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Research Article

Improved Delivery Through Biological Membranes. XXIV. Synthesis, *in Vitro* Studies, and *in Vivo* Characterization of Brain-Specific and Sustained Progestin Delivery Systems[1]

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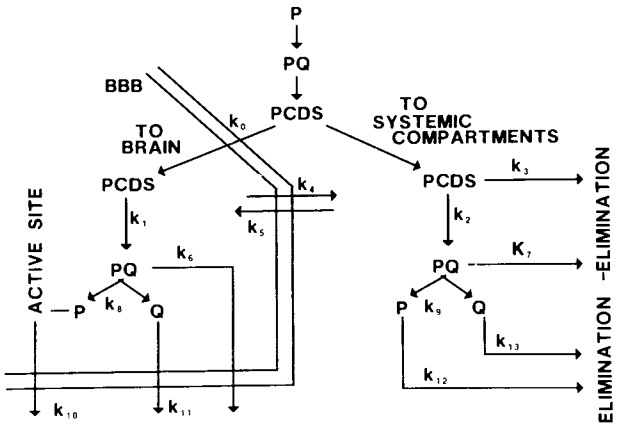
Dihydropyridine ⇄ pyridinium salt-based brain-selective delivery systems were synthesized for the progestins, ethisterone, norethindrone, and norgestrel. After initial lipophilicity and *in vitro* studies indicated the feasibility of applying these compounds to brain-specific delivery, *in vivo* distribution studies were performed on one of the redox delivery systems. After systemic administration of the chemical delivery system based on norethindrone, sustained and selective delivery of the oxidized form of the drug–carrier complex was observed in the brain. In addition, a slow and sustained release of the parent steroid, norethindrone, occurred. This release produced substantially higher levels of norethindrone for more prolonged periods than the administration of norethindrone itself.

KEY WORDS: progestins; norethindrone; sustained delivery of drugs; blood–brain barrier.

INTRODUCTION

Progestins (P) are synthetic steroid derivatives that exert progesterone-like activity in the central nervous system (CNS) and in the periphery. Central actions of these agents are important in their estrogen-associated contraceptive effects (2), in their apparent ability to mitigate the severity of premenstrual tension (3), and in their ability to alter behavior (4). Unfortunately certain progestins, especially those derived from testosterone, exert androgenic, estrogenic, and antihormone effects as well as progestogenic actions (5). Compounds such as ethisterone (1), norethindrone (2), and norgestrel (3) elicit certain unwanted peripherally manifested effects such as alterations in metabolism, hypertension, and weight gain because of these actions (2). A method for specifically delivering progestins to the brain may be useful in mitigating these peripheral untoward actions. If this specific delivery were sustained, then dosing intervals could be decreased and the amount of progestin administered could be greated reduced. Such a system could also be a powerful neuroendocrine probe for separating central and peripheral progestogenic actions.

The method chosen to accomplish this delivery is based on a dihydropyridine ⇄ pyridinium salt redox system (6–8). This brain-targeting chemical delivery system (CDS) was recently shown to be capable of delivering the gonadal steroids estradiol (9) and testosterone (10) to the CNS. The major aim in applying the CDS to lipophilic compounds such as steroids, which readily pass the BBB, is to produce sustained levels of these steroids in the CNS. Thus while the administration of the parent steroid produces significant levels of that steroid in the brain, these levels rapidly fall, causing frequent dosing to maintain therapeutically significant levels. This system is theoretically described in Scheme I.



Scheme I

As shown, a P is derivatized to form a steroid nicotinate, followed by quaternization to give a progestin trigonellinate ion (PQ^+) and reduction to give the corresponding 1,4-dihydropyridine or progestin chemical delivery system (PCDS). Upon systemic administration of the PCDS, an extensive distribution occurs because of the relatively high lipophilicity of the dihydropyridine derivative. This metabolically labile species should then ubiquitously oxidize to form the physiologically inactive PQ^+. The inactivity of the PQ^+ is

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predicted based on reports that 17-esters of norethindrone, in themselves, lack significant progesterone receptor affinity and biological action and require hydrolysis for activity (2). The hydrophilic salt should then rapidly be eliminated from the periphery but retained in the CNS. This rapid peripheral elimination and central retention of the quaternary salts formed *in vivo* is well documented in other systems (7). The retention is due to the blood–brain barrier (BBB), which prevents rapid reequilibration out of the brain of polar species. With time, the "locked-in" quaternary salt hydrolyzes to produce the pharmacologically active steroid in a sustained manner. In this system, peripheral toxicity should be reduced by preventing significant accumulation of the parent steroid. In addition, central toxicity is also attenuated since the majority of the steroid is present in the form of an inactive carrier.

In the present report, lipophilicity and *in vitro* studies were performed to determine the feasibility of P delivery by this method. Detailed *in vivo* studies were then performed to demonstrate oxidation of the CDS, rapid peripheral elimination of the pyridinium salts, and sustained central delivery of the parent steroid.

MATERIALS AND METHODS

Chemistry

Uncorrected melting points (MP) were obtained using an Electrothermal melting-point apparatus. Microcombustion analysis was performed by Atlantic Microlabs, Inc., Atlanta, Ga. Proton nuclear magnetic resonance spectra (NMR) were recorded on a Varian EM 360 or EM 390 spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to an internal standard, tetramethylsilane. Ultraviolet spectra (UV) were determined using a Hewlett Packard 8451A diode array spectrophotometer. Thin-layer chromotography (TLC) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60.

General Procedure for Synthesizing the Nicotinates

Five grams of the appropriate steroid was dissolved in 125 ml of dry, freshly distilled pyridine. To this solution were added 9 g (1.5 molar excess) of nicotinic anhydride (11,12) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). The reaction mixture was stirred at room temperature for several days protected from moisture. When TLC indicated that the reaction was completed, the solution was poured over 800 ml of ice water. The resulting solid was collected by filtration, dried over P_2O_5 *in vacuo,* and then recrystallized.

17β-[(3-Pyridinylcarbonyl)oxy]pregn-4-en-20-yn-3-one (4). Ethisterone (**1**) (5 g, 0.016 mol) was reacted with nicotinic anhydride in pyridine for 7 days. The solid obtained was recrystallized from aqueous methanol. The yield was 88%. MP 203–204.5°C. TLC 60:40 hexane:ethyl acetate R_f = 0.11 (H_2SO_4/char). UV (MeOH) nm 230, 242. NMR ($CDCl_3$) δ 1.03 (s, 3H, angular CH_3); 1.20 (s, 3H, angular CH_3); 1.36–2.63 (m, 20H, skeletal protons); 2.70 (s, 1H, alkynyl proton); 5.73 (s, 1H, α,β-unsat. proton); 7.43 (m, 1H, pyridine C-5 proton); 8.30 (m, 1H, pyridine C-4 proton); 8.80 (m, 1H, pyridine C-6 proton); 9.20 (m, 1H, pyridine C-2 proton). Analysis calculated for $C_{27}H_{31}NO_3$: C, 77.40; H, 7.43; N, 3.36. Found: C, 77.57; H, 7.52; N, 3.35.

17β-[3-Pyridinylcarbonyl)oxy]-19-norpregn-4-en-20-yn-3-one (5). Norethindrone (**2**) (5 g, 0.017 mol) was stirred for 7 days in a pyridine solution of nicotinic anhydride and DMAP. After initial workup, **5** was recrystallized from aqueous methanol. The yield was 84%. MP 199–201°C. TLC 60:40 hexane:ethyl acetate R_f = 0.09 (H_2SO_4/char). UV (MeOH) nm 242. NMR ($CDCl_3$) δ 1.07 (s, 3H, angular CH_3); 1.0–3.33 (m, 20H, skeletal protons); 2.70 (s, 1H, alkynyl proton); 5.85 (s, 1H, α,β-unsat. proton); 7.29–7.53 (m, 1H, pyridine C-5 proton); 8.17–8.4 (m, 1H, pyridine C-4 proton); 8.70–8.93 (m, 1H, pyridine C-6 proton); 9.23 (s, 1H, pyridine C–2 proton). Analysis calculated for $C_{26}H_{29}NO_3$: C, 77.42; H, 7.20; N, 3.47. Found: C, 77.32; H, 7.22; N, 3.44.

13-Ethyl-17β-[(3-pyridinylcarbonyl)oxy]-18,19-dinorpregn-4-en-20-yn-3-one (6). Norgestrel (**3**) (2 g, 0.006 mol) was stirred in a solution of nicotinic anhydride and DMAP for 20 days. Compound **6** was recrystallized from aqueous ethanol. The yield was 70%. MP 202–206°C. UV (MeOH) nm 230, 242. NMR δ ($CDCl_3$) 0.73–3.32 (m, 2H, skeletal protons + 13-ethyl group); 2.80 (s, 1H, alkynyl proton); 5.80 (s, 1H, α,β-unsat. proton); 7.23–7.57 (m, 1H, pyridine C-5 proton); 8.13–8.40 (m, 1H, pyridine C-4 proton); 8.67–8.87 (m, 1H, pyridine C-6 proton); 9.17 (s, 1H, pyridine C-2 proton). Analysis calculated for $C_{27}H_{31}NO_3$: C, 77.70; H, 7.43; N, 3.36. Found: C, 77.72; H, 7.49; N, 3.30.

General Procedure for Quaternization

The steroid-17-trigonellinate iodides were obtained by dissolving 2 g of the appropriate steroid nicotinate in acetonitrile. Two milliliters of methyl iodide was added to the solution, which was then heated at reflux for 12 hr. The resulting solid was collected by filtration and dried.

1-Methyl-3-{[(pregn-4-en-20-yn-3-one-17β-yl)oxy]carbonyl}pyridinium Iodide (7). Compound **4** (2 g) was derivatized as described above. The yield was 98% (2.62 g). MP 226–227°C. UV (MeOH) nm 224. NMR (d_6-DMSO) δ 1.05 (s, 3H, angular CH_3); 1.20 (s, 3H, angular CH_3); 0.70–2.97 (m, 20H, skeletal protons + 1H alkynyl proton); 4.50 (s, 3H, N^+-CH_3); 5.63 (s, 1H, α,β-unsat. proton); 8.13–8.5 (m, 1H, pyridinium C-5 proton); 8.83–9.13 (m, 1H, pyridinium C-4 proton); 9.15–9.40 (m, 1H, pyridinium C-6 proton); 9.46 (bs, 1H, pyridinium C-2 proton). Analysis calculated for $C_{28}H_{34}NO_3I$: C, 60.11; H, 6.08; N, 2.50; I, 22.72. Found: C, 60.39; H, 6.14; N, 2.45; I, 22.55.

1-Methyl-3-{[(19-norpregn-4-en-20-yn-3-one-17β-yl)oxy]carbonyl} pyridinium Iodide (8). Compound **5** (2 g) was derivated as described above. The yield was 95%. MP 207–210°C. UV (MeOH) nm 224. NMR (d_6-DMSO) δ 1.03 (s, 3H, angular CH_3); 0.66–2.70 (m, 20H, skeletal protons); 3.73 (s, 1H, alkynyl proton); 4.48 (s, 3H, N^+-CH_3); 8.10–8.46 (m, 1H, pyridinium C-5 proton); 8.90–9.13 (m, 1H, pyridinium C-4 proton); 9.15–9.37 (m, 1H, pyridinium C-6 proton); 9.47 (bs, 1H, pyridinium C-2 proton). Analysis calculated for $C_{27}H_{32}NO_3I \cdot \frac{1}{2}H_2O$: C, 58.48; H, 5.78; N, 2.53; I, 22.94. Found: C, 58.19; H, 5.99, N, 2.49; I, 22.64.

1-Methyl-3-{[(13-ethyl-18,19-dinorpregn-4-en-20-yn-3-one-17β-yl)oxy]carbonyl}pyridinium Iodide (9). Compound

6 (420 mg) was derivatized as described above. The yield was 89%. MO 185–187°C. UV (MeOH) nm 224. NMR (d_6-DMSO) δ0.66–2.90 (m, 25H, skeletal protons + 13-ethyl group); 3.83 (s, 1H, alkynyl protons); 4.50 (s, 3H, N^+-CH_3); 5.75 (s, 1H, α,β-unsat. proton); 8.00–8.57 (m, 1H, pyridinium C-5 proton); 8.77–9.63 (m, 3H, pyridinium C-4,6,2 protons). Analysis calculated for $C_{28}H_{34}NO_3I$: C, 60.11; H, 6.08; N, 2.50. Found: C, 60.00; H, 6.20; N, 2.49.

General Procedure for Reduction

One gram of the appropriate trigonellinate iodide was suspended in 150 ml of cold, degassed aqueous ethanol. To this mixture were added 770 mg (4 molar excess) of sodium hydrogen carbonate and 940 mg (2 molar excess) of sodium dithionite. After stirring for 1 hr under N_2, the solution was removed and extracted with chloroform. The organic layer was dried over $MgSO_4$ and removed *in vacuo* yielding a yellow foam. The material was then chromatographed on a neutral alumina column (Brockman activity 1, 80–200 mesh) which had been prepared with methylene chloride. The resulting solution was removed under reduced pressure yielding a yellow solid.

17β-{[(1-Methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}pregn-4-en-20-yn-3-one (10). Compound 7 (1 g) was reduced as described above yielding 521 mg (56%) of a yellow foam. MP darkens at 134°C; dec. at 217–220°C. UV (MeOH) nm 362, 242. NMR ($CDCl_3$) δ0.73–2.77 (m, 19H, skeletal protons); 0.90 (s, 3H, angular CH_3); 1.17 (s, 3H, angular CH_3); 2.57 (s, 1H, alkynyl proton); 2.90 (s, 3H, N-CH_3); 3.0–3.17 (bd, 2H, pyridine C-4 protons); 4.57–4.87 (m, 1H, pyridine C-5 proton); 5.47–5.77 (m, 2H, α,β-unsat. proton + pyridine C-6 proton); 6.80–7.0 (s, 1H, pyridine C-2 proton). Analysis calculated for $C_{28}H_{35}NO_3 \cdot H_2O$: C, 74.50; H, 8.20; N, 3.10. Found: C, 74.65; H, 8.11; N, 3.06.

17β-{[(1-Methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}19-norpregn-4-en-20-yn-3-one (11). Compound 8 (1 g) was reduced as described above. The yield was 60%. MP 201–203°C. UV (MeOH) nm 242, 362. NMR ($CDCl_3$) δ0.73–2.67 (m, 20H, skeletal protons), 0.97 (s, 3H, angular CH_3); 2.67 (s, 1H, alkynyl proton); 2.97 (s, 3H, N-CH_3); 3.03–3.20 (m, 2H, pyridine C-4 protons); 4.6–5.0 (m, 1H, pyridine C-5 proton); 5.5–5.93 (m, 2H, pyridine C-6 proton + α,β-unsat. proton); 6.9–7.03 (s, 1H, pyridine C-2 proton). Analysis calculated for $C_{27}H_{33}NO_3 \cdot H_2O$: C, 74.14; H, 8.01; N, 3.20. Found: C, 74.01; H, 7.79; N, 3.10.

13-Ethyl-17β-{[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]oxy}-18,19-dinorpregn-4-ene-20-yn-3-one (12). Three hundred thirty milligrams of 9 was reduced as described above. The yield was 56%. UV (MeOH) nm 242, 360. NMR ($CDCl_3$) δ0.7–2.73 (m, 25H, skeletal protons + 13-ethyl group); 2.60 (s, 1H, alkynyl proton); 2.90 (s, 3H, N-CH_3); 4.67–4.83 (bs, 2H, pyridine C-4 protons); 4.53–4.93 (m, 1H, pyridine C-5 proton); 5.43–5.93 (m, 2H, pyridine C-6 proton + α,β-unsat. proton); 6.07–7.0 (bs, 1H, pyridine C-2 proton). Analysis calculated for $C_{28}H_{35}NO_3 \cdot H_2O$: C, 74.50; H, 8.20; N, 3.10. Found: C, 74.59; H, 8.15; N, 3.16.

Analytical Studies

In all determinations a Toya Soda ODS-120T C-18 reverse-phase column was used. The analytical column was fitted with a guard column which contained pellicular octadecyl-phase coated packing. The column was calculated to contain 15622 theoretical plates. In all cases, the mobile phase flow rate was 1 ml/min and determinations were made at ambient temperature. The chromatographic system consisted of a Perkin–Elmer Series 4 pump, a Kratos 757 spectroflow variable-wavelength absorbance detector, and a Perkin–Elmer LCI-100 integrator. For automated analyses, a Perkin–Elmer ISS-100 autosampler was used. Two analytical systems were developed. Method A, which consisted of a mobile phase containing 60% acetonitrile and 40% 0.05 *M* KH_2PO_4 buffer (pH 4.6), was used in the detection and quantitation of the parent steroids (**1**, **2**, and **3**) and the quaternary salts (**4**, **5**, and **6**). Under the conditions used for the analysis, the retention time for **1** was 8.41 min; for **2**, 7.41 min; for **3**, 8.35 min; for **4**, 4.67 min; for 7, 4.67 min; for 8, 4.65 min; and for 9, 4.66 min. The compounds in these series were detected at 242 nm. Method B, which was used to quantitate the dihydropyridines (**10**, **11**, and **12**), consisted of a mobile phase of 90:10 acetonitrile:H_2O. The analysis was monitored at 360 nm, and in this system, **10** eluted at 7.2 min, **11** at 6.3 min, and **12** at 7.01 min.

Octanol:Water Partition Coefficient (log*P*) Determinations

Saturated solutions of **1**, **2**, 7, **8**, **10**, or **11** were prepared in octanol. Two milliliters of each solution was then partitioned against 2 ml of water saturated with octanol at ambient temperature for 1 hr. The concentration of each compound in both phases was then determined by methods A and B. The log ratio of the peak height of the compound in octanol and in water was determined and reported as the log *P*. Each determination was done in duplicate and data are reported as the average of the two values.

Extrapolated log *k'* Determinations

In determining lipophilicity by a chromatographic method, the change in retention time for **1**, **2**, **10**, and **11** with changes in the acetonitrile:water composition of the mobile phase was recorded. The range of mobile phase concentrations was 55:45 water:acetonitrile to 75:25 water:acetonitrile. For each compound, at each mobile phase composition, the capacity factor (log *k'*) was determined. The log *k'* is given by the equation

$$\log k' = \log\left[\frac{t_r - t_o}{t_o}\right]$$

where t_r is the retention time of the compound and t_o is the retention time of an unretained peak (formaldehyde). The log *k'* values were then plotted against the percentage H_2O in the mobile phase and a straight line was obtained. Extrapolation to 100% H_2O gave the extrapolated log *k'*. The correlations obtained for these lines were 0.993 for **1**, 0.993 for **2**, 0.995 for **10**, and 0.996 for **11**.

In Vitro Studies

The stability of **10**, **11**, and **12** in human and rat whole blood and in 40% rat brain homogenate was determined. Human blood was obtained by venipuncture and collected

into heparinized tubes. Rat blood was obtained by cardiac puncture from animals anesthetized with ether and was heparinized. Rat brain homogenate was prepared by homogenizing 4 g of freshly obtained rat brain in 10 ml of pH 7.4 phosphate-buffered saline. All tissues were handled in essentially the same way, so that 20 μl of a (5×10^{-3} *M*) DMSO solution of the appropriate dihydropyridine was pipetted into 5 ml of the appropriate biological matrix maintained at 37°C. At various times after this introduction, 100 μl of the medium was removed and treated with 400 μl of ice-cold acetonitrile. The resulting suspension was centrifuged at 13,000*g* for 5 min in a Beckman Microfuge 12. The supernatant was removed and filtered through 0.45-μm nitrocellulose filters (Millipore) and 20 μl was analyzed by methods A and B. The decrease in the log peak height with time gave the pseudo first-order rate constants for disappearance. The appearance of the quaternary salts was also investigated. In all cases the rates of appearance and disappearance were the same within experimental error. In addition, the number of moles of dihydropyridine consumed at the end of each experiment was the same, within experimental error, as the number of moles of quaternary salt produced. For examining hydrolysis, a 5×10^{-3} *M* solution in DMSO of **7**, **8**, or **9** was added to the appropriate biological matrix and samples were processed as before. Method A was used in this analysis. In these analyses, the decrease in the peak height of the quaternary salt was examined. Since the peak height did not significantly change in the experimental time course, the appearance of the parent steroid was used as an indication of hydrolysis.

In Vivo Studies

Conscious male Sprague–Dawley rats (BW, 190–210 g) were restrained and injected iv (tail vein) with a dose of either 10 mg/kg **11**, 7.1 mg/kg **2**, or 0.5 ml/kg vehicle (DMSO). The dose of vehicle was the same in all administrations. Animals were sacrificed at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 7 hr, 24 hr, 48 hr, and 72 hr posttreatment in the case of **11** and at 15 min, 24 hr, and 48 hr in the case of **2**. For each time point, six or seven animals were used. Animals were decapitated and trunk blood was collected into heparinized tubes. Brain, liver, lung, testis, heart, and mesentary fat were removed, weighed, and immediately frozen on dry ice within 90 sec of death. In preparing the organs for analysis, each organ or pair of organs was homogenized in 1 ml of water. To this were then added 4 ml of ice-cold acetonitrile containing 5×10^{-6} *M* internal standard [ethisterone (**1**)] and 1 ml of saturated sodium chloride. The mixture was then rehomogenized, centrifuged, and cooled at −15°C. The acetonitrile layer, which separates in these circumstances, was removed, filtered through 0.45-μm nitrocellulose membranes, and stored in autosampler vials. The sealed vials were stored at −15°C until they were analyzed. A slight variation of the above procedure was required in the case of the liver. Rather than using the whole organ, the liver was dissected and only 3 g was homogenized. In preparing the blood for analysis, 1 ml of water, 4 ml of acetonitrile, and 1 ml of saturated sodium chloride were added to 1 ml of blood and the samples treated as before. Detection and quantitation of **2**, **8**, and **11** were accomplished using methods A and B. In the case of the blood, and interfering peak required alteration of system A. This application required gradient elution beginning with a mobile phase of 50:50 acetonitrile:0.05 *M* KH_2PO_4 buffer which ramped to 65:35 acetonitrile:buffer and then reequilibrated at 50:50 for the next analysis. In these circumstances, **8** eluted at 5.1 min, **2** at 10.5 min, and **1** (i.e., the internal standard) at 12.49 min. The interfering peak eluted at 4.68 min. This peak was not found in chromatographs of other tissues and did not interfere with analysis of **11** in blood. Standard curves were constructed in the appropriate organ homogenates and blood and peaks measured as a ratio to the internal standard. The standard curves for **11** were prepared by adding different concentrations of **11** to various organs and then preparing the samples as described for the unknowns. The standard curves were linear with correlation coefficients >0.9999. These standards were analyzed for **8** and **2**. Neither of these materials was present. This is an indication of the stability of **11** during workup. For **8** a similar procedure was followed. In this case the samples were analyzed for **2**. None was detected. In these assays, the limit of detection was approximately 1.0 μg/g for **11**, 0.2 μg/g for **8**, and 0.3 μg/g for **2**. The levels of the parent steroid **2** in animals treated with **11** were too low to be calculated adequately using the above sample preparation. The assay sensitivity was increased by concentrating the samples. Acetonitrile extracts of brain or blood corresponding to a particular time point were pooled and the solvent was removed. The residue was reconstituted with 100 μl of acetonitrile, yielding a sample approximately 100 times more concentrated than the starting extracts. The reconstitute was analyzed by system A and stardard curves were constructed by processing known quantities of **2** in the same manner as the samples. A small quantity of **8** was also carried through this procedure. No hydrolysis was detected. In these assays the limit of detection was 5 ng/g for **2**.

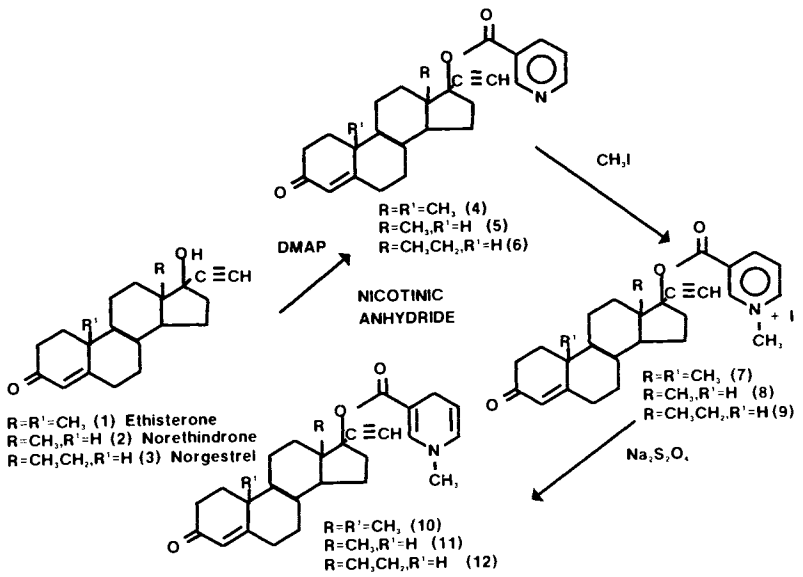
RESULTS AND DISCUSSION

Chemistry

Synthesis of the various progestin chemical delivery systems, **10**, **11**, and **12**, is summarized in Scheme II. Optimal yields were obtained by the reaction of **1**, **2**, or **3** with nicotinic anhydride in pyridine to which a trace of 4-(dimethylamino)pyridine (DMAP) had been added. The resulting nicotinates, **4**, **5**, and **6**, were then quaternized with methyl iodide to give the trigonellinate iodides **7**, **8**, and **9**. Reduction of the quaternary salts in basic aqueous alcoholic sodium dithionite afforded the corresponding dihydropyridines **10**, **11**, and **12**.

Lipophilicity Studies

One of the primary factors that determines whether the CDS will function as described in Scheme I is the lipophilicity of the various system components. The PCDS is expected to be more lipophilic than the parent steroid, while the oxidized carrier (PQ^+) is expected to be far less lipophilic. These changes must occur if increased distribution of the PCDS and increased peripheral elimination of PQ^+ are to occur. To investigate these parameters, the octanol:water partition coefficients (log *P*) (13) and HPLC capacity factors



Scheme II

(log k') (14,15) were determined for various components of the delivery scheme.

The log P values determined for **1**, **2**, **7**, **8**, **10**, and **11** are given in Table I. The data indicate that the PCDSs are an order of magnitude more lipophilic than the parent steroids. The data also show that the quaternary salts are at least 4.5 orders of magnitude more hydrophilic than the corresponding PCDS and more than 3 orders of magnitude more hydrophilic than the parent steroids.

The log P also proved to be fairly sensitive in exposing subtle differences in structure between the 1 series and their 19-nor analogues. Ethisterone (**1**) was found to be 1.4 times more lipophilic than **2**. The two corresponding quaternary salts **7** and **8** and dihydropyridines (**10**) and (**11**) demonstrated a relatively small difference in lipophilicity.

In further examining the relative differences in lipophilicities, a modified log k' method (12,13) based on HPLC elution was used. In these determinations, the change in capacity factors of **1**, **2**, **10**, and **11** was determined as a function of the percentage water in the mobile phase. The values were then extrapolated to 100% water. In this application a C-18 (octadecyl based packing) rather than C-8 (octyl based packing) column, as previously reported, was used so that the absolute values of the partition coefficients were not as important as the relative differences between values. Since an aqueous acetonitrile mobile phase was not compatible with elution of the quaternary salts, they were not investigated by this method. The relative differences in lipophilicities, between various components of the delivery scheme determined by the log k' method, generally agreed with log P values (Table I). The lipophilicity difference between **1** and **2** agreed quite well with the log P values. Interestingly, the log k' determinations indicated a similar relative difference in lipophilicity between the parent steroid (**1** and **2**) and the dihydropyridines (**10** and **11**).

Table I. Calculated log P and Extrapolated log k' Data for Various Progestins and Their Corresponding Trigonellinate Salts and Dihydrotrigonellinates

Compound	log P	Extrapolated log k'
1	3.11	0.476
2	2.97	0.374
7	−0.03	—
8	0.02	—
10	4.49	1.36
11	4.44	1.24

In Vitro Studies

Having shown that the components of the delivery scheme possess the appropriate physicochemical properties necessary for successful CDS application, their various chemical transformations were examined. The delivery scheme requires oxidation of the PCDS and subsequent hydrolysis of the PQ^+ in order for specific and sustained central drug delivery to occur. Oxidation studies in human and rat whole blood and rat brain homogenates were therefore completed (Table II). The dihydropyridine compounds oxidized in a pseudo first-order fashion, with a concomitant quantitative increase in the concentration of the quaternary salts. Interestingly **10** seemed to be the least stable of the

Table II. Pseudo First-Order Rate Constants (k) ± SE, Half-Lives ($t_{1/2}$), and Average Correlation Coefficients (r) for the Disappearance of **10**, **11**, and **12** from Various Biological Media

Medium	Compound	$k \times 10^{-3}$ min^{-1}	$t_{1/2}$ (min)	r
Whole rat blood	**10**	11.5 ± 0.9	60.3	0.974
	11	5.86 ± 0.05	118.2	0.994
	12	5.97 ± 0.06	116.2	0.984
Whole human blood	**10**	4.26 ± 0.05	162.7	0.969
	11	5.48 ± 0.10	126.4	0.960
	12	4.79 ± 0.08	144.6	0.984
40% rat brain homogenate	**10**	73.9 ± 2.3	9.4	0.998
	11	60.9 ± 1.6	11.37	0.996
	12	43.5 ± 3.7	15.93	0.993

Table III. Concentration of Norethindrone (2) (μg/g or μg/ml) in Various Tissues and Blood after a 7.1-mg/kg iv Dose of Norethindrone (2)

	Tissue concentration ± SE							
Time	Brain[a]	Blood[a]	Heart	Liver	Kidney	Fat	Testis	Lung
15 min	2.31 ± 0.11	1.35 ± 0.08	2.19 ± 0.13	0.207 ± 0.013	6.65 ± 0.85	—[b]	0.93 ± 0.17	8.25 ± 1.69
24 hr	—	—	—	—	—	—	—	—
48 hr	—	—	—	—	—	—	—	—

[a] For 24- and 48-hr time points the samples were pooled and concentrated as described in the text.
[b] Below detection limit.

three compounds in rat blood and the most stable in human blood. In rat brain homogenates, the rate of disappearance of **10** was almost twice that of **12**. The steric hindrance of the 13-ethyl group may be important in slowing this enzymatic reaction (7).

The stability of the quaternary salts (PQ^+) is important to the overall delivery scheme. The PCDS must be oxidized before ester hydrolysis and the rate of hydrolysis should be slow to allow for a sustained delivery. The PQ^+ were found to be relatively stable *in vitro*. When the disappearance of the PQ^+ was examined by measuring the disappearance of the HPLC peak corresponding to this compound, significant changes did not occur. When, however, the parent steroid was assayed, a small but significant amount was produced. This was extensively studied in brain homogenate. In this tissue, the levels of parent steroid increased steadily yielding, at the end of the experiment (2 hr), a molar concentration equivalent to 2.2% of the initial quaternary salt concentration. This small conversion was consistent when the insignificant change in the peak height of the quaternary salt since the coefficient of variation for the salt in the above system was approximately 2%. This is similar to previous studies with the estradiol-CDS. In this case, *in vitro* hydrolysis in organ homogenates was minimal. When, however, the compound was systemically administered, not only was *in vivo* hydrolysis confirmed but also sustained pharmacological action was observed (8).

In Vivo Distribution

The widely used progestin **2** and the corresponding CDS **11** were selected for tissue distribution studies. A dose of 23.8 μmol/kg of **2**, **11**, or vehicle was administered iv (tail vein) into conscious Sprague–Dawley male rats. The results of these studies are given in Tables III and IV and in Figs. 1–3.

Administration of **2** produced a distribution consistent with the lipophilic nature of the molecule (Table III). At 15 min, relatively high levels of the steroid were found in all tissues (except fat) and blood. The steroid readily passed the BBB, yielding a brain/blood ratio of 1.7 at 15 min, and similarly passed into other organs, producing organ/blood ratios of 1.6, 4.9, and 6.1 in heart, kidney, and lung, respectively. The lung contained the highest level of free steroid. At the second sampling point of 24 hr, the levels of **2** in all tissues had fallen to undetectable levels. The inability of the steroid to accumulate in the brain is consistent with its lipophilicity. While the steroid can pass the BBB easily, it can also easily be lost from the CNS.

After iv administration of the CDS, **11** rapidly disappears from most tissues, while the oxidized quaternary salt **8** is ubiquitously produced. Sustained levels of **8** in the brain are necessary for prolonged specific CNS drug action. As Fig. 1 and Table IV indicate, **8** is rapidly lost from blood and liver and is present only in small quantities in the testis and fat. These levels, close to the limit of detection, disappear by 24 hr. The low concentrations of **2**, **8**, or **11** in mesentery fat shows that the CDS does not accumulate there and is indicative of the fact that the CDS does *not* operate via a peripheral depot mechanism. The levels of **8** in the kidney disappear by 72 hr. By 24 hr, the level of **8** was higher in the brain than in all other organs or blood. In addition, the rate of pseudo first-order loss of **8** from tissues, calculated by a logarithmic transformation of the terminal portion of the tissue concentration versus time curves, was slowest in the brain. As Fig. 2 indicates, the half-life of disappearance of **8**

Table IV. Concentration of **8** (μg/g or μg/ml) in Various Tissues and Blood after a 10-mg/kg iv Dose of **11**

	Tissue concentration ± SE							
Time	Brain	Blood	Lung	Heart	Liver	Kidney	Fat	Testis
5 min	3.16 ± 0.80	2.47 ± 0.19	14.99 ± 5.84	5.36 ± 1.95	2.11 ± 0.69	11.94 ± 2.50	0.42 ± 0.08	0.22 ± 0.22
15 min	2.59 ± 0.61	0.84 ± 0.09	7.85 ± 3.66	5.29 ± 2.02	1.85 ± 0.66	4.88 ± 1.29	0.27 ± 0.06	0.18 ± 0.02
30 min	5.01 ± 0.82	0.70 ± 0.03	24.13 ± 1.90	9.54 ± 1.45	0.67 ± 0.24	8.23 ± 0.85	0.36 ± 0.06	0.22 ± 0.03
60 min	5.32 ± 0.30	0.71 ± 0.04	23.92 ± 2.52	9.95 ± 0.49	0.59 ± 0.22	6.11 ± 0.41	0.41 ± 0.11	0.26 ± 0.02
2 hr	5.47 ± 0.49	—[a]	31.58 ± 2.87	10.30 ± 0.72	0.64 ± 0.09	5.18 ± 0.16	—	0.32 ± 0.02
4 hr	4.99 ± 0.88	—	11.24 ± 2.06	9.36 ± 1.32	0.58 ± 0.07	4.43 ± 0.34	—	0.32 ± 0.02
7 hr	4.85 ± 0.60	—	6.46 ± 1.59	8.40 ± 0.95	0.55 ± 0.11	3.24 ± 0.46	—	0.19 ± 0.02
24 hr	4.86 ± 0.43	—	4.15 ± 0.68	3.26 ± 0.37	0.31 ± 0.07	1.65 ± 0.21	—	—
48 hr	3.52 ± 0.48	—	2.76 ± 0.72	1.16 ± 0.20	—	0.75 ± 0.08	—	—
72 hr	1.82 ± 0.18	—	0.86 ± 0.30	0.35 ± 0.07	—	—	—	—

[a] Below detection limit.

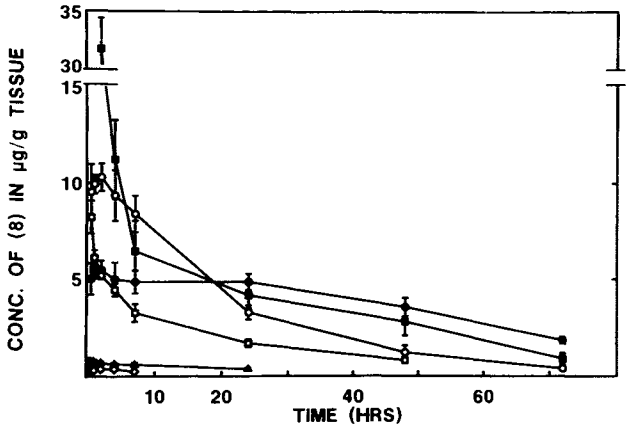


Fig. 1. Concentration of 8 in brain (●), lung (■), heart (○), kidney (□), liver (▲), and testis (◇) after iv administration of 10 mg/kg (11).

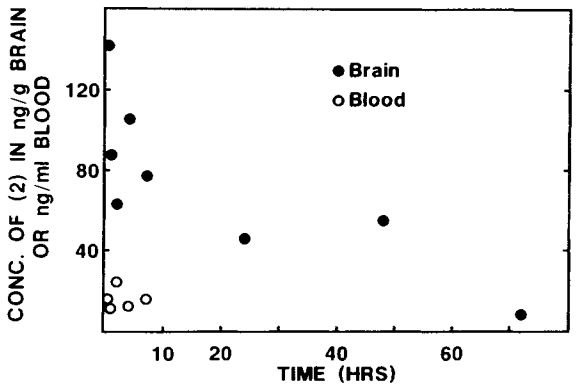


Fig. 3. Concentration of 2 in pooled brain (●) and blood (○) samples after iv administration of 10 mg/kg (11).

in the brain was 33.8 hr, while in the kidney, lung, and heart the values were 19.5, 18, and 14.4 hr, respectively. Blood and liver showed rapid elimination, while testis and fat accumulated only small amounts of 8. The data clearly show the differential retention of 8 in the CNS.

Pools from brain and blood samples obtained from animals administered either 11 or 2 (at 24 or 48 hr posttreatment) were concentrated to analyze levels of the parent steroid (2). Figure 3 gives these results. The brain levels of 2 after administration of 11 were sustained through 48 hr at between 50 and 100 ng/g, dropping to approximately 8 ng/g at 72 hr. Blood levels, on the other hand, were substantially lower and disappeared by 24 hr. These low blood levels indicate that 2 found in the CNS is derived from centrally delivered 8 and *not* from peripheral sources. No 2 was detected in the brain or blood of animals 24 or 48 hr after iv administration of 2.

In comparing delivery of 2 to the brain after administration of either 2 or 11, the PCDS was found to be superior. Systemic administration of 2 gave blood and brain levels that were undetectable at 24 hr even after concentrating the samples, while significant brain levels of 2 were attained with the CDS for the duration of the experiment (3 days). In addition, these concentrations are sustained and are not typified by rapid increases and decreases, which characterize multiple dosing. This delivery scheme could, therefore, allow a decreased dosing interval, which would produce levels of drug which do not increase to possible toxic concentrations. Peripheral effects would be minimized and central side effects, likewise, would decrease since the majority of the steroid delivered is tied up in an inactive carrier. We have recently demonstrated (16) that one single dose of a similar CDS for estradiol produces prolonged (over 30 days) profound (LH suppression, antiovulatory, etc.,) effects, clearly centrally, as the circulating estradiol levels are no different from control after the first 4–6 days. This progestin system could be useful as a contraceptive, in conjunction with estrogens, or in the treatment of central progesterone deficiencies such as premenstrual stress (2,3). In addition, certain aberrant behavior conditions may be successfully treated with the CDS (4). In summary, several progestin chemical delivery systems were synthesized. Initial lipophilicity and *in vitro* studies indicated that these systems possessed the appropriate characteristics for successful CDS application. After selecting one of the delivery systems, *in vivo* distribution studies were done. These showed that after a single administration of 11, relative retention of the oxidized carrier 8 in the brain with a resulting slow release of the active steroid occurred. On the other hand, blood levels were undetectable. In addition, more sustained and substantially higher levels of 2 were delivered centrally after 11 administration compared with injection of the parent steroid itself.

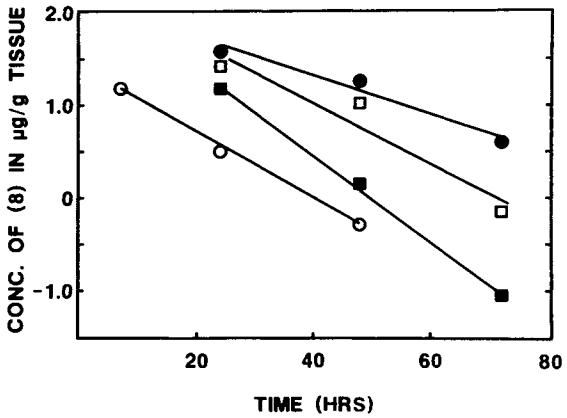


Fig. 2. Log disappearance of 8 from brain (●), lung (□), heart (■), and kidney (○) after an iv dose of 10 mg/kg (11).

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