*Journal of Chromatography*, 130 (1977) 129–138


CHROM. 9211

# PREPARATION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF IODINATED DIETHYLSTILBESTROLS AND SOME RELATED STEROIDS*

DUSICA MAYSINGER, CAROLE S. MARCUS and WALTER WOLF

*School of Pharmacy, Radiopharmacy Program, University of Southern California, Los Angeles, Calif. 90033 (U.S.A.)*

and

MARKO TARLE** and JOSEPH CASANOVA

*Department of Chemistry, California State University at Los Angeles, Los Angeles, Calif. 90032 (U.S.A.)*

(First received March 15th, 1976; revised manuscript received April 20th, 1976)

## SUMMARY

Diethylstilbestrol and estradiol have been converted to their mono- and poly-iodinated derivatives by electrophilic substitution of positive iodine into the activated aromatic rings. Both chemical and electrochemical procedures have been employed for the iodination, and the latter procedure proved to be superior. These iodinated derivatives have been purified by preparative high-performance liquid chromatography, and characterized by ultraviolet and mass spectroscopy.

## INTRODUCTION

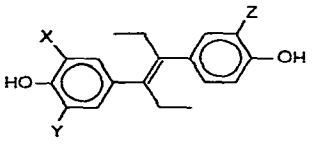
The role of steroid hormones and of their analogs in the treatment of various types of cancer has been well established[1], and repeated suggestions have been made that some of these same agents may also be of interest in the diagnosis of some such disorders. Tubis *et al.*[2] had proposed radioiodinated diethylstilbestrol diphosphate for tumor diagnosis, and prepared the labeled agent by $^{131}$I-Cl addition to the central double bond. While the preliminary results were somewhat disappointing, recent work on the *in vitro* binding of [$^{3}$H]estradiol to estrogen receptors in tumor tissue as a predictor of tumor response to hormone therapy has reawakened interest in such steroids. Other authors have also reported, parallel to this work, interest in related radioiodinated steroids and analogs for tumor diagnosis and therapy[3–6].

The iodine atom is bulky, and the addition of I-Cl to the central double bond of diethylstilbestrol removes the conjugation between the two benzene rings, and it may also affect the stereochemistry of this molecule. Inasmuch as the spatial configu-

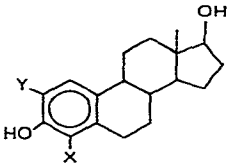
* Preliminary report of electrochemical iodination[1].

** To whom correspondence should be addressed.

ration of diethylstilbestrol is critical to steroidometic activity, loss of such a conformation may explain the rather poor results attained by previous authors. A program was instituted to synthesize radioiodinated diethylstilbestrol whose conformation was to be retained, and the present report summarizes the work performed on the synthesis, isolation, purification, and identification of iodinated diethylstilbestrol. During the course of this work, high-performance liquid chromatography (HPLC) became a technique of choice, allowing also for the measurement of other physicochemical parameters. The present report is the first of a series dealing with the synthesis, chemical and biological properties, and potential clinical interest of diethylstilbestrol analogs. Moreover, quantitative measurements of iodinated diethylstilbestrol and estradiol make possible rapid and accurate determination of hydrophobic partition coefficients. Calculations of log *P* values for these compounds based upon the calibration graphs and retention volumes reported herein will be published elsewhere.




| | *X* | *Y* | *Z* |
|---|---|---|---|
| **1** | H | H | H |
| **6** | I | H | H |
| **7** | I | H | I |
| **8** | I | I | I |




| | *X* | *Y* |
|---|---|---|
| **2** | H | H |
| **3** | I | H |
| **4** | H | I |
| **5** | I | I |

## EXPERIMENTAL

### *Chemicals*

*trans*-Diethylstilbestrol (**1**) and 17$\beta$-estradiol (**2**) were reagent grade (Sigma, St. Louis, Mo., U.S.A.). *trans*-Diethylstilbestrol was crystallized from benzene seven times to remove the *cis* isomer[7]. The purity of both compounds was checked by NMR, IR spectroscopy and thin-layer chromatography (TLC), and HPLC prior to use. **1** and **2** were stored in the dark at +6°. All chemicals used in the synthesis were reagent grade. Acetonitrile used as a solvent in electrochemical oxidations was purified as previously described[8]. Tetraethylammonium fluoroborate was commercially available (Southwestern Analytical Chemicals, Austin Texas, U.S.A.) and was used without further purification. Degassed reagent-grade chloroform served as an eluant in analytical and preparative HPLC. Spectrograde chloroform was washed with water, dried over sodium sulfate and distilled before use for quantitative HPLC determination of **1**, **2**, and their iodinated analogues.

### *Preparation of 4-iodoestradiol (**3**), 2-iodoestradiol (**4**), 2,4-diiodoestradiol (**5**), iododiethylstilbestrol (**6**), diiododiethylstilbestrol (**7**), and triiododiethylstilbestrol (**8**) using the electrochemical method and the chemical procedure*

N-Iodoacetonitrilium ion, $CH_3C{\equiv}NI^+$, was generated on a platinum gauze anode in a three-compartment cell as described previously by Miller *et al.*[9]. The cell compartment volumes were: 75 ml anode, 15 ml cathode, and 2–3 ml reference. Anhydrous acetonitrile and 0.2 *M* tetraethylammonium fluoroborate were used as solvent and supporting electrolyte, respectively. Electrooxidation was carried out at

a controlled potential* of 2.2–2.4 V (*vs.* saturated calomel electrode). The reaction stoichiometry was followed chronoamperometrically**. The solvent was preoxidized at 2.4–2.6 V until the residual current had dropped to approximately 1 mA. An amount of iodine equivalent to the amount of substrate to be oxidized (2–5 mg-atom), in the appropriate stoichiometry, was oxidized using a working potential of 2.4–2.6 V. Initial currents were 600–900 mA, and current was passed until the solution was pale yellow. At this time the current had fallen to 10–15 mA. The total current passed varied from run to run, but was between 1.6–1.8 mF/mg-atom of iodine. The pale yellow anolyte was transferred to an ethanolic solution of **1** or **2**. Iodination of substrates occurred rapidly. The solvent was removed under reduced pressure and a solid residue was taken in ethyl acetate. The organic layer was washed with water and saturated sodium chloride solution. After drying the solution over magnesium sulfate, solvent was removed and reaction products were examined by TLC and HPLC.

Chemical iodination of **1** and **2** was performed in a manner described by Katzenellenbogen *et al.* for hexestrol iodination[10]. To a stirred ethanolic solution containing substrate (**1** or **2**) and ammonia, iodine was added dissolved in absolute ethanol or in dry tetrahydrofuran. The addition was dropwise over a 1-h period. The resulting solution was worked up as described in the iodination of **1** and **2** via N-iodoacetonitrilium ion. Product analysis was performed by TLC and HPLC.

*Thin-layer chromatography*

TLC of **1–8** and their mixtures was performed on 20 cm × 20 cm plates uniformly covered with silica gel [Eastman-Kodak (Rochester, N.J., U.S.A.) Chromagram sheet with fluorescent indicator No. 6060]. Plates were deactivated by prewashing in absolute ethanol prior to use. Samples were applied with a micropipette and chromatograms were developed in equilibrated tanks at room temperature to a height of 18 cm. Several solvent systems were used, *viz.* chloroform, methylene chloride, ethyl acetate–benzene (1:1), and benzene–methanol (9:1). Neither benzene nor benzene–butanol (1:1) as solvents gave satisfactory separations. Spots were visualized with a UV lamp and/or with iodine vapors.

*High-pressure liquid chromatography*

A constant flow pump M-6000 (Waters Assoc., Milford, Mass., U.S.A.) was used to deliver chloroform as an eluant at a pressure of 6000 p.s.i. and at a rate of 1 ml/min (analytical performance) or 3 ml/min (preparative procedure) to a prepacked column (1 ft. × 1/8 in., analytical; 4 ft. × 3/8 in., preparative) of μ-Porasil (Waters Assoc.). Silica gel columns were conditioned by passing dry degassed chloroform at high flow-rate for several hours. A low-pressure mercury photometric detector (254 nm) and a refractive index detector (Waters Assoc.) were used. The liquid chromatograph was connected to a recorder with a sensitivity selector (1–1000 mV) (Linear Instrument). Outputs from both detectors were obtained in all measurements. Injections were made with 10-μl, 20-μl, 50-μl and 250-μl high-pressure syringes in the U6K septumless injector (Waters Assoc.). Retention volumes were calculated from

* PAR Model 373 Potentiostat-Galvanostat, Princeton Applied Research Corp., Princeton, N.J., U.S.A.

** Simpson Multirecorder 605, Simpson Electric Co., Chicago, Ill., U.S.A.

the point of injection. The void volume from the UV cell in the detector to the outlet was determined to be 0.95 ml and it was taken into account in preparative separations. Samples of material isolated on the preparative column were examined analytically and were repurified two to three times as required to obtain at least 98% purity.

*HPLC calibration graphs*

Calibration solutions of **1–7** were prepared by dissolving the repurified samples in spectrograde chloroform. Concentrations of compounds **2**, **3**, **4**, and **5** ranging from $10^{-4}$–$10^{-5}$ moles/l were used. Concentrations of **2** and **6** were approximately ten times lower. Measurements were performed using the described liquid chromatograph at the highest sensitivity limit. Peak areas were obtained planimetrically in $mm^2$.

*Spectrometric data*

Mass spectra were recorded at 70 eV on Varian CH-5 and AEI MS-9 spectrometers. The temperature of the ion source was maintained at 200° and the sample temperature was held at 35°. An accelerating voltage of 1.7 and 2.2 kV was applied in two consecutive measurements. Molecular peaks were observed from all compounds examined, with the following relative intensities (10–20%):

**6**, 394 ($M^+$, 19%); **7**, 520 ($M^+$, 11%); **8**, 646 ($M^+$, 10%); **3**, 398 ($M^+$, 16%); **4**, 398 ($M^+$, 15%); **5**, 524 ($M^+$, 20%)

The fragmentation patterns in all mass spectra were in accordance with the proposed structures. Prominent masses corresponding to $I^+$ or $HI^+$ were not found. UV spectral data were measured using a Beckman DB-GT spectrophotometer equipped with the Beckman "$10^{11}$" recorder. Spectrometric-grade chloroform was used as a solvent as well as 95% aqueous ethanol prepared from reagent-grade absolute ethanol. Extinction coefficients at $\lambda_{max}$ were calculated from UV spectra of **1–6***:

**2**, (chl) $\lambda_{nm}$ = 239, 277, and 283 ($\varepsilon$ = 2988, 2464, and 2320); (EtOH) $\lambda_{nm}$ = 205, 218, 278, and 284 ($\varepsilon$ = 12813, 3065, 3377, and 3024)

**3**, (chl) $\lambda_{nm}$ = 237, 278, and 286 ($\varepsilon$ = 5000, 4625, and 4500); (EtOH) $\lambda_{nm}$ = 213, 226, 283, and 289 ($\varepsilon$ = 28750, 15500, 3775, and 3750)

**4**, (chl) $\lambda_{nm}$ = 237, 282, and 289 ($\varepsilon$ = 4800, 4100, and 4000); (EtOH) $\lambda_{nm}$ = 214, 230, 285, and 292 ($\varepsilon$ = 14162, 8198, 3168, and 3043)

**5**, (EtOH) $\lambda_{nm}$ = 216, 238, 284, and 294 ($\varepsilon$ = 38250, 13500, 4250, and 3875);

**1**, (chl) $\lambda_{nm}$ = 245 and 273 ($\varepsilon$ = 15800 and 8700); (EtOH) $\lambda_{nm}$ = 203, 235, and 273 ($\varepsilon$ = 28056, 23046, and 10020)

**6**, (chl) $\lambda_{nm}$ = 240, 255, and 279 ($\varepsilon$ = 16000, 15800, and 8000); (EtOH) $\lambda_{nm}$ = 217, 237, and 272 ($\varepsilon$ = 27500, 17500, and 10625).

Extinction coefficients at 254 nm were calculated for all these compounds and the results are tabulated in Table VII.

## RESULTS AND DISCUSSION

According to published literature, chemical iodination of estradiol carried out in several ways leads to the formation of **5** and one monoiodinated derivative[3,11,12] or only diiodinated product[13]. It has been found recently[14] that three iodinated compounds are formed in all of these reactions. They are **3**, **4**, and **5**. It appears that one

* Abbreviations: chl = chloroform; EtOH = ethanol.

TABLE I

HPLC PRODUCT ANALYSIS IN CHEMICAL AND ELECTROCHEMICAL IODINATION OF ESTRADIOL

$\mu$-Porasil column, 1 ft. × 1/8 in.; solvent, chloroform; flow-rate, 1 ml/min; working pressure, 1500 p.s.i.; UV detector sensitivity, 02; recorder sensitivity, 10 mV; chart speed, 0.5 in./min.

| *Compound* | $V_R$ *(ml)* | **2** *vs. iodine or* $CH_3CNI^+$ *reaction molar ratio* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Chemical iodination* | | | *Electrochemical iodination* | | |
| | | *1:0.5* | *1:1* | *1:2* | *1:0.5* | *1:1* | *1:2* |
| **2** | 13.59 | 70.3* | 40.4 | 24.6 | 69.2 | 38.6 | 20.7 |
| **3** | 7.64 | 8.4 | 13.7 | 12.9 | 7.9 | 17.0 | 19.9 |
| **4** | 6.72 | 14.2 | 21.8 | 20.1 | 18.2 | 29.1 | 34.2 |
| **5** | 5.90 | 7.1 | 24.1 | 42.4 | 4.7 | 15.3 | 25.2 |

* Relative peak area ratio, measured by a cut-and-weight method and calculated from the calibration graphs given in Figs. 1 and 2.

TABLE II

TLC $R_F$ VALUES OF ESTRADIOL, DIETHYLSTILBESTROL, AND THEIR IODINATED DERIVATIVES

Solvent systems: (A) ethyl acetate–benzene (1:1), (B) chloroform; (C) methylene chloride; (D) benzene–methanol (9:1).

| *Compound* | *Solvent system* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *A** | *A* | *B** | *B* | *C** | *C* | *D** |
| **2** | 0.59 | 0.71 | 0.29 | 0.10 | 0.32 | 0.29 | 0.72 |
| **3** | 0.71 | 0.79 | 0.47 | 0.24 | 0.58 | 0.49 | 0.83 |
| **4** | 0.76 | 0.79 | 0.56 | 0.36 | 0.74 | 0.58 | 0.83 |
| **5** | 0.81 | 0.84 | 0.73 | 0.49 | 0.80 | 0.68 | 0.85 |
| **1-*cis*** | 0.32 | | 0.04 | | | | |
| **1** | 0.55 | | 0.04 | | | | |
| **6** | 0.75 | | 0.30 | | | | |
| **7** | 0.82 | | 0.30 | | | | |
| **8** | 0.85 | | 0.30 | | | | |

* Silica gel plate prewashed in absolute ethanol.

TABLE III

RETENTION VOLUMES OF **1**, **6**, **7**, **8**, **2**, **3**, **4**, AND **5** ON A HPLC COLUMN IN CHLOROFORM

Silica gel analytical $\mu$-Porasil column, 1 ft. × 1/8 in; flow-rate 1 ml/min; applied pressure, 6000 p.s.i.; UV detector sensitivity, 02; recorder sensitivity, 3 mV.

| *Compound* | $V_R$ *(ml)* |
|---|---|
| **1** | 10.586 ± 0.020 |
| **6** | 6.260 ± 0.002 |
| **7** | 5.092 ± 0.016 |
| **8** | 3.714 ± 0.012 |
| **2** | 13.586 ± 0.020 |
| **3** | 7.636 ± 0.028 |
| **4** | 6.716 ± 0.020 |
| **5** | 5.902 ± 0.022 |

of the monoiodinated products was previously unobserved in TLC analyses, probably due to the use of improper solvent systems. Our investigations were directed towards developing the most facile method for the separation of iodinated estradiols as well as the most suitable method for their detection and quantitative analysis. Therefore, two different synthetic routes for estradiol iodination were examined to ascertain that which would lead to more selective monoiodination. Significantly greater biological activity has been achieved with monoiodinated estradiols **3** and **4** in comparison with **5**, so that a selective monoiodination is highly desirable. Electrochemical and chemical procedures were applied and molar ratios of **2** *vs.* iodine were systematically varied. The resulting product mixtures were analyzed by HPLC and TLC (Tables I and II).

It appears that the electrochemical procedure is slightly more selective than the chemical procedure with respect to estradiol monoiodination, but a small amount of **5** is still found. Isolation of **3**, **4**, and **5** from the reaction mixtures obtained by both the chemical and the electrochemical process yielded 30–40% of crude products. Usually two to three further consecutive preparative separations by HPLC were sufficient to permit the isolation of a reasonably pure product (>98% purity). Recyclization procedures applied in these separations gave identical results. The retention volumes of **1**, **3**, **4**, and **5** are given in Table III.

A chemical method for tetraiodination of **1** was described earlier[15] and that procedure was used in the attempted synthesis of **7** (ref. 16). Efforts to prepare pure polyiodinated diethylstilbestrol using this method were unsuccessful in our hands and a complex mixture of products was observed on TLC and HPLC. The same product composition was obtained using the chemical procedures described in Experimental[10]. The assignments of signals from HPLC and spots observed on TLC in product analysis are shown in Tables IV and II, respectively. Variations in the ratio of **1** to iodine failed to furnish more information about the reaction products.

In contrast, the electrochemically generated $I^+$ complex led to the formation of four major reaction products as long as the ratio of iodine was 1:2 or lower (Table V). More complex mixtures were obtained using larger amounts of iodine, although previously observed major products were still present in substantial amounts. Compounds corresponding to the major peaks from liquid chromatograms were isolated on a preparative column. Their purification was carried out as described in Experimental. Possible decomposition of iodinated diethylstilbestrols caused by UV light during preparative repurifications was avoided by using only the refractive index detector. Iodine content and molecular formulas of these products came from mass spectral data and made the structural assignments possible. UV spectra confirmed the phenolic character of isolated products. UV bands corresponding to quinones or fused aromatic structures were not found. Slight bathochromic shift in absorption maxima observed in **6** (in comparison with **2**) is consistent with the auxochromic effect of the iodine atom.

The retention volumes of **2**, **6**, **7**, and **8** are listed in Table III. These values correlate qualitatively with the expected decrease in acidity and with the additivity of the Hansch $\pi$ value accompanying progressive iodination. The reaction mixtures obtained from the chemical and electrochemical iodination of **1** contained several variable minor products having a $V_R$ value greater than **1**. The retention volumes of these substances are not listed in Tables IV and V, and their structures are not known.

TABLE IV

HPLC PRODUCT ANALYSIS IN CHEMICAL IODINATION OF DIETHYLSTILBESTROL

Silica gel analytical $\mu$-Porasil column, 1 ft. × 1/8 in.; solvent, chloroform; flow-rate, 1 ml/min; working pressure, 1500 p.s.i.; detector sensitivity, 02 UV; recorder sensitivity, 10 mV; chart speed, 0.5 in./min.

| $V_R$ (ml) | 1 *vs. iodine reaction molar ratio* | | | | |
|---|---|---|---|---|---|
| | *1:1* | *1:2* | *1:3* | *1:4* | *1:6* |
| 6.26* | 37.1** | 33.9 | 40.0 | 19.0 | 19.3 |
| 5.74 | | | | | 5.3 |
| 5.36 | 10.8 | 10.3 | 33.8 | 35.8 | 35.1 |
| 5.00 | 3.2 | 4.1 | | 1.3 | 1.1 |
| 4.82 | 7.5 | | | | |
| 4.66 | | 32.4 | | 4.8 | |
| 4.50 | 16.2 | | | | 2.3 |
| 4.36 | | | 2.0 | | |
| 4.16 | | 0.3 | 1.4 | 3.2 | 2.9 |
| 3.76 | | 1.7 | | 28.4 | |
| 3.62 | | 6.3 | 17.5 | | 19.9 |
| 3.42 | 16.1 | 0.7 | | 1.9 | |
| 3.22 | 0.8 | | 0.7 | 4.9 | |
| 3.10 | | 7.6 | 1.4 | 0.7 | 13.5 |
| 2.90 | 4.8 | 1.4 | 2.8 | | 0.6 |
| 2.78 | 2.7 | | 0.4 | | |
| 2.54 | | 1.0 | | | |
| 2.36 | 0.8 | 0.3 | | | |

* Monoiodinated diethylstilbestrol, see Table V.

** Relative peak area ratio, measured by a cut-and-weight method and calculated from the calibration graphs given in Figs. 1 and 2.

TABLE V

HPLC PRODUCT ANALYSIS IN ELECTROCHEMICAL IODINATION OF DIETHYLSTILBESTROL

Silica gel analytical $\mu$-Porasil column, 1 ft. × 1/8 in.; solvent, chloroform; flow rate, 1 ml/min; working pressure, 1500 p.s.i.; detector sensitivity, 02 UV; recorder sensitivity, 10 mV; chart speed, 0.5 in./min.

| *Product* | $V_R$ (ml) | 1 *vs.* $CH_3CNI^+$ *reaction molar ratio* | | | | |
|---|---|---|---|---|---|---|
| | | *1:1* | *1:2* | *1:3* | *1:4* | *1:6* |
| **6** | 6.26 | 57.6** | 36.4 | 34.0 | 23.2 | 16.7 |
| **7** | 5.08 | 25.1 | 45.2 | 36.0 | 24.7 | 17.9 |
| | 4.64* | | | | 3.3 | 2.0 |
| | 4.00* | | | 1.0 | 23.2 | 23.6 |
| **8** | 3.72 | 8.2 | 10.8 | 22.0 | 19.3 | 10.7 |
| | 3.52* | | | | | 19.4 |
| | 3.04* | 1.3 | 3.8 | 3.2 | 4.0 | 1.4 |
| | 2.72* | 7.8 | 3.8 | 4.0 | 2.3 | 8.3 |

* Unidentified product.

** Relative peak area ratio, measured by a cut and-weight method and calculated from the calibration graphs given in Figs. 1 and 2.

TABLE VI

QUANTITATIVE DETERMINATION OF PROPORTIONS* OF **2**, **3**, **4**, **5**, **1**, AND **6** ON HPLC

Silica gel $\mu$-Porasil column, 4 ft. × 3/8 in.; eluent, chloroform; working pressure, 2000 p.s.i.; detector sensitivity, 02 UV; recorder sensitivity, 3 mV; chart speed, 0.5 in./min.

| *Volume ($\mu$l)* | *Relative peak area ($mm^2$)* | | | | | |
|---|---|---|---|---|---|---|
| | **2** | **3** | **4** | **5** | **1** | **6** |
| 25 | 19.1 | 49.3 | 33.0 | 35.0 | 19.4 | 30.9 |
| 50 | 39.3 | 99.5 | 67.0 | 69.3 | 39.7 | 61.5 |
| 100 | 78.7 | 197.0 | 133.4 | 140.5 | 79.4 | 123.7 |
| 150 | 120.0 | 297.0 | 202.2 | 209.5 | 121.8 | 191.0 |
| 250 | 199.0 | 493.0 | 333.9 | 349.2 | 201.1 | 314.2 |
| 500 | 395.0 | 988.2 | 675.0 | 704.5 | 400.4 | 620.1 |

* Concentrations: (**2**) 4.982 × $10^{-5}$ moles/l, 13.55 ng/$\mu$l; (**3**) 3.719 × $10^{-5}$ moles/l, 14.8 ng/$\mu$l; (**4**) 2.488 × $10^{-5}$ moles/l, 9.90 ng/$\mu$l; (**5**) 1.25 × $10^{-6}$ moles/l, 6.55 ng/$\mu$l; (**1**) 1.8 × $10^{-6}$ moles/l, 0.482 ng/$\mu$l; (**6**) 2.25 × $10^{-6}$ moles/l, 0.8865 ng/$\mu$l.

TLC of **1**, *cis*-**1**, **6**, **7**, and **8** was performed with several solvent systems. The only solvent system which led to satisfactory separation was ethyl acetate–benzene (1:1) (Table II). However, preparative TLC separation using this solvent mixture was not satisfactory for product isolation. It has been found during this study that retention of iodinated diethylstilbestrols (especially polyiodinated derivatives) on silica gel results in product decomposition and formation of **1** along with several unknown compounds. HPLC, as a more rapid method, was adopted as a suitable isolation technique.

Crude reaction mixtures and purified **6**–**8** were stored at −12° in the absence of light. Even these conditions did not prevent decomposition of **8** within eight days, and **7** was partially decomposed (5–6%) during the same storage time. Compound **6** was reasonably stable under the same conditions (1–3% decomposition to diethylstilbestrol and unidentified compounds). Iodinated estradiols 3–5 could be kept at −12° for six months without any observed decomposition. However, storage at dry-ice temperature is highly recommended for the samples described here.

Table VI gives the relative peak areas of diethylstilbestrol, estradiol, and their odinated derivatives. Data for the calibration curves given in Figs. 1 and 2 were derived from these values. From the data of Figs. 1 and 2 it is apparent that the UV spectral data for **1**–**6** not only provided information on the structure of iodinated

TABLE VII

EXTINCTION COEFFICIENTS AT 254 nm FROM UV SPECTRA OF **1**, **6**, **2**, **3**, **4**, AND **5**

| *Compound* | *Solvent* | |
|---|---|---|
| | *Chloroform* | *95% aq. ethanol* |
| **1** | 12500 | 13200 |
| **6** | 15630 | 15200 |
| **2** | 457 | 1280 |
| **3** | 1470 | 1480 |
| **4** | 1570 | 1440 |
| **5** | 3360 | 3330 |

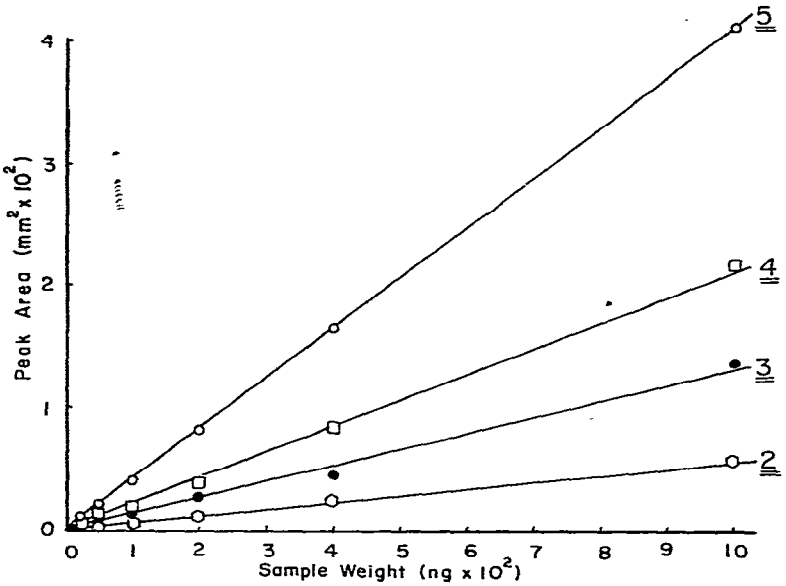



Fig. 1. Calibration graph of peak area *versus* amount of substrate (in ng) injected. **2** = Estradiol; **3** = 4-iodoestradiol; **4** = 2-iodoestradiol; **5** = 2,4-diiodoestradiol.

compounds but were used to confirm the accuracy of calibration curves derived from quantitative measurements of these compounds.

Linear relationships between values of $\varepsilon_{254}$ in chloroform (Table VII) and peak areas obtained from equimolar quantities of **1–6** (Figs. 1 and 2) were established with-

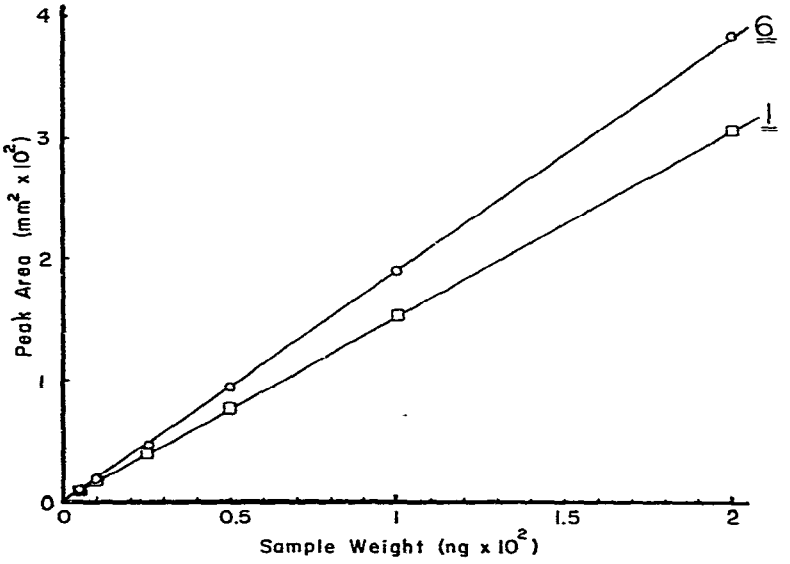



Fig. 2. Calibration graph of peak area *versus* amount of substrate (in ng) injected. **1** = *trans*-Diethylstilbestrol; **6** = monoiododiethylstilbestrol.

in an error of 0.2–1.2%. The utility of these calibration graphs was confirmed in the determination of log *P* values in the chloroform/water system for all compounds reported herein.

## CONCLUSION

It was found that chemical and electrochemical iodination of estradiol led to similar product distributions, but electrochemical preparation of iodinated diethylstilbestrol produced a less complex mixture of reaction products. Therefore, this procedure is preferable to other synthetic routes respecting the ease of product isolation. HPLC was the most convenient technique for this purpose and for the analytical determination of iodinated estrogens. Spectral data obtained on isolated iodinated products permitted their structural assignments. Calibration graphs for the quantitative determination of all pure isolated products and their parent compounds have been made. The stability of iodo derivatives of estradiol is greater than the stability of iodinated diethylstilbestrols. This work demonstrates the possibility for isolation of the most desirable monoiodinated diethylstilbestrol and estradiol derivatives, especially those that could be labelled with radioiodine for their further application in biological and clinical studies.

## ACKNOWLEDGEMENTS

The authors are indebted to Ms. Sally Wenceslao and Ms. Janet Warner, students at California State University at Los Angeles, for their technical assistance. One of us (D.M.) was a Fulbright scholar during part of this work and was partially supported by Grant 5-POI-CA-14089 of the National Cancer Institute.

## REFERENCES

1 D. Maysinger, M. Terle, C. S. Marcus, J. Casanova and W. Wolf, *Chem. Congr. N. Amer. Continent, 1st, Mexico City, Nov. 30–Dec. 5, 1975.*
2 M. Tubis, J. S. Endrow and W. H. Blahd, *Nucl. Med.*, 6 (1966) 1.
3 R. Ghanadian, S. L. Waters, M. L. Thakur and G. D. Chisholm, *Int. J. Appl. Radiat. Isot.*, 26 (1975) 1.
4 M. Miki, T. Machida, A. Ishibashi, T. Minami and T. Minami, *Jap. J. Urol.*, 61 (1970) 592.
5 Z. Szendröi, L. Kocsar, Zs. Karika, M. Fuzi, Gy. Tarjan, Gy. Reischl and S. Ickardt, *Int. Urol. Nephrol.*, 5 (1973) 311.
6 Z. Szendröi, L. Koczar, Z. Karika and S. Eckhardt, *Lancet*, 1 (1973) 1253.
7 V. W. Winkler, M. A. Nyman and R. S. Egan, *Steroids*, 17 (1971) 197.
8 J. F. Coetzee, G. P. Cunningham, D. K. McGuire and G. R. Padmanathan, *Anal. Chem.*, 34 (1962) 1139.
9 L. L. Miller, E. P. Kujawa and C. B. Campbell, *J. Amer. Chem. Soc.*, 92 (1970) 2821.
10 J. A. Katzenellenbogen, H. J. Johnson, K. E. Karlson and H. N. Meyers, *Biochemistry*, 13 (1974) 2968.
11 S. Albert, R. D. H. Heard, C. P. Leblond and J. Saffran, *J. Biol. Chem.*, 177 (1949) 247.
12 D. Exley and A. Dutton, *Steroids*, 14 (1969) 575.
13 H. Takikawa, S. Miyashita and K. Imai, *Gunma J. Med. Sci.*, 15 (1966) 15.
14 S. Archimandritis, H. E. Rowher, J. A. Berman and W. Wolf, *Chem. Congr. N. Amer. Continent, 1st, Mexico City, Nov. 40–Dec. 5, 1975.*
15 S. Bodanszky, *Chem. Zentralbl.*, 132 (1961) 2032.
16 S. Bodanszky, E. Boumassar and G. Giuliani, *Arch. Ital. Patol. Clin.*, 10 (1967) 131.