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Improved delivery through biological membranes. XXII *. Synthesis and distribution of brain-selective estrogen delivery systems

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Summary

1,4-Dihydrotrigonelline derivatives of the estrogens, estradiol (E_2), estrone (E_1) and estradiol-3-methyl ether ($3MeE_2$) were synthesized as potential brain-selective delivery forms for these steroids. Initial in vivo screening showed that the estradiol chemical delivery system (E_2CDS) was most appropriate for further in vivo study. Accordingly, when E_2CDS was systemically administered to rats, sustained levels of the oxidized, trigonelline ester delivery system (E_2Q^+) were found in the brain while peripheral levels of E_2Q^+ rapidly fell. In addition, radioimmunoassay (RIA) and spectroscopic methods detected E_2 in the brain after administration of E_2CDS. The undetectable levels of E_2 in the blood suggest that the E_2 found in the central nervous system (CNS) was derived from centrally delivered E_2Q^+ and not from peripheral sources.

Introduction

Synthetic estrogens such as those incorporated in oral contraceptives or those used in the treatment of postmenopausal estrogen withdrawal are relatively safe and effective. Several factors, however, argue for the development of other estrogenic agents. First, the use of oral contraceptives has been associated with increased incidence of cancer, thromboembolic episodes, hypertension and altered metabolism (Ross, 1985; Kaplan, 1978; Silverberg and Makawski, 1975). Postmenopausal use of estrogens has been linked to increased incidence of cystic hyperplasia of the endometrium, a premalignant condition (Ross, 1985). Some reports attribute these deleterious side-effects to the synthetic estrogenic component of steroid combinations used to treat these conditions. Arguments have been made that the neural steroids lack some or all of the detrimental characteristics responsible for these side effects (Astedt, et al., 1977; Mahesh, et al., 1977). The inclusion of natural estrogens into these preparations is not indicated since their metabolic lability both in the gut and in the periphery leads to unpredictable and inconsistent biological results (Bolt, 1979;

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Spona and Schneider, 1977).

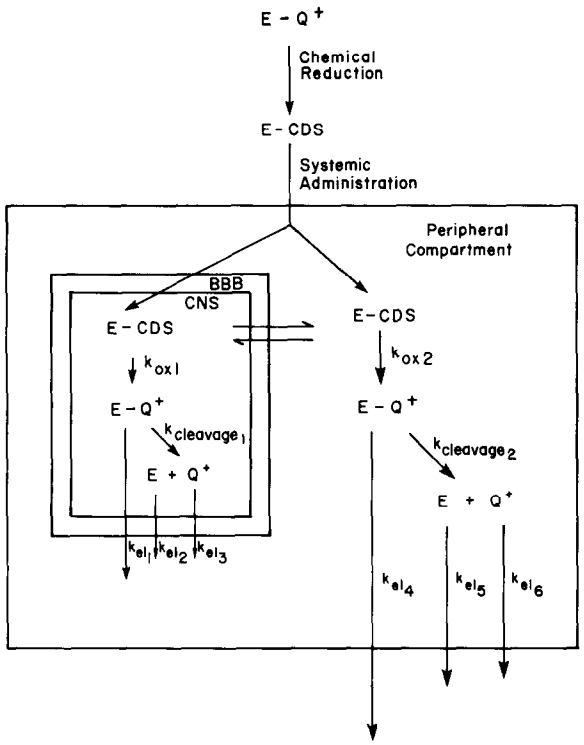
Second, evidence suggests that the primary locus of action of estrogens acting as contraceptives or acting to counter the effects of estrogen withdrawal syndrome is in the central nervous system (CNS) (Goodman and Knobil, 1984; Kalra and Kalra, 1983; Barraclough and Wise, 1982). More definite statements concerning this subject are difficult to make because of the inability to non-invasively administer estrogen specifically to the periphery or specifically to the CNS. In any case, estrogens can exert contraceptive action by interacting at the hypothalamus, a structure inside the blood–brain barrier (BBB), to affect luteinizing hormone releasing hormone (LHRH) secretion, or at the anterior pituitary, a structure outside the BBB, to alter luteinizing hormone (LH) secretion. Both or either of these events may interfere with the ovulatory cascade to result in contraception. In postmenopausal women the depletion of estrogen can give rise to a number of CNS-mediated events including vasomotor "hot flushing" (Lauritzen, 1973). Estrogens are effective in alleviating these symptoms (Upton, 1984).

Third, steroid hormones are generally highly fat-soluble and demonstrate large log P values (Leo et al., 1971). While this high lipophilicity allows these steroids to easily pass into the CNS through the BBB to exert their effects, there is little to prevent their loss from the CNS, and consequently they are not retained. This then forces either frequent dosing of the steroids to maintain therapeutically important levels in the brain or the administration of a depot form of estrogen. This latter method is not ideal since it is not possible to easily stop the estrogen administration once it has begun. In addition the time of onset of estrogenic action is variable.

Based on these 3 factors: (1) estrogens, especially synthetic metabolically-stable estrogens, may have dangerous peripheral actions; (2) the main site of action of contraceptive estrogens and estrogens used to treat "hot flushing" is probably within the CNS; and (3) estrogens are not retained in the CNS after peripheral administration, the possibility of delivering naturally occurring estrogens with some selectivity to the CNS was examined. The method chosen to address this problem was the dihydropyridine $\rightleftarrows$ pyridinium salt redox system (Bodor et al., 1981) which has clearly been demonstrated to accomplish brain-specific delivery when applied to a number of pharmacologically active species including phenethylamine (Bodor and Farag, 1983a), dopamine (Bodor and Farag, 1983b) and testosterone (Bodor and Farag, 1984). This delivery system has recently been reviewed (Bodor and Brewster, 1983; Bodor, 1985).

The chemical delivery system (CDS) is based on the facile interconversion of a lipophilic dihydropyridine and a hydrophilic pyridinium salt, similar to the endogenous NADH $\rightleftarrows$ NAD^+ system, and on the peculiar characteristics of the BBB. The capillaries which make up the cerebral vasculature differ from capillaries found in the periphery in that cerebral capillaries are tightly joined to one another (Rapoport, 1976; Pardridge et al., 1975). This prevents materials from moving between the capillaries and forces compounds to move *through* the lipoidal cell membranes if they are to gain access or leave the brain parenchyma. This barrier restricts the passage of molecules into and out of the brain to highly lipophilic compounds although specialized mechanisms are available for the movement of nutrients, metabolic wastes and other polar species (Pardridge, 1981). In addition, the bulk flow of cerebrospinal fluid (CSF) provides a slow avenue of escape for compounds in the CNS (Schanker, 1965).

The CDS is carrier-mediated in that a molecular carrier must be attached to the agent to be transported. The carrier chosen for the present application, as well as for many others, is the non-toxic natural product 1-methylnicotinic acid or trigonelline. In practice, the steroid is condensed with nicotinic acid and further quaternized to give the trigonellinate (EQ^+). This quaternary salt is highly polar. The EQ^+ is then chemically reduced to give the 1-methyl-1,4-dihydronicotinate steroid or the estrogen-chemical delivery system (ECDS). This species is expected to be more lipoidal than the parent steroid and many times more lipoidal than the corresponding EQ^+. The ECDS is administered systemically as illustrated in Scheme I. The dihydropyridine, because of its high lipophilicity, partitions into the brain and many other body locations represented in Scheme



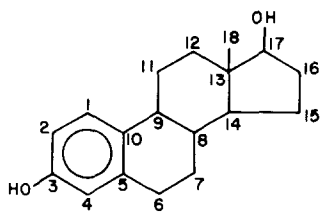
Scheme I

I as one peripheral compartment. The metabolically unstable ECDS is then ubiquitously oxidized to the hydrophilic EQ^+ which is rapidly lost from the periphery (i.e. $k_{el_4} \gg k_{cleavage_2}$). In the brain, however, the polar species is trapped within the BBB, that is k_{el_1} is slow. In the CNS, the EQ^+ will slowly hydrolyse to yield the parent steroid and the small trigonelline carrier (i.e. $k_{el_1} < k_{cleavage_1}$). This carrier can be rapidly eliminated from the CNS because of the occurrence of active molecular pumps which have specificity for this type of molecule while the steroid is left to exert its effect ($k_{el_3} > k_{el_1}$) either at the hypothalamus or via the hypothalamic pituitary portal system at the pituitary.

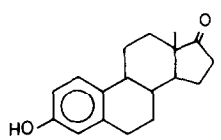
Previous studies have shown that 17-esters of estradiol interact poorly with the estrogen receptor and, thus, predict that both ECDS and EQ^+ are not estrogenic (Dusterberg and Nishino, 1982). Estrogenic effects obtained by using this system are, therefore, assumed to be due to the free estrogen.

This method as outlined allows for: (1) the direct and specific delivery of naturally occurring estrogen to the CNS in the form of an inactive carrier; (2) rapid clearance of this species from the periphery; and (3) slow hydrolysis of the inactive EQ^+ to release the active species in the CNS. Pharmacological ramifications of these effects include decreased peripheral and central toxicity, increased efficacy and decreased dosing interval.

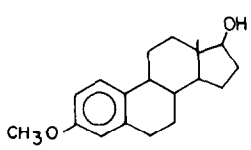
Three estrogens were chosen for initial examination. They were estradiol (estra-1,3,5 (10)-triene,3,17-diol, E_2), estrone (3-hydroxyestra-1,3,5(10)-triene-17-one, E_1) and estradiol-3-methyl ether (3-methoxyestra-1,3,5(10)-triene-17-ol, $3MeE_2$).



β - Estradiol



Estrone



Estradiol-3-methyl ether

Estradiol is the most potent natural estrogen (Murad and Haynes, 1980). Chemically it is a diol containing a phenolic 3-moiety and a 17-alcohol group. Three possible delivery systems could therefore be considered, the 3-ester, the 17-ester or the 3,17-bis ester. All of these compounds should behave differently kinetically due to the nature of

the ester(s) formed. Estrone is a less potent estrogen than E_2 but can be biochemically transformed to E_2 by 17β-hydroxysteroid dehydrogenase (Bolt, 1979). In E_1, the 17-hydroxy group of E_2 is oxidized to the 17-ketone so that only a delivery system based on the 3-ester is possible. Estradiol-3-methyl ether is demethylated in vivo to give the active E_2 (Willams et al., 1976). This demethylation is mediated by mixed function oxidases in the liver. In the case of $3MeE_2$, only the 17-position is free for esterification. These three steroids, therefore, allow examination of both chemical properties of the delivery systems, i.e. phenolic versus alkyl esters, and the pharmacological properties of the various estrogens involved. It was the aim of this work to synthesize various of the possible delivery systems and then screen them using in vitro tests for one which would be subjected to more detailed physiochemical and pharmacokinetic studies.

Materials and Methods

Chemistry

Elemental analyses of compounds synthesized were performed by Atlantic Microlabs, Atlanta, GA. Uncorrected melting points were determined using either a Mel-Temp or Electrothermal melting point apparatus. Ultraviolet spectroscopy (UV) was performed on either a Cary 210 or Hewlett Packard 8451A Diode array spectrophotometer. For kinetic analysis an Apple II Plus microprocessor equipped with kinetic software was dedicated to the Cary 210 instrument. Infrared spectra (IR) were recorded on either a Backman Acculab 1 or Microlab 620 MX spectrophotometer. Samples were analyzed as potassium bromide pellets or as a thin film on sodium chloride windows. Nuclear magnetic resonance spectra (NMR) were recorded on either a Varian T60 or EM360A spectrometer. The samples were dissolved in an appropriate deuterated solvent and chemical shifts (δ) reported relative to an internal standard (tetramethylsilane, TMS). Thin-layer chromatography (TLC) was performed on Redi-Plate silica gel plates coated to a thickness of 0.25 mm. All chemicals were of reagent grade. Steroids were obtained from Sigma Co. Pyridine was routinely distilled over CaH_2 before use.

3-(Chlorocarbonyl)pyridinium hydrochloride (1). To nicotinic acid (41g, 0.33 mol) at 0°C were slowly added 115 ml (1.58 mol) of thionyl chloride. The mixture was heated to reflux while stirring for 1 h. The white crystalline material produced was collected by filtration and washed with cold dry benzene. Excess thionyl chloride was azeotroped off with dry benzene. Yield 90% (53.67 g). Physical data were identical with the literature (Chemical Dynamics, 1984).

3,17β-Bis[(3-pyridinylcarbonyl)oxy]estra-1,3,5(10)-triene (2). To (**1**) (5.3 g, 0.03 mol) in 30 ml of dry pyridine at 0°C were added 2 g (0.0073 mol) of β-estradiol. After refluxing for 1 h, the mixture was poured over 100 ml of ice water and the resulting precipitate collected by filtration. The precipitate was dried over P_2O_5 in vacuo. Yield 90% (3.18 g); M.p. 148–150°C. UV (MeOH) 222, 262 nm. IR (KBr) 1750, 1725 ν (2 C=O stretch). NMR ($CDCl_3$) [1] δ 9.2–9.0 (br s, 2H, C-2,2′ pyridine protons); 8.7–8.3 (m, 2H, C-6,6′ pyridine protons); 8.4–8.0 (m, 2H, C-4,4′ pyridine protons); 7.5–7.1 (m, 3H, C-5,5′ pyridine protons + C-1 E_2 proton); 6.9–6.7 (m, 2H, C-2,4 E_2 protons); 5.0–4.7 (t, 1H, C-17α E_2 proton); 3.2–1.3 (m, 15, skeletal E_2 protons); 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{30}H_{31}N_2O_4$. Theory: C, 74.50; H, 6.47; N, 5.79. Found: C, 74.40; H, 6.32; N, 5.75.

3-Hydroxy-17β-[(3-pyridinylcarbonyl)oxy]estra-1,3,5(10)-triene (3). To (**2**) (0.5 g, 0.0010 mol) were added 60 ml of a 0.5% $KHCO_3$ solution in 95% aqueous methanol. The suspension was allowed to stir overnight at room temperature. After addition of 60 ml of H_2O, the suspension was extracted repeatedly with chloroform. The organic layer was separated, dried over $MgSO_4$, and removed in vacuo. The resulting pinkish solid was suspended in cold methanol and filtered to give 0.36 g (94%) of the desired product. M.p. 216–217°C. UV(MeOH) 222, 264 nm. IR (KBr) 3500–3000 ν (phenolic OH stretch), 1735–1725 ν (C=O stretching); NMR ($CDCl_3$) 9.1–8.9 (d, 1H, C-2 pyridine proton); 8.7–8.5 (dd, 1H, C-6 pyri-

[1] Primes indicate protons on the 3-nicotinic acid ester (i.e. the phenolic ester).

dine proton); 8.2–8.1 (dt, 1H, C-4 pyridine proton); 7.4–6.9 (m, 3H, C-5 pyridine proton + C-1 E_2 proton + phenol OH (exchangeable with D_2O)); 6.7–6.4 (m, 2H, C-2,3 E_2 protons); 5.1–4.7 (m, 1H, C-17α E_2 protons); 3.0–1.2 (m, 15, E_2 protons); 1.0–0.9 (s, 3H, C-18 E_2 proton). Anal. $C_{24}H_{27}NO_3$. Theory: C, 76.36; H, 7.22; N, 3.71. Found: C, 76.20; H, 7.25; N, 3.70.

*1-Methyl-3-{[(3-hydroxyestra-1,3,5(10)triene-17β-yl)oxy]carbonyl}pyridinium iodide (**4**, E_2Q^+).* To (**3**) (2.095 g, 0.006 mol) in 200 ml of acetone were added 2 ml (0.032 mol) of methyl iodide. The solution refluxed overnight yielding a yellow solid which was collected by filtration, washed and dried in vacuo. The yield was 83% (2.42 g) M.p. 255–261°C (dec.). UV (MeOH) 222, 268 nm. IR (KBr) 3600–3150 ν (phenolic OH stretch), 1740 ν (C=O stretch). NMR (d_6-DMSO) 9.2–9.0 (s, 1H, C-2 pyridinium proton); 9.0–8.5 (m, 3H, C-4,6 pyridinium protons + phenolic OH (exchangeable)); 8.2–7.8 (m, 1H, C-5 pyridinium proton); 7.0–6.7 (m, 1H, C-1 E_2 proton); 6.5–6.2 (m, 2H, C-2,4 E_2 proton); 5.0–4.7 (t, 1H, C-17α E_2 proton); 4.5–4.3 (s, 3H, N-CH_3); 2.9–1.2 (m, 15H, E_2 skeletal proton); 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{25}H_{29}NO_3I$. Theory: C, 57.92; H, 5.65; N, 2.70. Found: C, 57.70; H, 5.73; N, 2.68.

*3-Hydroxy-17β-{[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}estra-1,3,5(10)-triene (**5**, E_2CDS).* To (**4**) (1.09 g, 0.0021 mol) in 150 ml of 50:50 *t*-butanol: H_2O was added 1.06 g (0.0126 mol) of $NaHCO_3$ and 1.46 g (0.0084 mol) of $Na_2S_2O_4$. The mixture stirred at 0°C under N_2 for 1 h. The solution was then extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and reduced in vacuo yielding a yellow foam. Yield 64% (0.24 g). M.p. 115–130°C (dec.) UV (MeOH) 222, 220, 356 nm. IR (KBr) 3600–3150 ν (phenolic OH stretching), 1700 ν (C=O stretching). NMR ($CDCl_3$) 7.0–6.8 (m, 2H, C-1 E_2 proton + C-2 pyridine proton); 6.7–6.4 (m, 2H, C-2,4 E_2 protons); 5.7–5.4 (br d, 1H, C-6 pyridine proton); 5.0–4.5 (m, 3H, C-17α E_2 proton + C-5 pyridine proton + phenolic OH (exchangeable)); 3.2–3.0 (m, 2H, C-4 pyridine protons); 3.0–2.9 (s, 3H, N-CH_3); 2.8–1.1 (m, 15H, E_2 skeletal protons); 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{25}H_{30}NO_3 \cdot 1/2\ H_2O$. Theory: C, 74.59; H, 8.03; N, 3.48. Found: C, 74.57; H, 8.04; N, 3.40.

*1,1′-Dimethyl-3,3′-{[(estra-1,3,5(10)-trien-3,17β-diyl)dioxy]dicarbonyl}dipyridinium diiodide (**6**, $E_2(Q^+)_2$).* Two grams of (**2**) (0.004 mol) were added to 50 ml of acetone and 2 ml (0.032 mol) methyl iodide. The solution was heated at reflux overnight. The resulting precipitate was filtered, washed with acetone and dried. The yield was 88% (2.75 g). M.p. 251–252°C. UV (MeOH) 222, 268 nm. NMR ($CDCl_3$ + d_6-DMSO) 9.87–9.73 (bs, 1H, C-2′ pyridinium proton); 9.6–8.90 (m, 5H, C-2,6,6′,4,4′ pyridinium protons); 8.57–8.17 (m, 2H, C-5, 5′ pyridinium protons); 7.60–7.0 (m, 3H, C-1,2,4 E_2 protons); 5.23–4.8 (bt, 1H, C-17α E_2 proton); 4.73–4.4 (bs, 6H, 2N$^+$-CH_3); 3.10–1.10 (m, 15H, E_2 skeletal protons); 1.10–0.97 (s, 3H, C-18 E_2 protons). Anal. $C_{32}H_{39}N_2O_4I_2$. Theory: C, 50.13; H, 4.70; N, 3.66; I, 33.16. Found: C, 50.04; H, 4.75; N, 3.56; I, 33.09.

*1-Phenylmethyl-4-(aminocarbonyl)-1,2-dihydropyridine (**7**).* Compound (**7**) was synthesized according to the literature (Nuvole et al., 1984). 1-Benzylisonicotinamide bromide was prepared by quaternizing isonicotinamide with benzyl bromide. Ten grams of 1-benzylisonicotinamide bromide were added to a solution of 50 ml of methanol and 25 ml of 2 M NaOH at −25°C in N_2 atmosphere. To this solution was then added 0.323 g (0.0085 mol) of $NaBH_4$. The solution darkened and after 30 min was filtered yielding a greenish crystalline material. The solid gave appropriate spectral analysis and was used without further purification. The material was stored at −90°C under argon. UV (MeOH) 370 nm.

*3,17β-Bis{[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}estra-1,3,5(10)-triene (**8**, $E_2(CDS)_2$).* One gram (1.31 mmol) of (**6**) was dissolved in 100 ml of dry acetonitrile. To this solution, which was flushed with N_2, were added 0.28 g (1.31 mmol) of (**7**) and the reaction stirred at 0°C for 1 h. The solvent was then removed under reduced pressure leaving a solid. The solid was suspended in methylene chloride, filtered, and the filtrate chromatographed several times on a neutral alumina column prepared with methylene chloride. A solid foam was obtained after purification and evaporation of the solvent in vacuo. UV (MeOH) 216, 364 nm. NMR ($CDCl_3$) 7.23–7.10 (br s, 2H, C-2,2′

pyridine protons); 6.97–6.73 (m, 3H, C-1,2, 4 E_2 protons); 5.77–5.53 (dt, 2H, C-6,6′ pyridine protons); 4.93–4.57 (m, 3H, C-5,5′ pyridine protons and C-17α E_2 proton); 3.27–3.03 (bs, 4H, C-4,4′ protons); 3.00–2.73 (d, 6H, 2N-CH_3); 2.53–1.13 (m, 15H, E_2 skeletal protons); 0.97–0.77 (s, 3H, C-18, E_2 protons). Anal. $C_{32}H_{38}N_2O_4$. Theory: C, 74.71; H, 7.39; N, 5.45. Found: C, 74.60; H, 7.21; N, 5.33.

*3-[(3-Pyridinylcarbonyl)oxy]estra-1,3,5(10)-trien-17-one (**9**).* To (**1**) (2.65 g, 0.015 mol) in 20 ml of pyridine at 0°C were added 2.0 g (0.0073 mol) of estrone. After refluxing for 1 h, the mixture was poured over ice water and the resulting precipitate filtered and dried in vacuo. The yield was 72% (2.01 g). M.p. 207–210°C. UV (MeOH) 220, 266 nm. IR (KBr) 1750–1730 ν (2 broad C = 0 stretches). NMR ($CDCl_3$) 9.3–9.10 (br s, 1H,C-2 pyridine proton); 8.8–8.6 (br d, 1H, C-6 pyridine proton); 8.4–8.2 (dt, 1H, C-4 pyridine proton); 7.5–7.1 (m, 2H, C-5 pyridine proton + C-1, E_1 proton); 7.0–6.7 (m, 2H, C-2, 4 E_1 protons); 3.2–1.3 (m, 15H, E_1 skeletal protons); 1.0–0.9 (s, 3H, C-18, E_1 protons). Anal. $C_{24}H_{25}NO_3$. Theory: C, 76.76; H, 6.72; N, 3.73. Found: C, 76.37; H, 6.96; N, 3.76.

*1-Methyl-3-{[(estra-1,3,5(10)-triene-17-one-3-yl) oxy]carbonyl}pyridinium iodide (**10**, E_1Q^+).* One ml of methyl iodide (0.016 mol) was added to 0.5 g (0.0013 mol) of (**9**) in 20 ml of acetone. The solution was heated at reflux overnight. The precipitate formed was filtered, washed with acetone and dried. The yield was 0.62 g or 90% M.p. 245–249°C (dec.). UV (MeOH) 222, 268 nm. IR (KBr) 1755–1740 ν (2 broad C=O stretches). NMR (d_6-DMSO) 9.8–9.7 (br, s 1H, C-2 pyridinium proton); 9.4–9.0 (m, 2H, C-4, 6 pyridinium protons); 8.4–8.0 (m, 1H, C-5 pyridinium proton); 7.4–7.2 (m, 1H, C-1, E_1 proton); 7.1–6.9 (m, 2H, C-2,4 E_1 protons); 4.6–4.4 (s, 3H, N^+-CH_3); 3.2–1.3 (m, 15H, skeletal E_1 protons); 1.0–0.9 (s, 3H, C-18 E_1 protons). Anal. $C_{25}H_{28}NO_3I$. Theory: C, 58.03; H, 5.47; N, 2.71. Found: C, 58.16; H, 5.51; N, 2.67.

*3-{[(1-Methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}estra-1,3,5(10)-trien-17-one (**11**, E_1CDS).* To (**10**) (0.6 g, 1.16 mmol) in 50:50 aqueous methanol were added 0.58 g (7.0 mmol) of $NaHCO_3$ and 0.98 g (4.6 mmol) of $Na_2S_2O_4$. The mixture stirred at room temperature for 2 h and was then extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and removed in vacuo to give 0.303 g (67%) of a yellow solid. M.p. 130–180°C (dec.). UV (MeOH) 360 nm. IR (KBr) 1745–1740 ν (2 C=O stretches). NMR ($CDCl_3$) 7.2–7.0 (m, 2H, C-1 E_1 proton + C-2 pyridine proton); 6.8–6.6 (m, 2H, C-2,4 E_1 protons); 5.8–5.3 (m, 1H, C-6 pyridine proton) 5.0–4.6 (m, 1H, C-5 pyridine proton); 3.2–3.0 (m, 2H, C-4 pyridine protons); 3.0–2.8 (s, 3H, N-CH_3); 2.5–1.2 (m, 15H, E_1 skeletal protons); 1.0–0.9 (s, 3H, C-18 E_1 protons). Anal. $C_{25}H_{29}NO_3 \cdot 1/2H_2O$. Theory: C, 74.96; H, 7.56; N, 3.50. Found: C, 75.44; H, 7.27; N, 3.38.

*3-Methoxy-17β-[(3-pyridinylcarbonyl)oxy]estra-1,3,5(10)-triene (**12**).* To (**1**) (3.15 g, 0.017 mol) in 20 ml of pyridine at 0°C were added 2 g (0.007 mol) of estradiol-3-methyl ether. After refluxing for 1 h, the solution was poured into 100 ml of ice water, the solid formed has then collected by filtration and dried in vacuo. The yield was 2.07 g (76%) M.p. 140–142°C. UV (MeOH) 222, 266 nm. IR (KBr) 1725 ν (C=O stretch). NMR ($CDCl_3$) 9.3–9.0 (br s, 1H, C-2 pyridine proton); 8.8–8.6 (m, 1H, C-6 pyridine proton); 8.4–8.1 (br d, 1H, C-4 pyridine proton); 7.5–7.0 (m, 2H, C-5 pyridine and C-1 E_2 proton); 6.8–6.5 (m, 2H, C-2,4 E_2 proton); 5.1–4.7 (m, 1H, C-17α E_2 proton); 3.8–3.6 (s, 3H, O-CH_3); 3.0–1.2 (m, 15H, E_2 skeletal protons); 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{25}H_{29}NO_3$. Theory: C, 76.68; H, 7.48; N, 3.58; Found: C, 76.49; H, 7.50; N, 3.55.

*1-methyl-3-{[(3-methoxyestra-1,3,5(10-triene-17β-yl)oxy]carbonyl}pyridinium iodide (**13**, $3MeE_2Q^+$).* To 1.0 ml of methyl iodide (0.016 mol) in 20 ml acetone were added 1.5 g of (**12**) (0.0038 mol) and the solution refluxed overnight. The precipitate obtained was isolated by filtration, washed and dried in vacuo. The yield was 1.6 g or 76%. M.p. 230–234°C (dec.). UV (MeOH) 224, 268 nm. IR (KBr) 1745 ν (C=O stretching). NMR (d_6-DMSO) 9.5–9.3 (br s, 1H, C-2 pyridinium proton); 9.2–8.8 (m, 2H, C-4,6 pyridinium protons); 8.3–8.0 (m, 1H, C-5 pyridinium proton); 7.2–7.0 (m, 1H, C-1 E_2 proton); 6.8–6.5 (m, 2H, C-2,4 E_2 protons); 5.2–4.8 (m, 1H, C-17α E_2

proton); 4.6–4.4 (s, 3H, N^+-CH_3); 3.8–3.6 (s, 3H, O-CH_3); 3.0–1.2 (m, 15H, E_2 skeletal protons); 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{26}H_{32}NO_3I$. Theory: C, 58.53; H, 6.06; N, 2.63. Found: C, 58.25; H, 6.07; N, 2.54.

*3-Methoxy-17β-{[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}estra-1,3,5(10)-triene (**14**, 3Me-E_2CDS).* To (**13**) (0.6 g, 1.12 mmol) in 50% aqueous methanol were added 0.57 g of $NaHCO_3$ (6.7 mmol) and 0.78 g of $Na_2S_2O_4$. The mixture stirred for 2 h at which time it was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo. The yield was 74% or 0.34 g. M.p. 120–170°C (dec.). UV (MeOH) 360 nm. IR (KBr) 1705 ν (C=O stretch). NMR ($CDCl_3$) 7.3–7.2 (m, 1H, C-1 E_2 proton); 7.0–6.9 (s, 1H, C-2 pyridine proton); 6.8–6.6 (m, 2H, C-2,4 E_2 protons); 5.8–5.6 (m, 1H, C-6 pyridine proton); 5.0–4.6 (m, 2H, C-5 pyridine proton + C-17α E_2 proton); 3.9–3.7 (s, 3H, O-CH_3); 3.2–3.0 (m, 2H, C-4 pyridine protons); 3.0–2.8 (s, 3H, N-CH_3); 2.4–1.2 (m, 15H, E_2 skeletal protons; 1.0–0.9 (s, 3H, C-18 E_2 protons). Anal. $C_{26}H_{30}NO_3$. Theory: C, 76.61; H, 8.18; N, 3.44. Found: C, 76.75; H, 8.43; N, 3.37.

In vitro characterization

The stability of (**5**), (**11**) and (**14**) in human plasma, rat brain and liver homogenates was determined. Human blood was freshly drawn in heparinized tubes and plasma was separated by centrifugation at $3000 \times g$. The plasma was diluted 50% with pH = 7.4 phosphate buffer. Rat liver homogenate was prepared by homogenizing 2 g of freshly obtained rat liver in 5 ml of pH = 7.4 phosphate buffer and diluting the homogenate to 100 ml giving a 2% w/v suspension. Similarly 2 g of freshly obtained rat brain were homogenized with buffer and diluted to give a 4.0% w/v homogenate. In all cases, the homogenates or plasma were warmed to 37°C and then placed in a cuvette in a thermostated cell-holder. A small volume (50 μl) of a concentrated solution of the appropriate dihydropyridine was then added to the cuvette and the disappearance of the 360 nm absorbance measured. When the log of the absorbance was plotted with time, a straight line was obtained. The negative of the slope gave a pseudo-first-order rate constant.

In determining octanol–water partition coefficients (log P), 4 ml of a 1×10^{-2} M solution of E_2, E_2Q^+ or E_2CDS was prepared in octanol. This solution was partitioned against 4 ml of octanol saturated water for 2 h. After equilibration, the aqueous and organic phases were analyzed for appropriate steroid. A ratio consisting of the peak height of the steroid in octanol divided by the peak height of the steroid in water was developed. The log of this ratio was the log P. The method of analysis was HPLC, the details of which are given later.

In examining hydrolysis of E_2Q^+, 50 μl of a 5×10^{-3} M solution of (**4**) in DMSO were added to 5 ml of either a 20% rat brain homogenate, a 20% rat liver homogenate, rat plasma or whole rat blood. The homogenates were prepared using phosphate-buffered saline (pH = 7.4). In all cases the homogenates or plasma or blood was maintained at 37°C. At various times after the addition of (**4**), 100 μl of the appropriate biological matrix was removed and immediately treated with 400 μl of ice-cold acetonitrile in the case of brain, liver and plasma and 6% DMSO in acetonitrile in the case of blood. The DMSO prevents clumping of the whole blood. The samples were then centrifuged at $13{,}000 \times g$ and filtered through 0.45 μm nitrocellulose membranes. Analysis of (**4**) and E_2 is described in the following section. Compound (**4**) proved to be relatively stable in that significant changes in the HPLC peak due to E_2Q^+ did not occur during the time course of the experiment. When, however, the appearance of E_2 was assayed, a small but progressive increase in the concentration of E_2 was detected throughout the time course of the experiment (usually 2 h). Data are presented as percent formation of the E_2 from (**4**) at the termination of the experiment.

In vivo characterization

White male Sprague–Dawley rats, separated in groups of eight and weighing between 200–250 g, were anesthetized intramuscularly with Inovar (0.13 ml/kg). A dose of 60 mg/kg of E_2CDS (**5**) in dimethylsulfoxide (DMSO) was administered in the external jugular vein which had previously been exposed. At 5, 30, 60, 120, 240, 360, 480 and 1440 min after the injection, blood was collected

via heart puncture and the animals were sacrificed by decapitation. Brain, lung, liver and kidney were removed and frozen immediately. In preparing the organs for analysis, each was homogenized in 1 vol. of H_2O and then extracted with 4 ml of acetonitrile. One ml of blood was used for analysis and to this 4 ml of acetonitrile was added. The samples were centrifuged and frozen and the supernatant removed and analyzed. Standard curves were constructed by adding various concentrations of (**4**) to brain, liver, kidney, lung and blood and then treating the samples exactly as the unknowns. The linearity of the curves produced was good giving correlation coefficients greater than 0.999. In addition, no hydrolysis of (**4**) was observed in these standards. Similar standards were prepared for (**5**) to examine the stability of (**5**) during work-up. In these samples no (**4**) of E_2 was detected. The quaternary salt (**4**) was quantitated by HPLC on an Altex Ultrasphere C_{18} reverse-phase column. The mobile phase was 70% acetonitrile : 30% water containing 0.2% sodium 1-pentane sulfonate. The flow rate was 2 ml/min and the retention time of (**4**) was approximately 4 min. In analyzing the E_2CDS (**5**) or E_2, a Toya-Soda ODS-120T column was used. The mobile phase used to elute the dihydropyridine (**5**) was 90 : 10 acetonitrile : water while the parent steroid eluted in 55 : 45 acetonitrile : 0.05 M KH_2PO_4 buffer. The flow rate in these cases was 1 ml/min. The retention time under these conditions was 6 min for the E_2CDS while the free steroid eluted at 8 min. The half-life of (**4**) in each tissue was determined by a logarithmic transformation of the terminal portion of the tissue concentration versus time curve. The negative of the slope of this straight line gave the first-order rate constant. Several system configurations were used for this work. They consisted of: (1) a Waters Associate system equipped with a Model U6K injector, a Model 6000A solvent delivery system and a Model 440 absorbance detector (254 nm); (2) a Beckman system consisting of a Model 112 solvent delivery system, a Model 160 absorbance detector and a Model 421 controller; and (3) a Perkin-Elmer system containing a Perkin-Elmer Series 4 pump, a Kratos Spectroflow 757 variable wavelength detector and an LCI-100 integrator.

In analyzing rat brain and blood for E_2 produced from E_2Q^+ in vivo, two methods were employed: radioimmunoassay (RIA) and a sample pooling and concentrating HPLC technique. For the RIA method, conscious Sprague–Dawley male rats were injected with either 3 or 15 mg/kg of (**5**) in DMSO or 0.5 ml/kg DMSO i.v. (tail vein). Four rats were used in each drug treatment group while 9 were included in the vehicle control group. After 30 min, the animals were sacrificed and the brains removed and each homogenized in phosphate-buffered saline (3 ml buffer/g tissue). One ml of this homogenate from each brain was extracted 3 times with 6 ml of diethyl ether. The collected ether fractions from each brain were then evaporated at 37 °C under a stream of nitrogen. Analysis was made by RIA as previously described (V. Shille et al., 1984). The cross-reactivity of E_2Q^+ for the E_2 antibody was examined by calculating the amount of E_2 from known amounts of E_2Q^+. In addition the extraction of E_2Q^+ into ether was examined by measuring immunoreactive E_2 in samples of E_2Q^+ analyzed in the same way as the unknown.

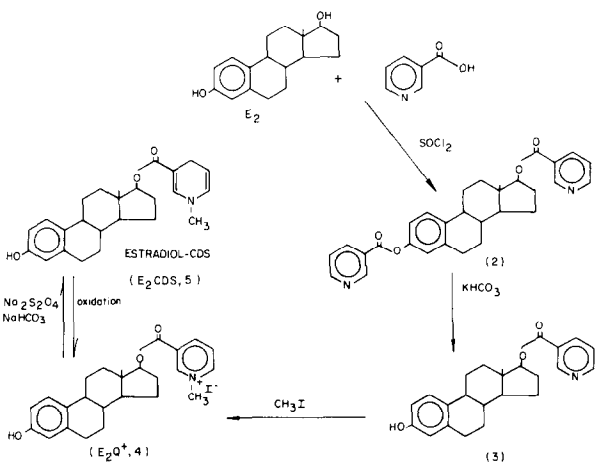
In determining E_2 concentration from animals treated with (**5**, E_2CDS) by HPLC, Sprague–Dawley male rats ($n = 6$) were injected with 30 mg/kg (**5**) in DMSO. After 30 min, the animals were sacrificed and the brains and 1 ml of blood were removed and frozen immediately on dry-ice. In preparing the tissues for analysis the brain or 1 ml of blood was homogenized in 1 ml of H_2O. To this homogenate were then added 1 ml of a saturated sodium chloride solution and 4 ml of acetonitrile. After remixing and centrifugation, the samples were maintained at −15 °C for 3 h. The separated acetonitrile layers were then removed. Extracts from blood samples were combined as were extracts from brain samples and the solvent removed under argon at 40 °C. The residue was then reconstituted with 100 μl of acetonitrile. The brain and blood reconstitutes were then centrifuged at 13,000 × *g* and filtered through 0.45 μm nitrocellulose membranes. The samples (40 μl) were then analyzed by HPLC as previously described. Standards were prepared by adding known concentrations of E_2 to brain homogenates and then treating the standards as unknowns. The limit of detec-

tion for this method was 1.5 ng/g tissue or ml blood.

Results and Discussion

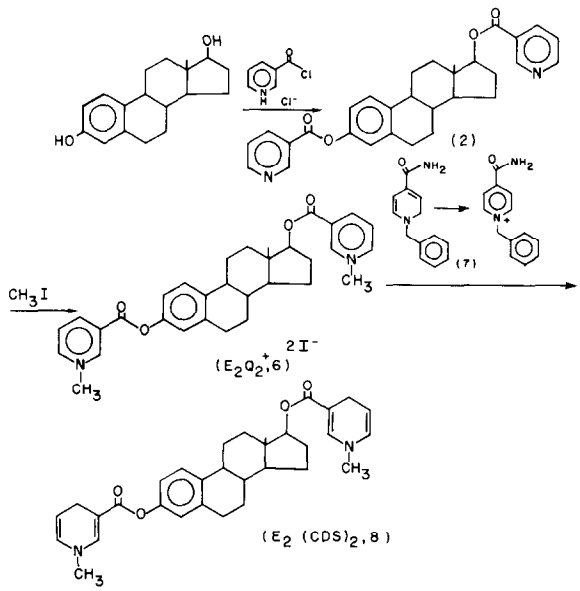
Chemistry

Two of the three possible delivery systems for E_2 were synthesized. In preparing the delivery system based on the 17-ester, Scheme II was followed. The 3,17-bis nicotinate of E_2 (**2**) was obtained by treating E_2 with nicotinoyl chloride hydrochloride (**1**) in pyridine. Specific hydrolysis of the phenol ester occurred in methanolic potassium bicarbonate yielding the 17-nicotinate of E_2 (**3**). Quaternization with methyl iodide gave the iodide salt (**4**, E_2Q^+) which was reduced in basic aqueous sodium dithionite to give the corresponding dihydropyridine (**5**, E_2CDS).



Scheme II

Synthesis of the delivery system based on the 3,17-bisester (**8**, $E_2(CDS)_2$) proved more difficult. The 3,17-bisnicotinate (**2**) was quaternized with methyl iodide to give the bistrigonellinate diiodide (**6**) (Scheme III). This bis salt did not give the expected product when treated with basic aqueous sodium dithionite in that cleavage of the 3-ester was invariably observed. The use of other reducing agents such as sodium borohydride or sodium cyanoborohydride and other reducing conditions did not alter this result. The desired $E_2(CDS)_2$ was finally obtained by resorting to a different class of reducing agent. The activated dihydropyridine, 1-benzyl-1,2-dihydroisoicotinamide (**7**) (Nuvole et al., 1984) rapidly reduced the bisquaternary salt (**6**) at room temperature in acetonitrile to the bisdihydropyridine (**8**). This compound is included for completeness although biological evaluation has not been completed.



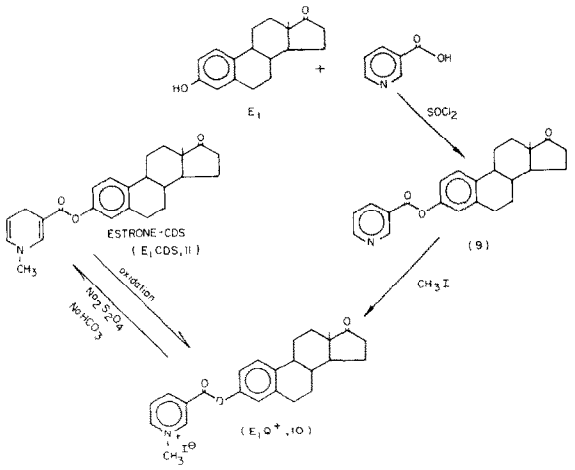
Scheme III

The CDS for E_1 and $3MeE_2$ were obtained in a straightforward manner (Scheme IV and V, respectively) by reacting the appropriate steroid with (**1**) in pyridine. The appropriate nicotinates (**9**) and (**12**) were then quaternized with methyl iodide to give the salts (E_1Q^+, **10** and $3MeE_2Q^+$, **13**). Reduction of the pyridinium salts gave the E_2CDS (**11**) and the $3MeE_2$CDS (**14**) in good yields.

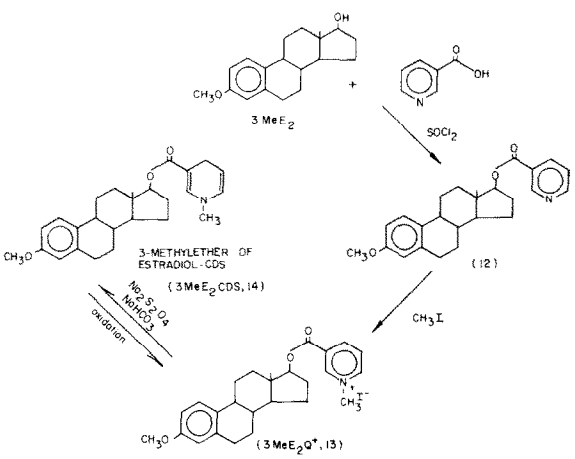
In vitro examination

The stability of E_2CDS (**5**), E_2CDS (**11**) and $3MeE_2$CDS (**14**) were assayed in human plasma and in rat brain and liver homogenate.

The results of this examination are given in Table 1. These data indicate the similarity in stability of the E_2CDS and the $3MeE_2$CDS as well as the lability of the phenolic E_1CDS. Since the method used to assess stability was UV, these data



Scheme IV



Scheme V

are gross indications of metabolism, mainly oxidation. These data tend to exclude E_1CDS from further consideration since it may be too unstable to properly participate in the delivery scheme. From the remaining two systems, the E_2CDS was chosen because of its closer relationship to the active E_2.

According to the overall delivery scheme, the E_2CDS should be more lipophilic than the parent E_2 while the E_2Q^+ should be far less lipophilic than either the parent steroid or the dihydropyridine. To examine this, the 1-octanol : water partition coefficient were determined for E_2, E_2Q^+ and E_2CDS. The log P calculated for E_2 was 3.76, that for E_2CDS was 4.50 and that for the E_2Q^+, -0.144. This indicates that the quaternary salt is 8000 times more hydrophilic than the parent E_2 and 44,000 times more hydrophilic than the E_2CDS. In addition, the data show that the E_2CDS is approximately 5 times more lipophilic than E_2.

The stability of the E_2Q^+ was next examined. The E_2Q^+ must hydrolyze to release the parent compound but the rate of this cleavage should be slow to allow sustained delivery. In general, 17-esters of estradiol such as estradiol-17-valerate revert to the parent compound with relative ease in vivo (Dustenberg and Nishino, 1982). This condition would not be conducive to the proper functioning of the described delivery system. The stability of E_2Q^+ was therefore assayed in rat brain and liver homogenate and in rat blood and plasma. In all cases E_2Q^+ proved to be relatively stable. In

TABLE 1

Pseudo-first-order rate constants (k), half-lives and correlation coefficients (r) for the disappearance of E_2CDS (5), E_1CDS (11) and 3MeE_2CDS (14) from various biological media

Media	Compound	Rate constant $k(\times 10^{-4})\ s^{-1} \pm$ S.E.M.	Half-life (min)	r
Plasma	E_2CDS	0.738 ± 0.09	156.6	0.983
	E_1CDS	2.18 ± 0.07	53.0	0.970
	3MeE_2CDS	0.908 ± 0.04	127.3	0.991
Liver homogenate	E_2CDS	3.87 ± 0.40	29.9	0.991
	E_1CDS	6.73 ± 0.21	17.2	0.949
	3MeE_2CDS	4.31 ± 0.61	26.8	0.993
Brain homogenate	E_2CDS	4.03 ± 0.59	29.2	0.997
	E_1CDS	15.4 ± 1.1	7.5	0.999
	3MeE_2CDS	4.05 ± 0.58	28.8	0.998

the case of brain, liver and blood no significant changes in the peak height of the E_2Q^+ were observed in the time course of these experiments. A slight decrease was observed in the case of plasma. To analyze hydrolysis, the appearance of E_2 was used. In all cases, a small but measurable increase in the concentration of E_2 occurred throughout the experiments. In the brain, at 120 min, the molar amount of E_2 produced was 1.2% of the initial molar concentration of E_2Q^+. In blood this value was 3.1%, in liver 2.3% and in plasma 20.1%. In the case of plasma the molar amount of E_2Q^+ lost and E_2 produced was the same within experimental error. The inability to significantly measure the degradation of E_2Q^+ in brain, liver and blood is consistent with the small amounts of E_2 produced and the similar error (coefficient of variation = 3.5%) for the E_2Q^+ in the analysis. This slow production of E_2 is consistent with the delivery scheme although this in vitro behavior does not necessarily give an indication of in vivo performance.

In vivo distribution

The preceding data indicate that the E_2CDS possesses the characteristics which would allow it to act as a chemical delivery system for E_2. To prove this delivery scheme, a tissue distribution study was performed in rats. In this study, a dose of 60 mg/kg of E_2CDS was administered i.v. and at various times after injection the animals were sacrificed, blood and organs collected, and the samples analyzed for the E_2Q^+ (**4**). The results of this experiment are given in Table 2 and in Figs. 1–4. Examination of the terminal portion of these tissue distribution curves allowed the half-life of loss to be calculated. These first-order values were found to be 46 min in the case of the liver, 5.5 h in the lung, 8 h in the kidney and 23 h in the brain. This evidence clearly shows the differential elimination predicted by the delivery scheme and the retention of the E_2Q^+ in the brain. At 24 h the level of the E_2Q^+ is high in the brain, second only to the kidney in concentration. The levels of the E_2Q^+ in the kidneys are not surprising since this organ is a major site for its elimination.

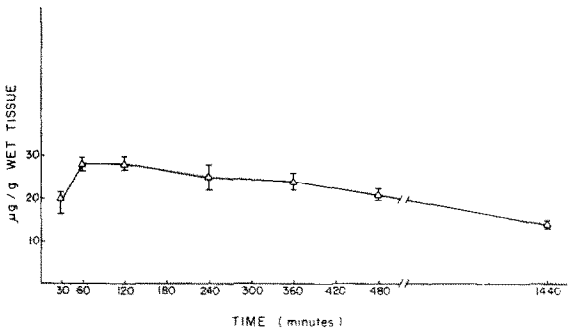


Fig. 1. Distribution of E_2Q^+ (**4**) in the brain after i.v. administration of 60 mg/kg E_2CDS (**5**).

The release of E_2 in the CNS was next examined. Simple HPLC techniques could not detect released estradiol. Since, however, the limit of detection of E_2 in this method was 5 μg/g tissue, it was possible that significant E_2 was released although not detected. To prove E_2 release two, more sensitive, assays were employed. Using an RIA method, 17.90 ± 0.88 ng/g tissue E_2 and

TABLE 2

In vivo distribution of E_2Q^+ (4) following intravenous administration of 60 mg/kg E_2CDS (5)

Time (min)	Wet tissue concentration (μg/g or μg/ml ± S.E.M.)				
	Brain	Liver	Kidney	Lung	Blood
5	*	121 ± 14	248 ± 12	98 ± 5.4	*
30	19 ± 2.6	152 ± 15	302 ± 10	107 ± 13	*
60	28 ± 1.5	110 ± 8.5	201 ± 9	79 ± 14	*
120	28 ± 1.5	20 ± 6.2	108 ± 10	55 ± 11	*
240	25 ± 2.9	7 ± 0.55	58 ± 3.5	39 ± 4.6	*
360	24 ± 2.1	*	52 ± 1.8	37 ± 3.0	*
480	21 ± 1.3	*	53 ± 8.6	40 ± 4.4	*
1440	14 ± 1.2	*	17 ± 1.5	*	*

* Below detection limit

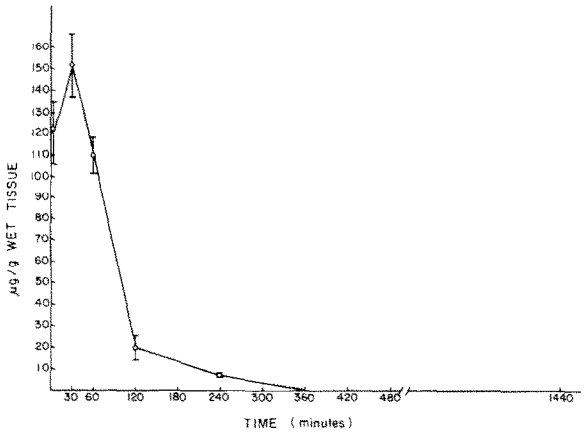


Fig. 2. Distribution of E_2Q^+ (**4**) in the liver after i.v. administration of 60 mg/kg E_2CDS (**5**).

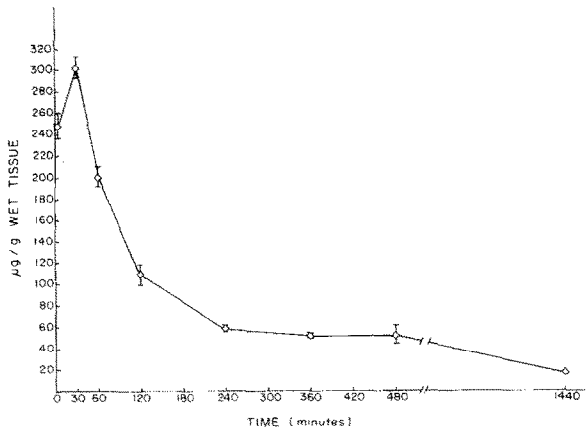


Fig. 4. Distribution of E_2Q^+ (**4**) in the kidney after i.v. administration of 60 mg/kg E_2CDS (**5**).

5.29 ± 0.64 ng/g tissue E_2 were detected in the brain of male rats 30 min after administration of either 15 or 3 mg/kg (**5**), respectively. Estradiol could not be detected (< 30 pg/g tissue) in animal given vehicle only. Since, however, a small amount of cross-reactivity (< 1%) was found between the E_2-antibody and the E_2Q^+ and since some of the E_2Q^+ extracted into ether, a portion of the E_2 detected may be attributed to the E_2Q^+. To clarify this point, a more selective HPLC assay was developed. To obtain the appropriate sensitivity, it was necessary to pool and concentrate samples. Using this technique, 11.2 ng/g tissue E_2 was found in the brain of rats treated with 30 mg/kg (**5**) at 30 min post-treatment. Estradiol was not detected in the blood of these animals indicating levels were less than 1.5 ng/g. This absence indicates that E_2 in the brain is derived from centrally delivered E_2Q^+ and not from peripheral sources. In both the brain and blood determinations, a small amount of E_2Q^+ was carried through the assay. No hydrolysis was detected. The value of 11.2 ng/g obtained from animals treated with 30 mg/kg E_2CDS by this HPLC method was lower than the value of 17.9 ng/g obtained after administration of 15 mg/kg E_2CDS by the RIA method. This difference may be due to some RIA cross-reactivity. Pharmacological studies of this delivery system corroborates the delivery of estradiol to the CNS (Estes et al., 1985).

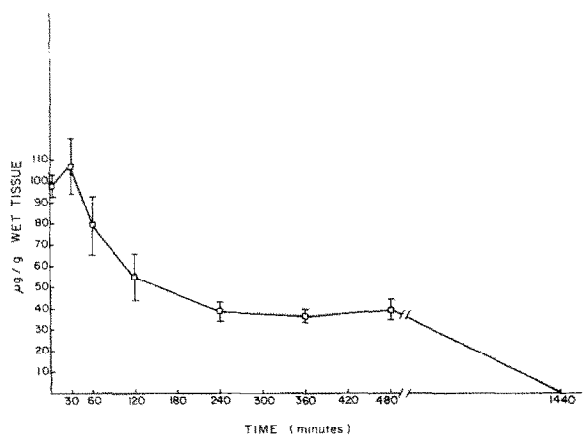


Fig. 3. Distribution of E_2Q^+ (**4**) in the lung after i.v. administration of 60 mg/kg E_2CDS (**5**).

In conclusion, a delivery system for the potent natural estrogen, estradiol, was developed. The data presented showed that, after a single E_2CDS administration, the E_2Q^+ delivery form was retained in the brain for a longer period than in the periphery. In addition, free estradiol was detected in the brains of animals receiving E_2CDS. Estradiol could not be detected in the blood indicating that the E_2 delivered was derived from central sources of E_2Q^+ and not peripheral ones. Such a system could be useful as a contraceptive or in the treatment of estrogen withdrawal associated with menopause or hysterectomy. In addition it provides a tool for examining the neuroendocrine functions of estrogen separate from its peripheral

effects. This system has several advantages over direct systemic administration of synthetic or natural estrogens. First, by the system design the concentration of the carrier decreases rapidly in the periphery which prevents the free steroids from attaining significant peripheral concentrations. This should mitigate any peripheral toxicities. Second, the major portion of the steroid which is "locked" into the CNS is in the form of an ideally inactive complex so that central toxicity of the steroids should be lessened.

Acknowledgements

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References

Astedt, B., Svanberg, L., Jeppsson, S., Liedholm, P. and Rannevik, G., The natural oestrogenic hormone oestradiol as a new component of combined oral contraceptives. *Br. Med. J.*, 1 (1977) 269.

Barraclough, C.S. and Wise, P.M., The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle stimulating hormone secretion. Endocr. Rev., 3 (1982) 91–119.

Bodor, N., Chemical drug delivery systems. In *Theory and application of bioreversible carriers in drug design. Sym. Proc. of Acad. Pharm. Sci.*, 1986, in press.

Bodor, N., and Brewster, M.E., Problems of delivery of drugs to the brain. *Pharmacol. Ther.*, 19 (1983) 337–386.

Bodor, N. and Farag, H., Improved delivery through biological membranes XI. A redox chemical delivery system and its use for brain specific delivery of phenethylamine. *J. Med. Chem.*, 26 (1983a) 313–318.

Bodor, N. and Farag, H., Improved delivery through biological membranes XIII. Brain specific delivery of dopamine with a dihydropyridine-pyridinium salt type redox delivery system. *J. Med. Chem.*, 26 (1983b) 528–534.

Bodor, N. and Farag, H., Improved delivery through biological membranes XIV. Brain specific, sustained delivery of testosterone using a redox chemical delivery system. *J. Pharm. Sci.*, 73 (1984) 385–389.

Bodor, N., Farag, H. and Brewster, M., Site-specific, sustained release of drugs to the brain. *Science*, 214 (1981) 1370–1372.

Bolt, H.M., Metabolism of estrogens—natural and synthetic. *Pharmacol. Ther.*, 4 (1979) 155–181.

Chemical Dynamics Corp., *Chemalog Catalog/Handbook of Biochemicals and Organic Chemicals*, Chemical Dynamic Corp., South Plainfield, NJ, 1984, p. 311.

Dusterberg, B. and Nishino, Y., Pharmacokinetic and pharmacological features of oestradiol valerate. *Maturitas*, 4 (1982) 315–324.

Estes, K., Brewster, McCornack, J., Simpkins, J. and Bodor, N., Evidence for sustained release of estradiol using a redox delivery system, *Endocr. Soc. Abstr.*, Baltimore, Md., no. 205 (1985).

Goodman, R.L. and Knobil, E., The sites of action of ovarian steroids in the regulation of LH secretion. *Neuroendocrinology*, 32 (1981) 57–63.

Kala, S.P. and Kalra, P.S., Neural Regulations of luteinizing hormone secretion in the rat. *Endocr. Rev.*, 4 (1983) 311–351.

Kaplan, N.M., Cardiovascular complications of oral contraceptives. *Annu. Rev. Med.*, 29 (1978) 31–40.

Lauritzen, C., The management of the pre-menopausal and post-menopausal patient. In van Keep, P.A. and Lauritzen, C. (Eds.), *Ageing and Estrogens*, Karger, Basel, 1973.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. *Chem. Rev.*, 71 (1971) 525–616.

Mahesh, V.B., Mills, T.M., Lin, T.J., Ellengood, J.C. and Braselton, W.E., Metabolism, Metabolic clearance rate, blood metabolites and blood half-life of norethindrones and mestranol. In Garentline S. and Berendes H.W. (Eds.), *Pharmacology of Steroid Contraceptive Drugs*, Raven Press, New York, 1977, pp. 117–130.

Nuvole, A., Paglieh, G., Sanna, P. and Acheson, R.M., Selective reduction of pyridinium, quinolinium and pyrazinium salts to the dihydro stage with 1-benzyl-1,2-dihydro-isonicotinamide. *J. Chem. Res.*, (S) (1984) 356–357.

Pardridge, W., Transport of nutrients and hormones through the blood–brain barrier, *Diabetologia*, 20 (1981) 246–254.

Pardridge, W.M., Connor, J. and Crawford, I.L., Permeability changes in the blood-brain barrier: causes and consequences. *CRC Crit. Rev. Toxicol.*, 3 (1975) 159–199.

Rapoport, S.I., *The Blood–Brain Barrier in Physiology and Medicine*, Raven Press, New York, 1976.

Ross, G.T., The Ovaries, In: Wyngaarden, J.N. and Smith, L.H. (Eds.) *Textbook of Medicine*, Philadelphia, PA, 1985, pp 1379–1393.

Schanker, J., Passage of drugs into and out of the central nervous system. *Antimicrob. Agents Chemother.*, (1965) 1044–1050.

Shille, V.M., Thatcher, M.E. and Simmons, K.J., Efforts to induce estrus in the bitch, using pituitary gonadotropins. *J. Am. Vet. Med. Assoc.*, 184 (1984) 1469–1473.

Silverberg, S.O. and Makawski, E.L., Endometrial carcinoma in young women taking oral contraceptive agents. *Obstet. Gynecol.*, 46 (1965) 503–506.

Spona, J. and Schneider, W., Bioavailability of natural estrogens in young females with secondary amenorrhea. *Acta Obstet. Gynecol Scand.*, Suppl. 65 (1977) 33–38.

Upton, V., Therapeutic considerations in the management of the climacteric. *J. Reprod. Med.*, 29 (1984) 71–79.

Williams, J., Longcope, C. and Williams, K., Metabolism of radioactive 17β-estradiol 3-methyl ether by humans. *Steroids*, 27 (1976) 17–28.