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SYNTHESIS OF 1,4-DIHYDROPYRIDINES HAVING AN N-ALKYLPYRIDINIUM SUBSTITUENT AT THE 4-POSITION AND THEIR AFFINITY TOWARDS LIPOSOMAL MEMBRANES

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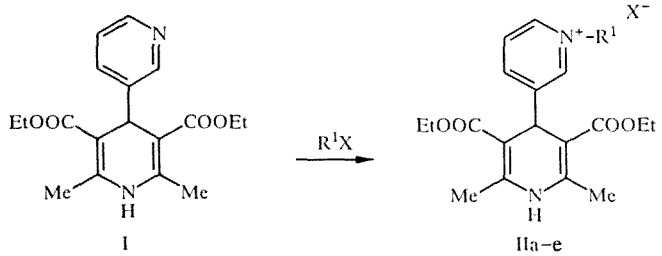
There has been synthesized a series of 1,4-dihydropyridines having an N-alkylpyridinium substituent at position 4 with a varying length of hydrocarbon chain. Their affinity to model (liposomal) membranes has been studied. It was found that this affinity increased with lengthening of the hydrocarbon chain on the N-pyridinium substituent at the 4-position of the 1,4-dihydropyridine ring. However, lengthening of the hydrocarbon chain in the 3,5-ester groups of the 1,4-dihydropyridine ring led to a decrease in the binding to the liposome when a 4-(N-hexadecylpyridinium) substituent was present.

Among the broad spectrum of novel pharmacologically active compounds major attention has been drawn to 1,4-dihydropyridines (1,4-DHP) [1-4]. In many cases, compound physiological activity depends on localization in the cell or the cell membrane [5], hence a study of compound binding to the membrane allows one to infer features of pharmacological activity. It has been shown that alkyl and cycloalkyl esters of 4-(2-difluoromethoxyphenyl)-1,4-DHP, having a marked affinity for the liposomal membrane, show hypotensive activity [6]. It is known that the calcium channel antagonist, amlodipine, binds well to model membranes [5] but other 1,4-DHP's having a positive charge in the molecule have not been systematically studied. There is only a single report [7] of the affinity to calcium channels of 1,4-DHP's having different length hydrocarbon chains in the 3,5-ester groups and containing quaternary nitrogen atom on the terminal carbon atom of these chains. In this work it was shown that both the length of the alkyl chain and the presence of the positive charge can influence the affinity of these compounds to the membrane.

With the aim of clarifying the effects of carbon chain lengths in substituents with different localizations on the ability of compounds having a positive charge to bind to liposomal membranes we have synthesized two series of 1,4-DHP's, viz. IIa-e and IVa-c:



I IIa–e

IIa R^1 = Me, X = I; b R^1 = C_3H_7, X = I; c R^1 = C_4H_9, X = Br; d R^1 = C_9H_{19}, X = Br; e R^1 = $C_{16}H_{33}$, X = Br

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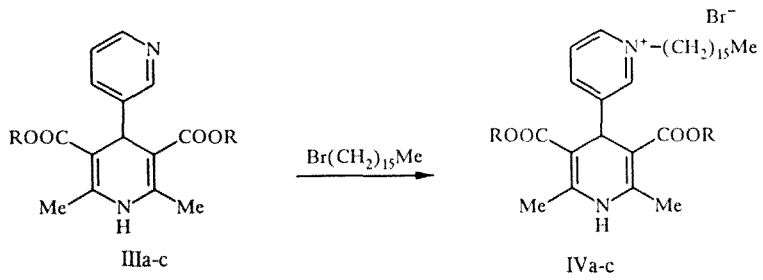

IIIa-c IVa-c

IVa R = Me, b R = C_4H_9, c R = $C_{14}H_{29}$

Compounds IIa-e and IVa-c were synthesized from the corresponding 4-(3-pyridyl) 1,4-DHP's (I and IIIa-c) by refluxing the starting material with the appropriate alkyl halide in acetone solution. For IIa-c an increase in the reaction time was observed with lengthening of the hydrocarbon chain of the alkyl halide.

The affinity of the 1,4-DHP to the liposomal membrane was assessed from the intensity of quenching of the fluorescence of anthracene. The nonpolar anthracene probe interacts with the membrane in the region of the glyceryl fatty acid ester bonds, i.e., near the boundary of the hydrophobic and polar regions of the glycerophospholipids. According to literature data [8-10] it is precisely here that the 1,4-dihydropyridines are localized. The mechanism of fluorescence quenching is characterized by the presence of a connection between the quenching and the concentration of the membrane bound compound (quencher) as follows [11]:

$$(F_0 - F) / F = K_q \cdot r / N , \quad (1)$$

where F_0 and F are the fluorescence intensities of the probe in the absence and presence of the quencher respectively, K_q the apparent quenching constant, r the concentration of the membrane bound quencher, and N the number of sites of membrane binding.

If the compound absorbs light in the fluorescence region for the membrane bound probe the fluorescence of the latter may be quenched as a result of radiationless (inductive–resonance or singlet–singlet) transfer of energy (in the donor an $S_1 \rightarrow S_0$ and in the acceptor an $S_0 \rightarrow S_1$ transfer). The radiationless transfer can be simply described as a dipole–dipole interaction between acceptor and donor [12].

The method of measuring the affinity of the substance to the membrane is carried out as follows. The 1,4-DHP is added to the liposome containing the fluorescent anthracene measured in the absence (F_0) and presence (F) of the quencher. The proportional concentration of the quencher bound to the membrane (r) can be calculated from the value of $\ln(F_0/F)$ in order to assess the affinity of the 1,4-DHP to the membrane [16].

$$r \sim A \cdot \ln (F_0/F) \quad (2)$$

Liposomes from egg phosphatidylcholine were used as the model membrane.

Transfer of energy from the donor to the acceptor molecules is possible under conditions where the maximum absorption of the acceptor falls within the maximum fluorescence of the donor. Hence, in order to correlate $\ln(F_0/F)$ for different compounds it was necessary to compare the absorption spectra for overlap of the spectrum for the investigated compounds with the fluorescence spectrum of anthracene.

All of the compounds studied have a long wave absorption in the region 360-364 nm (Table 2), hence the results obtained can be compared amongst themselves. The dependence of the compound affinity to the lipid membrane on the structure of the substituent in the 1,4-DHP molecule is illustrated in Table 1 and in Fig. 1.

An increase in the alkyl chain on the pyridyl quaternary nitrogen at position 4 of the 1,4-DHP ring leads to an increase in the affinity of the compound to the liposomal membrane (see Fig. 1), compound IIe having the highest affinity. With this in mind and keeping the 4-substituent unchanged, we increased the length of the hydrocarbon chain at positions 3 and 5 of the 1,4-DHP ring. However, lengthening of the alkly chain at these positions (compound IVb, IVc) causes the binding to the liposome to decrease.

TABLE 1. Effect of an Alkyl Substituent (R) in the 3 and 5 Positions of the 1,4-Dihydropyridine Ring on the Affinity of the Compounds to a Liposomal Membrane

Compound	R	ln (F_0/F)	Compound	R	ln (F_0/F)
IVa	CH_3	0,37	IVb	C_4H_9	0,30
IIe	C_2H_5	0,42	IVc	$C_{14}H_{29}$	0,10

TABLE 2. Parameters for the Compounds Synthesized

Compound	mp, °C	UV Spectrum (in ethanol), λ_{max}, nm (log ε)	Empirical formula	Found, % / Calculated, %			Yield, %
				C	H	N	
IIa	195...196	228 (4,44), 269 (3,87), 360 (3,62)	$C_{19}H_{25}N_2O_4I$	48,1 / 48,3	5,2 / 5,3	5,8 / 5,9	90
IIb	201...203	230 (4,48), 268 (3,91), 360 (3,69)	$C_{21}H_{29}N_2O_4I$	50,2 / 50,4	5,8 / 5,8	5,8 / 6,0	76
IIc	243...245	233 (4,39), 269 (3,88), 362 (3,66)	$C_{22}H_{31}N_2O_4Br$	56,5 / 56,6	6,7 / 6,7	6,0 / 5,8	60
IId	142...143	232 (4,38), 268 (3,89), 364 (3,67)	$C_{27}H_{41}N_2O_4Br$	60,1 / 60,3	7,6 / 7,7	5,0 / 5,2	88
IIe	135...136	233 (4,42), 269 (3,90), 362 (3,68)	$C_{34}H_{55}N_2O_4Br$	64,0 / 64,2	8,7 / 8,7	4,2 / 4,4	63
IVa	171...173	231 (4,42), 266 (3,89), 363 (3,67)	$C_{32}H_{51}N_2O_4Br$	63,1 / 63,3	8,6 / 8,5	4,5 / 4,6	75
IVb	125...127	232 (4,41), 268 (3,89), 360 (3,67)	$C_{38}H_{63}N_2O_4Br$	65,8 / 66,1	9,5 / 9,2	3,9 / 4,1	53
IVc	133...135	233 (4,46), 268 (3,95), 362 (3,75)	$C_{58}H_{103}N_2O_4Br$	71,3 / 71,6	10,6 / 10,7	2,6 / 2,9	89

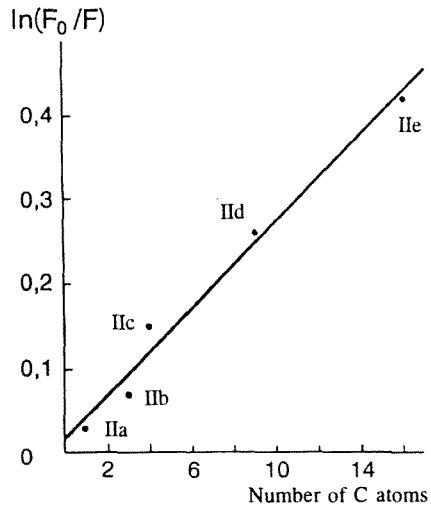

Fig. 1. Binding of IIa-e with the liposomal membrane.

EXPERIMENTAL

PMR spectra were recorded on a Bruker WH-90/DS (90 MHz) spectrometer using TMS as internal standard. UV spectra were taken on a Hitachi 557 UV-vis spectrophotometer. The basic parameters for the compounds obtained are given in Tables 2-4.

TABLE 3. PMR Spectra of IIa-d

Compound	Chemical shifts, δ (ppm) and spin-spin coupling constants, J, (Hz)								
	4-Py				N-H, br. s	4-H, s	2,6-CH_3, s	3,5-C_2H_5	N-R^1
	6-H, d, J_{65} = 6	2-H, s	4-H, d, J_{45} = 8	5-H, dd, J_{54} = 8, J_{56} = 6					
IIa	8,99	8,74	8,39	7,83	7,55	5,10	2,55	4,13 (q, J = 7), 1,30 (t, J = 7)	4,68 (c)
IIb	9,27	8,59	8,35	7,94	7,96	5,06	2,52	4,05 (q, J = 7), 1,20 (t, J = 7)	4,76 (t, J = 7), 2,25...1,88 (m), 1,02 (t, J = 7)
IIc	9,35	8,80	8,58	8,32	7,89	5,09	2,53	4,06 (q, J =7), 1,21 (t, J = 7)	4,80 (t, J = 7), 2,10...1,18 (m), 0,97 (t, J = 7)
IId	9,00	8,36	8,33	7,90	8,33	5,09	2,50	4,07 (q, J =7), 1,99 (t, J = 7)	4,72 (t, J = 7), 1,99...1,13 (m), 0,87 (t, J = 7)

TABLE 4. PMR Spectra of II IVa-c

Compound	Chemical shifts, δ (ppm) and spin-spin coupling constants J, (Hz)										
	4-Py				N—H, br. s	4-H, s	N—$C_{16}H_{33}$			2,6-CH_3, s	R
	6-H, d, J_{65} = 6	2-H, s	4-H, d, J_{45} = 8	5-H, dd, J_{54} = 8, J_{56} = 6			N—CH_2, t, J = 7	$(CH_2)_{14}$, m	CH_3, t, J = 7		
IVa	9,22	8,89	8,30	7,88	8,52	5,06	4,77	2,14...1,25	0,87	2,54	3,62 (6H, s)
IIe	9,26	8,72	8,33	7,90	8,69	5,06	4,75	2,00...1,21	0,86	2,52	4,05 (4H, q, J = 7), 1,22 (6H, t)
IVb	9,25	8,77	8,34	7,90	8,63	5,08	4,75	1,99...1,20	0,82	2,50	4,00 (4H, t, J = 7), 2,0...1,2 (14H, m)
IVc	9,15	8,66	8,32	7,87	8,61	5,05	4,75	2,0...1,05	0,87	2,51	3,99 (4H, t, J = 7), 2,0...1,05 (48H, m), 0,87 (6H, t, J = 7)

The method for determining the affinity of the 1,4-DHP to the model phospholipid membrane has been described in [6]. Fluorescence measurements were performed on a Hitachi 850 spectrofluorimeter. The error in the method did not exceed 5%.

1-Methyl-3-(2′,6′-dimethyl-3′,5′-dicarbethoxy-1′,4′-dihydropyridyl-4′)pyridinium Iodide (IIa). Compound I (1 g, 0.003 mole) was dissolved with heating in acetone (20 ml) and methyl iodide (1.3 ml, 2.2 g, 0.015 mole) was added two to three aliquots over 20 min. The product was refluxed for 1 h. After cooling, the filtered precipitate was recrystallized from acetone to give 1.3 g of yellow crystalline product.

1-Propyl-3-(2′,6′-dimethyl-3′,5′-dicarbethoxy-1′,4′-dihydropyridyl-4′)pyridinium Iodide (IIb). Compound I (1 g, 0.003 mole) was dissolved with heating in acetone (20 ml) and propyl iodide (1.47 ml, 2.6 g, 0.015 mole) was added. The product was refluxed for 8 h. After cooling, the filtered precipitate was recrystallized from acetone to give 1.2 g of yellow crystalline product.

1-Butyl- or 1-Nonyl-3-(2′,6′-dimethyl-3′,5′-dicarbethoxy-1′,4′-dihydropyridyl-4′)pyridinium Bromides (IIc, IId). Compound I (1 g, 0.003 mole) wad dissolved in acetone (50 ml) and butyl or nonyl bromide (0.045 mole) added. The product was refluxed for 40-48 h, cooled, and the filtered precipitate was recrystallized from acetone to give IIc (0.9 g) or IId (1.4 g).

1-Hexadecyl-3-(2′,6′-dimethyl-3′,5′-dicarbalkoxy-2′,4′-dihydropyridyl-4′)pyridinium Bromides (IVa-c, IIe). The corresponding esters (IIIa-c, 0.003 mole) were dissolved with heating in acetone or a 1:1 mixture of acetone and chloroform (10-20 ml) and hexadecyl bromide (1.8 ml, 1.8 g, 0.006 mole) was added. The product was refluxed for 45-50 h, cooled to −15°C, and the precipitate filtered and washed on the filter with hot hexane. Recrystallization from acetone gave IIe (1.2 g), IVa (1.5 g), IVb (1.0 g), or IVc (1.3 g).

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