Improved Delivery through Biological Membranes. 11.[1] A Redox Chemical Drug-Delivery System and Its Use for Brain-Specific Delivery of Phenylethylamine[2]

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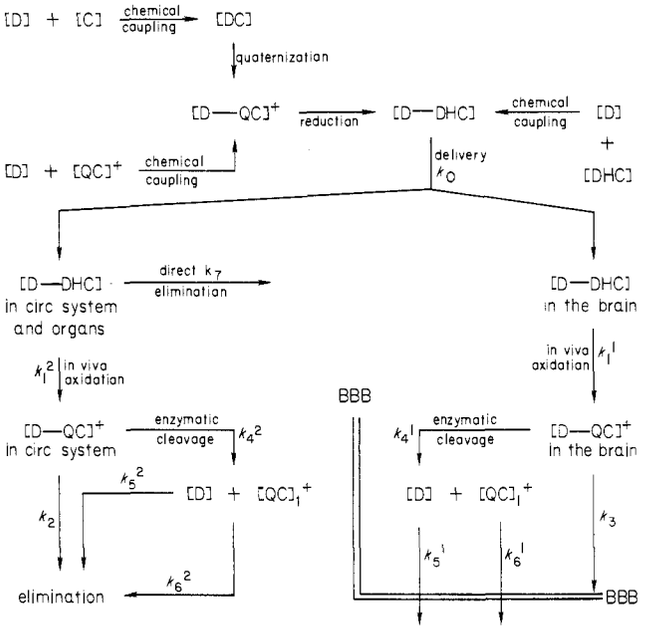
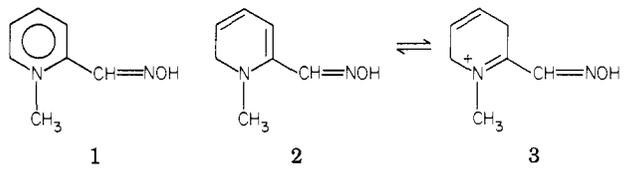
A dihydropyridine ⇄ pyridinium salt type redox system is described as a general and flexible method for site-specific and sustained delivery of drugs to the brain. According to this, a biologically active compound linked to a lipoidal dihydropyridine carrier easily penetrates the blood–brain barrier. Oxidation of the carrier part in vivo to the ionic pyridinium salt prevents its elimination from the brain, while elimination from the general circulation is accelerated. Subsequent cleavage of the quaternary carrier–drug species results in sustained delivery of the drug in the brain and facile elimination of the carrier part. The concept is illustrated with phenethylamine as the drug and trigonelline as the quaternary carrier. One injection to rats of 1-methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine resulted in continuous build up in the brain of the corresponding 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium salt, reaching a maximum at about 80 min. At this time, the general circulation was practically void of either compound, while the accumulated carrier–drug species provided a source for sustained drug delivery only in the brain.

The concept of developing methods for site-specific delivery of biologically active agents is highly desirable to improve efficacy and decrease toxicity. Although a lot of work has been done in this area, whether by the use of drug delivery systems,[4] carriers,[5] and/or transient derivatization and prodrug approach,[6–9] the concept is still in its primordial phase. Very few examples have promised simple and successful solutions to the many problems encountered. The site-specific and/or sustained release of drugs to the brain is even more difficult. The delivery of drugs to the brain is often seriously limited by transport and metabolism factors and more specifically by the functional barrier of the endothelial brain capillary wall called the blood–brain barrier (BBB).[10] It is generally accepted that the ability of the molecule to pass the blood–brain barrier is a function of its partition coefficient between lipid and water. Lipid insoluble or highly ionized compounds fail to achieve a cerebrospinal fluid (CSF)/plasma distribution ratio of 1[11,12] unless they are actively transported.[13] This is due to the fact that their rate of entry is relatively slower than their rate of exit from the CSF. The approach of derivatizing the compound and forming a prodrug that exhibits improved physicochemical properties for the transport through the blood–brain barrier[10] should be treated with caution, since while one can improve delivery of the drug to the brain in the same time, the drug may exhibit improved transport to other tissues and deep depots, thus increasing the incidence of systemic side effects. Hence, delivering drugs exclusively or preferentially to the brain is very difficult, and until this time, no simple and general method to achieve this goal has been known.

Scheme I



Based on our earlier observations[14,15] that *N*-methylpyridinium 2-carbaldoxime (2-PAM), a quaternary salt type drug (1), can be delivered to the brain as its 5,6-di-



1 2 3

hydropyridine derivative (Pro-2-PAM; 2 and 3), where the

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active species (2-PAM) is then formed, as well as, in view of our studies, on the bidirectional time-dependent movement of this 2-PAM ⇌ Pro-2-PAM system through the BBB,[16] a general delivery concept based on dihydropyridine ⇌ pyridinium salt carrier was developed, which can function as a specific and sustained release method, as shown by Scheme I.

According to this, a drug [D] is either coupled to a tertiary carrier [C] and then quaternized or coupled to a quaternary carrier $[QC]^+$ directly, and the obtained $[D–QC]^+$ is reduced chemically to the lipoidal dihydro form [D–DHC]. Alternately, the drug [D] can directly be coupled with the dihydro carrier [DHC]. After in vivo administration of this [D–DHC] compound, it is quickly distributed (k_0) throughout the body, including the brain. The dihydro form [D–DHC] is then oxidized in the brain (k_1^1) and in the body (k_1^2) (i.e., the NAD ⇄ NADH system) to the original $[D–QC]^+$ (ideally inactive) quaternary salt. [Superscript 1 refers to processes in the brain, while superscript 2 indicates similar processes in the body. These latter processes are given an overall rate (i.e., k_1^2 for oxidation), although the actual process takes place with different rates in the various organs.] $[D–QC]^+$, due to its ionic hydrophilic character, should be eliminated fast from the body, while the BBB should prevent its elimination from the brain ($k_2 \gg k_3$; $k_2 \gg k_7$). Enzymatic cleavage of the $[D–QC]^+$ that is "locked in" the brain will result in a sustained delivery of the drug species [D], following its normal elimination (k_5^1), metabolism. A properly selected carrier, $[QC]^+$, will also be eliminated fast from the brain ($k_6^1 \gg k_3$). Due to the facile elimination of $[D–QC]^+$ from the general circulation, only small amounts of drug are released in the body ($k_2 \gg k_4^2$), [D] will be released primarily in the brain ($k_4^1 > k_3$). The overall result, ideally, will be a *brain-specific sustained release of the target drug.*

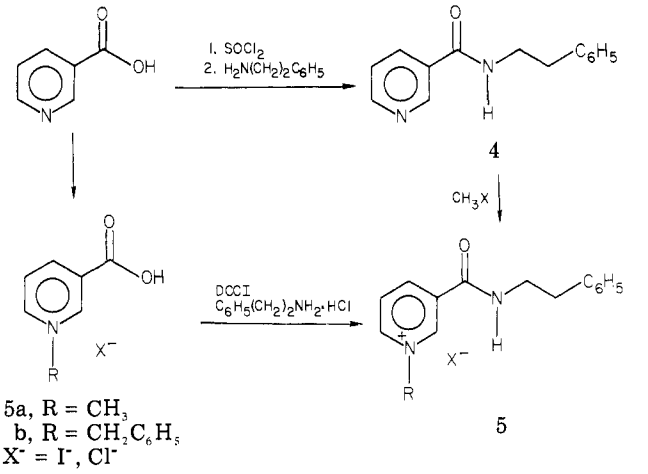
A quick analysis of the concept indicates that this is not a simple prodrug type design, but rather a "*chemical delivery system*" (CDS). That is, the $[D–QC]^+$ species can be considered a prodrug of [D], but the reduced [D–DHC] form, which is actually delivered, is not a prodrug; rather, it is a special pro-prodrug with additional properties resulting in a site-specific and sustained release. It is evident, that simple prodrug approaches cannot solve the variety of transport-delivery problems[17] they are expected to overcome. Multistep optimization of the drug delivery is required in many cases, particularly when site-specific or site-enhanced delivery is the objective. In general terms, in these cases, the drug [D] is transformed via several chemical steps into the chemical delivery system form [CDS], which will then, after administration, undergo several enzymatic and/or chemical transformations, for example, via a prodrug, in order to achieve the desired optimal delivery.

$$[CS]' + [D] \xrightarrow{\text{chemical reactions}} [CDS] \xrightarrow{\text{delivery to body}} [CDS]\ \text{at site} \xrightarrow{\text{enzymatic reaction [1]}}$$

$$[PD]\ \text{at site} \xrightarrow[\text{reaction [2]}]{\text{enzymatic}} [D] + [P]'\ \text{drug at site}$$

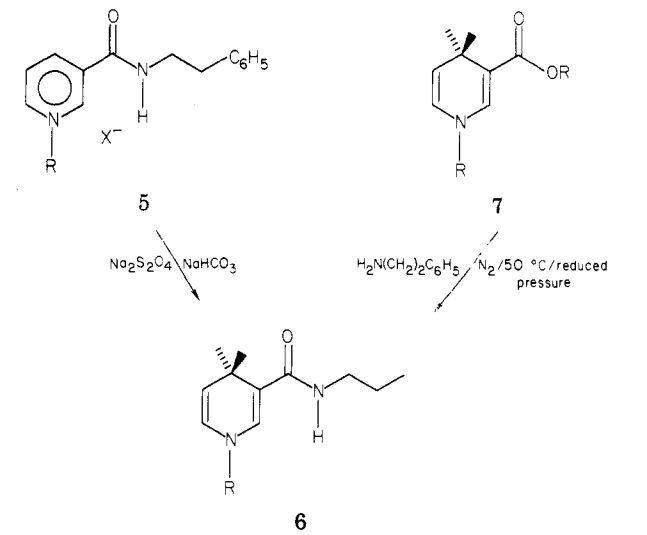
A variant of this concept was successfully applied to the delivery of hydrocortisone and other steroids[18,19] and most recently by a very interesting alternate way for delivery of some drugs to the eye.[20]

Scheme II



5a, R = CH_3
b, R = $CH_2C_6H_5$
X^- = I^-, Cl^-

Scheme III[a]



[a] R = CH_3 (a); R = $CH_2C_6H_5$ (b)

One additional, very significant aspect of the present redox delivery system relates to toxicity: It is expected to significantly reduce systemic toxicity by accelerating the elimination of the drug–quaternary carrier system from the general circulation. On the other hand, even the central toxicity should be reduced by providing a low level, sustained release of the active species in the brain. One main factor in this whole picture is the choice of the quaternary carrier, which must be of low toxicity alone and in combination with the drug.

It is important to note that this method will provide the desired level of a drug in the CNS, without requiring high circulatory concentrations. The drug blood level has virtually no effect on the brain levels, after the last oxidation step and the "lock in" process takes place.

The present paper describes an application of the concept by using phenethylamine, which, besides its own activity is an adequate model drug for a variety of CNS amines. *N*-Alkylnicotinic and 3,5-pyridinedicarboxylic acids were investigated as precursors for the carrier systems.

Chemistry. Alternate routes for the synthesis of the designed quaternary derivatives, $[D–QC]^+$ (**5**) (Schemes

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Table I. Rates of Oxidative Conversion in Biological Fluids[a] of 1-Methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6a) and 1-Benzyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6b) to the Corresponding Quaternary Pyridinium Salts 5a and 5b

	6a → 5a (methyl)				6b → 5b (benzyl)			
medium	*N*	$k \times 10^{-4}$, s^{-1}	*r*	$t_{1/2}$, min	*N*	$k \times 10^{-4}$, s^{-1}	*r*	$t_{1/2}$, min
human plasma	13	1.80 ± 0.34	0.998	64.2 ± 12.1	12	0.74 ± 0.11	0.999	156 ± 2.3
whole blood	5	8.40 ± 0.94	0.952	13.7 ± 1.9	5	4.74 ± 0.93	0.974	24.4 ± 5.1
brain homogenate	8	4.12 ± 0.28	0.996	28.2 ± 2.0	13	2.10 ± 0.14	0.999	55 ± 4.3
liver homogenate	7	8.13 ± 0.96	0.999	14.4 ± 2.1	5	7.50 ± 0.60	0.998	15.3 ± 1.2

[a] At 37 °C, undiluted heparinized human whole blood, 20% fresh human plasma, and 2% rat brain and liver homogenates were used. The conversions of 6a to 5a and 6b to 5b were followed by the changes in their characteristic UV spectra ($\lambda_{max} \simeq 350$ nm for 6a and 6b; 262 nm for 5a and 5b), against appropriate reference samples.

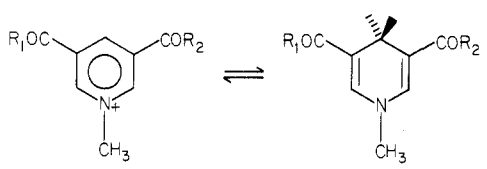
Table II. Rates of Oxidative Conversion in Blood and Brain Homogenate[a] of Selected 3,5-Disubstituted Dihydropyridine Derivatives, 9a and 9b

	9a → 8a				9b → 8b			
medium	*N*	$k \times 10^{-5}$, s^{-1}	*r*	$t_{1/2}$, h	*N*	$k \times 10^{-5}$, s^{-1}	*r*	$t_{1/2}$, h
brain homogenate	6	0.84 ± 0.03	0.997	22.9 ± 0.0	6	1.74 ± 0.03	0.983	11.1 ± 0.02
blood	5	4.94 ± 0.6	0.949	3.9 ± 0.5	5	11.30 ± 0.47	0.972	1.17 ± 0.4

[a] At 37 °C, undiluted heparinized human blood and 2% fresh brain homogenate were used.

II and III), have been tested in order to select the one with the highest yield and purity of the final compounds. Phenethylamine was either coupled with nicotinic acid, followed by quaternization to the salt [D–QC]$^+$ (**4** and **5**), or coupled directly with trigonelline. The corresponding 1,4-dihydropyridine derivative [D–DHC] (**6**) was prepared either by reduction of the corresponding quaternary (**5**) with sodium dithionite or by coupling of the reduced trigonelline ester (**7**) with phenethylamine (Schemes II and III).

In order to select the best carrier system, we also synthesized a number of 3,5-disubstituted quaternary model compounds (**8**), where the stability of the corresponding



8 9

8a, 9a, $R_1 = R_2 = NHCH_2CH_2C_6H_5$
8b, 9b, $R_1 = C_2H_5O$
$R_2 = NHCH_2CH_2C_6H_5$

dihydropyridine derivatives (**9**) is enhanced by extending the conjugation using an electron-attracting substituent capable of resonance interaction at C5.[21]

Physiocochemical and in Vitro Studies. First, the kinetics and reaction products of the oxidation reaction of the 1,4-dihydropyridine derivatives (**6a,b**, and **9a,b**) were studied with alcoholic silver nitrate and hydrogen peroxide solutions. The in vitro rates of oxidation of the 1,4-dihydropyridine derivatives (**6a,b** and **9a,b**) in 20% plasma, 2% brain homogenate, 2% liver homogenate, and in whole blood were also determined. To have an idea about the site-specific conversion of the quaternary **5a** in the brain, we determined the kinetics of its disappearance in the brain homogenate.

In Vivo Brain-Delivery Studies. The dihydropyridine derivative **6a** was selected for the in vivo study in order to demonstrate the concept. A solution of **6a** in Me_2SO was injected through the jugular vein to a group of male Sprague–Dawley rats, which were then sacrificed at various time intervals following administration, and the blood and the brain were analyzed for the quaternary compound (**5a**).

Results and Discussion

It was found that the synthesis of the quaternary salt (**5a**) via coupling first nicotinic acid with phenethylamine, followed by quaternization (Scheme II), is the best choice. An overall yield of about 66% calculated on the phenethylamine was thus obtained. Coupling trigonelline hydrochloride with phenylethylamine hydrochloride by using DCCI in pyridine gave a comparable yield of a crude product, which, however, needed to be crystallized several times. For the preparation of the 1,4-dihydropyridine derivative (**6a**), reduction of the corresponding quaternary salt gave purer products than coupling 1,4-dihydrotrigonelline ester (**7**) with phenethylamine. The reduction of the quaternary salts (**5**) have been done by using the selective and ambivalent[22] reducing agent sodium dithionite, in slightly alkaline medium. It is known that reduction of 3-substituted or 3,5-disubstituted pyridinium salts with sodium dithionite in mildly basic solutions affords exclusively the corresponding 1,4-dihydropyridines,[21] whose structures were also established for the present compounds (**5a,b** and **9a,b**) based on their spectral (UV and NMR) and chemical properties.[21,22] The dihydro derivatives obtained were either oily or semisolid compounds. Attempts to prepare the β-protonated enamine salts were unsuccessful, since some nucleophilic addition and dimerization reactions occur. The dihydro derivatives **6a** and **6b** were found to be relatively stable and can be quantitatively oxidized back to the corresponding quaternary derivatives by H_2O_2 or $AgNO_3$ (based on spectral data and characterization of the isolated products), while **9a** and **9b** were found to be very stable toward air-oxidation and, as expected, require a longer reaction time, with H_2O_2 or $AgNO_3$, to get oxidized quantitatively to the corresponding quaternary salts **8a** and **8b**.

In Vitro Studies. The in vitro kinetics in biological fluids indicated facile oxidative conversion of the dihydro forms **6a** and **6b** to the corresponding quaternary derivatives, as indicated in Table I, while the rate of interconversion of compounds **9a** and **9b**, Table II, indicates that such dihydro forms are significantly more stable under these conditions, as well.

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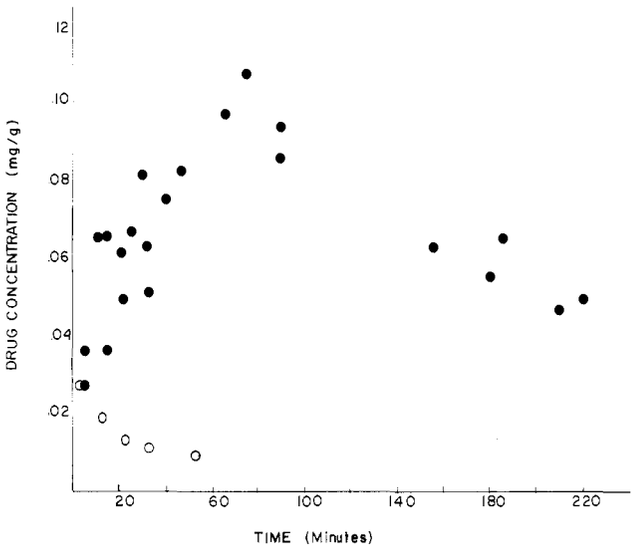

Figure 1. The concentration of 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium (ion) (**5a**) in the brain (●) and the blood (○) of rats following administration of 1-methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (**6a**).

In Vivo Studies. The *N*-methyl dihydro derivative **6a** was selected for in vivo study due to its relative ease of interconversion to **5a**. The results summarized in Figure 1 very strongly support the concept shown in Scheme I. Thus, the quaternary salt **5a** (ion) disappears quickly from the blood. On the contrary, the concentration of **5a** (ion) is increasing in the brain steadily, reaching a maximum at about 80 min following administration. The next, descending portion corresponds to a half-life of 2.35 ($k = 4.92 \times 10^{-3}$ min^{-1}; $r = 0.962$). The rate of site-specific conversion of **5a** to phenethylamine and trigonelline was found to have a half-life of 3 h. Thus, the descending portion in Figure 1 corresponds mainly to the sustained delivery of phenethylamine in the brain. Thus, all criteria set forth in Scheme I were met: One, iv injection of a drug coupled to a dihydropyridine carrier system resulted in an accumulation of the corresponding drug–quaternary carrier species in the brain, followed by a sustained release of the drug in the brain, while the drug–quaternary carrier system was rapidly eliminated from the blood. When the quaternary derivative **5a** was administered at equivalent dose levels, no amount could be detected in the brain of rats.

These successful brain-delivery and "locking-in" experiments made it possible to study the unique bidirectional movement of the complex [D–DHC] $\rightleftarrows$ [D–QC]$^+ \rightarrow$ [D] + [QC]$^+_1$ system. The kinetics of appearance and disappearance of the [D–QC]$^+$ species in the brain and blood showed dramatic differences and suggest the usefulness of this kind of system for studies of some *important basic transport mechanisms in the brain.* It appears that the uptake of [D–DHC] in the brain is a passive transport, while elimination of small [QC]$^+$ species is suggested to be by an active transport, as shown before[16] in the case of another small quaternary compound.

The overall significance of the proposed carrier system is multiple, since it should lead to highly improved treatment of numerous disease states, reduce toxicity of a number of drugs currently used, make available to the brain a variety of important drugs, and should lead to important information and understanding of the transport and enzyme systems in the BBB, as well as in the brain.

Experimental Section

Chemistry. All melting points were taken on a Mel-Temp apparatus and are not corrected. Elemental analyses were performed at Atlantic Microlab, Inc., Atlanta, GA. Infrared spectra were determined with a Beckman Acculab 1 double-beam recording spectrophotometer. NMR spectra were determined by means of a Varian T60A spectrometer. All chemical shifts reported are in δ units (parts per million) relative to tetramethylsilane. Ultraviolet absorbance spectra were determined with a Cary Model 219 spectrophotometer.

***N*-Phenethylnicotinamide** (4). To a solution of 14.7 g (0.083 mol) of nicotinoyl chloride hydrochloride in 150 mL of dry and freshly distilled pyridine was dropped 10.45 mL (0.083 mol) of phenethylamine while stirring over 15 min. The mixture was then heated on a water bath for 2 h. Pyridine was removed by rotary evaporation. The brown oily residue was poured onto crushed ice. The cream-white solid separated and was filtered by suction, washed with cold water, and crystallized from 50% aqueous methanol to yield 4 (13.3 g 70%): mp 79–80 °C; IR (KBr) 3320 (NH), 1630 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.66 (br s, 1 H, C_2 pyridine proton), 8.46 (br s, 1 H, C_6 pyridine proton), 8.0–7.6 (m, 1 H, C_4 pyridine proton), 7.33–6.90 (br s, 6 H, C_6H_5 + C_5 pyridine proton), 7.0–6.57 (broad, 1 H, CONH), 3.73 (q, 2 H, NCH_2), 2.97 (t, 2 H, CH_2Ph). Anal. ($C_{14}H_{14}N_2O$) C, H, N.

1-Methyl-3-(*N*-phenethylcarbamoyl)pyridinium Iodide (5a). To a solution of 2.26 g (0.01 mol) of *N*-phenethylnicotinamide in 5 mL of methanol was added 1.3 mL (0.02 mol) of methyl iodide. The mixture was refluxed for 3 h. The methanol was removed, and the yellow oily residue was cooled and, upon scratching the flask, transformed to a yellow gritty solid. The solid was crystallized from acetone to yield **5a** (3.5 g, 95%): mp 134–136 °C; UV max (buffer pH 7.4) 210, 225, 226 nm; IR (KBr) 3240 (NH), 1665 (C=O) cm^{-1}; NMR ($CDCl_3$/Me_2SO-d_6) δ 9.63 (s, 1 H, C_2 pyridine proton), 9.4–8.9 (m, 2 H, C_4 and C_6 pyridine protons), 8.32–8.06 (m, 1 H, C_5 pyridine proton), 4.6 (s, 3 H, N^+CH_3), 3.9–3.46 (m, 2 H, NCH_2), 3.2–2.8 (m, 2 H, CH_2Ph). Anal. ($C_{15}H_{17}IN_2O$) C, H, N.

1-Benzyl-3-(*N*-phenethylcarbamoyl)pyridinium Bromide (5b). To a solution of 2.26 g (0.01 mol) of *N*-phenethylnicotinamide (4) in 5 mL of methanol was added 1.4 mL (0.0114 mol) of benzyl bromide. The mixture was refluxed for 3 h. The solvent was removed, and the yellow oily residue was transformed into a buff gritty material upon scratching. The solid was crystallized from acetone/ether to yield **5b** (3.7 g, 95%): mp 142–144 °C; UV max (buffer pH 7.4) 210 and 260 nm; IR (KBr) 3180 (NH), 1670 (C=O) cm^{-1}; NMR ($CDCl_3$/Me_2SO-d_6) δ 10.26 (br s, 1 H, C_2 pyridine proton), 9.53–8.90 (m, 2 H, C_6 and C_4 pyridine protons), 8.16–7.13 (m, 12 H, $2C_6H_5$ + CONH + C_5 pyridine proton), 6.13 (s, 2 H, N^+CH_2), 3.96–3.50 (m, 2 H, NCH_2), 3.26–2.83 (m, 2 H, CH_2Ph). Anal. ($C_{21}H_{21}BrN_2O$) H, N; C: calcd, 63.48; found, 63.99.

1-Methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6a). To a solution of 3.68 g (0.01 mol) of compound **5a** in 200 mL of deaerated water were added 5.0 g (0.06 mol) of sodium bicarbonate and 200 mL of ether. The mixture was stirred in an ice bath, and 7.1 g (0.04 mol) of sodium dithionite was added gradually over a period of 5 min. The mixture was stirred for 3 h under nitrogen. The ether layer was then separated, washed with water, dried with Na_2SO_4, and distilled under vacuo. A yield of 1.8 g (76%) of **6a** as a bright yellow viscous oil was obtained, which gives a positive test for dihydropyridine with alcoholic silver nitrate solution: UV max (buffer pH 7.4) 210, 290, and 360 nm; NMR ($CDCl_3$) δ 7.2 (s, 5 H, C_6H_5), 6.9 (br s, 1 H, C_2 pyridine proton), 5.6 (d of d, 1 H, J = 8 and 2 Hz, C_2 pyridine proton), 5.3–5.1 (broad, 1 H, CONH), 4.5–4.7 (m, 1 H, C_5 pyridine proton + NCH_3 + CH_2Ph). Anal. ($C_{15}H_{18}N_2O$) C, H, N.

1-Benzyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6b). Using the same method described above, we reduced compound **5b** (3.97 g, 0.01 mol) with sodium dithionite (7.1 g, 0.04 mol) and sodium bicarbonate (5.0 g, 0.06 mol) to yield 2.3 g (72%) of a bright yellow viscous oil, which reduces alcoholic silver nitrate solution: UV max (buffer pH 7.4) 210 and 355 nm; NMR ($CDCl_3$) two overlapping singlets at 7.2 (10 H, $2C_6H_5$), 7.1 (br s, 1 H, C_2 pyridine proton), 5.68 (d of d, 1 H, J = 8 and 2 Hz, C_6 pyridine proton), 6.4–5.0 (broad, 1 H, CONH), 4.84–4.60 (m, 1 H, C_5 pyridine proton), 4.35 (s, 2 H, NCH_2), 3.5 (q, 2 H, J = 7.0 Hz, NCH_2), 3.0 (br s, 2 H, C_4 pyridine proton), 2.8 (t, 2 H J = 7.0 Hz, CH_2Ph).

3,5-Bis(*N*-phenethylcarbamoyl)pyridine (10a). To a solution of 2.53 g (0.01 mol) of diethyl 3,5-pyridinedicarboxylate

in 10 mL of methanol was added 3.0 g (0.025 mol) of phenethylamine. The mixture was refluxed overnight, and then the solvent was removed. The residue was washed with very dilute HCl solution and water, dried, and crystallized from ethanol to yield 2.9 g (80%) of **10a**: mp 189–190 °C; NMR ($CDCl_3$) δ 9.00 (d, J = 2 Hz, 2 H, 2,6-dipyridyl), 8.33 (t, J = 2 Hz, 1 H, 4-pyridyl), 7.30 (s, 10 H, 2 C_6H_5), 6.93–6.40 (broad, 2 H, 2 CONH), 3.83 (q, J = 7 Hz, 4 H, 2 NCH_2), 3.00 (t, J = 7 Hz, 4 H, 2 CH_2Ph). Anal. ($C_{23}H_{23}N_3O_2$) C, H, N.

5-Carbethoxy-3-(*N*-phenethylcarbamoyl)pyridine (10b). To 10 g (0.05 mol) of 5-carbethoxy-3-pyridinecarboxylic acid[23] was added 30 mL of thionyl chloride, and the mixture was warmed on a water bath while stirring until clear (~3 h). The excess thionyl chloride was distilled under reduced pressure. The residue was cooled to room temperature, and 50 mL of sodium-dry benzene was added. The solution was cooled in an ice bath, a solution of 6.2 g (0.051 mol) of phenethylamine and 4 mL of pyridine in 50 mL of dry benzene was dropped in while stirring over 1 h, and the mixture was left overnight at room temperature. The mixture was then washed with water until free from Cl^- ion. The organic layer was dried with Na_2SO_4, and the solvent was removed. The residue was crystallized from ether/petroleum ether mixture to yield **10b** (9.0 g, 67%): mp 159–161 °C; IR (KBr) 3300 (NH), 1725 (ester CO), 1650 cm^{-1} (amide CO); NMR ($CDCl_3$) δ 9.13–8.96 (two doublets, 2 H, J = 2 Hz, C_2 and C_6 pyridine protons), 8.53 (t, 1 H, J = 2 Hz, C_4 pyridine proton), 7.16 (s, 6 H, C_6H_5 + CONH), 4.36 (q, 2 H, J = 7 Hz, OCH_2), 3.4 (q, 2 H, J = 7 Hz, NCH_2), 2.9 (t, 2 H, J = 7 Hz, CH_2Ph), 1.33 (t, 3 H, J = 7 Hz, CH_3). Anal. ($C_{17}H_{18}N_2O_3$) C, H, N.

1-Methyl-3,5-bis(*N*-phenethylcarbamoyl)pyridinium Iodide (8a). To a solution of 2.0 g (5.3 mmol) of the diamide **10a** in 10 mL of acetone was added 2 mL of methyl iodide, and the mixture was refluxed for 24 h. The yellow crystalline solid that separated was filtered, washed with acetone, and dried. Obtained were 1.4 g (51%) of **8a**: mp 186–188 °C; UV spectrum of a solution in phosphate buffer, pH 7.4, showed a plateau at 275 nm, a shoulder at 225 nm, and a sharp peak at 203 nm (ϵ 67 356); IR (KBr) 3240 (NH), 1665, 1650 cm^{-1} (twin band, C=O); NMR ($CDCl_3/D_2O$) δ 9.35 (d, 2 H, J = 2 Hz, C_2 and C_6 pyridine protons), 8.56 (d, 1 H, J = 2 Hz, C_4 pyridine proton), 7.20 (s, 10 H, 2 C_6H_5), 4.56 (s, 3 H, N^+CH_3), 3.66 (t, 4 H, J = 7 Hz, 2 NCH_2), 2.96 (t, 4 H, J = 7 Hz, 2 CH_2Ph). Anal. ($C_{24}H_{26}IN_3O_2$) C, H, N.

5-Carbethoxy-1-methyl-3-(*N*-phenethylcarbamoyl)-pyridinium Iodide (8b). To a solution of 2.9 g (0.01 mol) of compound **10b** in 5 mL of acetone was added 3 mL of methyl iodide. The mixture was refluxed while stirring for 8 h and then left overnight. The yellow crystalline solid that precipitated was filtered, washed with acetone, dried, and crystallized from acetone to yield **8b** (3.5 g, 82%): mp 168–170 °C; IR (KBr) 3250 (NH), 1725 (ester CO) 1670 (amide CO) cm^{-1}; UV max (buffer pH 7.4) 268 (weak plateau) and 206 nm (ϵ 53 667); NMR (Me_2SO-d_6) δ 9.53 (br s, 2 H, C_2 and C_6 pyridine protons), 9.33–9.10 (m, 1 H, C_4 pyridine proton), 7.16 (s, 5 H, C_6H_5), 4.63–4.26 (complex m, 5 H, N^+CH_3 + OCH_2), 3.56 (q, 2 H, J = 6 Hz, NCH_2), 2.90 (t, 2 H, J = 6 Hz, CH_2Ph), 1.4 (t, 3 H, J = 7 Hz, CH_3). Anal. ($C_{18}H_{21}IN_2O_3$) C, H, N.

1-Methyl-3,5-bis(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (9a). The title compound was prepared by the same procedure as compound **6a**, with 1 g (0.002 mol) of **8a**, 1.0 g (0.012 mol) of sodium bicarbonate, and 1.4 g (0.008 mol) of sodium dithionite to yield 0.65 g (86%) of an orange-yellow semisolid, which could not be crystallized. Its alcoholic solution shows a slow reduction to alcoholic silver nitrate solution: UV max (buffer pH 7.4) 388, 210 nm; NMR ($CDCl_3$) δ 7.13 (s, 5 H, C_6H_5), 6.76 (s, 1 H, C_2 pyridine protons), 3.51 (q, 4 H, J = 7 Hz, 2 NCH_2), 3.06–2.60 (m, 9 H, OCH_2 + C_4 pyridine proton + NCH_3).

5-Carbethoxy-1-methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (9b). This compound was prepared following the same procedure as compound **6a** with 1.0 g (0.002 mol) of **8b**, 1.0 g (0.012 mol) of sodium bicarbonate, and 1.42 g (0.008 mol) of sodium dithionite to yield 0.60 g (84%) of an orange-yellow viscous oil, which reduces alcoholic silver nitrate, but very slowly: UV max (buffer pH 7.4) 205, 390 nm; NMR ($CDCl_3$) δ 7.33 (s, 5 H, C_6H_5), 7.0 (s, 2 H, C_2 and C_6 pyridine protons), 5.8–5.3 (broad, 1 H, CONH), 4.2 (q, 2 H, J = 7 Hz, OCH_2), 3.66 (q, 2 H, J = 7 Hz, NCH_2), 3.16 (br s, 2 H, C_4 pyridine protons), 3.0 (q, 2 H, J = 7 Hz, CH_2Ph), 1.4 (5, 3 H, J = 7 Hz, CH_3).

Oxidation by Hydrogen Peroxide. To 10 mL of 30% H_2O_2 was added 0.2 g of the dihydropyridine derivative (**6a,b** and **9a,b**). The mixture was stirred, and samples were taken to check the UV spectrum.

Oxidation by Silver Nitrate. To 5 mL of saturated methanolic $AgNO_3$ solution was added 1 mL of 5% methanolic solution of the dihydropyridine derivative. The mixture was shaken, left for 5 min to complete precipitation of silver and then centrifuged, and an aliquot was taken to check the UV spectrum.

Calibration Curves. A UV study of compounds **5a,b**, **6a,b**, **7a,b**, and **9a,b** revealed that they obey Beer's Law with good correlation coefficients (not less than 0.999) and at a wide range of dilution levels (10^{-4}–10^{-8} M) in both methanol and 2% aqueous methanol. The study was done at 350 nm for the dihydro derivatives and at 262 nm for all the quaternary salts.

Kinetics of Oxidation of the Dihydro Derivatives (Table I). In Plasma. A freshly prepared solution of the dihydro derivative (0.2 mL, 6.25×10^{-4} M) in methyl alcohol was diluted to 10 mL with 20% plasma (diluted with phosphate buffer, pH 7.4). The solution was kept at 37 °C and the UV spectrum was scanned from 400 to 250 nm every 10 min for 2 h against a reference sample made by dilution of 0.2 mL of methyl alcohol with 20% plasma to 10 mL.

In Whole Blood. In each of five tubes containing 0.1 mL of a 10×10^{-4} M methanolic solution of the freshly prepared dihydro derivative was added 2 mL of fresh heparinized whole human blood, and the tubes were kept at 37 °C in a water bath. At the end of the time period to be investigated, 8 mL of acetonitrile was added, and the tubes were then shaken vigorously and centrifuged. The absorption of the supernatant solution at 350 nm was measured. A reference sample was made by addition of 0.1 mL of methyl alcohol instead of the sample solution following the same procedure.

In Brain Homogenate. Rat brain tissue (2.0 g) was homogenized in 10 mL of phosphate buffer, pH 7.4. The homogenate was centrifuged for 15 min at 3000 rpm, decanted, heated in a water bath at 50 °C for 5 min, and then centrifuged again. The supernatant solution was diluted to 100 mL with phosphate buffer, pH 7.4. To 10 mL of the freshly prepared homogenate was added 0.2 mL of a 6.25×10^{-4} M methanolic solution of the freshly prepared dihydro derivative. The mixture was scanned at 37 °C from 400 to 250 nm every 10 min for 2 h on a Cary 219 spectrophotometer.

Reference Sample. Methyl alcohol (0.2 mL) was diluted to 10 mL with the brain homogenate solution, and the mixture was used to record the base line on a Cary 219 spectrophotometer and as a reference of the dihydro derivative sample solution.

In Liver Homogenate. Liver Homogenate Solutions. Sprague–Dawley rat liver tissue (5.0 g) was homogenized in 50 mL of phosphate buffer, pH 7.4. The homogenate was centrifuged, decanted, heated in a water bath at 50 °C for 5 min, and then centrifuged again. The supernatant homogenate was diluted to 250 mL with phosphate buffer, pH 7.4.

Reference Sample. Methyl alcohol (0.2 mL) was diluted to 10 mL with the liver homogenate solution, and the mixture was used to record the base line on a Cary 219 spectrophotometer and as a reference for the dihydro derivative sample solution.

Dihydro Derivative Sample Solution. A solution of the freshly prepared dihydro derivative (0.2 mL, 6.25×10^{-4} M) in methyl alcohol was diluted to 10 mL with liver homogenate solution. The mixture was scanned at 37 °C from 400 to 250 nm every 5 min for 1 h.

Analytical Methods. A high-pressure liquid chromatography method was developed for the analysis of the quaternary **6a**. The chromatographic analysis was performed on a component system consisting of a Waters Associates Model 6000A solvent-delivery system, a Model U6K injector, and a Model 440 dual-channel absorbance detector operated at 254 and 280 nm; a 30 cm × 3.9 mm (internal diameter) reverse-phase μBondapak C_{18} column

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(Waters Associates), operated at ambient temperature, was used. The mobile phase used consisted of a mixture of acetonitrile and 0.055 M phosphate buffer, pH 6.2 (3:2). At a flow rate of 2 mL/min, **6a** has a retention time of 2 min.

Kinetics of the Disappearance of the Quaternary Salt (5a) from the Brain Homogenate. A freshly perfused Sprague–Dawley rat brain (2.0 g) was homogenized in 20 mL of phosphate buffer, pH 7.4. A solution of 10.0 mg of 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium iodide (**5a**) in 2 mL of aqueous methanol (1:1) was added, and the thoroughly mixed mixture was kept at 37 °C in a water bath. At each time period, 1 mL of the mixture was taken, shaken thoroughly with 8 mL of acetonitrile, centrifuged, and injected into the HPLC. The amount of quaternary compound in the sample was determined from a standard calibration curve and percentage recovery of a sample taken at time 0.

In Vivo Study of 1-Methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6a). A group of 22 male Sprague–Dawley rats of average weight of 300 ± 50 g was anesthetized with Inovar, and the freshly prepared dihydro compound (**6a**) was injected through the external jugular as a solution in Me_2SO (0.5 g/mL) at a dose of 125 mg/kg animal body weight. The amount of Me_2SO thus injected is less than 0.2 g/kg, way below the dose of 1 g/kg that was shown[25] as still *not* affecting the permeability of the blood–brain barrier. At appropriate time periods, 1 mL of blood was withdrawn from the heart and added to 3 mL of saline in a tube; then the animal was perfused with 20 mL saline solution and decapitated, and the brain was obtained. The brains were weighed and, together with the blood samples, immediately placed in the freezer for overnight storage. Each brain was homogenized in 2 mL of water; then 8 mL of acetonitrile was added, and the mixture was homogenized again and centrifuged. To the blood samples was added 16 mL of acetonitrile, and the mixture was shaken vigorously and centrifuged. The supernatants from the brain and the blood samples were analyzed in duplicate by using HPLC. The amounts of the quaternary compound were determined from a standard calibration curve, and a recovery experiment was made by injection of a certain amount of the quaternary compound directly into a freshly perfused rat brain or 1 mL of blood and then treated in the same manner as the brain and blood samples.

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Registry No. 4, 24303-08-0; **5a**, 84254-38-6; **5b**, 84254-40-0; **6a**, 80703-25-9; **6b**, 39713-12-7; **8a**, 84278-95-5; **8b**, 84254-42-2; **9a**, 84278-96-6; **9b**, 84254-43-3; **10a**, 84254-39-7; **10b**, 84254-41-1; nicotinoyl chloride hydrochloride, 20260-53-1; phenethylamine, 64-04-0; 5-carbethoxy-3-pyridinecarboxylic acid, 84254-37-5; trigonelline hydrochloride, 6138-41-5; phenethylamine hydrochloride, 156-28-5; diethyl 3,5-pyridinedicarboxylate, 4591-56-4; 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium, 80703-26-0.

Structure–Activity Relationships in the Antiinflammatory Steroids: A Pattern-Recognition Approach

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A pattern-recognition technique has been used to determine structure–activity relationships for antiinflammatory steroids. Experimental results using the human vasoconstrictor test of McKenzie and Stoughton and the rat granuloma cotton pellet method of Meier were correlated with the various substructural descriptors. Steroids were classified into two categories according to potency, and a pattern-recognition method was applied to determine their relative ranking. The resulting structure–activity relationships obtained and the relative contributions of the various structural variables for both bioassays are discussed. A synergistic effect was predicted to be in operation between certain pairs of substituents.

The introduction and developoment of glucocorticosteroids have been the major therapeutic advances in dermatology during the past 50 years. Early work in corticosteroid research was directed to the synthesis of compounds with high antiinflammatory potency and to the reduction of side effects such as sodium retention. These attempts have been partially successful, since some corticosteroids have been synthesized that are locally active but that have reduced systemic activity.

Topical antiinflammatory activity has been enhanced by various modifications of the steroid nucleus, the most important being the removal or masking of the hydroxy groups and fluorination at the 6- and 9-positions. However, it is now believed[2] that the initial importance attached to fluorination has been overestimated, since a number of nonfluorinated steroids exhibit high potency, for example, hydrocortisone 17-butyrate and budesonide, and some fluorinated steroids display relatively low activity, such as betamethasone and dexamethasone.

In an attempt to rationalize the large volume of data that exists on the antiinflammatory steroids, we have applied a pattern-recognition technique in order to determine structure–activity relationships. Such relationships attempt to rationalize the connection between the molecular structure of a chemical compound and its measured biological activity. If such relationships could be determined for the steroids, then they would be of considerable practical and theoretical importance because of the significant role that steroids play in medicinal chemistry. For example, the relationships would allow the chemist to predict the biological activity of untested, or unsynthesized, steroids and, hence, adopt a more rational approach to drug design.

Structure–activity studies employed in medicinal chemistry in the past have used the empirical method of Hansch,[3] the mathematical model of the Free–Wilson

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