Pergamon

Tetrahedron 55 (1999) 9923–9932

TETRAHEDRON

Separation of Amino Acid Enantiomers using Supported Liquid Membrane Extraction with Chiral Phosphates and Phosphonates

Paweł Dżygiel,[a] Piotr Wieczorek*,[a] Jan Åke Jonsson,[b] Małgorzata Milewska[c] and Paweł Kafarski[c]

[a] Institute of Chemistry, University of Opole, ul. Oleska 48, 45-052 Opole, Poland,

[b] Department of Analytical Chemistry, University of Lund, S-221 00, P.O.Box 124 Lund, Sweden,

[c] Institute of Organic Chemistry, Biochemistry and Biotechnology, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

Received 12 April 1999; revised 25 May 1999; accepted 10 June 1999

Abstract: A series of dialkyl and monoalkyl phosphates, phosphites and phosphinates based on (-)-menthol and (-)-nopol were synthesized and used as carriers for transport of aromatic amino acids through supported liquid membranes. Although all the compounds were found to be effective carriers (with transport rate dependent on their structure) the enantioselectivity of the process obtained was low or moderate.

INTRODUCTION

It is widely known that chirality of molecules plays a vital role in most chemical and biochemical processes where specific and selective interactions between chemical species take place. Thus, obtaining and identifying enantiopure compounds is one of the most important and often difficult demands in fields such as drug chemistry, agrochemistry or food chemistry. To fulfil these requirements the methods of asymmetric synthesis, biotransformation and chiral separation have been developed. Chiral separation still remains the most popular approach because it is relatively inexpensive, simple to carry out and has reasonably low time demands.

One of the techniques scarcely applied for chiral separation is the use of the supported liquid membrane (SLM) technology. SLM offers several advantages as an extraction method,[1] but the most important feature is that only minute amounts of the expensive chiral carriers are required in order to achieve resolution of enantiomers. The possibility of the enantiomeric separation of amino acids with SLMs has attracted significant attention because of the great biological relevance of this class of compounds.

Creating optimal conditions for chiral separation of amino acid enantiomers with SLM extraction is usually achieved by incorporating a chiral selector in the hydrophobic liquid membrane phase [2] or using a chiral organic liquid as a membrane phase.[3] Various types of carrier molecules have been designed and applied for this purpose. The most important group consists of macrocyclic compounds derived from crown ethers.[4] Other possibilities include the use of chiral complexes of transitions metals,[5] carriers with porphyrin or

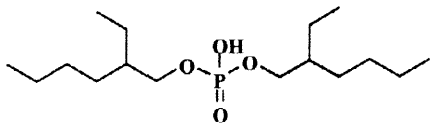
0040-4020/99/$ - see front matter
PII: S0040-4020(99)00531-1

sapphyrin rings [6] as well as macrocyclic pseudopeptides.[7] Although these carriers turned out to be more or less effective chiral selectors, their further application seems to be limited due to their moderate solubility in organic solvents. This prevents their use in high concentrations, which might give the possibility of simultaneous separation and enrichment.

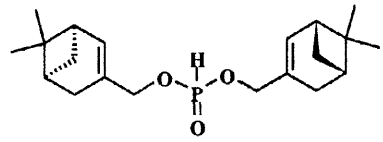
Supported liquid membrane extraction has also been successfully applied as a sample preconcentration technique for biomedical and environmental analysis.[1] In this case the main objective is to obtain as high an enrichment of sample as possible. Various types of biologically active compounds including amino acids have been analyzed using this approach. One of the most popular means for the extraction of polar compounds is utilization of supported liquid membrane technology with di-(2-ethylhexyl)phosphoric acid (compound **1**, DEHP) as a carrier.[8] Therefore we decided to use carriers derived from phosphoric and phosphonic acids for the separation of enantiomeric amino acids by means of SLM. Six esters of these acids (compounds **2**-**7**) with two chiral alcohols, namely (-)-menthol and (-)-nopol were synthesized and, together with commercially available compound **9**, have been applied as carriers in a supported liquid membrane system for the transport of aromatic amino acids. The influence of the carrier structure and carrier concentration on the flux and the chiral discrimination between D and L enantiomers of different amino acids have been investigated.

RESULTS AND DISCUSSION

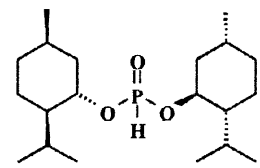
A typical SLM system consists of a hydrophobic liquid phase (membrane phase), immobilized in pores of a polymeric support separating two hydrophilic water phases. Chemical species pass through the membrane phase from one aqueous phase (donor phase) to another (acceptor phase). The mechanism of amino acid transport with di-(2-ethylhexyl) phosphate (compound **1**, DEHP) acid as a carrier has been described previously.[8]



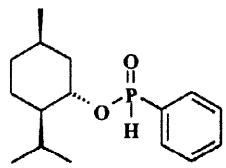
(1)



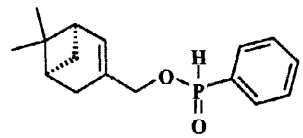
(2)



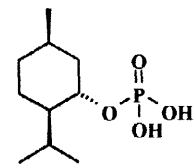
(3)



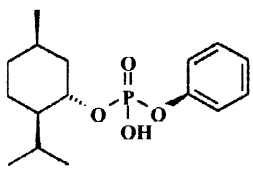
(4)



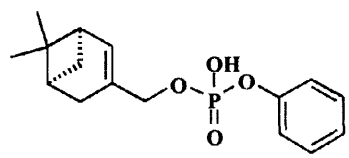
(5)



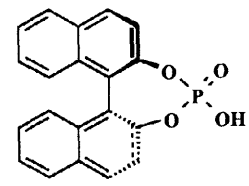
(6)



(7)



(8)



(9)

Hereby, it is only important to mention that this transport is proton-driven and the H^+ gradient is built over the membrane from the acceptor (pH = 1) to the donor phase (pH = 3.3). In a hydrophobic environment phosphate diesters exist mainly as dimers due to the their highly acidic nature and the strong polarization of P=O double bond.[9] The formation of a complex of amino acid with the carrier is governed by interaction between the protonated amino acid amino group and the negatively charged phosphate (i.e. by formation of ion-pair soluble in membrane phase). Such a complex is stabilized by hydrogen bonds that form preferentially in apolar media. The ion pair diffuses from the interface of the donor and membrane phases to the interface of the membrane phase and the acceptor phase. The amino acid is released there while the carrier undergoes protonation and carries a proton in reverse direction. Having this in mind, we concentrated on the influence of the structure of the alcohol fragment of the carrier molecule on the chiral discrimination and effectiveness of amino acids transport.

We have synthesized seven analogues (compounds **2-8**) of DEHP differing in acidity (phosphates, phosphinates and phosphites) and in the structure of the chiral fragment which derives from (-)-menthol or (-)-nopol. In actual transport experiments only six of them were used because compound **8** underwent decomposition in organic solvents at room temperature. For comparison the commercially available binaphthalene-2,2'-diyl hydrogen phosphate (compound **9**) was also used as carrier.

Table 1. The Influence of Carrier Type on Flux (J) and Discrimination (α) of Tryptophan.

Carrier	$J \times 10^{-10}$ [mol/m^{2}s]		α
	L-Trp	D-Trp	
[a]**1**	10.00	10.00	1.00
[b]**2**	8.20	8.00	1.04 (L)
[a]**3**	5.40	6.60	1.22 (D)
[b]**4**	0.86	1.11	1.30 (D)
[b]**5**	0.16	0.17	1.06 (D)
[a]**6**	6.10	6.70	1.09 (D)
[b]**7**	0.31	0.35	1.13 (D)
[a]**9**	8.32	8.12	1.02 (L)

[a] tri-(2-ethylhexyl) phosphate (TEHP) used as membrane phase

[b] dihexyl ether (DHE) used as membrane phase

D or L indicates the preferential form of enantiomers

As seen from Table 1 the use of optically active carriers resulted in lower or comparable fluxes of tryptophan through the membrane, compared with standardly used DEHP (compound **1**). Quite interestingly, the kind of optically active alcohol used for the preparation of the chiral carriers only has a small influence on

chiral discrimination of the transport rate. The value of α varies form 1.04 to 1.30. Thus, there is little contribution of the alcohol part of the carrier molecule to the discrimination of the tryptophan enantiomers, even if those moieties bear at least two chiral centers in their structures. This could be related to the weak steric interactions of those substituents with the amino acid molecule caused by too great a distance between them. Moreover, the carriers (structures **2-7**) are quite flexible and might readily change their conformations. There is no clear-cut dependence of either the rate of transport or its stereoselectivity on the chemical nature of carrier. Thus, the flux of tryptophan in the presence of highly acidic phosphate monoester **6** has nearly the same value as that observed for the less acidic phosphate diester **9** or those for far less acidic dialkyl phosphites **2** and **3**. Introduction of a phenyl group into the carrier molecule (compounds **4**, **5** and **7**) resulted in a significant decrease of tryptophan flux. This might be explained by changes in geometry of the ion pair in comparison to other carriers. It is well established that, in the case of complexes where hydrogen bonds play the most important role any slight conformational distortions influence complex stability.[10] Consequently, those changes could result in a weakening of the bonds between the amino acid $-NH_3^+$ group and the phosphate or phosphite. The fact that, in the case of carrier **9**, a molecule of rigid structure, the transport rate is highest seems to confirm this assumption.

The introduction of a phenyl group instead of one of the ester groups also affects the chiral differentiation by changes in the preference towards one of the enantiomers (as seen when comparing compound **2** with **4**) or in the significant decrease of the α value (**3** and **5**). This could be attributed to π-π interactions between the phenyl moiety of the carrier and the aromatic fragment of tryptophan. These interactions are of little importance in apolar media but in this case they seem to be strong enough to have an impact on the discrimination.[10]

Table 2. The Influence of Amino Acid Structure on the Flux (J) and Enantioselectivity (α) of Transport.

Amino Acid	$J \times 10^{-10}$ [mol/m^{2}s][a]		α		V [Å^3][b]	π*[b]
	carrier **3**	Carrier **9**	carrier **3**	carrier **9**		
L-Trp D-Trp	5.40 6.60	8.32 8.12	1.22 *(D)*	1.02 *(L)*	185.90	1.85
L-Phe D-Phe	1.20 1.66	6.63 6.60	1.38 *(D)*	1.10 *(L)*	155.80	1.56
L-Tyr D-Tyr	3.42 3.83	1.30 1.12	1.11 *(D)*	1.16 *(L)*	162.70	0.89

[a] DEHP was used as membrane phase

[b] hydrophobibicity (π*) and molecular volume (V) taken from Ref. 11

D or L indicates the preferential form of enantiomers transported

We have also studied the influence of the amino acid structure on enantiomeric discrimination by carriers **3** and **9**. These molecules differ significantly in their structures and have shown different enantioselectivities towards tryptophan. Results shown in Table 2 clearly demonstrate that carrier **3** is a considerably better selector for tryptophan and phenylalanine than molecule **9**. Both carriers differentiate similarly between the enantiomers of tyrosine. These variances probably stem from the different type of the

interaction responsible for chiral discrimination. When the flexible carrier **3** is applied, steric repulsion plays the most important role. With a more bulky amino acid the repulsive interactions between its side chain and carrier are stronger. Thus, the highest fluxes are observed for tryptophan and they decrease with the reduction of amino acid molecular volume (V). These interactions also caused smaller changes in the hydrogen bonds geometry and the complex stability is therefore greater. The chiral discrimination does not follow this pattern and the highest enantioselectivity was observed for phenylalanine and the smallest for tyrosine. Thus, they are not correlated with the V value. In the case of the rigid, aromatic-like carrier **9** the most important interactions seem to be those of liphopilic character. This conclusion comes from the fact that the highest fluxes were obtained in the case of tryptophan (the most liphopilic amino acid) and they decline with decreasing amino acid hydrophobicity (π^*). The enantiomer preference increased in reverse order. Tyrosine having the smallest π^* value, is bonded stronger to carrier, probably due to the stronger stacking forces between the binaphthyl moiety and the aromatic side chain. As a consequence, the flux is smaller (tyrosine is not released to the acceptor phase as easily as other amino acids) but the chiral discrimination is only slightly higher.

Table 3. The influence of concentration of carrier **4** on rate and enantioselectivity of tryptophan transport through a DHE membrane.

Carrier concentration [mM]	***L*-Trp**	***D*-Trp**	α
50	0.86	1.11	1.30
150	2.06	2.14	1.03
300	5.30	5.60	1.05

The influence of the concentration of carrier **4**, which exhibited the highest enantioselectivity in tryptophan transport, on the flux and chiral discrimination of this amino acid is shown in Table 3. Obviously, the transport rate increased with carrier concentration on expense of enantioselectivity of the transport which significantly decreased. This indicates that the source or enantioselectivity is rather of kinetic origin (the two diasteromeric complexes formed at the interface between donor and membrane phase move at different rate across the interface) than a thermodynamic one (the complexes have different formation constant).[12]

CONCLUSIONS

(-)-Menthyl and (-)-nopyl organophosphonate esters (compounds **2-7**), analogues of standardly used di-(2-ethylhexyl) phosphate (compound **1**), appeared to be an interesting group of newly designed carriers for extraction of amino acids by means of liquid membranes. Amino acids were quite efficiently transported through tri-(2-ethylhexyl) phosphate and dihexyl ether membranes containing these esters and supported in PTFE matrix. The enantioselectivity of the transport was moderate and dependent on both structure of carrier and transported amino acid. Anyway, compounds **2-7** may be considered as a leads for the design of new carriers belonging to this class of compounds.

EXPERIMENTAL

Materials

All reagents used for preparation of carriers were of analytical purity and were purchased either from Aldrich (Milwaukee, Wisconsin, USA) or Merck (Darmstadt, Germany). (-)-Menthol and (-)-nopol were obtained from Fluka (Buchs, Switzerland). Binaphthalene-2,2'-diyl hydrogen phosphate (carrier **9**) was purchased from Fluka, whereas enantiomers of tryptophan, phenylalanine and tyrosine were obtained from Sigma (St. Louis, MO, USA). Organic solvents used as a liquid membrane phase, namely tri-(2-ethylhexyl) phosphate (TEHP) and dihexyl ether (DHE) were obtained from Fluka and Aldrich, respectively. Water was purified with a Millipore Q system.

NMR measurements

Proton and phosphorus NMR spectra were recorded in deuterated $CDCl_3$ on a Bruker DRX spectrometer operating at 300.13 MHz for ^1H and 121.50 MHz for ^{31}P. Chemical shifts are given in relation to $SiMe_4$, 85% and H_3PO_4 respectively. All downfield shifts are denoted as positive.

Membrane unit

The membrane unit was described previously.[13] It consisted of two PTFE blocks (diameter 120 mm and thickness 8 mm) with grooves arranged as an "Archimedes" spiral (depth 0.25 mm; width 1.5 mm and length 2.5 m). The grooves serve as a reservoirs (channels) of acceptor and donor phases. Aluminum blocks with 6 mm thickness were used on both sides of the PTFE blocks to stabilize the set-up. A porous PTFE membrane with polyethylene backing (porosity 0.7; pore size 2 μm and effective membrane area 37,5 cm^2) purchased from Millipore (Bedford, MA, USA) was impregnated with typically 50 mM carrier solutions in DHE or TEHP for 30 min. Then the membrane was placed between two PTFE blocks and the whole construction was clamped together. After installation of the membrane, the excess of the carrier solution present on its surface was removed by pumping 20 ml of water through both channels. The donor phase was pumped with a peristaltic pump (Minipuls 3, Gilson Medical Electronic, Viller-le-Bel, France) using acid resistance tubes (Acid Mainfold Tubing, Elkay Products, Shrewsbury, MA, USA) connected to the membrane unit. The acceptor phase was stagnant.

Transport experiments

The 1 mM solutions of enantiomeric mixtures of amino acid hydrochlorides were prepared by dissolving equimolar quantities of *D,L*-amino acid and hydrochloric acid. They were pumped through the donor phase channel with flow rate 0.1 ml/min for 12 h or 24 h. After this time, the acceptor phase (0.1 M HCl) containing transported amino acids was transferred into 2 ml flask. The collected sample was analyzed by capillary electrophoresis with α-cyclodextrin as a chiral selector according to a previously described procedure.[14] The donor and acceptor channels were washed with 20 ml of water before the membrane unit was used for the next experiment. The fluxes were calculated from the equation $J = dc/dt \times V/A$ where A is an effective membrane area and V is a volume of collected sample. The chiral discrimination factor α was determined directly from the electrophoretic measurements. Each experiment was repeated 2-3 times.

Synthesis of carriers

Although the carriers were synthesized according to standard procedures the syntheses are somewhat tricky and require the use of specific separation and purification procedures in each case. Their NMR spectra were interpreted based on the spectra of substrates[15] and spectra of similar compounds.[16]

Di-(-)-menthyl phosphite (compound 2). To a solution of (-)-menthol (23.7 g; 0.15 mole) in 20 ml of benzene, phosphorus trichloride (6.85 g, 0.05 mole) was added dropwise while cooling the solution to 10°C. The volatile substances were removed on a rotary evaporator and then by distillation under reduced pressure (0.5 mm Hg) collecting fractions boiling up to 60°C. The residue was dissolved in ethyl acetate and washed three times with saturated sodium bicarbonate and then with water. The organic solution was dried over anhydrous magnesium sulphate. Evaporation of the solvent yields an oily yellowish product of satisfactory purity with 40% yield. $[\alpha]_D^{23}$ –45 (c 1.00, $CHCl_3$). IR (film): 2433 (PH), 1179(PO), 1017 (POC) cm^{-1}. ^{31}P NMR ($CDCl_3$) δ (ppm): 8.57. ^1H NMR ($CDCl_3$) δ (ppm): 0.7 and 0.86 (d, *J* 7.0 Hz, 6H each, $CHCH_3$); 0.83 (d, *J* 6.4 Hz, 6H, CCH_3); 0.6-1.05 (m, 4H, CH_2CH_2); 1.10 (d-q, *J* 11.4, 4.4 Hz; 2H, $CHCHCH_3$); 1.3-1.45 (m, 4H, CH_2CH_2, CH_2CHCH_3); 1.56 (d, *J* 11.2 Hz, 4H, CH_2CHO); 1.85-2.15 (m, *J* 12.7, 7.0, 2.5 Hz, 4H, CH_2CH_2, $CHCHCH_3$); 4.14 (d-q-q, *J* 10.2, 4.8 Hz, J_{PH} 10.2 Hz, 2H, CHOP); 6.76 (d, J_{PH} 701.3 Hz, 1H, PH). Microanalysis calculated for $C_{20}H_{39}O_3P$ (358.51): 8.64% P, 67.01% C, 10.97% H; found: 8.94 % P, 66.85% C, 11.13% H.

Di-(-)-nopyl phosphite (compound 3). To the solution of (-)-nopol (24.9g, 0.15 mole) in 20 ml of benzene, phosphorus trichloride (6.85g, 0.05 mole) in 10 ml of benzene was added dropwise at room temperature. Volatile substances were then removed by passing air through the solution under reduced pressure maintaining the temperature below 80°C. The residue was purified by distilling off solvents under reduced pressure (0.5 mm Hg, 120°C). The oily residue was dissolved in ethyl acetate, washed three times with a solution of sodium bicarbonate and then with water. The organic solution was dried over anhydrous magnesium sulphate and the residue was purified by means of silica-gel column chromatography using a mixture of ethyl acetate and acetone (10:0.25 v/v). Phosphite **3** was obtained as a dense colourless oil. Yield 30%. $[\alpha]_D^{23}$ –23 (c 1.00, $CHCl_3$). IR (film): 2428 (PH), 1262 (PO), 974 (POC) cm^{-1}. Because of formation of new chiral center at phosphorus two isomers are seen in NMR spectra. Efforts to separate these isomers were unsuccessful. ^{31}P NMR ($CDCl_3$) δ (ppm): 8.61 and 8.69 (stereoisomer ratio 2:1). ^1H NMR ($CDCl_3$) δ (ppm): 0.76 and 1.20 (s, major isomer, CCH_3); 0.85 and 0.89 (s, minor isomer, CCH_3); 0.94 (d, *J* 20.5 Hz, major isomer, $CHCH_2CH$); 1.07 (d, *J* 8.5 Hz, major isomer, $CHCH_2CH$); 1.28 (d-d, *J* 14.0, 4.2 Hz, minor isomer, $CHCH_2CH$); 2.43 (t-t, *J* 14.9, 4.5 Hz, minor isomer, $CHCH_2CH$); 1.60 (t, *J* 4.6 Hz, minor isomer, $CHCH_2CH$); 1.97 (t, *J* 4.6 Hz, major isomer, $CHCH_2CH$); 1.6-1.75 (m, major isomer, $CHCH_2CH$); 2.0-2.1 (m, minor isomer, $CHCH_2CH$); 2.1-2.2 (m, *J* 5.6 Hz, minor isomer, $CH_2CH{=}$, CH_2CH_2O); 2.2-2.3 (m, *J* 5.6 Hz, major isomer, $CH_2CH{=}$, CH_2CH_2O); 3.9-4.0 (m, major isomer, CH_2O); 4.1-4.2 (m, minor isomer, CH_2O); 5.1-5.15 (m, major isomer, $=CH$); 5.4-5.5 (m, minor isomer, $=CH$); 6.72 (d, J_{PH} 694.5 Hz, major isomer, PH); 6.75 (d, J_{PH} 692.7 Hz, minor isomer, PH). Microanalysis calculated for $C_{20}H_{31}O_3P$ (350.44): 8.84 % P, 68.55% C, 8.92% H; found: 9.04 % P, 68.40% C, 8.79% H.

*(-)-Menthyl phenylphoshinite (compound **4**).* To the solution of (-)-menthol (10.0 g; 0.064 mole) and pyridine (5.06 g; 0.064 mole) in 20 ml of toluene, phenyl dichlorophosphine (11.45 g, 0.064 mole) in 15 ml of toluene was added dropwise at room temperature. The reaction mixture was then stirred for 1 hour at 80°C. After cooling and removal of pyridine hydrochloride (by filtration) the organic layer was washed several times with water and dried over anhydrous magnesium sulphate. Removal of solvents yields crude phosphinite **4** as a colourless oil of satisfactory purity. Yield 24%. $[\alpha]_D^{23}$ –56 (c 1.00, $CHCl_3$). IR (film): 2337 (PH), 1236 (PO), 960 (POC) cm^{-1}. Because of formation of new chiral center at phosphorus two isomers are seen in NMR spectra. Efforts to separate these isomers were unsuccessful. ^{31}P NMR ($CDCl_3$) δ (ppm): 25.89 and 22.52 (stereoisomer ratio 5:3). ^1H NMR ($CDCl_3$) δ (ppm): 0.64 and 0.82 (d, *J* 6.9 Hz, 3H together, $CHCH_3$); 0.86 and 0.91 (d, *J* 6.4 Hz, 3H together, $CHCH_3$); 0.84 and 0.90 (d, *J* 6.9 Hz, 3H together, CCH_3); 0.75-1.20 (m, 2H, CH_2CH_2); 1.22 (q-q, *J* 11.9 Hz, 1H, $CHCHCH_3$); 1.3-1.5 (m, 2H, $CCHCH_3$, CH_2CH_2); 1.5-1.75 (m, 2H, CH_2CHOP); 2.0-2.65 (m, 2H, CH_2CH_2, $CHCHCH_3$); 4.22 (q-q, *J* 10.4, 4.5 Hz, major isomer, CHOP); 4.24 (q-q, *J* 8.1, 4.4 Hz, minor isomer, CHOP); 7.61 (d, J_{PH} 553.3 Hz, major isomer, PH); 7.63 (d, J_{PH} 556.6 Hz, minor isomer, PH); 7.548 and 7.553 (t, *J* 7.3 Hz, 2H, C_6H_2); 7.47 (m, 1H, C_6H); 7.73 (d-d, *J* 6.8, 13.9 Hz, 2H, C_6H_2). Microanalysis calculated for $C_{16}H_{25}O_2P$ (280.35): 11.05 % P, 68.55% C, 8.99% H; found: 8.89 % P, 68.27% C, 9.01% H.

*(-)-Nopyl phenylphosphinite (compound **5**).* To the solution of (-)-nopol (10.0 g; 0.06 mole) and pyridine (4.75 g; 0.06 mole) in 20 ml of toluene phenyl dichlorophosphate (10.74 g, 0.06 mole) in 15 ml of toluene was added dropwise at room temperature. The reaction mixture was then stirred for 1 hour at 80°C. After cooling and removal of pyridine hydrochloride (by filtration) the organic layer was washed several times with 5% solution of sodium hydroxide and with water, the toluene layer was dried over anhydrous magnesium sulphate. Removal of solvents yielded compound **5** of satisfactory purity as a dense colourless oil. Yield 18%. $[\alpha]_D^{23}$ –30 (c 1.00, $CHCl_3$). IR (film): 2342 (PH); 1237 (PO), 962 (POC) cm^{-1}. Because of formation of new chiral center at phosphorus two isomers are seen in NMR spectra. Efforts to separate these isomers were unsuccessful. ^{31}P NMR ($CDCl_3$) δ (ppm): 26.4 and 25.93 (1:1 mixture). ^1H NMR ($CDCl_3$) δ (ppm): 0.74 and 1.18 (s, 3H, $CHCH_3$); 1.06 (d-d, *J* 8.5, 2.4 Hz, 1H, $CHCH_2CH$); 1.04 (q-q, *J* 7.2 Hz, 1H, $CHCH_2CH$); 2.0-2.15 (m, $CHCH_2CH$); 2.15 (bd, *J* 7.4 Hz, 1H, $CHCH_2CH$); 2.1-2.35 (m, 4H, CH_2CH_2O, CH_2CH=); 4.06 (m, *J* 6.8 Hz, J_{PH} 17.1 Hz, 2H, CH_2O); 5.2-5.25 bs, 1H CH=); 7.51 (d, *J* 562..3 Hz, 1H PH); 7.44 (t-t, *J* 7.6, 3.4 Hz, 2H, C_6H_2); 7.52 (t-t, *J* 7.4, 1.6 Hz, 1H, C_6H); 7.71 (d-d, *J* 6.9 Hz, J_{PH} 13.8 Hz, 2H, C_6H_2). Microanalysis calculated for $C_{16}H_{21}O_2P$ (276.32): 11.21 % P, 69.55% C, 7.66% H; found: 11.33 % P, 69.32% C, 7.82% H.

*Mono-(-)-menthyl phosphate (compound **6**)* was prepared according to the known procedure.[17] Thus, (-)-menthol (26.27 g; 0.168 mole) was dissolved in 40 ml of toluene and phosphorus oxychloride (8.6 g; 0.056 mole) in 20 ml of toluene was added dropwise maintaining the temperature around 30°C. Then the mixture was stirred in water bath for 1.5 hour at 50°C and cooled to room temperature. The toluene solution was then washed with water (controlling the presence of phosphates in aqueous layer) and the aqueous phase was alkalized to pH 9 with 1 M sodium hydroxide solution. Alkaline solution was acidified with 1.8 M nitric acid and left in a refrigerator in order to obtain a crystalline product. This product was dried in dessicator over phosphorus pentaoxide, dissolved in ethyl acetate and left in refrigerator in order to precipitate pyrophosphate.

Monomenthyl phosphate was obtained by removal of ethyl acetate under reduced pressure and crystallization from a water-ethanol mixture. The product was obtained as a white crystalline hydrate with 15% yield melted at 82.5°C (lit. 82-84°C), whereas compound dried in dessicator over phosphorus pentaoxide melted at 130-131° C (lit. 133-134° C). $[\alpha]_D^{23}$ –75 (c 1.00, $CHCl_3$). IR (film): 3700-2000(hydrogen bondings), 1178 (PO), 1062 (POC) cm^{-1}; ^{31}P NMR ($CDCl_3$) δ (ppm): 2.12. ^1H NMR ($CDCl_3$) δ (ppm): 0.7 and 0.9 (d, *J* 6.8 Hz, 3H each, $CHCH_3$); 0.88 (d, *J* 6.4 Hz, 3H, CCH_3); 0.75-1.0 (m, 2H, CH_2CH_2); 1.09 (bq, *J* 11.6 Hz, 1H, $CHCHCH_3$); 1.25-1.45 (m, 2H, CH_2CH_2, CH_2CHCH_3); 1.59 (bd, *J* 10.9 Hz, 2H, CH_2CHO); 1.9-2.15 (m, 2H, CH_2CH_2, $CHCHCH_3$); 3.9-4.1 (m, 1H, CHOP); 7.61 (bs, 2H, PO_3H_2). Microanalysis calculated for $C_{10}H_{21}O_4P$ (233.27): 13.11 % P, 50.84% C, 8.96% H; found: 13.09 % P, 50.97% C, 9.13% H.

(-)-Menthyl phenyl phosphate (compound 7). To the solution of (-)-menthol (10.0 g; 0.064 mole) and pyridine (5.06 g; 0.064 mole) in 20 ml of toluene phenyl dichlorophosphate (13.50 g, 0.064 mole) in 15 ml of benzene was added dropwise at room temperature. The reaction mixture was then stirred for 1 hour at 80°C. After cooling and removal of pyridine hydrochloride (by filtration) the organic layer was washed several times with water and dried over anhydrous magnesium sulphate. Removal of solvents yielded colourless oil of phosphate **7** of satisfactory purity. Yield 20%. $[\alpha]_D^{23}$ –55 (c 1.00, $CHCl_3$). IR (film): 1294, 1199 (PO), 1014 (POC) cm^{-1}; ^{31}P NMR ($CDCl_3$) δ (ppm): 0.52. ^1H NMR ($CDCl_3$) δ (ppm): 0.81 (d, *J* 6.4 Hz, 3H, $CHCH_3$); 0.92 and 0.93 (d, *J* 6.4 Hz, 1.5H each, $CHCH_3$); 0.90 and 0.91 (d, *J* 7.1 Hz, 1.5H, CCH_3); 0.8-1.35 (m, 2H, CH_2CH_2); 1.32 and 1.35 (d-q, *J* 11.4, 4.6 Hz, 0.5H each, $CHCHCH_3$); 1.35-1.45 (m, 1H, CH_2CH_2);.1 50-1.75 (m, 1H, $CCHCH_3$); 2.33 (bd, *J* 11.9 Hz, 2H, CH_2CHOP); 2.0-2.5 (m, 2H, CH_2CH_2, $CHCHCH_3$); 4.3-4.55 (m, 1H, CHOP); 7.0-7.5 (m, 6H, C_6H_5, PO_3H). Microanalysis calculated for $C_{16}H_{25}O_4P$ (312.35): 9.92 % P, 61.53% C, 8.07% H; found: 9.64 % P, 61.78% C, 7.88% H.

(-)-Nopyl phenyl phosphate (compound 8). To the solution of (-)-nopol (10.0 g; 0.06 mole) and pyridine (4.75 g; 0.06 mole) in 20 ml of toluene, phenyl dichlorophosphate (11.7 g, 0.06 mole) in 15 ml of toluene was added dropwise at room temperature. The reaction mixture was then stirred for 1 hour at 80°C. After cooling and removal of pyridine hydrochloride (by filtration) the organic layer was washed several times with sodium bicarbonate and water, and dried over anhydrous magnesium sulphate. Removal of solvents yields crude phosphate **8** as a dense yellow oil of satisfactory purity. Yield 18%. $[\alpha]_D^{23}$ –29 (c 1.00, $CHCl_3$). IR (film): 1288, 1214 (PO), 1023 (POC) cm^{-1}; ^{31}P NMR ($CDCl_3$) δ (ppm): -5.2. ^1H NMR ($CDCl_3$) δ (ppm): 0.71 and 1.17 (s, 3H, $CHCH_3$); 0.82 (d, *J* 10.2 Hz, 1H, $CHCH_2CH$); 1.04 (d-d, *J* 5.7, 1.4 Hz, 1H, $CHCH_2CH$); 1.91 (t-t, *J* 5.5, 1.4 Hz, $CHCH_2CH$); 1.9-2.0 (m, 1H, $CHCH_2CH$); 2.2-2.3 (m, 4H, CH_2CH_2O, CH_2CH=); 4.02 (t-t, *J* 7.2 Hz, 2H, CH_2O); 5.1-5.2 (m, 1H CH=); 7.0-7.2 (m, 5H, C_6H_5). Microanalysis calculated for $C_{16}H_{21}O_4P$ (308.32): 10.05 % P, 62.33% C, 6.87% H; found: 10.33 % P, 62.12% C, 7.00% H.

Compound **8** underwent decomposition (most possibly *via* pyrophosphates, as indicated by NMR) upon storage either as pure compound or in its solutions.

ACKNOWLEDGEMENTS

This work was supported in part by Komitet Badań Naukowych and in part by a grant for P. Dżygiel from the Swedish Instiute.

REFERENCES

1. Jönsson, J.Å.; Mathiasson L. *Trends Anal. Chem.* **1999**, in press
2. Bryjak, M.; Wieczorek, P.; Kafarski, P.; Lejczak, B. *J. Membr. Sci.* **1988**, 37, 287-291.
3. Bryjak, M. Kozłowski, J.; Wieczorek, P.; Kafarski, P. *J. Membr. Sci.* **1993**, 85, 221-228.
4. Pietraszkiewicz, M.; Kożbiał, M.; Pietraszkiewicz O. *J. Membr. Sci.* **1998**, 138, 109-113.
5. Tsukube, H.; Shinoida S.; Uenishi, J.; Kanatani, T.; Itoh, H.; Shiode, M.; Iwachido, T.; Yonemitsu, O. *Inorg.Chem.* **1998**, 37, 1585-1591.
6. Sessler, J. L.; Andrievsky, A. *Chem. Eur. J.* **1998**, 4, 159-167.
7. Miyake, H.; Yamashita, T.; Kojima, Y.; Tsukube, H. *Tetrahedron Lett.* **1995**, 36, 7669-7672.
8. Wieczorek, P.; Jönsson, J.Å.; Mathiasson L. *Anal. Chim. Acta* **1997**, 346, 191-197.
9. Chu, F.; Flatt, L. S.; Anslyn, E.V. *J. Am. Chem. Soc.* **1994**, 116, 4194-4204.
10. Webb, T.H.; Wilcox, C.S. *Chem. Soc. Rev.* **1993**, 383-395.
11. El Tayar, N.; Tsai, R.-S.; Carrupt, P.-A.; Testa B. *J. Chem. Soc. Perkin Trans. 2* **1992**, 79-84.
12. Scrimin, P.; Tecilla, P.; Tonellato, U. *Tetrahedron* **1995**, 51, 217-230.
13. Wieczorek, P.; Jönsson, J.Å.; Mathiasson L. *Anal. Chim. Acta*, **1997**, 337, 183-189.
14. Dżygiel, P.; Wieczorek, P.; Jönsson, J.Å. *J. Chrom. A.* **1998**, 793, 414-418.
15. (a) Farooq, M.; Hanson, J.R.; *Phytochemistry* **1995**, 40, 815-818. (b) Chapuis, Ch.; Brauchli, R.; *Helv. Chim. Acta* **1992**, 75, 1527-1546.
16. Shi, M.; Inoue, Y. *J. Chem. Soc. Perkin Trans 1* **1998**, 2421-2427.
17. (a) Miłobędzki, T.; Janczak, W. *Roczniki Chemii* **1931**, 11, 840-854; (b) Tagaki W.; Hashizume, T. *J. Org. Chem.* **1961**, 26, 3038-3040.