem. Soc.*, 1578 (1952).

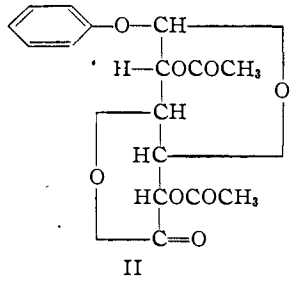
oxidation method using reduced platinum oxide as a catalyst. Apparently adsorbed hydrogen gas on platinum prevented initiation of the oxidation, since we encountered no difficulty in obtaining a 32% yield using platinum black. The identity of synthetic I to the natural product was shown by its melting point, rotation value, properties of the benzylamine salt, and the fine structure of its ultraviolet absorption spectrum.[4] Infrared spectrum data in dioxane is given here for the first time. Enzymatic hydrolysis of I by β-D-glucuronidase commercially prepared and in normal rat liver homogenate, proceeded readily at *p*H 5.0 in phosphocitrate buffer. Less enzymatic hydrolysis occurred in 4 hours with this substrate than with 6-bromo-2-naphthyl β-D-glucopyruronoside. The method of synthesis and susceptibility to enzymatic hydrolysis by β-D-glucuronidase[8] establish the pyranose structure of I.

We have shown that acetobromoglucuronolactone has a furanose structure,[8] and since this was the starting material for the Neuberg and Neimann synthesis,[5] the phenyl β-D-glucofururonoside which they reported could not be the same as the natural product (pyranose). Synthesis of their intermediate, phenyldiacetyl-β-D-glucofururonolactone (II) was accomplished in 70% yield by fusion of phenol and triacetyl-β-D-glucofururonolactone[8] in the presence of *p*-toluenesulfonic acid at 100° *in vacuo*. In the course of a previous study,[11] it was found that when pentaacetyl-α-D-glucose was used in a similar procedure, it was recovered unchanged.[12] This reaction therefore probably involved a participation of the neighboring C_2-acetoxyl group and thus it would either lead to II through a double inversion, or to an orthoester (IIa) through the intermediate ion  .[13] That

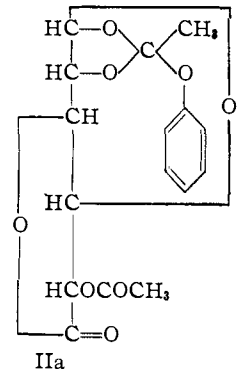
II did not have such a structure was shown by its stability in slightly acid solution, a procedure which was used to demonstrate the orthoester structure of methyldiacetyl-β-D-glucuronolactone.[14] The β-configuration of II was assigned on the basis of its rotation value. The glycosidic linkage of compound II, however, was hydrolyzed very rapidly



I



II



IIa

in alkali. Attempts to deacetylate II in methanol with either sodium, barium or ammonia gave phenol and a small amount of methyl-β-D-glucofururonolactone. Similar sensitivity to alkali of other aryl diacetyl-β-D-glucofururonolactones has been observed[8,12] and explains why Neuberg and Neimann's results have been difficult to repeat.

Experimental[15]

Phenyl β-D-Glucopyruronoside (I).—An aqueous suspension of phenyl β-D-glucopyranoside[16] (1.0 g. in 150 cc.) and 0.3 g. of platinum black,[17] was vigorously stirred in a three-necked flask, equipped with a mechanical stirrer and two gas bubblers fitted with fritted glass discs. Oxygen was introduced into the mixture while it was maintained at 90 to 100° on the steam-bath, and 0.15 g. of sodium bicarbonate was added in portions to keep the solution slightly alkaline. At the end of 3 to 3.5 hours, the amber colored solution was filtered from the catalyst and concentrated *in vacuo* to about 25 cc. The solution was acidified to *p*H 5 to 6 with 3 *N* hydrochloric acid, and extracted with 5 portions (25 cc. each) of ethyl acetate. The ethyl acetate solution was dried with anhydrous sodium sulfate, filtered and evaporated at reduced pressure to give 0.6 g. of the crude product which was crystallized from benzene–ethanol in fine, fluffy needles, 0.35 g. (32%). The sample was dried in a vacuum desiccator over anhydrous calcium chloride followed by desiccation in a pistol at 100°[4]; m.p. 160–161° (lit. 161–162°)[4]; $[\alpha]^{29}D$ −87.5° (*c* 2.10, water) (lit. $[\alpha]^{23}D$ −90.5°).[4]

Anal. Calcd. for $C_{12}H_{14}O_7$ (270.23): C, 53.33; H, 5.22. Found: C, 53.35; H, 5.38.

The fine structure of the ultraviolet absorption spectrum, determined with a Beckman DU spectrophotometer was identical to that reported (λ_{max} 265.5 and 271.5 mμ, ε 864 and 673, respectively; lit.[4] λ_{max} 264.5 and 271 mμ, ε 810 and 690, respectively). The infrared absorption in dioxane showed a 5.80 μ band, a broad diffuse band at 3.2 and 3.8 mμ, characteristic of carboxyl group and a 6.3 μ band due to the phenyl group. A positive naphthoresorcinol test for glucuronic acid was obtained.

The dihydrate of I was obtained as fine needles by slow crystallization from a minimum amount of water, but the sintering point varied from 67 to 109°, depending upon the bath temperature when inserted. Sintering was reported at 110°.[4]

Benzylamine salt of I crystallized from ethanol in fine needles and decomposed at 203–204°, when it was inserted at a bath temperature of 190° (lit. m.p. 206–207° (dec.)), $[\alpha]^{30}D$ −63.3° (*c* 2.1, water) (lit. $[\alpha]^{25}D$ −62.3° in water).

Anal. Calcd. for $C_{19}H_{23}NO_7$ (377.38): C, 60.47; H, 6.14; N, 3.71. Found: C, 60.59; H, 6.35; N, 3.83.

Enzymatic Hydrolysis of I.[18]—The comparative rates of hydrolysis of phenyl β-D-glucopyruronoside (I) (0.6 mg.) and

(11) K.-C. Tsou and A. M. Seligman, THIS JOURNAL, **74**, 3066 (1952).

(12) K.-C. Tsou, unpublished observation.

(13) For details of similar discussion, see S. Winstein, C. Hanson and E. Grunwald, THIS JOURNAL, **70**, 812 (1948).

(14) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **110**, 707 (1935).

(15) All melting points are corrected. Microanalyses by Dr. S. M. Nagy and Associates, Microchemical Laboratory, M.I.T., Cambridge, Mass.

(16) E. M. Montgomery, N. K. Richtmeyer and C. S. Hudson, THIS JOURNAL, **64**, 690 (1942).

(17) Purchased from Baker and Co., Newark, N. J. If the reduced form of platinum oxide is used, care must be taken to remove all adsorbed hydrogen gas prior to its use.

(18) These experiments were performed in collaboration with Dr. Selma H. Rutenburg.

6-bromo-2-naphthyl β-D-glucopyruronoside[19] (1.0 mg.) were determined as follows: Equimolar amounts of each substrate in 4 cc. of 10% methanol were adjusted with 0.15 *M* phosphocitrate buffer (1.0 cc.) to *p*H 5.0 and incubated with 0.5 cc. of the supernatant of a centrifuged homogenate (5 mg. per cc. in water) of rat liver and 0.5 cc. of a solution containing 10 mg. of a commercial preparation of bovine β-glucuronidase[20] for 4 hours at 37°. Phenol was measured by adjustment of the *p*H to 9.5 with 0.2 *M* trisodium phosphate (0.5 cc.) followed by addition of 0.5 cc. of a 30% alcoholic solution of 2,6-dibromo-N-chloro-*p*-quinoneimine (1.0 mg. per cc.). The blue color density was measured in a photoelectric colorimeter (Klett) with a 590 mμ filter and conversion to micrograms of phenol was accomplished with an appropriate calibration curve. 6-Bromo-2-naphthol was measured by adjustment of the *p*H to 7.6 with 0.2 *M* trisodium phosphate (0.3 cc.) followed by coupling to an azo dye with tetrazotized diorthoanisidine. The dye was extracted into chloroform (10 cc.) and the color density was measured in a photoelectric colorimeter with a 540 mμ filter. The procedure and calibration curve are given elsewhere.[21] Control tubes without substrate and without enzyme were also prepared.

In 4 hours, commercial β-glucuronidase had hydrolyzed the phenyl compound 7% and the naphthyl compound 52% (0.024 μM. per hour and 0.31 μM. per hour, respectively). Hydrolysis of these substrates by rat liver in 4 hours was 13 and 57%, respectively (0.05 μM. per hour and 0.35 μM. per hour). Apparently bovine glucuronidase hydrolyzes the phenyl compound less readily than does rat glucuronidase, whereas no significant difference in hydrolysis rate was found with the naphthyl compound.

Phenyldiacetyl-β-D-glucofururonolactone (II).—A mixture of triacetyl-β-D-glucofururonolactone[8] (13.5 g.), phenol (12.6 g.) and *p*-toluenesulfonic acid (0.1 g.) was fused *in vacuo* for 25 minutes at 100°. The melt was triturated with 20 cc. of 95% ethanol and the crude product was collected and washed with ethanol; yield 12.0 g., m.p. 178–181°. The product crystallized from methyl cellosolve in fine needles; yield 10.2 g. (70%), m.p. 188–189°, $[\alpha]^{30}D$ +74.5° (*c* 1.85, in chloroform). Its infrared absorption spectrum in chloroform had a 5.59 μ band for γ-lactone, a 5.75 μ band for ester, and 6.30 μ band for the phenyl group.

Anal. Calcd. for $C_{16}H_{16}O_8$ (336.29): C, 57.14; H, 4.80. Found: C, 57.17; H, 4.89.

A sample of II was dissolved in 0.1% hydrochloric acid in dioxane and its rotation of polarized light was observed not to change for 2 hours. Thus, the possibility of an ortho-ester structure (IIa) was excluded.

Deacetylation of II.—A small piece of metallic sodium (about 1–2 mm.[3]) was added to a suspension of II (0.1 g.) in 10 cc. of absolute methanol. The suspension was shaken manually, and in one hour a light yellow solution resulted. After standing for an additional hour, the solution was acidified with acetic acid and evaporated to dryness *in vacuo*. Phenol was sublimed from the mass at 1 mm. and was found to react positively with 2,6-dibromo-N-chlorp-*p*-quinoneimine and sodium carbonate. The brown residue was free of phenol. It was extracted with absolute methanol. On concentrating the decolorized alcoholic solution and after 2 days at 4°, a small amount of white clusters of crystals (*ca.* 10 mg.) separated; m.p. 138–140°, $[\alpha]^{30}D$ −49.7° (*c* 1.25, water). This was probably identical with methyl-β-D-glucofururonolactone (lit. m.p. 139°), $[\alpha]^{23}D$ −59°,[22] contaminated with some of the α-isomer. When the reaction was conducted at 0° overnight, only phenol and 50% of the starting material recovered. Only phenol was obtained and no product was isolated when deacetylation was performed in absolute methanol with dry ammonia at 0°, or in absolute methanol with barium methoxide at 0°.

(19) This substrate was synthesized for the histochemical demonstration of the enzyme and its synthesis will be published by Tsou and Seligman elsewhere.

(20) Purchased from Viobin Corp., Monticello, Illinois.

(21) R. B. Cohen, K.-C. Tsou, S. H. Rutenburg and A. M. Seligman, *J. Biol. Chem.*, **195**, 239 (1952).

(22) E. M. Osman, K. C. Hobbs and W. E. Walston, THIS JOURNAL, **73**, 2726 (1951).

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

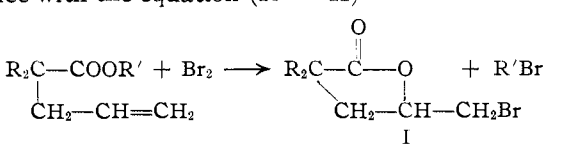
Participation of a Neighboring Carboxyl Group in Addition Reactions. I. The Mechanism of the Reaction of Bromine with γ,δ-Unsaturated Acids and Esters[1]

BY RICHARD T. ARNOLD, MARCELLO DE MOURA CAMPOS[2] AND KENNETH L. LINDSAY

RECEIVED OCTOBER 16, 1952

The mechanism of the reaction between bromine and γ,δ-unsaturated acids or esters, which leads to the formation of δ-bromo-γ-pentanolactones, has been investigated in some detail. The formation of alkyl bromides during the bromination of γ,δ-unsaturated esters has been shown to involve essentially complete inversion of the alkyl group. The effect of the structure of the unsaturated acid on the course of the reaction is discussed.

It has long been recognized that γ,δ-unsaturated acids frequently react with bromine to form δ-bromo-γ-pentanolactones and hydrogen bromide instead of the simple addition product, in accordance with the equation (R′ = H)



The bromination of 2,2-diphenylpenten-4-oic acid,[3] diallylmalonic acid[4] and oleanolic acid[5] represent typical examples of this transformation, and several new examples are reported in the Experimental section of this paper.

The rapid formation of δ-bromo-γ-pentanolactones from esters of γ,δ-unsaturated acids is well established in a few cases,[3,4] although the reaction has received little attention and no one has, as yet, reported the isolation of an alkyl halide (R′Br) which might be anticipated as the other product, although Craig[6] has obtained indirect evidence for the formation of methyl bromide during the bromination of methyl 2,2-diphenylpenten-4-oate. The major objective of the present study was to investi-

(1) (a) Presented before the Division of Organic Chemistry during the 122nd Meeting of the American Chemical Society at Atlantic City, N. J., September, 1952; (b) taken in part from the Ph.D. Thesis of Kenneth L. Lindsay, 1952.

(2) Rockefeller Postdoctoral Fellow from the University of Sao Paulo, Brazil.

(3) P. N. Craig and I. H. Witt, THIS JOURNAL, **72**, 4925 (1950).

(4) R. Fittig and E. Hjelt, *Ann.*, **216**, 52 (1883).

(5) A. Winterstein and W. Hammerle, *Z. physiol. Chem.*, **199**, 56 (1931).

(6) P. N. Craig, THIS JOURNAL, **74**, 129 (1952).