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Note

Aromatic Iodination of Diethylstilbestrol Diphosphate[1]

D. Maysinger and W. Wolf

School of Pharmacy, Radiopharmacy, University of Southern California, Los Angeles, California 90033, USA,

and

*J. Casanova and M. Tarle**

Department of Chemistry, California State University, Los Angeles, California 90032, USA

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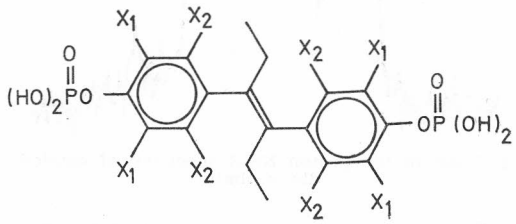
Sterically crowded estrogenic compound diethylstilbestrol diphosphate was successfully polyiodinated on the aromatic rings applying an electrochemical procedure. The reported synthesis also opens the way for the preparation of potentially useful radiolabelled cancer chemotherapeutics.

Diethylstilbestrol diphosphate **1** (DES-2P) is a synthetic estrogen which is used widely in the treatment of prostatic carcinoma. It was observed that ^{131}I-labeled DES-2P, prepared by chemical[2] and electrochemical[3] methods, accumulates both in the human prostate and in some methastatic tissue. Sterically crowded but moderately activated DES-2P can be radioiodinated using weak eletrophiles to give low specific activity of the labeled substance[2,3]. One of us, (D. M.) has found[4] that DES-2P cannot be satisfactorily iodinated using the chemical method adequate for hexestrol polyiodination[5]. Employing the electrochemical method used for iodination of simple unhindered aromatic rings[6], we have synthesised tetraiodo DES-2P(**2**) and octaiodinated diethylstilbestrol diphosphate (**3**). Control over the amount of iodine incorporated could be achieved by varying the iodine/DES-2P mole ratio.



1 (DES-2P) $X_1 = X_2 = H$
2 (DES-2P-4I) $X_1 = I$, $X_2 = H$
3 (DES-2P-8I) $X_1 = X_2 = I$

* Correspondance should be addressed to this author at Clinic of Nuclear Medicine and Oncology, Clin. Hospital »Dr. M. Stojanović«, 41000 Zagreb.

The ^{13}C NMR spectrum of **3** did not show signals from aromatic non-protonated carbon atoms probably due to ^{127}I quadrupole peak broadening and lower nuclear Overhouser enhancement. Carbon-13 spin-lattice relaxation time for this kind of nuclei is extremly long[7]. In our studies peaks corresponding to aromatic carbons in **3** have not been seen even when the time delay of 50 seconds was inserted after the data aquisition following each pulse. Chemical shifts of ethyl groups signal in proton NMR spectrum of **3** are in accord with both inductive and resonance effects of eight iodine atoms located on aromatic rings. The methylene groups signal from **3** was observed as the simple quartet while the peak corresponding to methyl groups was split further into three sharp lines with the coupling constant $J = 2$ Hz (Figure 1).

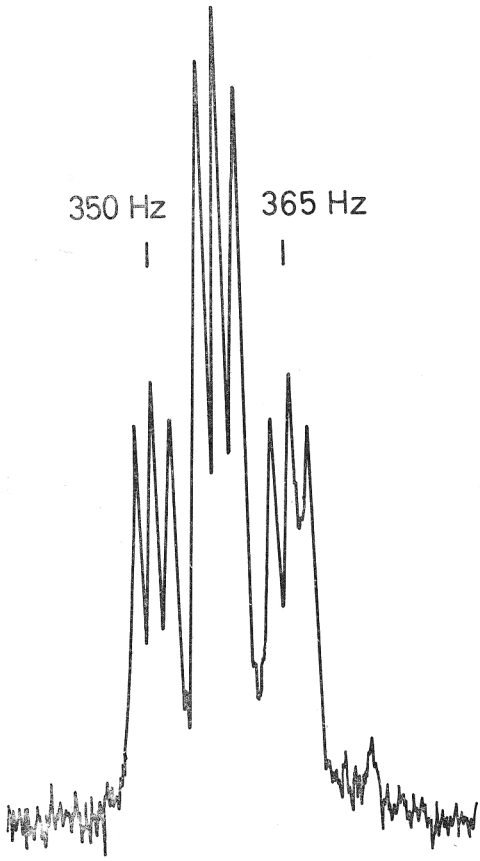


Figure 1. Methyl groups signal in the proton NMR spectrum of octaiodinated diethylstilbestrol diphosphate

The magnitude of this coupling constant was independent upon the strength of the external field. Decoupling of CH_3 groups signal with methylene protons causes the collapse of the former to a line still split into three others of equal hights ($J = 2$ Hz). The heteronuclear spin decoupling by irradiating the sample at the frequency equal to the resonance frequency of phosphorus did not change the original spectrum.

Since this highly unusual splitting is field independent, it appears to be a coupling and not a manifestation of slow conformational equilibria. However, we cannot offer an adequate explanation for this multiplet pattern observed for the methyl groups in the octaiodinated diethylstilbestrol diphosphate[8].

Attempts to shed more light on this problem by studying the NMR spectrum of octaiodo diethylstilbestrol have failed due to the instability of this compound[9]. From the NMR spectra of **2** and **3** it can be tentatively concluded that the rate of substitution is much lower on the hindered and less activated carbons *meta* to the phosphate groups.

The present synthetic procedure is not only interesting as a chemical method for the preparation of sterically hindered polyiodinated aromatic systems, but also opens the way for the synthesis of the potentially useful radiolabelled compounds.

EXPERIMENTAL

Proton NMR spectra were obtained at 60 and 100 MHz on a Varian Model A-60 or on a Varian HA-100 spectrophotometer, respectively, using alkaline D_2O solutions (pH = 10) with dioxane or methylene chloride as internal standards. Carbon-13 NMR spectra came from a Brucker HX-90 spectrometer. Infrared spectra were recorded on a Beckman IR-12 spectrophotometer. Analytical high-pressure liquid chromatography was performed on a Model ALC/GPC 244 Liquid Chromatograph (Waters Associates) using a μ-Porasil (silica gel), 1 ft × 0.125 inch column and hexane-absolute ethanol 9:1 as a solvent. Working potential in exhaustive electrolysis was held constant by means of a PAR Model 373 Potentiostat-Galvanostat. Coulometric data were obtained from Simpson Multirecorder 605. Polarographic analyses were performed using a PAR 174-A Polarographic Analyzer.

Electrooxidation of iodine (4 mM) was carried out in the three compartment cell at a controlled potential of 2.4—2.6 V (*vs* saturated calomel electrode) on a platinum gauze anode. Using 70 ml of anhydrous acetonitrile and 0.2 M tetraethylammonium fluoroborate the *N*-iodoacetonitrilium ion $CH_3—\overset{+}{C}=NI$ was produced[6]. Reaction progress was followed chronoamperometrically. Transfer of the pale yellow anolyte to an ethanolic solution of **1** (1 mM) resulted in an instant formation of brown color. After removal of solvent *in vacuo*, the solid residue was repeatedly extracted with absolute ethanol. Crude iodinated compound obtained from the extracts by evaporation was converted to its salt in aqueous NaOH (pH = 10). Acidification of the aqueous layer (pH = 2) gave a pale brown precipitate purified further through centrifugation[2] and drying in high vacuum, 40—60%, mp. 147 °C (decomp)[10].

Anal. $C_{18}H_{14}I_8O_8P_2$ (1435.14) calc'd.: C 15.05; H 0.98; I 70.74%
found: C 15.21; H 1.11; I 70.48%

Octaiodinated product showed in the proton NMR spectrum signals corresponding to methyl groups (δ 1.17, m, 6 H, J_{AB} = 7.5 Hz, J_{BC} = 2 Hz) and methylene protons (δ 3.18, q, 4 H, J_{AB} = 7.5 Hz) but no peaks in aromatic region. Each line of the methyl triplet was split further into three lines of equal heights. Diethylstilbestrol diphosphate showed different chemical shifts and splitting patterns (δ 0.74, t, 6 H, J_{AB} = 7.5 Hz; δ 2.19, q, 4 H, J_{AB} = 7.5 Hz; δ 7.15, s, 8H). The change in the reaction molar ratio of **1** and iodine to 1:2 yielded tetraiodo DES-2P (2). In the proton NMR spectrum of **2** a singlet of aromatic protons was seen (δ 7.15, 4 H) as well peaks corresponding to methyl (δ 1.15, t, 6 H, J_{AB} = 7.5 Hz) and methylene groups (δ 3.17, q, 4 H, J_{AB} = 7.5 Hz). In the carbon-13 NMR spectrum of **1** peaks corresponding to carbons of the conjugated π-system (δ 120.6, 130.0, 137.2, 139.2 and 153.0), methyl (δ 13.98) and methylene (δ 29.19) carbons were observed. Octaiodinated **3** gave in ^{13}C NMR spectrum peaks of methyl (δ 7.48) and methylene (δ 52.82, broad) carbons. Signals corresponding to the conjugated aromatic skeleton were not present in the spectrum.

The heteronuclear decoupling on phosphorus and homonuclear decoupling on the frequency corresponding to the resonance frequency of aromatic protons (in 1)

did not change the original proton NMR spectrum of **3**. The double resonance NMR experiment performed on the octaiodinated parent showed, after irradiation of methylene protons, the signal of methyl groups as three lines ($J_{BC} = 2$ Hz) of equal hights.

The C-I bond streching vibrations of **3** were seen in the far ir spectrum at 510 and 540 cm^{-1} (nujol mull).

Paper chromatography of **2** and **3** was performed using the upper layer of a mixture *n*-butanol:acetic acid:water (4:1:5) as a solvent. Spots were visualized by spraying chromatograms with phenolphtalein solution and 0.02% aq. sodium hydroxide. Rf values corresponding to both **2** and **3** were within the range 0.30—0.35. These data together with the Rf values of the starting material (0.31—0.35) and diethylstilbestrol itself (0.92—0.95), confirm that both phosphate groups were retained in the iodinated substrates. Liquid chromatogram of **3** showed two peaks: v_R 1.30 ml (rel area 99.65%) and 1.48 ml (0.35%). The latter signal corresponds to diethylstilbestrol diphosphate.

Polarographic studies of DES-2P and its periodinated derivative were carried out in DMF and Et_4NBF_4. DES-2P shows two polarographic waves ($E_{1/2}{}^1 = -1.58$ V, $E_{1/2}{}^2 = -2.07$ V *versus* sce) due to reduction of the conjugated aromatic system. The polarogram of **3** exhibits three waves ($E_{1/2}{}^1 = -0.24$ V, $E_{1/2}{}^2 = -1.96$ V, $E_{1/2}{}^3 = -2.45$ V). We have assigned the first wave to the reduction of vicinal C-I bonds[11], while the other two waves are assigned to the reduction of aromatic nuclei.

REFERENCES

1. Preliminary report on electrochemical iodination: D. Maysinger, M. Tarle, C. Marcus, J. Casanova, and W. Wolf, paper presented at *The 1st Chemical Congres of the North American Continent*, Mexico City, 1975.
2. M. Tubis, J. S. Endow, and W. H. Blahd, *Nucl. Medizin*, **6** (1966) 1.
3. Z. Szendroi, L. Koczar, Z. Karika, and S. Eckhardt, *Lancet*, **1** (1973) 1253.
4. D. Maysinger, *Ph. D. Thesis*, University of Southern California, Los Angeles, 1976.
5. J. A. Katzenellenbogen, H. J. Johnson, K. E. Karlson, and H. N. Myers, *Biochem.*, **13** (1974) 2986.
6. L. L. Miller and B. F. Watkins, *J. Amer. Chem. Soc.*, **98** (1976) 1515.
7. G. C. Levy, *Chem. Commun.*, (1972) 47.
8. The explanation of the 2 Hz coupling as an unusual through space coupling by a Fermi contact mechanism appears untenable. Iodine 127 has a spin quantum number of 5/2, which implies that coupling to a single iodine by a simple Fermi contact interaction would result in a sextet.
9. D. Maysinger, C. S. Marcus, W. Wolf, M. Tarle, and J. Casanova, *J. Chromat.* **130** (1977) 129.
10. The product was stable in the form of the sodium salt after storing at 6 °C for four months.
11. S. Wawzonek and J. H. Wagenknecht, *J. Electrochem. Soc.*, **110** (1963) 420.

SAŽETAK

Aromatsko jodiranje dietilstilbestrol-difosfata

D. Maysinger, W. Wolf, J. Casanova i M. Tarle

Sintetski estrogen dietilstilbestrol-difosfat uspješno je polijodiran na aromatskim prstenima u reakciji s elektrokemijski generiranim *N*-jodoacetonitrilijevim ionom. Opisana sinteza otvara put za pripravu preparata markiranih radioaktivnim jodom, koji se mogu primijeniti u kemoterapiji tumora.

SCHOOL OF PHARMACY, RADIOPHARMACY,
UNIVERSITY OF SOUTHERN CALIFORNIA,
LOS ANGELES, CALIFORNIA 90033
DEPARTMENT OF CHEMISTRY, CALIFORNIA
STATE UNIVERSITY, LOS ANGELES,
CALIFORNIA 90032

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