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Communications to the Editor

Extension of a Redox-Based Chemical Delivery System to α,β-Unsaturated Ketones†

Sir:

Brain-enhanced drug delivery has often been made possible through the use of redox chemical-delivery systems (CDS).[1-5] This carrier-based approach involves formation of a dihydronicotinate conjugate of a drug or neurotransmitter. The manipulation described greatly enhances the tissue permeability of the conjugate and allows it to penetrate into many body compartments inaccessible to the unmanipulated agent. The carrier is specially designed to undergo enzyme-catalyzed oxidation resulting in the formation of a nicotinate salt. This now-polar species is readily lost from the systematic circulation but is trapped behind the lipoidal blood–brain barrier. The deposited salt can subsequently hydrolyze, releasing the active drug. This scheme provides for rapid peripheral elimination of the administered drug thereby reducing nontarget-site toxicities. In addition, since the majority of the drug delivered to the CNS is in the form of an inactive conjugate, central toxicities may also be mitigated. Attendant improvements associated with this method include the potential for lower drug doses and longer dosing intervals.

The CDS has been applied to a wide variety of pharmacologically active agents.[6-10] Chemically, alcohols, amines, and carboxylic acids have been considered and the only limitation in extending this approach to drugs is that they contain in their structure a suitable chemical point for attachment of the carrier. In some cases, this criterion is not satisfied. Progesterone (1) is an endogenous steroid which exerts a variety of centrally mediated physiological effects.[11-13] The high lipophilicity associated with this compound allows it to readily enter the CNS. This same characteristic, however, also provides for rapid elimination, with the result being a short duration of action. It may be desirable, however, to exploit the central actions of progesterone such as its effect on LHRH separate from peripheral manifestations. Thus, application of the CDS to progesterone may be useful. While synthetic progestagens have been considered, these compounds have in their structure an acylatable 17-hydroxy group.[14] Progesterone is acetylated at the 17-position and contains an

Scheme I

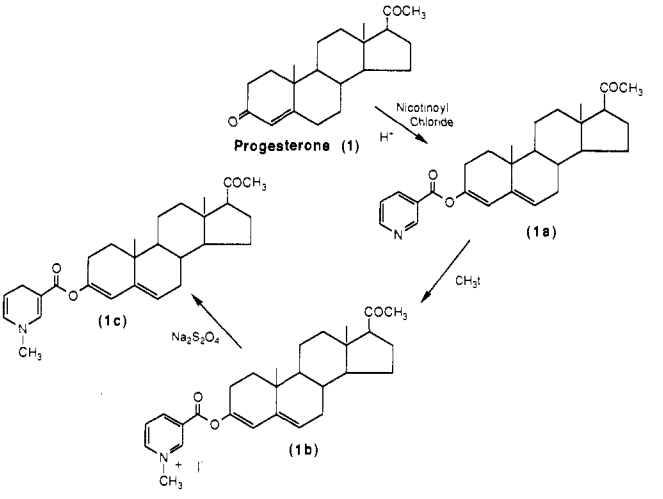

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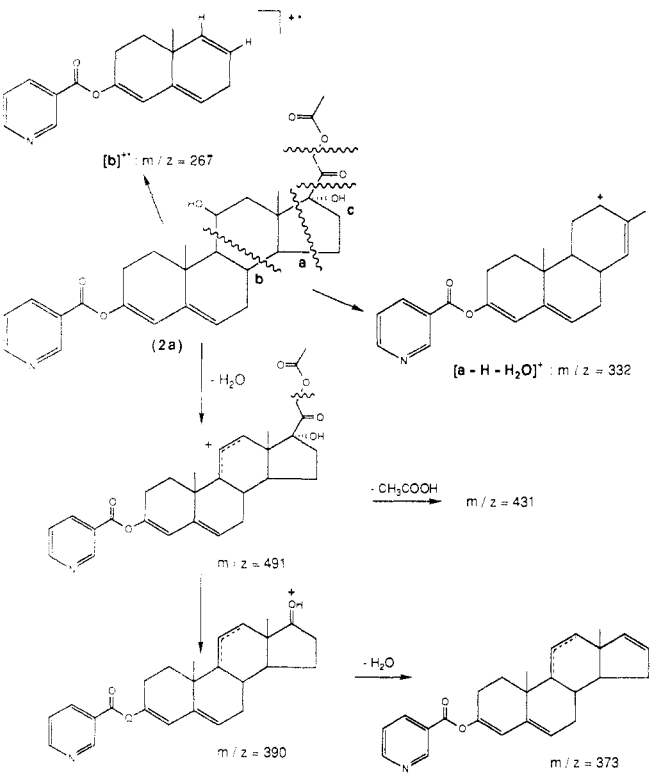
Table I. In Vitro Rate Constants of the Disappearance of 1c and the Conversion of **1b** to Progesterone

	half-life, min	corr coeff
1b → 1		
whole human blood	26.9	0.996
whole rat blood	8.7	0.998
rat liver homogenate	13.7	0.994
1c		
whole human blood	stable	
rat liver homogenate	16.0	0.999
rat brain homogenate	106.0	0.980

α,β-unsaturated ketone function in its A ring. Thus, preparation of nicotinic acid conjugates would seem to be inappropriate. In this communication, we extend the CDS approach to progesterone and other α,β-unsaturated ketones including medroxyprogesterone acetate (**2**) (Depo-Provera) and hydrocortisone 21-acetate (**3**).

The approach chosen to apply the CDS to progesterone and other α,β-unsaturated ketones was enolization followed by trapping of the enolate as the corresponding nicotinate ester. Simple enol esters of progesterone such as the enol acetate have been used as depot forms of this steroid.[15,16] Progesterone was treated with a slight excess of nicotinoyl chloride hydrochloride in a pyridine solvent with pyridinium tosylate as a catalyst. The obtained 3-enol 3-pyridinecarboxylate[17] **1a** was subsequently quaternized with methyl iodide to provide the 3-enol 1-methyl-3-(carbonyloxy)pyridinium salt[18] **1b** and was reduced in aqueous sodium dithionite to yield the 3-enol 1-methyl-3-(carbonyloxy)-1,4-dihydropyridine derivative of progesterone **1c**[19] (Scheme I). Studies were subsequently conducted on the generality of this reaction. Medroxyprogesterone acetate (**2**) and hydrocortisone 21-acetate (**3**) were both treated with the acid chloride of nicotinic acid in the presence of an acid catalyst to yield the corresponding 3-enol esters (**2a** and **3a**, respectively).[20,21]

Scheme II. Proposed Mass Fragmentation Pattern (EI, 70 eV) for **2a**



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(17) In the preparation of **1a**, 5g (0.016 mol) of progesterone (1) was dissolved in 100 mL of freshly distilled pyridine. To this solution were added 6 g of nicotinoyl chloride hydrochloride and a catalytic amount of pyridinium tosylate. After 3 h, the solution was poured over ice and the resulting solid was collected by filtration. The brown powder was recrystallized from methanol to give pale brown crystals: yield 87%; mp = 187–196 °C; TLC (silica, 80:20 $CHCl_3$–acetone) R_f = 0.54; UV (methanol) λ_{max} 230, 266 nm; IR (KBr) 1698 (21-(CC=O)), 1733 ((pyridine-C=O)); ^1H NMR ($CDCl_3$) 9.35 (bs, 1 H, pyridine C-2 proton), 8.87 (m, 1 H, pyridine C-6 proton), 8.40 (m, 1 H, pyridine C-4 proton), 7.47 (m, 1 H, pyridine C-5 proton), 5.93 (s, 1 H, alkenic proton (C-4)), 5.50 (s, 1 H, alkenic proton (C-6)), 2.83–0.63 (m, 18 H, skeletal protons), 2.13 (s, 3 H, acetyl protons), 1.08 (s, 3 H, C-18 protons), 0.68 (s, 3 H, C-19 protons); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) 208.94 (COCH_3), 163.41 (ester carbonyl), 153.59 (pyridine C-2), 150.71 (pyridine C-6), 146.72 (pregnane C-3), 138.98 (pregnane C-5), 137.17 (pregnane C-4), 137.34 (pyridine C-4), 125.57 (pyridine C-3), 124.47 (pyridine C-5), 117.30 (pregnane C-6), 63.25 (pregnane C-17), 56.62 (pregnane C-14), 47.57 (pregnane C-9), 43.80 (pregnane C-13), 38.46 (pregnane C-10, C-16), 34.72 (pregnane C-12), 33.57 (pregnane C-1), 31.51 (pregnane C-8), 31.39 (pregnane C-2), 24.66 (pregnane C-7), 24.19 (pregnane C-21), 22.58 (pregnane C-15), 21.02 (pregnane C-11), 8.71 (pregnane C-19), 13.20 (pregnane C-18). Anal. ($C_{27}H_{33}NO_2$) C, H, N.
(18) In the preparation of **1b**, 1 g of 3-[(3-pyridinylcarbonyl)oxy]-pregn-3,5-dien-20-one (**1a**) (2.4 mmol) was dissolved in 25 mL of acetone. To this mixture was added 2 mL of methyl iodide and the solutions refluxed overnight. The solid produced was collected by filtration: yield 91% (1.2 g); mp = 222–225 °C; UV (methanol) λ_{max} 208, 228, 266 nm; ^1H NMR (DMSO-d_6) 9.68 (bs, 1 H, pyridinium C-2 proton), 9.30 (m, 1 H, pyridinium C-6 proton), 9.07 (m, 1 H, pyridinium C-4 proton), (m, 1 H, pyridine C-5 proton), 5.98 (s, 1 H, alkenic proton (C-4)), 5.53 (bs, 1 H, alkenic proton (C-6)), 4.50 (s, 3 H, N^+CH_3), 2.80–0.87 (m, 18 H, skeletal protons), 2.07 (s, 3 H, acetyl protons), 0.98 (s, 3 H, C-18 protons), 0.57 (s, 3 H, C-19 protons). Anal. ($C_{28}H_{36}NO_3I$) C, H, N, I.
(19) In the preparation of **1c**, 2 g (3.6 mmol) of 1-methyl-3-[[(pregn-3,5-dien-20-on-3-yl)oxy]carbonyl]pyridinium iodide (**1b**) was mixed with 100 mL of 70% aqueous methanol to which 1.5 g (18 mmol) of $NaHCO_3$ had been added. A 1.9-g portion (10.3 mmol) of $Na_2S_2O_4$ was then added while the mixture cooled at 0 °C. After 1 h of mixing, the reaction mixture was extracted with CH_2Cl_2 several times. The organic layer was washed, dried over $MgSO_4$, reduced in volume, and chromatographed on a neutral alumina column prepared with CH_2Cl_2: yield 89% (1.3 g); UV (methanol) 210, 230, 362 nm; ^1H NMR ($CDCl_3$) 7.03 (s, 1 H, pyridine C-2 proton), 5.63 (m, 2 H, pyridine C-6 proton and C-4 alkenic proton), 5.38 (bs, 1 H, alkenic proton, C-6, 4.8 (m, 1 H, C-5 pyridine proton), 3.13 (bs, 2 H, pyridine C-4 pyridine protons), 2.95 (s, 3 H, NCH_3), 2.75–0.95 (m, 18 H, skeletal protons), 2.18 (s, 3 H, acetyl protons), 1.03 (s, 3 H, C-18 protons), 0.65 (s, 3 H, C-19 protons). Anal. ($C_{28}H_{37}NO_3$) C, H, N.
(20) ^1H and ^{13}C NMR assignments for **2a** were given as follows: ^1H NMR (300 MHz, $CDCl_3$) δ 9.28 (d, 1 H, pyridine C-2), 8.81 (m, 1 H, pyridine C-6), 8.36 (m, 1 H, pyridine C-4), 7.45 (m, 1 H, pyridine C-5), 6.25 (2, 1 H, alkenic proton), 2.12 (s, 3 H, 17-acetate), 2.06 6α-methyl), 1.05 (s, 3 H, 18-methyl), 0.683 (s, 3 H, 19-methyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.13 (20-carbonyl carbon), 170.75 (17-acetate carbonyl carbon), 163.89 (enol nicotinate carbonyl carbon), 153.63 (pyridine C-2), 151.08 (pyridine C-6), 147.05 (pregnane C-3), 137.34 (pyridine C-4), 131.52 (pregnane C-5), 128.51 (pregnane C-4), 125.88 (pyridine C-3), 123.39 (pyridine C-5), 113.56 (pregnane C-6), 96.92 (pregnane C-17), 51.92 (pregnane C-14), 47.45 (pregnane C-9), 46.71 (pregnane C-13), 38.70 (pregnane C-10 and C-16), 35.19 (pregnane C-12), 33.88 (pregnane C-1), 31.58 (pregnane C-8), 31.08 (pregnane C-2), 30.45 (6α-methyl), 26.35 (pregnane C-15), 26.32 (pregnane C-7), 24.53 (pregnane C-21), 21.24 (acetate methyl), 20.91 (pregnane C-15), 18.87 (pregnane C-11), 18.04 (pregnane C-19), 14.36 (pregnane C-18).

Proof of the assigned structures took the form of infrared (IR), ^1H and ^{13}C NMR, and mass spectrometric analyses. Upon enolization, the long-wavelength carbonyl stretching of progesterone (1672 cm^{-1}) underwent a hypsochromic shift to 1732 cm^{-1}, consistent with the formation of **1a**. The 17-acetyl moiety is unaffected by this manipulation. In the ^1H NMR spectra, the proton attached to the central carbon of the α,β-unsaturated ketone is shifted downfield by δ 0.23 and a second proton (δ 5.44) attached to an unsaturated carbon appeared. The ^{13}C NMR reveals alterations consistent with the assigned conversion including the appearance of an ester carbonyl absorbance at δ 163.4, shielding of the 3-pregnane carbon (δ 197.3 → 146.7), and appearance of an additional unsaturated (pregnane C-6) carbon (δ 117.3). Similar changes were manifested upon enolization in medroxyprogesterone acetate and hydrocortisone 21-acetate. Compound **2a** was also examined by electron impact (EI) mass spectrometry. Proposed fragmentation patterns are given in Scheme II. On the basis of the spectral interpretation of the parent steroid,[22] a mass shift of m/z 105 was observed for structurally significant ions, consistent with the structure proposed for the 3-enol nicotinate. Spectroscopic analyses of **1b**[18] and **1a**[19] were consistent with their assigned structures.

The conversion of **1b** to the parent compound was examined in various biological matrices including whole human blood, whole rat blood, rat liver homogenate, and rat brain homogenate.[23] As shown in Table I, hydrolysis of **1b** to **1** occurred at varying rates in various media. Conversion of **1b** was faster in rat blood than human blood, consistent with the high enzymatic activity of the rodent systems. Compound **1c** was relatively stable in human blood but converted to the quaternary salt with a $t_{1/2}$ of 16 min in rat liver homogenate and about 1.7 h in brain homogenate.

These studies indicate that enol esters of α,β-unsaturated ketones are viable bridges for chemical delivery systems. These compounds can be prepared from the ketone and nicotinoyl chloride hydrochloride in the presence of an acid catalyst. The dihydropyridines were shown to be oxidized in biological media to yield the quaternary salts. Likewise, the salts were quickly hydrolyzed to yield the parent compound.

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† This is contribution no. 36 in the series Improved Delivery through Biological Membranes.

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(21) ^1H and ^{13}C NMR and MS assignments for **3a** were given as follows: ^1H NMR (300 MHz, DMSO-d_6) δ 9.16 (d, 1 H, pyridine C-2), 8.84 (m, 1 H, pyridine C-6), 8.34 (d, 1 H, pyridine C-4), 7.57 (m, 1 H, pyridine C-5), 5.93 (s, 1 H, alkenic (4) proton), 5.50 (s, 1 H, alkenic (6) proton), 4.95 (q, 2 H, 21-CH_2, J = 8 Hz), 2.10 (s, 3 H, CH_3CO), 1.18 (s, 3 H, 19-CH_3), 0.52 (s, 3 H, 18-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) 205.45 (pregnane C-20), 169.69 (acetate carbonyl)*, 163.17 (nicotinate carbonyl)*, 153.04 (pyridine C-2), 150.33 (pyridine C-6), 146.84 (pregnane C-3), 137.09 (pregnane C-4), 136.98 (pyridine C-4), 136.82 (pregnane C-5), 125.18 (pyridine C-3), 123.77 (pyridine C-5), 116.38 (pregnane C-6), 67.79 (pregnane C-11), 48.99 (pregnane C-21), 46.35 (pregnane C-9); MS m/z (EI, 70 eV) 491 ($M^+ - H_2O$), 390 ($M^+ - H_2O - COCH_2OCOCH_3$), 372 (390 – H_2O), 332 (M^+ – D ring – H_2O), 267 (M^+ – C and D ring). An asterisk indicates exchangeable assignments.

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(23) Sprague–Dawley rats, Charles Rivers Lboratories, were the source of brain, liver, and blood samples. Freshly obtained brain or liver was homogenized with pH 7.4 phosphate-buffered saline to give a final concentration of 20% w/v. These matrices, as well as rat and human blood, were maintained at 37 °C. The test compound (5×10^{-3} M) was then introduced in a small volume (25 μL) of dimethyl sulfoxide to 2.5 mL of the matrix. After various time intervals, samples (100 μL) of homogenate were withdrawn, treated with cold acetonitrile (400 μL), and centrifuged at 13000g for 5 min. The supernatant was then analyzed by HPLC. The analytical method utilized a Toya Soda ODS 120T C-18 reversed-phase column operating at ambient temperature. The mobile phase consisted of 75:25 acetonitrile–0.05 M KH_2PO_4 and the flow rate was 1.0 mL/min. Under this condition, progesterone (1) eluted at 9.5 min and **1b** eluted at 5.7 min. Compounds were quantitated with ultraviolet detection at 266 nm. In the case of **1c**, a mobile phase containing 90:10 acetonitrile–water was required and the compound had a retention time of 13.1 min. The compound was detected at 360 nm.

A Radiotracer for Mapping Cholinergic Neurons of the Brain

Sir:

A γ-emitting radiotracer that localizes selectively in vivo in brain cholinergic neurons could provide an externally detectable map of regional neuronal degeneration in Alzheimer's disease. Such a radiopharmaceutical might also be used to monitor the neuronal-sparing efficacy of future drugs developed for treating this devastating disease. Vesamicol is a potent inhibitor of the vesicular sequestration of acetylcholine (Ach).[1-3] Structural studies and kinetic data suggest that vesamicol binds to the outside of the storage vesicle membrane at a locus distinct from the active site of the Ach transporter. The inhibition of Ach storage by vesamicol is stereoselective and noncompetitive, suggesting that a specific receptor exists which can allosterically modulate the Ach transporter.[2] Parsons and co-workers recently reported an elegant study of the Ach blocking activity of 84 analogues of vesamicol.[1] A number of derivatives in the benzovesamicol series were equipotent with vesamicol. Additionally, it was found that the edge of the benzo ring anti to the hydroxyl group in benzovesamicol was a territory of high bulk tolerance; seemingly this region of the compound points away from the receptor surface. In this communication we describe the synthesis and preliminary in vivo screening of racemic 5-[^{125}I]iodobenzovesamicol (IBVM) and its enantiomers as possible mapping agents for central cholinergic neurons.

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