Chemistry of Heterocyclic Compounds, Vol. 32, No. 10, 1996

SYNTHESIS OF 1,4-DIHYDROPYRIDINE DERIVATIVES HAVING AN OXY-, ALKOXY-, OR DIMETHYLAMINOPHENYL SUBSTITUENT IN THE 4 POSITION, THEIR ANTIOXIDANT ACTIVITY, AND THEIR BINDING TO PHOSPHOLIPID MEMBRANES

M. Plotnietse, G. Tirzitis, Ya. Uldrikis, Zh. Koronova, D. Tirzite, N. Makarova, and G. Duburs

The method of Hantzsch was used to synthesize esters and amides of 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylic acid, containing electron donor substituents in the phenyl ring. It was found that in addition to their antioxidant properties, 3,5-diamides and 4-(3',4'-dioxyphenyl) derivatives have an affinity for model phospholipid membranes.

It is well known that certain derivatives of 1,4-dihydropyridine (1,4-DHP) exhibit antioxidant activity (AOA) [1-4], which is expressed mainly in 4-unsubstituted 1,4-DHP [5-7]. In 4-substituted derivatives, the presence of an oxyphenyl substituent in the ester groups of 1,4-dihydropyridine-3,5-dicarboxylic acid leads to an increase in AOA [8], and certain 4-oxyphenyl-1,4-DHP exhibit anti-inflammatory [9] and anti-ischemic [10] activity. However, there have been no systematic studies of 1,4-DHP that contain a phenol substituent in the 4 position, this substituent being characteristic of many natural and synthetic antioxidants. From this standpoint it was desirable, to study AOA in the series 4-oxyphenyl-, 4-alkoxyphenyl-, and 4-dimethylaminophenyl-1,4-DHP, as well as their bonding to phospholipid membranes, since in the disperse systems constituted by all biological objects, the degree of activity of antioxidants depends on their properties of penetrating membranes.

According to Hantzsch's classical method of synthesis, a series of derivatives of 1,4-DHP (I-XXIX) were synthesized from substituted benzaldehydes, esters, or amides of acetoacetic acid and ammonia.

The yields, melting points, and ultimate analysis data are presented in Table 1, and the ESR spectral data are given in Table 2; the data on AOA and on bonding with phospholipid membranes are presented in Table 3. As a rule, the yields of compounds II-XII, XIV-XXII, and XXV are lower than those of 1,4-DHP that were synthesized from benzaldehydes containing electron-acceptor substituents. Among the synthesized compounds II-XII, XIV-XXII and XXV, the yields are higher in the case of alkoxybenzaldehydes (see compounds VI and XI), and also in the case of methyl esters of acetoacetic acid (see compounds VI and VII, XVII and XVIII). The data of ESR spectra fully confirm the structures of the synthesized derivatives of 1,4-DHP: the spectra of compounds II-XII, XIV-XXII and XXV show signals characteristic of the 2,6-CH_3 groups, with chemical shifts in the range 2.03-2.33 ppm; the signals of 2',3',5', and 6' protons of the p-substituted phenyl ring in the 4 position consist of a pair of doublets with chemical shifts in the region of 6.6 ppm and 7.2 ppm and a splitting constant of 9 Hz. In the UV spectra (not shown) of derivatives of 1,4-dihydropyridine-3,5-dicarboxylic acid, there are absorption peaks in the region 350-357 nm which are characteristic of esters, and in the region 343-345 nm, of amides. In the IR spectra (not shown), characteristic absorption bands of N−H groups in the region 3318-3425 cm^{-1} and of 3,5-carbonyl groups in the region 1680-1711 cm^{-1} are observed.

Latvian Institute of Organic Synthesis, Riga LV-1006. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 10, pp. 1358-1365, October, 1996. Original article submitted June 20, 1996.

 0009-3122/96/3210-1166$15.00

TABLE 1. Reaction Times, Yields and Characteristics of Synthesized Compounds

Compound	mp, °C (recrystallization solvent)	Empirical formula	Found, % / Calculated, %			Rection time, h	Yield, %
			C	H	N		
II	164...166 (4:1 benzen–hexane)	$C_{21}H_{27}NO_5$	67.3 67,5	7.5 7,3	3.5 3,8	5	39
III	180...182 (2:3 ethanol–water)	$C_{23}H_{31}NO_5$	68.6 68,8	7.9 7,8	3.5 3,5	5,5	31
IV	112...114 (3:1 ethanol–water)	$C_{43}H_{71}NO_5$	75.3 75,7	10.9 10,5	1.9 2,1	5,5	52
V	250 (dec.) (ethanol)	$C_{27}H_{25}N_3O_3$	73.7 73,8	5.5 5,7	9.5 9,6	1,5	41
VI	244...246 (1: 1 ethanol–hexane)	$C_{17}H_{19}NO_6$	61.5 61,3	5.8 5,7	3.8 4,2	4,5	47
VII	200...201 (1:2 ethanol–water)	$C_{19}H_{23}NO_6$	63.4 63,2	6.4 6,4	4.0 3,9	6	19
VIII	166...170 (1:2 ethanol–water)	$C_{27}H_{25}N_3O_4$	68.6 68,5	5.8 5,7	8.5 8,8	4,5	37
IX	191...194 (ethanol)	$C_{18}H_{21}NO_5$	65.4 65,2	6.5 6,4	4.0 4,2	2,5	60
X	57...59 (hexane)	$C_{36}H_{57}NO_5$	74.0 74,1	10.4 9,8	2.2 2,4	5	35
XI	141...143 (1:2 ethanol–water)	$C_{19}H_{23}NO_6$	63.3 63,2	6.4 6,4	3.6 3,9	4,5	65
XII	110...113 (*)	$C_{21}H_{27}NO_6$	64.6 64,8	7.2 7,0	3.7 3,6	32	17
XIV	135...138 (1:4 benzen–hexane)	$C_{20}H_{25}NO_5$	67.0 66,8	7.2 7,0	3.6 3,9	4,5	69
XV	54...57 (ethanol)	$C_{46}H_{77}NO_5$	75.9 76,3	11.3 10,7	1.6 1,9	5	22
XVI	280 (dec.) (1:1 ethanol–water)	$C_{30}H_{31}N_3O_3$	74.3 74,8	6.6 6,5	8.4 8,7	1,5	49
XVII	115...117 (1:3 benzen–hexane)	$C_{24}H_{33}NO_5$	69.5 69,4	8.2 8,0	3.5 3,4	4,5	87
XVIII	75...77 (hexane)	$C_{26}H_{37}NO_5$	70.3 70,4	8.8 8,4	3.0 3,2	5	21
XIX	49...52 (ethanol)	$C_{42}H_{69}NO_5$	75.2 75,5	10.9 10,4	1.9 2,1	5	64
XX	64...66 (ethanol)	$C_{50}H_{85}NO_5$	76.9 77,0	11.6 11,0	1.5 1,8	5	32
XXI	200 (dec.) (2:1 ethanol–water)	$C_{34}H_{39}N_3O_3$	75.4 75,9	7.4 7,3	7.8 7,8	2,5	46
XXII	102...105 (hexane)	$C_{32}H_{49}NO_5$	72.7 72,8	10.4 9,4	2.5 2,7	5	79
XXV	245 (dec.) (1:1 dioxane–water)	$C_{29}H_{30}N_4O_2$	73.3 73,2	6.5 6,6	11.4 11,8	4	26

*Purified chromatographically.

The AOA of the synthesized compounds was determined in a disperse system of joint oxidation of β-carotene and methyl linoleate. Peroxidation of the lipids was initiated with 2′,2′-azobis-2-amidinopropane hydrochloride. The oxidation rate was estimated from the decrease in β-carotene concentration [19-21]. The activities of the compounds studied were compared with the activity of the 4-unsubstituted derivative of 1,4-DHP, i.e., diludin (2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine, compound XXVI), which is used as an antioxidant in agriculture [15-16] and as a radiation protector for the skin in clinical practice [22]. As is evident from the data obtained (Table 3), among the compounds studied, amides (V, VIII, XVI, XXI, XXV, XXVII, XXIX) were found to have the greatest AOA. Antioxidant properties were exhibited by compounds VI and VII, which, like compound VIII, have a dioxyphenyl substituent in the 4 position. The 4-unsubstituted amide XXVII has an AOA similar to diludin, but lower than that of amides substituted in the 4 position, apparently because of intrinsic instability. Comparing compounds XXVI and XXVIII, which contain ethoxycarbonyl groups in the 3 and 5 positions, we see

TABLE 2. ESR Spectra of Synthesized Compounds

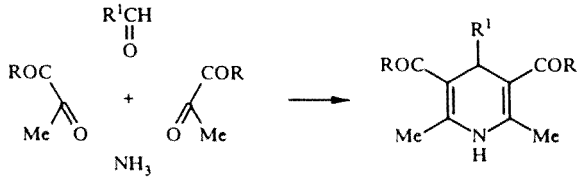
Compound	Chemical shifts, δ, ppm, and spin-spin coupling constants, J, Hz				
	R	2,6-CH_3, S, 6H	4-H, S	N-H, br.s	R^1
1	2	3	4	5	6
II	0,89 (6H, t, J = 7, CH_3); 1,40...1,85 (4H, m, CH_2CH_3); 4,01 (4H, t, J = 7, OCH_2)	2,32	4,92	5,57	5,11 (1H, s, 4'-OH); 6.60 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.10 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
III	0,60...1,80 (16H, m, $C(CH_3)$, C_2H_5); 4,82 (2H, m, OCH)	2,30	5,74	5,90	4.91 (1H, s, 4'-OH); 6,59 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.10 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
IV	0,87 (6H, t, J = 6, CH_3); 1,10...1,75 (48H, m, $(CH_2)_{12}$); 4,02 (4H, m, J = 6, OCH_2)	2,31	5,65	5,37	4.92 (1H, s, 4'-OH); 6,59 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.10 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
V	6,83...7,58 (10H, m, C_6H_5); 9,10 (2H, s, CONH)	2,03	4,93	7,93	6.53 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7,22 (2H, AA'XX', J_{AX} = 9, 3',5'-H); 9.02 (1H, s, 4'-OH)
VI	3,50 (6H, s, CH_3)	2,18	4,65	8,73	6.30 (1H, d.d, 6'-H, $J_{2',6'}$ = 3, $J_{5',6'}$ = 7.5); 6,49 (1H, d, $J_{5',6'}$ = 7.5, 5'-H); 6,50 (1H, d, $J_{2',6'}$ = 3, 2'-H); 8,42 and 8,50 (2 × 1H, two s, 3'-OH, 4'-OH)
VII	1,14 (6H, t, J = 7, CH_3); 3.96 (4H, q, J = 7, OCH_2)	2,21	4,67	8,61	6.30...6.55 (3H, m, $-C_6H_3-$); 8.45 and 8,52 (2 × 1H, two s, 3'-OH, 4'-OH)
VIII	6,30...7,60 (10H, m, C_6H_5); 9,03 (2H, s, CONH)	2,05	4,83	7,92	6.30...7.60 (3H, m, $-C_6H_3-$); 8.49 and 8,53 (2 × 1H, two s, 3'-OH, 4'-OH)
IX	3,60 (6H, s, CH_3)	2,30	4,94	5,72	3.71 (3H, s, 4'-OCH_3); 6.72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.17 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
X	0,87 (6H, t, J = 7, CH_3); 1,25 (28H, s, $(CH_2)_7$); 1.50...1,70 (4H, m, CH_2CH_3); 4.00 (4H, t, J = 7, OCH_2)	2,31	4,92	5,52	3.71 (3H, s, 4'-OCH_3); 6,69 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7,15 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XI	3,64 (6H, s, CH_3)	2,31	4,95	5,69	3,78 and 3,80 (6H, two s, 3' and 4'-OCH_3); 6,71 (1H, s, 2'-H); 6,78 (2 × 1H, two s, 5' and 6'-H)

TABLE 2. (Continued)

Compound	Chemical shifts, δ, ppm, and spin-spin coupling constants, J, Hz				
	R	2,6-CH_3, s, 6H	4-H, s	N-H, br.s	R^1
1	2	3	4	5	6
XII	1,14 (t, 6H, CH_3, J =7); 4,02 (4H, q, J = 7, OCH_2)	2,24	5,15	6,00	3,72 and 3,74 (6H, two s, 2', 4'-OCH_3); 6,31 (1H, d, J = 9, 6'-H); 6,39 (1H, s, 3'-H); 7,09 (1H, d, J = 9, 5'-H)
XIV	3.81 (6H, s, CH_3)	2,30	4,94	5,61	0.98 (3H, t, J = 6.8, 4'-CH_3); 1,55...1.95 (2H, m, 4'-β-CH_2); 3,82 (2H, t, J = 6.8, 4'-OCH_2); 6.72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.15 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XV	0,86 (6H, t, J = 6, CH_3); 1.44...1.95 (48H, m, $(CH_2)_{12}$); 3.98 (4H, t, J = 6, OCH_2)	2,30	4,92	5,51	0,98 (3H, t, J = 7.5, 4'-CH_3); 1.44...1.95 (2H, m, 4'-β-CH_2); 3.82 (2H, t, J = 7.5, 4'-OCH_2); 6.72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.16 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XVI	6.82...7.35 (12H, m, NHC_6H_5)	2,21	4,64	5,44	1.00 (3H, t, J = 7, 4'-CH_3); 1.60...1.98 (2H, m, 4'-β-CH_2); 3.87 (2H, t, J = 7, 4'-OCH_2); 6,82...7.35 (4H, m, C_6H_4)
XVII	3.63 (6H, s, CH_3)	2,33	4,93	5,64	0,90 (3H, t, J = 6, 4'-CH_3); 1,50 (8H, br.s, 4'-$(CH_2)_4$); 1,70...1.90 (2H, m, 4'-β-CH_2); 3.88 (2H, t, J = 6, 4'-OCH_2); 6.72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.15 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XVIII	1.22 (6H, t, J = 7, CH_3); 4.07 (4H, q, J = 7, OCH_2)	2,30	4,92	5,77	0.93 (3H, t, J = 7, 4'-CH_3); 1.32 (8H, br.s, 4'-$(CH_2)_4$); 1.60...1.93 (2H, m, 4'-β-CH_2); 3,88 (2H, t, J = 7, 4'-OCH_2); 6,72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7,15 (2H, AA'XX', J_{AX} = 9, 3',6'-H)
XIX	0,88 (6H, t, J = 6, CH_3); 1.45...1.85 (32H, m, $(CH_2)_8$); 4.02 (4H, t, J = 6, OCH_2)	2,28	4,92	5,57	0,88 (3H, t, J = 6, 4'-CH_3); 1,26 (8H, br.s, 4'-$(CH_2)_4$); 1,45...1,85 (2H, m, 4'-β-CH_2); 3.88 (2H, t, J = 6, 4'-OCH_2); 6.70 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.17 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XX	0.87 (6H, t, J = 6, CH_3); 1.10...1.85 (4H. m, CH_2CH_3); 1.26 (44H, br.s $(CH_2)_{11}$); 4.00 (4H, t, J = 6, OCH_2)	2,30	4,92	5,54	0.87 (3H, t, J = 6, 4'-CH_3); 1.10...1.85 (10H, m, 4'-$(CH_2)_4$); 3.85 (2H, t, J = 6, 4'-OCH_2); 6.71 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7.16 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XXI	6,80...7.33 (12H, m, NHC_6H_5)	2,21	4,63	5,37	0,87 (3H, t, J = 6, 4'-CH_3); 1.10...1.90 (10H, m, 4'-$(CH_2)_5$); 3.89 (2H, t, J = 6, 4'-OCH_2); 6.80...7.33 (4H, m, C_6H_4)
XXII	3.36 (6H, s, CH_3)	2,33	4,93	5,57	0,88 (3H, t, J = 7, 4'-CH_3); 1.26 (24H, br.s, 4'-$(CH_2)_{12}$); 1.60...1.90 (2H, m, 4'-β-CH_2); 3,88 (2H, t, J = 7, 4'-OCH_2); 6,72 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 7,15 (2H, AA'XX', J_{AX} = 9, 3',5'-H)
XXV	6.75...7.58 (12H, m, NHC_6H_5)	2,20	4,56	5,50	2.90 (6H, s, 4'-$N(CH_3)_2$); 6.69 (2H, AA'XX', J_{AX} = 9, 2',6'-H); 6.75...7.58 (2H, AA'XX', J_{AX} = 9, 3',5'-H)

TABLE 3. Parameters of Binding to Phospholipid Membranes and Antioxidant Activity of Compounds I-XXIX

Compound	R	R^1	ln (F_0 / F)	AOA
I	OC_2H_5	4-HOC_6H_4	0,29	1,4
II	OC_3H_7-n	4-HOC_6H_4	0,35	1,3
III	$OCH(CH_3)C_2H_5$	4-HOC_6H_4	0,30	1,1
IV	$OC_{14}H_{29}$-n	4-HOC_6H_4	0,11	1,2
V	NHC_6H_5	4-HOC_6H_4	0,26	5,7
VI	OCH_3	3,4-$(HO)_2C_6H_3$	0,19	7,4
VII	OC_2H_5	3,4-$(HO)_2C_6H_3$	0,29	6,7
VIII	NHC_6H_5	3,4-$(HO)_2C_6H_3$	0,27	10,3
IX	OCH_3	4-$CH_3OC_6H_4$	0,24	1,2
X	$OC_{10}H_{21}$-n	4-$CH_3OC_6H_4$	0,12	1,2
XI	OCH_3	3,4-$(CH_3O)_2C_6H_3$	0,16	1,1
XII	OC_2H_5	2,4-$(CH_3O)_2C_6H_3$	0,40	1,4
XIII	OCH_3	3,4,5-$(CH_3O)_3C_6H_2$	0,16	1,2
XIV	OCH_3	4-n-$C_3H_7OC_6H_4$	0,35	1,3
XV	$OC_{14}H_{29}$-n	4-n-$C_3H_7OC_6H_4$	0,10	1,1
XVI	NHC_6H_5	4-n-$C_3H_7OC_6H_4$	0,25	4,5
XVII	OCH_3	4-n-$C_7H_{15}OC_6H_4$	0,34	1,2
XVIII	OC_2H_5	4-n-$C_7H_{15}OC_6H_4$	0,37	1,4
XIX	$OC_{10}H_{21}$-n	4-n-$C_7H_{15}OC_6H_4$	0,13	1,1
XX	$OC_{14}H_{29}$-n	4-n-$C_7H_{15}OC_6H_4$	0,10	1,2
XXI	NHC_6H_5	4-n-$C_7H_{15}OC_6H_4$	0,25	3,6
XXII	OCH_3	4-n-$C_{15}H_{31}OC_6H_4$	0,08	1,4
XXIII	OCH_3	4-$(CH_3)_2NC_6H_4$	0,22	1,4
XXIV	$OCH(CH_3)_2$	4-$(CH_3)_2NC_6H_4$	0,40	1,4
XXV	NHC_6H_5	4-$(CH_3)_2NC_6H_4$	0,32	4,5
XXVI	OC_2H_5	H (diludin)	0,29	3,6
XXVII	NHC_6H_5	H	0,24	3,5
XXVIII	OC_2H_5	C_6H_5	0,34	1,4
XXIX	NHC_6H_5	C_6H_5	0,31	5,3



I-XXIX

I R = OC_2H_5, R^1 = 4-HOC_6H_4 [11]; II R = OC_3H_7-n, R^1 = 4-HOC_6H_4; III R = $OCH(CH_3)C_2H_5$, R^1 = 4-HOC_6H_4; IV R = $OC_{14}H_{29}$-n, R^1 = 4-HOC_6H_4; V R = NHC_6H_5, R^1 = 4-HOC_6H_4; VI R = OCH_3, R^1 = 3,4-$(HO)_2C_6H_3$; VII R = OC_2H_5, R^1 = 3,4-$(HO)_2C_6H_3$; VIII R = NHC_6H_5, R^1 = 3,4-$(HO)_2C_6H_3$; IX R = OCH_3, R^1 = 4-$CH_3OC_6H_4$; X R = $OC_{10}H_{21}$-n, R^1 = 4-$CH_3OC_6H_4$; XI R = OCH_3, R^1 = 3,4-$(CH_3O)_2C_6H_3$; XII R = OC_2H_5, R^1 = 2,4-$(CH_3O)_2C_6H_3$; XIII R = OCH_3, R^1 = 3,4,5-$(CH_3O)_3C_6H_2$ [12]; XIV R = OCH_3, R^1 = 4-n-$C_3H_7OC_6H_4$; XV R = $OC_{14}H_{29}$-n, R^1 = 4-p-$C_3H_7OC_6H_4$; XVI R = NHC_6H_5, R^1 = 4-n-$C_3H_7OC_6H_4$; XVII R = OCH_3, R^1 = 4-n-$C_7H_{15}OC_6H_4$; XVIII R = OC_2H_5, R^1 = 4-n,$C_7H_{15}OC_6H_4$; XIX R = $OC_{10}H_{21}$-n, R^1 = 4-n-$C_7H_{15}OC_6H_4$; XX R = $OC_{14}H_{29}$-n, R^1 = 4-n-$C_7H_{15}OC_6H_4$; XXI R = NHC_6H_5, R^1 = 4-n-$C_7H_{15}OC_6H_4$; XXII R = OCH_3, R^1 = 4-n-$C_{15}H_{31}OC_6H_4$; XXIII R = OCH_3, R^1 = 4-$(CH_3)_2NC_6H_4$ [13]; XXIV R = $OCH(CH_3)_2$, R^1 = 4-$(CH_3)_2NC_6H_4$ [14]; XXV R = NHC_6H_5, R^1 = 4-$(CH_3)_2NC_6H_4$; XXVI R = OC_2H_5, R^1 = H [15, 16]; XXVII R = NHC_6H_5, R^1 = H [17]; XXVIII R = OC_2H_5, R^1 = C_6H_5 [18]; XXIX R = NHC_6H_5, R^1 = C_6H_5 [17].

that the introduction of phenyl into the 4 position lowers the AOA (3.6 → 1.4), as has been shown previously in other oxidation systems [8]. Identical structural changes in compounds XXVII and XXIX, which have amide groups in the 3 and 5 positions, result in an increase in AOA (3.5 → 5.3), most probably because of an increase in the stability of the structure to autooxidation. In both cases (3,5-diethyl esters and 3,5-dianilides), the introduction of a hydroxyl group into the 4-aryl substituent did not change the AOA (compounds XXVIII and I; XXIX and V). However, the introduction of a second hydroxyl group results in a sharp increase in AOA (I and VII; V and VIII). As a result, compound VII is twice as active as diludin, and compound VIII, which contains both a dioxyphenyl substituent and an amide group, has the highest AOA, three times that of its 4-unsubstituted analog.

In the case of amides, the introduction of an alkoxy group into the p position of the 4-aryl substituent and the increase in its volume result in a gradual decrease in AOA (XXIX, XVI and XXI). It should be noted that all of the above compounds bind equally well to membranes.

The affinity of the compounds to phospholipid membranes was determined with the aid of fluorescence spectroscopy, where the probe used was anthracene [23, 24]. As a rule, compounds containing 5-ethoxycarbonyl substituents in the 3 and 5 positions bound better than the 3,5-dimethoxycarbonyl derivatives (VI and VII, XI and XII, XVII and XVIII). Interestingly, compounds having alkyls in both the 4-aryl substituent and 3,5-ester groups (IV, X, XV, XIX, XX, XXII) not only do not exhibit affinity to phospholipid membranes but also are not antioxidants. It is known from the literature that the affinity of compounds, including derivatives of 1,4-DHP, for membranes is conductive to the manifestation of their physiological activities [2, 25, 26]. Thus, the AOA of vitamin E derivatives depends on their ability to bind to biological membranes [27].

The results obtained have shown that all the compounds studied which possess AOA bind well to membranes. However, many compounds with a high affinity for membranes nevertheless are not antioxidants (II, III, XII, XIV, XVII, XVIII, XXIV). Thus, the binding of compounds to membranes is a necessary but not sufficient condition for the manifestation of AOA.

EXPERIMENTAL

The ESR spectra were recorded on a Bruker WH-90/DS NMR spectrometer in $CDCl_3$ or DMSO-D_6 solutions.

Compounds I-XXV were synthesized by a common procedure, whereby 0.05 mole of the corresponding benzaldehyde and 0.1 mole of the corresponding ester or amide of acetoacetic acid are dissolved in 10 to 20 ml of ethanol, and 0.06 mole of a 25% aqueous ammonia solution is added. The reaction mixture is boiled for 2 h to 32 h. The reaction is monitored by thin layer chromatography. After cooling, precipitate is filtered off, dried in air, and recrystallized from a suitable solvent (Table 1).

The method for determining the affinity of 1,4-DHP for model phospholipid membranes is described in [23, 24]. The binding of the compounds to liposomes was determined in accordance with the formula

$$I = \ln(F_0 / F),$$

where F_0 and F are the fluorescence intensities of anthracene in the absence and presence of 1,4-DHP. The fluorescence measurements were taken on a Hitachi 850 spectrofluorimeter. The error of the method does not exceed 5%.

Determination of AOA (19-21). Two milligrams of β-carotene is dissolved in 10 ml of chloroform. Into a round-bottom flask is transferred 1 ml of the solution, and 0.02 ml of methyl linoleate and 0.1 g of Twin-40 are added. The chloroform is driven off on a rotary evaporator at 40°C. Then, 50 ml of phosphate buffer (pH 7.4), freshly saturated with oxygen, is added in portions with vigorous stirring. The obtained aqueous emulsion of β-carotene and methyl linoleate is quickly dispensed in 5 ml portions into Bausch & Lomb cells into which were first added 20 μl of a $5 \cdot 10^{-4}$ molar solution of the studied derivative of 1,4-DHP in ethanol (control without 1,4-DHP) and 5 μl of a 0.5 molar aqueous solution of 2′,2′-azobis-2-amidinopropane hydrochloride as the oxidation initiator. The cells are closed, the solutions are incubated at 37°C, and the absorption is periodically measured at 460 nm (Spectronic-70). The AOA was calculated from the formula

$$AOA = \tau / \tau_0,$$

where τ and τ_0 are, respectively, the induction periods (time during which the concentration of β-carotene decreases by 1/3) in the presence and absence of the compound studied.

The authors thank the staff of the Physical Organic Chemistry Laboratory of the Latvian Institute of Organic Synthesis for plotting the IR and ESR spectra.

The work was conducted under the financial sponsorship of the Latvian Science Council, Grant No. 448.

REFERENCES

1. E. M. Marinova and N. V. Yanishlieva, Fat Sci. Technol., **94**, 428 (1992).
2. J. Lehuede, F. Huguet, B. Fauconneau, A. Piriou, and J. M. Vierfond, Eur. J. Med. Chem., **31**, 71 (1996).
3. W. B. Weglicki, I. T. Mak, and M. G. Simic, J. Mol. Cellul. Cardiol., **22**, 1199 (1990).
4. G. Ya. Dubur, Yu. A. Zilber, A. Kh. Velena, A. O. Kumerova, and G. D. Tirzit, Izv. Akad. Nauk LatvSSR, No. 7, 65 (1975).
5. G. Tirzitis, I. Kirule, and G. Duburs, Fat Sci. Technol., **90**, 411 (1988).
6. A. Kh. Velena, Author's abstract of dissertation for the degree of candidate of biological sciences, Riga (1975).
7. L. Kourimska, J. Pokorny, and G. Tirzitis, Nahrung, **37**, 91 (1993).
8. I. E. Kirule, Author's abstract of dissertation for the degree of candidate of chemical sciences, Riga (1987).
9. S. M. Jain, Kant Ravi, Devi Sunita, K. L. Dhar, Singh Surjeet, S. Bani, and G. B. Singh, Indian J. Chem., **29B**, 95 (1990).
10. Z. M. Yang and Y. M. Dong, Acta Pharm. Sinica, **26**, 661 (1991).
11. L. E. Hinkel and W. R. Mandel, J. Chem. Soc., **135**, 750 (1929).
12. Pat. 531,033 Span. ES; M. J. Verde Casanove and G. A. Galiano Ramos, Chem. Abstr., **105**, 226361 (1986).
13. A. Philips, J. Am. Chem. Soc., **71**, 4003 (1949).
14. Pat. 6,801,482 S. African; F. Bossert and W. Vater, Chem. Abstr., **70**, 96641 (1967).
15. Pat. 1,294,650 UK; S. Giller, G. Dubur, Y. Yldrikis, G. Tirzit, A. Valdman, I. Zakharchenko, Y. Spruz, V. Ronis, and A. Makarov, Chem. Abstr., **78**, 159440 (1979).
16. Pat. 1,521,913 UK; S. Giller, G. Dubur, G. Tirzitis, Y. Uldrikis, E. Kozlov, R. Ivanova, V. Yakovlek, N. Bogoslovsky, and Zh. Abramova, Chem. Abstr., **90**, 136453 (1979).
17. G. Ya. Dubur, Z. Ya. Ogle, and Ya. R. Uldrikis, Khim. Geterotsikl. Soedin., No. 12, 1642 (1974).
18. R. Schiff and J. Puliti, Ber., **16**, 1607 (1883).
19. H. E. Miller, J. Am. Oil Chem. Soc., **48**, 91 (1971).
20. G. J. Marco, J. Am. Oil Chem. Soc., **45**, 594 (1968).
21. I. Kirule, G. Tirzitis, and G. Duburs, LPSR ZA Vestis. Khim. Ser., No. 3, 354 (1990).
22. E. V. Ivanov, T. V. Ponomarjova, G. N. Merkusev, G. Ja. Dubur, E. A. Bisenieks, A. Z. Dauvarte, and E. M. Pilscik, Radiobiol. Radiother., **31**, No. 1, 69 (1990).
23. N. V. Makarova, G. V. Belevich, E. A. Bisenieks, M. M. Veveris, and G. Ya. Dubur, Khim.-Farm. Zh., No. 7, 810 (1988).
24. N. V. Makarova, Zh. V. Koronova, A. V. Plotnietse, D. Ya. Tirzite, G. D. Tirzit, and G. Ya. Duburs, Khim. Geterotsikl. Soedin., No. 8, 1112 (1995).
25. F. T. Van Amsterdam, A. Roveri, M. Maiorino, E. Ratti, and F. Ursini, Free Radic. Biol. Med., **12**, 183 (1992).
26. R. P. Mason, D. G. Rhodes, and L. G. Herbette, J. Med. Chem., **34**, 869 (1991).
27. J. S. Hinzmann, R. L. McKenna, T. S. Pierson, F. Han, F. J. Kezdy, and D. E. Epps, Chem. and Phys. Lipids, **62**, 123 (1992).