*Anal.* Calcd for $C_{18}H_{15}N_3O_4$: C, 64.15; H, 4.49; N, 12.47. Found: C, 63.93; H, 4.24; N, 12.54.

**Reaction of Pyridoxal with KCN at pH 6.0. 2,3*H*-2-Imino-3-hydroxy-4-(hydroxymethyl)furo[2,3-*c*]pyridine (4).**—To a solution of KOAc (1.0 g) and KCN (1.0 g) in water (10 ml), glacial acetic acid was added until pH 7.0 was reached (*ca.* 0.9 ml). Now pyridoxal HCl (0.5 g) was added to the solution, lowering the pH to about 6.0. The solution became darker and started depositing crystals. After cooling for 2 hr in ice, the resulting acetate salt of **4** was filtered, washed with acetone and ether, and dried. The yield was 0.49 g (79%): mp 149° dec (the compound could not be recrystallized, since it readily decomposes to 4-pyridoxic acid lactone); nmr (DMSO-$d_6$) 114 ($CH_3$, ionic acetate), 147 (2-$CH_3$), 298 (4-$CH_2$CH), 476 ($C_7$ H); ir $\lambda_{max}^{KBr}$ 1650 (C=N), 1750 $cm^{-1}$ (ionic acetate); uv $\lambda_{max}^{DMSO}$ 315 nm ($\epsilon$ 5700); $\lambda_{max}^{0.1\ N\ HCl}$ 249 nm ($\epsilon$ 8150), 352 (18,900); $\lambda_{max}^{pH\ 7.0}$ 251 nm ($\epsilon$ 6800), 357 (8350).

*Anal.* Calcd for $C_9H_{11}N_2O_3{}^+CH_3COO^-$: C, 51.98; H, 5.55; N, 11.08. Found: C, 52.25; H, 5.56; N, 11.08.

**Picrate of 4.**—With picric acid, **4** forms a violet picrate, mp 176° dec.

*Anal.* Calcd for $C_{15}H_{13}N_5O_{10}$: C, 42.55; H, 3.10; N, 16.54. Found: C, 42.84; H, 3.27; N, 16.80.

**Hydrobromide of 4.**—To a solution of **4** (acetate salt, 220 mg) in water (2.0 ml), 48% HBr (1.0 ml) was added. After a short time, brown crystals precipitated. The reaction mixture was kept in a refrigerator for 2 hr and filtered, and the precipitate was recrystallized from a mixture of ethanol and petroleum ether: mp 165–170° dec.

*Anal.* Calcd for $C_9H_{11}BrN_2O_3$: C, 39.30; H, 4.03; N, 10.19. Found: C, 39.41; H, 3.96; N, 10.50.

**Quinoxaline Derivative (7).**—Initially the reaction was carried out as for **5**, using the same amounts of reagents. After the addition of KCN (pH 7.4), about 5 min was allowed to elapse before *o*-phenylenediamine (0.56 g, 5.15 mmol) was added. Then the pH was lowered to 5 with 6 *N* HCl, and the reaction mixture was left standing in the cold for 4 hr. Since no precipitate formed, the pH was raised to 7.5 with NaOH solution, and the reaction mixture was left standing in a refrigerator overnight. The next day, 0.58 g of product had precipitated. The product was then washed with ethanol and ether. Additional material was isolated from the mother liquors, increasing the yield to 0.72 g (51%): mp 228–230° dec; tlc $R_f$ 0.3 (9:1 $CHCl_3$–$CH_3OH$); the Gibbs test gives a green spot, which changes to purple on standing; nmr (DMSO-$d_6$) 151 (2-$CH_3$), 265.5 (5-$CH_2OH$), 469–492 (multiplet of unresolved quinoxaline phenyl protons and of $C_6$ H of pyridoxine), 534 (quinoxaline diazine C–H); uv $\lambda_{max}^{EtOH}$ 215, 243, 323, 390 nm; $\lambda_{max}^{0.1\ N\ HCl}$ 211, 240, 260, 305–325 nm; $\lambda_{max}^{0.1\ N\ NaOH}$ 222, 239.5, 312, 386 nm.

*Anal.* Calcd for $C_{15}H_{13}N_3O_2\cdot 0.5H_2O$: C, 65.20; H, 5.10; N, 15.20. Found: C, 65.44; H, 5.07; N, 14.94.

The *p*-nitrobenzenesulfonyl derivative of the preceding compound was prepared by treating the compound (90 mg, 0.33 mmol) in ice-cold pyridine (4 ml) with *p*-nitrobenzenesulfonyl chloride (73 mg, 0.37 mmol). After the reaction mixture had been left standing overnight, pyridine was evaporated *in vacuo*, the residue was dissolved in chloroform, and the solution was washed with a bicarbonate solution and water. After drying ($Na_2SO_4$) and evaporating off the $CHCl_3$, the oil was dissolved in ethanol. The yield was 24 mg (30%): mp 186–188°; tlc (in ethyl acetate, $R_f$ 0.5) gave one spot, which was Gibbs-negative, indicating substitution on the phenolic hydroxyl. When the tlc plate was sprayed with an NaOH solution and heated, the Gibbs test was positive, indicating hydrolysis of the sulfonate ester. The nmr spectrum is consistent with the structure.

*Anal.* Calcd for $C_{21}H_{16}N_4O_6S$: C, 55.74; H, 3.56; N, 12.38. Found: C, 56.60; H, 3.70; N, 12.17.

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**Registry No.**—**1a**, 66-72-8; **4**, 41203-44-5; **4** picrate, 41203-45-6; **4** acetate salt, 41203-46-7; **4** HBr, 19839-38-4; **5**, 41203-47-8; **5** HBr, 41203-48-9; **5** triacetate, 41203-49-0; **7**, 41203-50-3; **7**, *p*-nitrobenzenesulfonyl derivative, 41296-58-6; KCN, 151-50-8; *p*-nitrobenzenesulfonyl chloride, 98-74-8.

**Supplementary Material Available.**—Figure 2 and a table of structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JOC-73-3793.

## 6α- and 6β-Hydroxyestradiol. Circular Dichroism and Substantiation of Configurational Assignments

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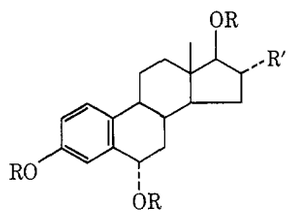
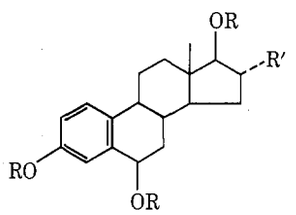
Recently we reported[2] the stereospecific syntheses of 6α- and 6β-hydroxyestriol[3] (**1a** and **2a**) and made configurational assignments by a comparison of the C-6 pmr signals of the respective tetraacetates (**1b** and **2b**). A broad, poorly defined triplet (spacing 8 Hz) was assigned to the pseudoaxial proton at C-6 in **1b**, while a narrow doublet (spacing 2.5 Hz) was assigned to the pseudoequatorial proton at C-6 in **2b**. The CD spectra of **1a** and **2a** show a strong and configurationally specific effect of the 6-hydroxyl substituent on the $^1L_b$ and $^1L_a$ dichroic absorption bands associated with the aromatic chromophore. While estradiol[4] and estriol each display $^1L_b$ and $^1L_a$ dichroic absorption of opposite sign (negative and positive, respectively), both CD maxima are negative for **1a** ($[\theta]_{283}^{EtOH}$ −1700 and $[\theta]_{229}^{EtOH}$ −5200) and positive for **2a** ($[\theta]_{280}^{EtOH}$ +620 and $[\theta]_{227}^{EtOH}$ +20,000). In the light of these data, a report[5] that the ORD spectrum of 6β-hydroxyestradiol (**2c**) shows a weak negative Cotton effect centered at 272 nm and a strong positive Cotton effect centered at 221 nm is puzzling. Since the C-16 hydroxyl group is so remote as to have little effect on the CD absorption within either the $^1L_b$ or the $^1L_a$ band,[6] one would predict that **2c** should display two positive Cotton effects, in strict analogy to **2a**.

The synthesis of the two epimeric 6-hydroxyestradiols, mp 239–249 and 191–195°, was reported by Wintersteiner and Moore in 1959,[7] and the higher melting triol was tentatively assigned the thermodynamically more stable 6α configuration on the basis of its method of preparation. It was noted, however, that the observed order of elution of the two triacetates **1d** and **2d** from alumina was opposite to that expected

(1) Supported by NIH Grant HD-05797.
(2) E. P. Burrows, D. L. Di Pietro, and H. E. Smith, *J. Org. Chem.*, **37**, 4000 (1972).
(3) Estriol is the trivial name for 1,3,5(10)-estratriene-3,16α,17β-triol.
(4) Estradiol is the trivial name for 1,3,5(10)-estratriene-3,17β-diol.
(5) P. Crabbé and W. Klyne, *Tetrahedron*, **23**, 3449 (1967).
(6) G. Snatzke and P. C. Ho, *Tetrahedron*, **27**, 3645 (1971).
(7) O. Wintersteiner and M. Moore, *J. Amer. Chem. Soc.*, **81**, 442 (1959).




1a, R = H; R′ = OH
b, R = Ac; R′ = OAc
c, R = R′ = H
d, R = Ac; R′ = H




2a, R = H; R′ = OH
b, R = Ac; R′ = OAc
c, R = R′ = H
d, R = Ac; R′ = H

on the basis of this assignment. The ORD spectrum of **2c** was reported by Crabbé and Klyne in 1967.[5] This sample of **2c** was obtained from Wintersteiner, but no physical constants or other spectral data were given for the material.

In order to remove these uncertainties and to establish unambiguously the configurations of the 6-hydroxyestradiols, we obtained pure samples of **1c** and **2c** by the methods and criteria described for **1a** and **2a**.[2] The physical constants of these samples are in good agreement with those reported earlier,[7] and the pmr spectra of triacetates **1d** and **2d** (Table I) demonstrate that the higher melting and the lower melting triols are **1c** and **2c**, respectively, in accord with the original tentative assignments. The CD spectra of triols **1c** and **2c** (Table II) are strictly analogous to those of **1a** and **2a**. The observed consistency of the Cotton effects (both negative for **1a** and **1c**, both positive for **2a** and **2c**) suggests a reliable means of configurational identification of minute amounts of the 6-hydroxyestradiol and the 6-hydroxyestriol isolated as metabolites in rats[8] and humans.[9]

TABLE I

PMR DATA FOR 6-HYDROXYESTRADIOLS IN CHLOROFORM-*d*

| Compd | δ, ppm[a] C-6 H | C-17 H[b] | C-3 $CH_3CO$[c] | C-6 $CH_3CO$[c] | C-17 $CH_3CO$[c] |
|---|---|---|---|---|---|
| 6α-Hydroxyestradiol triacetate (1d) | 6.08[d] | 4.74 | 2.28 | 2.13 | 2.05 |
| 6β-Hydroxyestradiol triacetate (2d) | 6.08[e] | 4.74 | 2.28 | 2.05 | 2.05 |

[a] Chemical shift downfield from TMS. [b] Multiplet. [c] Singlet. [d] Triplet, spacing 8 Hz. [e] Doublet, spacing 3 Hz.

### Experimental Section

Melting points were taken in open capillary tubes and are corrected. Tlc systems (silica gel HF-254) were 9:1 $C_6H_6$–EtOAc (system 1) or 4:1 $C_6H_6$–MeOH (system 2). Pmr spectra were determined with a JEOL MH-100 spectrometer and uv spectra with a Cary Model 14 spectrophotometer. CD spectra were measured using a Cary Model 60 spectropolarimeter equipped with a CD Model 6001 accessory.

**6-Oxoestradiol (4).**—Estradiol diacetate (4.83 g, 13.6 mmol) was oxidized as described previously for estriol triacetate[2] using $CrO_3$ (4.08 g, 40.8 mmol) in glacial HOAc (42 ml) and $H_2O$ (3.5 ml). The mixture of products (4.25 g) was combined with 4.77 g from a similar oxidation and chromatographed on 200 g of silica gel as described previously to give 1.13 g (10%) of crude 6-oxoestradiol diacetate (**3**), pure by tlc ($R_f$ 0.6, system 1). A sample recrystallized from MeOH had mp 170–171° (lit.[10] mp 173–175°). A solution of **3** (1.13 g, 3.05 mmol) in 0.5 *N* 95% methanolic KOH (12 ml) was allowed to stand for 10 hr at room temperature and then was evaporated to near dryness. The residue was diluted with $H_2O$ and acidified with 5% HCl, yielding **4** (0.77 g, 89%), pure by tlc ($R_f$ 0.5, system 2). A sample recrystallized from MeOH had mp 267–268° (lit.[10] mp 281–283°).

**6β-Hydroxyestradiol (2c).**—A solution of **4** (100 mg, 0.349 mmol) in absolute EtOH (15 ml) was hydrogenated for 18 hr over Pt (from 45 mg of $PtO_2$). Tlc of the residue after filtration through Celite and evaporation of the filtrate to dryness revealed a major component ($R_f$ 0.4, system 2) which was isolated by preparative tlc. This sample of **2c** (38 mg, homogeneous to tlc) would not crystallize but was converted to the readily crystalline triacetate **2d**: mp 173–176°; $[\alpha]^{25}D$ + 56° (*c* 1.05, absolute EtOH) [lit.[7] mp 176–178°; $[\alpha]^{24}D$ + 53° (*c* 0.860, $CHCl_3$)]. Alkaline hydrolysis (0.2 *N* 95% methanolic KOH) then yielded crystalline **2c**, which after recrystallization from MeOH melted at 125–135° followed by resolidification and melting at 195–200°, $[\alpha]^{25}D$ + 31° (*c* 0.64, absolute EtOH) [lit.[7] mp 126–134° followed by resolidification and melting at 191–195°, $[\alpha]^{25}D$ +29° (*c* 0.487, EtOH)].

**6α-Hydroxyestradiol (1c),** purchased from Steraloids, was homogeneous to tlc ($R_f$ 0.4, system 2): mp 230–235°; $[\alpha]^{25}D$ +85° (*c* 1.09, absolute EtOH) [lit.[7] mp 233–246°, $[\alpha]^{23}D$ +78° (*c* 0.746, EtOH)]. Acetic anhydride–pyridine treatment gave the triacetate **1d**: mp 141–143°; $[\alpha]^{25}D$ +35° (*c* 1.05, absolute EtOH) [lit.[7] mp 143–144°, $[\alpha]^{23}D$ +40° (*c* 0.944, $CHCl_3$)].

TABLE II

SPECTRAL DATA FOR ESTRADIOL DERIVATIVES IN ABSOLUTE ETHANOL[a]

| Compd | Uv max, λ, nm (ε) | CD max, λ, nm ([θ]) |
|---|---|---|
| 6α-Hydroxyestradiol (1c) | 288[b] (2000) | 289 (−1300) |
| | 282 (2200) | 283 (−1300) |
| | 229[b] (5900) | 230 (−3300) |
| | 222 (7700) | |
| 6β-Hydroxyestradiol (2c) | 288[b] (1900) | 288 (+500) |
| | 282 (2100) | 280 (+600) |
| | 228[b] (5900) | 228 (+20,000) |
| | 221 (7400) | |
| 6-Oxoestradiol diacetate (3) | | 366 (+850) |
| | | 352 (+4000) |
| | | 338 (+8300) |
| | | 326 (+10,000) |
| | 298 (2100) | 296 (−15,000) |
| | 247 (10,000) | 247 (−15,000) |
| 6-Oxoestradiol (4) | 327 (3000) | 345 (+22,000) |
| | | 311 (−20,000) |
| | 256 (8900) | 252 (−10,000) |
| | 222 (20,000) | 223 (+26,000) |

[a] *c* 0.0043–0.021 g/100 ml; *l* = 1 cm; temperature 25°. [b] Shoulder.

**Registry No.**—**1a**, 7291-49-8; **1c**, 1229-24-9; **1d**, 6626-42-2; **2a**, 36615-04-0; **2c**, 3583-03-7; **2d**, 6944-48-5; **3**, 3434-45-5; **4**, 571-92-6.

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## An Intramolecular Rearrangement Involving Neighboring Ether Oxygen

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This paper describes a molecular rearrangement involving neighboring-group participation by an ether oxygen. While investigating carbenoid reactions from