in the glucuronic acid–L-ascorbic acid cycle.[9-11] Although a colorimetric method was developed earlier in this laboratory[5] and was successfully applied to the determination of D-glucaric acid in normal mammalian urines as well as in human serum after loading of its precursors,[1] it was not applicable to the estimation of the normal serum level of the acid in man because of its insufficient sensitivity.

The normal serum levels of D-glucaric acid in man and the rat determined by the fluorometric method described above are given in Table II. Actually, a comparison between the colorimetric and the fluorometric methods with normal mammalian sera was unattainable, while a satisfactory coincidence between the two methods was observed with normal mammalian urines.[12]

TABLE II. Serum Level of D-Glucaric Acid in Man and the Rat

Species	D-Glucaric acid (μg/dl)[a]	Mean (±S.E.)
Man	50, 48, 47, 42, 40, 37, 33, 29, 24, 20, 17, 13, 10	32±14
Rat	216, 200, 150, 121, 111, 97, 83, 55, 40	119±60

a) Values were obtained by the Procedure I reported earlier.[5]

Concerning the drug-induced stimulation of the glucuronic acid pathways in man, the serum levels of D-glucaric acid in normal human adults and epilepsy patients chronically under treatment with diphenylhydantoin and phenobarbital, both are known as potential stimulator of drug metabolism, were determined by the presented fluorometric method and the results were reported previously.[9]

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C-Glycosyl Nucleoside. III.[1] Ethynylation of Glucosyl Bromide with Ethynylmagnesium Bromide

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In the reaction of sodium acetylide with tetra-*o*-acetyl-α-D-glucosyl bromide in liquid ammonia, Zelinski and Meyer[3] obtained an unknown compound in about 4% yield having mp 183—185.5°, $[\alpha]_D^{25}$ −68.8°, and an uncharacterized syrup in about 9% yield. On the other hand, Hurd and Holysz[4,5] reported the reaction of phenyllithium with tetra-*o*-acetyl-α-

1) Part II: H. Ogura and M. Ogiwara, *J. Org. Chem.*, in preparation.
2) Location: *Shirogane, Minato-ku, Tokyo, 108, Japan.*
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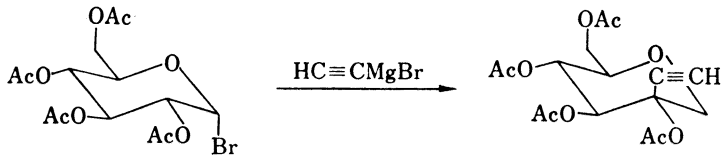
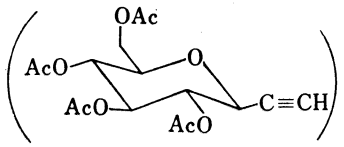
D-glucopyranosyl chloride in ether from which they obtained tetra-*o*-acetyl-*β*-D-glucopyranosyl and tetra-*o*-acetyl-*α*-D-glucopyranosylbenzene, and tetra-o-acetyl-1,5-anhydro-2-*β*-C-phenyl-D-glucitol.[5] They[4] also reported the reaction of ethyne bis(magnesium bromide) with tetra-o-acetyl-*α*-D-glucopyranosyl bromide to yield an uncharacterized tar.



II IV



III

We wish to report the reaction of ethynylmagnesium bromide (I) with tetra-*o*-acetyl-*α*-D-glucopyranosyl bromide (II) in tetrahydrofuran to obtain tetra-*o*-acetyl-*β*-D-glucopyranosylethyne (III) as an intermediate to C-nucleoside.[6] We obtained three other compounds, 1, 5-anhydro-tetra-*o*-acetyl-2-C-ethynyl-D-glucitol (IV) in about 4% yield, an unknown compound (V) having mp 88—90°, and 3-methyl-1,4-dipentyn-3-ol, as a by-product (20%) which has not been reported in literature.[1] The former compound (IV) showed mp 184.5—185.5° and its data are very similar to those of Zelinski's unknown compound.

Mass spectrum of IV showed *m/e* 356 (M^+: $C_{16}H_{20}O_9$), and infrared (IR) spectrum showed absorptions at 2132 (C≡C) and 1745 (C=O) cm^{-1}. From the nuclear magnetic resonance (NMR) spectrum (δ ppm: CD_3COCD_3, 100 MHz) of IV, with 3.25 (1H, singlet, C≡CH),[7] the ethynyl group should be attached to the tertiary carbon and therfore not in the anomer position. Further elucidation of NMR spectrum of IV, with 3.67 (doublet, H*a*-1), 4.61 (doublet, H*e*-1), and a J value (−11.3 Hz), showed the presence of geminal protons. A doublet at 5.38 (1H, $J_{3,4}$=9—6 Hz, 3-H) and a triplet at 5.16 (1H, $J_{4,5}$=9.5 Hz, 4-H) are attributed to the *trans*-diaxial protons. The acetoxyl groups may have an equatorial configuration from their chemical shift in deuteriochloroform (2.01—2.11) and in deuterioacetone (1.95—2.02).[8,9] This was confirmed by heating IV under reflux in acetic anhydride and pyridine, IV was recovered but not obtained any dehydration product. Therefore, the acetoxyl groups have not coplanar with any neighboring C-H bond,[5] and the ethynyl group may have an axial configuration.

Experimental[10]

A solution of ethynylmagnesium bromide (I), prepared from magnesium (12.0 g) and isopropyl bromide (52.0 g) in tetrahydrofuran (300 ml), was added dropwise to tetrahydrofuran (200 ml) solution saturated

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10) All melting points are uncorrected. Optical rotations were measured in a 0.1 dm tube with a JASCO automatic polarimeter DIP-SL. NMR spectra were recorded at 100 MHz with a Varian Associate H-100 spectrometer and tetramethylsilane was used as an internal reference. Mass spectra were taken with a Japan Electron Optics JMS-01S high-resolution spectrometer with a direct inlet system.

with acetylene at room temperature. A stream of acetylene was passed through the reaction mixture for 4 hr. To this solution, tetra-*o*-acetyl-α-D-glucopyranosyl bromide, (II, 17.4 g) in tetrahydrofuran (70 ml) was added dropwise with stirring at room temperature. After the reaction solution was stirred for 24 hr, ether (300 ml) was added, and which was treated with ice-water (200 ml) and acetic acid (10 ml). The organic layer was dried (Na_2SO_4) and evaporated to leave a syrup (3 g; 20%). Distillation of this syrupy residue gave 3-methyl-1,4-pentadiyn-3-ol, bp_{20} 45—47°, mp 54—56° (colorless prisms). Reported,[11] bp_{19} 44—46°, mp 58°.

The aqueous layer was evaporated to dryness under a reduced pressure and the residue was acetylated with acetic anhydride (150 ml) and pyridine (10 ml). There was obtained a brown oil which was chromatographed on silica gel. Elution with petroleum ether–benzene (2: 1) gave 1.2 g of an unknown compound (V) as colorless prisms (from hexane), mp 88—90°, $[\alpha]_D^{24}$ −2.8° ($CHCl_3$). *Anal.* Found: C, 53.38; H, 6.75. IR ν_{max}^{KBr} cm^{-1}: 3560, 3500, 3100, 2960, 2900, 2140, 1745, 1632, 1449, 1380, 1235, 1040, 905, 709.

Further elution of the column with benzene gave 0.54 g of IV as colorless needles (EtOH), mp 184.5—185.5°, $[\alpha]_D^{18}$ −64.5° ($CHCl_3$). *Anal.* Calcd. for $C_{16}H_{20}O_9$: C, 53.93; H, 5.66. Found: C, 53.94; H, 5.90. IR ν_{max}^{KBr} cm^{-1}: 3240, 2132 (C≡C), 1745 (C=O), 1440, 1380. NMR (CD_3COCD_3) δ (ppm): 1.95, 1.99, 2.01, 2.02 (12H, singlet, CH_3CO), 3.25 (1H, singlet, ≡CH), 3.67 (1H, doublet, Ja,e=11.3 Hz, 1-H*e*), 5.16 (1H, triplet, $J_{4,3}$=9.6 Hz, $J_{4,5}$=9.5 Hz, 4-H), 5.38 (1H, doublet, $J_{3,4}$=9.6 Hz, 3-H); ($CDCl_3$) δ (ppm): 2.01, 2.02, 2.07, 2.10 (CH_3CO), 2.75 (≡CH), 3.02 (1-H*a*), 3.52—3.75 (5-H), 4.16 (6-H), 4.23 (1-H*e*), 5.19 (4-H), 5.35 (3-H). Mass Spectrum (relative intensity) *m/e*: 356 (M^+). Calcd. for $C_{16}H_{20}O_9$: 356.111. Found: 356.115. 356 (2), 313 (2), 297 (2), 296 (3), 236 (6), 212 (11), 194 (24), 175 (20), 152 (34), 145 (18), 139 (20), 134 (22), 121 (25), 103 (13), 43 (100).

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