Rapid Paper

The Simultaneous Synthesis of 2- and 4-Iodoestradiol

Takuji TSUKAMOTO and Yukihiro YADA

Laboratory of Biological Chemistry, Faculty of Agriculture, Saga University, Saga 840, Japan

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Iodination of the A-ring of estradiol was performed by treating estradiol with iodine (I_2) in the presence of mercuric acetate. Although this procedure has generally been considered as a selective preparation method for 2-iodoestradiol, we found that two major products were obtained from the reaction. These compounds were separated clearly by silica gel medium-pressure liquid chromatography, using benzene–acetone (5 : 1, v/v) as a mobile phase. The two compounds were identified as 2-iodoestradiol (36.8% yield) and 4-iodoestradiol (21.5% yield) based on physicochemical data, and in particular on ^{1}H-NMR spectra.

A-ring-halogenated estrogens have been of interest in studies on the interaction between estrogens and target organs in biological systems.[1] In addition, such 2-halogenated estrogens as 2-iodoestradiol have often been synthesized as precursors for the preparation of [2-^{3}H]estradiol that was to be used as the substrate for a radiometric assay of estradiol 2-hydroxylase in various animal tissues.[2,3] In order to obtain accurate assay results in this radiometric assay, it is most important to prepare [2-^{3}H]estradiol from 2-iodoestradiol, which has the least amount of such concomitants as 4-iodoestradiol.

Hillman-Elies *et al.*[4] have reported a simple method for the selective preparation of 2-iodoestradiol (**2**) by treating estradiol (**1**) with iodine (I_2) in the presence of mercuric acetate in high yields (95%). When we prepared 2-iodoestradiol according to their method and guided by thin layer chromatography (TLC, silica gel–chloroform with 20% ethanol), we initially observed a major product of more than 90% yield with a small percentage of impurity after recrystallization from methanol–water. TLC of the product using other solvent systems such as benzene–ethanol (9 : 1, v/v), acetonitrile–water (9 : 1, v/v) and chloroform–water (4 : 1, v/v) also showed a single spot. However, the existence of two compounds in the major product was revealed by TLC using benzene–acetone (5 : 1, v/v) or chloroform–acetone (8 : 1, v/v) as the solvent system. Among 50 solvent systems tested, the best separation of the two compounds was obtained by benzene–acetone (5 : 1, v/v). The two compounds isolated by silica gel medium-pressure liquid chromatography, using benzene–acetone (5 : 1, v/v) as a mobile phase and recrystallized from methanol–water, were assigned as 2- and 4-iodoestradiol based on physicochemical data, and especially on ^{1}H-NMR spectra. In one case, a pair of singlets at δ 6.56 and 7.45, without discernible splitting of the *para* protons, is consistent with the 2-iodoestradiol (**2**) structure, whereas a pair of doublets at δ 6.68 (J=8.53 Hz) and 7.12 (J=8.53 Hz) is attributable to spin–spin coupling of the *ortho* protons of 4-iodoestradiol (**3**).

The final yield of analytically pure 2-iodoestradiol was 36.8% and that of 4-iodoestradiol was 21.5%. Thus, it should be noted that the reaction of estradiol with iodine in the presence of mercuric acetate yields the 2- and 4-iodo isomers of estradiol. In addition, the

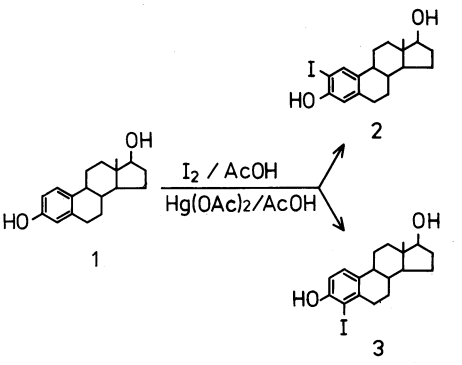

FIG. 1. Synthesis of 2- and 4-Iodoestradiol.

1: Estradiol, 1,3,5(10)-estratriene-3,17β-diol. **2**: 2-Iodoestradiol, 2-iodo-1,3,5(10)-estratriene-3,17β-diol. **3**: 4-Iodoestradiol, 4-iodo-1,3,5(10)-estratriene-3,17β-diol.

synthetic pathway and the isolation procedure described here could prove very convenient and practical for simultaneous synthesis of 2- and 4-iodoestradiol.

EXPERIMENTAL

Melting points (mp) determined on a Yanagimoto micro-melting-point apparatus were uncorrected. Prior to elemental analysis, all compounds were dried over phosphorus pentoxide until the weight remained constant at 60°C and 2 Torr. The elemental analyses were performed at the Central Analysis Room of Kyushu University. TLC was carried out on silica gel GF_{254} (Merck). Ultraviolet spectra were run on a JASCO UVIDEC-505 spectrophotometer. Optical rotations were measured with a JASCO J-20A polarimeter. ^{1}H-NMR spectra were recorded on JEOL PS-100 and JNM-GX 270 spectrometers with tetramethylsilane as the internal standard, chemical shifts being given in δ (ppm). Mass spectra were determined with an ESCO EMD-05A GC-MS spectrometer. Column chromatography was carried out with silica gel (Kieselgel 60, Merck, or CQ-3, Fuji).

Iodoestradiol (**2** and **3**). Estradiol (**1**, 272 mg, 1 mmol) in 10 ml of glacial acetic acid was mixed with mercuric acetate (165 mg, 0.52 mmol) in 10 ml of glacial acetic acid at 50°C. To this mixture, iodine (I_2, 260 mg, 102 mmol) in 10 ml of glacial acetic acid was added dropwise over a 5 minute period while the contents were stirred at 50°C. Subsequently, the reaction mixture was stirred for another 5 minutes at 50°C and then for 60 min at 27°C. The mixture was filtered through Whatman 50 filter paper by suction at room temperature, after which the filtrate was condensed to about 3.5 ml *in vacuo* at room temperature. To the condensed filtrate, potassium iodide (15 mg, 0.09 mmol) in glacial acetic acid (10 ml) was added and the mixture was filterd through Whatman 50 filter paper by suction. By adding 50 ml of 0.3% (w/v) sodium bisulfate solution to the filtrate at 0°C, crude crystalline iodoestradiol (pale yellow, 379 mg) was obtained in a yield of 95%. The crude compound (207 mg) was dissolved in *n*-hexane–chloroform–acetic acid (4:4:1, v/v) and subsequently chromatographed on a column (2.7 × 70 cm) of Kieselgel 60 using the same solvent system. There were two major peaks (S-3 and S-4), which were eluted immediately after one another and lacked clear separation, and three minor peaks, which were clearly separated (S-1, S-2 and S-5). The numbers of the peaks indicate the order in which they were eluted. S-5 was authentic estradiol. S-3 and S-4 (total 280 ml) were collected together, condensed to 3 ml *in vacuo* and recrystallized (142 mg, 68.6%) from methanol–water. The crystallized compound (20 mg) was dissolved in benzene–acetone (5:1, v/v) and clearly separated into two components (M-1 and M-2) by medium-pressure liquid chromatography (MPLC) using silica gel CQ-3, as shown in Fig. 2. Since the solvent contained benzene, which has strong absorption near 280 nm, the iodoestradiols contained in 0.2 ml of each fraction were monitored at 750 nm after color formation by the Lowry procedure,[5] which is routinely used for protein determinations.

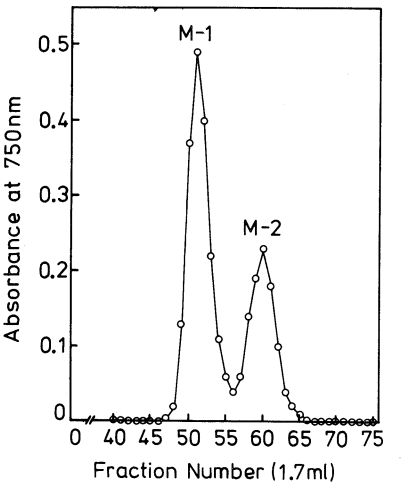

FIG. 2. Medium-pressure Liquid Chromatogram of 2- and 4-Iodoestradiol.

Conditions: column (0.8 × 10 cm ~ 1.5 × 30 cm), silica gel CQ-3; temperature, 20°C; mobile phase, benzene–acetone (5:1, v/v); flow rate, 1 ml/min.

2-Iodoestradiol (**2**). Peak M-1 (13.6 ml) fractionated by MPLC was collected and condensed to about 2 ml *in vacuo*, and then recrystallized from methanol–water to give 11.82 mg (59%) of a colorless crystal, which was analytically pure 2-iodoestradiol (**2**). The final yield of **2** through the entire synthesis procedure was 36.8%; mp 146 ~ 153°C (lit.[4] mp 130°C); $[\alpha]_{589}^{20}$ +114.6° (c=0.86, chloroform); TLC (silica gel–benzene with 20% acetone) showed a single spot (Rf 0.47); UV spectrum: λ_{max} (EtOH) 288 nm (ε 3820) and 295 nm (ε 3660); ^{1}H-NMR (DMSO-d_6): δ 0.651 (s, 3H, 18-CH_3), 6.56 (s, 1H, 4-H), 7.45 (s, 1H, 1-H); mass spectrum: m/z, 398 (M^+).

Anal. Found: C, 54.28; H, 5.57; O, 8.03. Calcd. for $C_{18}H_{23}O_2I$: C, 54.28; H, 5.82; O, 8.03%.

4-Iodoestradiol (**3**). Peak M-2 (13.6 ml) was collected and condensed to 2 ml *in vacuo* and recrystallized from methanol–water to give 6.6 mg (33%) of analytically pure 4-iodoestradiol (**3**). The final yield of **3** through the synthesis procedure was 21.5%; mp 165.0 ~ 171.5°C; $[\alpha]_{589}^{20}$ +61.5° (c=0.18, chloroform); TLC (silica gel–benzene with 20% acetone) showed a single spot (Rf 0.40); UV spectrum: λ_{max} (EtOH) 284 nm (ε 2780) and 292 nm (ε 2740); ^{1}H-NMR (DMSO-d_6): δ 0.647 (s, 3H, 18-CH_3), 6.68 (d, 1H, J=8.53 Hz, 2-H), 7.12 (d, 1H, J=8.53 Hz, 1-H); mass spectrum: m/z, 398 (M^+).

Anal. Found: C, 54.25; H, 5.76; O, 8.01. See above for the calcd. value.

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