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PRELIMINARY NOTE

SYNTHESIS OF 17β-D-GLUCOPYRANOSIDURONIC ACID OF 17α-ETHYNYLESTRADIOL

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SUMMARY

The synthesis of the 17β-D-glucuronide of 17α-ethynylestradiol was an unexpected product resulting from the use of $CdCO_3$ in the improved Koenigs–Knorr reaction between 17-alpha-ethynylestradiol and methyl (1α-bromo-triacetyl-glucuronate). Sephadex LH-20 was found to be effective in the purification of the synthetic product. Identification was obtained through mass spectrometry, infrared spectroscopy, ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, enzymatic and chemical analysis, and gas–liquid chromatography.

INTRODUCTION

The availability of authentic steroid conjugates has often been a limiting factor in the study of steroid hormone metabolism. The most common synthetic estrogen component of oral contraceptives, 17α-ethynylestradiol (EE_2), has been reported to be excreted in the human as a β-glucuronide conjugate(s) [1–5]. Definitive identification of these glucuronides has not been accomplished. To this end, we undertook the synthesis of the 3β-D-glucuronide of EE_2.

The synthesis of steroid glucuronides by the Koenigs–Knorr reaction traditionally has employed Ag_2CO_3 as the acid acceptor. The yields of estrogen glucuronides particularly those occurring through the phenolic hydroxy group, have always been low [6]. Conrow and Bernstein[7] examined this aspect of the synthesis of the 3-glucuronides of estrone and estradiol, and upon employing $CdCO_3$ in place of Ag_2CO_3, obtained a dramatic increase in yield of the initial condensation product. It appeared that this metal carbonate dictated a specificity of the reaction towards the more acidic phenolic hydroxy group. No reaction has been reported between aliphatic hydroxyl and the 1α-bromo sugar derivatives promoted with $CdCO_3$.

Herein reported is an anomalous product resulting from the reaction between 17α-ethynylestradiol-17β and methyl (1α-bromo-triacetyl-glucuronate) with $CdCO_3$ as the acid acceptor, being the 17β-glucuronide.

EXPERIMENTAL

The reaction was carried out as described by Conrow and Bernstein[7]: to a 500 ml r.b. 3-necked flask equipped with a magnetic stirrer, a reflux condenser, a dropping funnel, and Dean–Stark moisture trap was added 4.32 gms EE_2 (Research Plus) (14.6 mM), 7.53 gms $CdCO_3$ (Fisher) (43.8 mM) and 300 ml toluene. Azeotropic distillation was carried out for 30 min to dry the reaction milieu. There was then added a solution of 17.40 gms of methyl (1α-bromo-triacetyl-glucuronate) [8] (43.9 mM) in 200 ml of toluene at a rate of about 2–3 ml per min, the distillation rate being maintained the same. During the course of the reaction the salmon-pink color of the reaction mixture was observed as described by Conrow and Bernstein[7]. After completion of the addition, the distillation was continued for an additional 20 min. The workup of the reaction mixture was as previously described [7], yielding 5.7 gms of the crude conjugated product. Purification of the reaction products (benzene–ethanol) was achieved on Sephadex LH-20 column with benzene–methanol (95:5) as the solvent system. Separation of the products were monitored by TLC (silica gel, benzene–ether, 4:1) using spray reagent (conc. H_2SO_4), followed by heating in the oven at 150°C.

RESULTS

Structure determination

A pure product obtained from LH-20 chromatography had a U.V. max 283 nm (methanol), indicating the phenolic hydroxy group was free. Infrared spectra (KBr): 3500 cm^{-1}, 3290 cm^{-1}, 2929 cm^{-1}, 2870 cm^{-1} (broad), 1620 cm^{-1} (with shoulder 1580 cm^{-1}), 1220 cm^{-1} (broad), 1040 cm^{-1}. The 3290 cm^{-1} peak demonstrated the acetylenic H and 1622 cm^{-1} the aromatic H's. Carbazole test for glucuronic acid was positive [9]. Mass spectral analysis using Dupont 21-4908 showed mass peak (M^+) of 613, in accord with the calculated value 612.6.

De-acylation was performed as follows: 40 mg of K_2CO_3 was added to 50 mg of acylated product dissolved in 3 ml of 95% methanol. After stirring overnight at room temperature, the product showed I.R. spectra: marked increase of absorption at 3400 cm^{-1} (broad), and 1620 cm^{-1} (broad) and absence of 1750 cm^{-1} and 1210 cm^{-1}. The U.V. spectra of the de-blocked conjugate in methanol was 281–2 nm. Table 1 compares the ultraviolet spectra of other related compounds.

Table 1. U.V. spectra of other related compounds

Compound	Absorption max (nm)	Molar absorptivity (nm)
EE_2	281–2	1776
E_2-17-Acetate	281–2	1884
EE_2-17-Gluc. (product)	281–2	850
E_1-acetate	268.5, 274.5	735, 811
E_2-3-methyl ether	279.0, 287.5	1664, 1749

Table 2. Protonic signals

Compound	18-CH_3	H 1,2	H 3
Ethynyl estradiol	0.77	6.38	7.01
		6.43	6.98
		6.48	
Ethynyl estradiol glucuronide	0.85	6.38	7.00
		6.43	6.97
		6.48	

ppm downfield from TMS = 0. DMSO reference at 2.48 ppm.

The NMR spectra of the synthetic EE_2-glucuronide was taken on the Varian HR-220 MHz spectrometer in DMSO-d_6 solvent. Table 2 lists the relevant protonic signals for EE_2 and the 17-glucuronide.

Mass spectroscopy of the EE_2-glucuronide was obtained on the Atlas/Varian CH-7 through probe introduction of the methyl ester-trimethylsilyl derivative. The calculated molecular formula $C_{39}H_{66}O_8Si_4$ gave an exact mass of 774.3835 a.m.u.; a nominal mass of 774 was obtained from the spectra.

Enzymic hydrolysis

Twenty mg of the EE_2-glucuronide was treated with β-glucuronidase (*Helix pomatia*). A portion of the $CHCl_3$ extract (1%) was converted to the TMS derivative with 150 μl each of *bis* (trimethylsilyl) acetamide and trimethylsilylimidazole for 2 h at 120°C. Comparison of the derivative with an authentic EE_2-TMS by GLC (6 ft. OV-17 column, 235°, F & M model 400) showed the two were identical.

DISCUSSION

The evidence supporting the locus of the glucuronic acid at C-17 was from interpretation of the U.V. spectra and NMR spectra. The single U.V. peak maximum at 281–2 nm, unshifted from that of ethynylestradiol or the estradiol 17-acetate, indicated the phenolic hydroxyl was unsubstituted. The NMR spectra showed the protons at carbon 1, 2 and 4 to have identical chemical shifts for both the EE_2 and the glucuronide; the chemical shift of the proton signals of the C-18 CH_3 exhibited a significant downfield shift of the glucuronide compared to the EE_2. These two mutually supportive physical constants were further supported by the enzymic hydrolysis data, permitting the conclusion that a principal product of the synthesis was the EE_2-17β-glucuronide. This was not anticipated since the use of the $CdCO_3$ appeared to have a strongly phenolic directing activity in the Koenigs–Knorr reaction. That the 17β-hydroxyl group, considered to be in a strongly stereochemically hindered position, as opposed to the more readily accessible phenolic hydroxyl group, was a principle focus of glucuronidation reaction, adds interest to the organic and biological chemistry of groupings at C-17.

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