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Communication

Synthesis of a quaternary polyprenyl ammonium salt★

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Reaction of primary C_{55}-allylic alcohol moraprenol ($WT_3C_{7\text{-}9}$-OH, a polyprenol from mulberry leaves) with triethylamine in the presence of phosphorus oxychloride leads to a quaternary ammonium chloride with a good yield (72%) and high *cis*-stereoselectivity of the terminal isoprene unit. Cationic polyprenyl derivatives may be useful for transfection and immunological studies.

Keywords: polyprenol, quaternary ammonium salts, cationic lipids

INTRODUCTION

The key role of dolichyl and polyprenyl phosphates in the biosynthesis of polysaccharide and glycoconjugate carbohydrate chains in prokaryotic and eukaryotic cells is definitely established (for reviews see: Bugg & Brandish, 1994; Krag, 1998). It has been shown that polyprenyl phosphates display high antiviral and immunostimulating activity (Danilov *et al*., 1996). Phosphorylated polyprenols generally possess higher activity than free polyprenols. Therefore, of substantial interest is the study of polyprenyl and dolichyl phosphate analogues modified at the anionic group, in particular, a comparison of their biological activity.

Phosphorylation of dolichols (2,3-dihydropolyprenols) with phosphorus oxychloride in the presence of triethylamine was developed in 1981 by Danilov and Chojnacki (1981) as a simple and effective procedure for the preparation of dolichyl monophosphates. This method was successfully used in several laboratories (Jaenicke & Siegmunt, 1986; Warren *et al*., 1987; Sagami & Lennarz, 1987). Phosphorus oxychloride is certainly the simplest and the most readily available synthetic equivalent for phosphoryl cation. Application of this procedure to α-allylic polyprenols did not result in the desired phosphorylation products, probably due to a nucleophilic attack of the chloride ion formed or the amine molecule on the intermediate phosphorodichloridate. Polyprenyl phosphates were synthesized later by phosphorylation with tetra-*n*-butylammonium dihydrogen phosphate and trichloroacetonitrile (Danilov & Shibaev, 1991).

As part of our studies on the synthesis of polyprenyl and dolichyl phosphate derivatives (Danilov & Shibaev, 1991; Shibaev & Danilov, 1997), we earlier described the synthesis of first analogues of this type, namely dolichyl *H*-phosphonate and dolichyl thiophosphate (Danilov *et al*., 1991). The goal of later studies was the synthesis of similar derivatives bearing a diphosphate (Maltsev *et al*., 1995), sulfate (Maltsev *et al*., 2001), *H*-phosphonate and phosphorofluoridate groups (Sizova *et al*., 2003).

The growing interest in polyprenyl-containing compounds promted us to attempt chemical synthesis of alternative polyprenyl analogues bearing a positive charge. Cationic polyprenyl derivatives have not been found in Nature yet, but some of them were chemically prepared. Synthetic strate-

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Abbreviations: Et, ethyl; Mor, moraprenyl; THF, tetrahydrofuran.

gies for introduction of the amino functionality have been suggested for secondary and tertiary prenylamines. A family of isoprenylamines was synthesized by reacting polyprenylcarboxylic acid or polyprenylacetone with amines of the formula $H_2N–R$ or $HN–R_2$, wherein the R-substituent was a lower alkyl, cycloalkyl, aryl or aralkyl group (Takigawa *et al*., 1986). The amidation of polyprenylcarboxylic acid with an amine can be conducted using a dehydration-condensation agent (*N,N′*-dicyclohexylcarbodiimide is the most convenient). Reduction of the prenylamide to prenylamine was performed under complex metal hydrides action. The yield of secondary or tertiary prenylamines was about 23%. In addition, a polyprenyltrimethylammonium iodide was synthesized by reacting prenylamine with methyl iodide with 11% yield. These polyprenylamines can be converted to dolichols and therefore may be useful as intermediates for dolichol synthesis. The polyprenyl compounds can also be used for the synthesis of dolichol analogs and various other polyprenyl derivatives by taking advantage of their functional terminal groups.

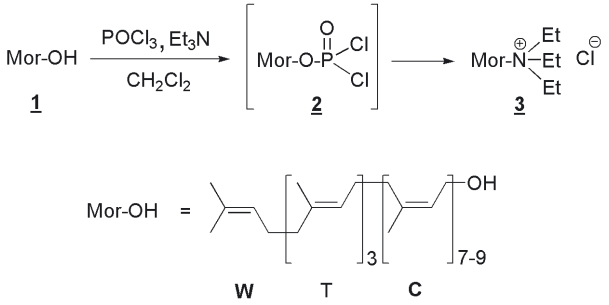
For the preparation of isoprenylpolyamine derivatives (Tahara *et al*., 1986) isoprenyl alcohol was first converted into corresponding bromide or arylsulfonic acid ester, which was then allowed to react with a polyamine (e.g. triethylenetetramine, diethylenetriamine). The reaction was carried out by using a large excess of the amino compound, or at temperature ranging from room tepmerature up to 100°C in the presence of a base. Secondary and tertiary prenylpolyamines were obtained with 12% yield. Prenyl-N-heterocyclic amines (Tahara *et al*., 1988) were synthesized from corresponding prenyl halides or arylsulfonic esters with heterocyclic compounds having at least one secondary amino group.

The obtained prenylamines revealed anti-tumor (Tahara *et al*., 1986), anti-vaccinia virus and human interferon inducing activity (Tahara *et al*., 1988). These derivatives may be useful for controlling virus infection of vertebrate animals. It is expected that such compounds could also be useful for transfection and immunological studies. In this paper, we describe the synthesis of triethylmoraprenylammonium chloride — a representative of quaternary cationic lipids.

MATERIALS AND METHODS

We used moraprenol ($WT_3C_{7\text{-}9}$-OH), a mixture of C_{55}-, C_{60}- and C_{65}-polyprenols obtained from mulberry leaves (the ratio of oligomer homologues, according to mass spectra data, was 35.7 : 56.3 : 8%, respectively) as the starting material (Vergunova *et al*., 1977). Triethylamine and phosphorus oxychloride were purchased from Fluka. The equilibrium mixture of *n*-butanol and water was prepared by mixing (shaking) equal volumes of both components. The upper layer of the equilibrium mixture contained *n*-butanol and water in a ratio of 80 : 20 (v/v) and the bottom layer of the mixture contained *n*-butanol and water in a ratio of 7 : 93 (v/v) (Hill & Malisoff, 1926, www.sou.edu/chem/ch205/waterbutanol.htm). TLC was performed on silica gel 60 F_{254} plates (Merck) developed with chloroform/methanol/water (80 : 20 : 2, by vol., solvent A). Unsaturated compounds were detected on TLC plates with iodine vapor with subsequent charring by spraying with 4% sulfuric acid in methanol and heating. ^1H and ^{13}C NMR spectra (^1H at 500 MHz, ^{13}C at 125 MHz) were recorded on a Bruker AMX-500 spectrometer for solutions in $CDCl_3 : CD_3OD$ (3 : 1). Chemical shifts (δ in ppm) are given relative to those for tetramethylsilane, *J* values are given in Hz. ESI-MS spectra were obtained with a Finnigan LCQ spectrometer.

Triethylmoraprenylammonium chloride (3). Triethylamine (183 μl, 1.3 mmol) was added to a solution of moraprenol (167.7 mg, 218.7 μmol) in dichloromethane (3 ml). The reaction mixture was cooled (−60°C), 100 μl 10% solution of $POCl_3$ (20 μl, 218.7 μmol) in dichloromethane was added to the mixture with stirring, and the solution was stirred at −60°C for 2 h. Then a mixture THF-H_2O (1.05 ml, 20 : 1) was added, stirring was continued for 1 h and the solvents were evaporated to dryness. Heptane (8 ml) was added to the residue and the solution was kept at −20°C overnight and then filtered through a PTFE filter (0.22 μm, Acrodisc). The residue was resuspended in heptane (8 ml), the solution was kept at −20°C overnight and filtered through a PTFE filter. The combined heptane solutions were concentrated, dissolved in the upper layer of the equilibrium mixture *n*-butanol–water (3 ml) and the solution was washed with the lower layer of the same equilibrium mixture (3 × 0.6 ml). The upper layer solution was concentrated, heptane (1 ml) was added to the residue, and the mixture was extracted with acetonitrile (6 × 2 ml). The combined acetonitrile extracts were concentrated to give desired triethylmoraprenylammonium chloride (**3**) as oil. Yield 139.7 mg (157.6 μmol), 72%, R_F (A) 0.5. ^1H-NMR ($CDCl_3 : CD_3OD$, 4 : 1): 5.14 (m, 1 H, =CH-2), 4.98 (m, 10.8 H, =CH), 3.44 (d, 0.5 H, $J_{1,2}$ 5, CH_2N-1, *trans* unit), 3.42 (d, 1.5 H, $J_{1,2}$ 5.5, CH_2N-1, *cis* unit), 3.15 (m, 6 H, CH_3CH_2N), 2.03 (m, 4 H, CH_2-5 and CH_2-, *W* unit), 1.91 (m, 36.5 H, CH_2), 1.85 (m, 4 H, CH_2-4 and CH_2-, *trans* unit), 1.75 (s, 3 H, CH_3-C-3), 1.54 (s, 24.5 H, CH_3-, *cis* unit), 1.47 (s, 3 H, CH_3-, *trans* and *W* units), 1.46 (s, 9 H, CH_3-, *trans* unit), 1.21 (m, 9 H, CH_3CH_2N). ^{13}C-NMR ($CDCl_3 : CD_3OD$, 4 : 1): 136.6 and 135.3 (CH_3C=, *cis* unit), 135.0 (CH_3C=, *trans* unit), 134.9 (CH_3C=, *W* unit), 124. 7-123.0 (=CH),



Scheme 1. Synthesis of triethylmoraprenylammonium chloride.

110.0 (=CH-2), 52.6 (CH_2N-1 and CH_3CH_2N), 39.4 (CH_2-4), 32.4 (CH_2-5), 31.9 and 31.7 ($CH_2C(CH_3)$=, *cis* unit), 26.3 (CH_2CH=, *trans* unit), 26.1 (CH_2CH=, *cis* unit), 25.6 (CH_3C=, *cis* unit), 25.2 (CH_3C=, *cis* unit), 23.5 (CH_3-5), 23.0 (CH_3CH=, *cis* and *W* units), 17.2 (CH_3C=, *trans* and *W* units), 15.6 (CH_3C=, *trans* unit), 7.2 (CH_3CH_2N). *Cis : trans* = 85 : 15. ESI–MS(+): *m/z* 850.6; 918.6; 986.6, calc. for $C_{61}H_{104}N$, M = 851.5; for $C_{66}H_{112}N$, M = 919.61; for $C_{71}H_{120}N$, M = 987.73.

RESULTS AND DISCUSSION

In this work we used moraprenol (**1**), a polyprenol from mulberry leaves ($WT_3C_{6\text{-}8}$-OH). It was found that triethylamine efficiently quaternized in the presence of moraprenol (**1**) and phosphorus oxychloride (Scheme 1). The reaction was completed within 2 h at about −60°C. After the decomposition of the excessive $POCl_3$ by addition of aqueous THF, triethylammonium salts of inorganic acids were removed by precipitation from heptane followed by extraction with an equilibrium mixture *n*-butanol-water. Triethylmoraprenylammonium chloride (**3**) was then extracted with acetonitrile from the heptane solution. The desired product was obtained in 72% yield that is several times higher than for secondary and tertiary prenylamines described earlier (Takigawa *et al*., 1986; Tahara *et al*., 1986; 1988). The product was stored without perceptible decomposition (TLC control) in a heptane solution at 4°C for several months.

The most precise and detailed information on the structure of polyprenyl derivatives could be obtained by analysis of the NMR spectra to determine stereochemistry and mutual arrangement of isoprene units in the molecule. NMR spectra of the product showed the characteristic shifts of some signals of the atoms of the α-terminal isoprene unit. The ^{1}H NMR spectrum virtually coincided with that of moraprenol, apart from a characteristic upfield shift of the signal for H-1 (δ 3.44 for CH_2N- *cis* unit and 3.42 for CH_2N- *trans* unit instead of 4.05 for the CH_2O group signal of the starting moraprenol). The presence of diagnostic signals at δ 3.15 and 1.21 (methylene and methyl groups of triethylammonium group, respectively) clearly confirmed the introduction of a triethylamine residue into the molecule.

The ^{13}C NMR spectrum of (**3**) was very similar to that of the starting alcohol (**1**) except for the signals of the α-terminal unit. Introduction of the triethylammonium group into the prenol derivative resulted in a large upfield shift of the C-1 signal (δ 52.6 for (**3**) as compared with 58.2 for (**1**)) and the presence of the signals of methylene and methyl groups of triethylammonium group at δ_C 52.6 and 7.2, respectively.

Signals of pseudo-molecular ions of compound (**3**) (*m/z* 850.6 for C_{55}-; 918.6 for C_{60}- and 986.6 for C_{65}-oligomer homologues) in the mass spectrum were close to the calculated values of M, 851.5; 919.61 and 987.73, respectively.

Formation of the product (**3**) may result from nucleophilic substitution of the dichlorophosphoryloxy group at the C-1 atom in the proposed intermediate (**2**) with Et_3N, thus forming the reaction product (Scheme 1). In this reaction, it was essential to find conditions that maximally favor S_N2-nucleophilic substitution; otherwise the formation of a quaternary moraprenyl ammonium salt with desired (*cis*) configuration would predominate. We have studied the influence of solvents on the ratio of the final *cis*/*trans* isomers. The use of toluene or THF instead of dichloromethane resulted in an increase of the amount of the *trans* derivative. Only the use of dichloromethane as a solvent led to the formation of a mixture of *cis* and *trans* derivatives with a high predominance of the former isomer (*cis* : *trans* = 85 : 15). There was no information about the *cis* : *trans* ratio for previously synthesized secondary and tertiary prenylamine derivatives (Takigawa *et al*., 1986; Tahara *et al*., 1986; 1988).

In conclusion, we have developed a one-pot procedure for the synthesis of quaternary polyprenylammonium chloride, which can be regarded as an example of a new class of cationic lipids.

The quaternary polyprenylammonium derivative thus obtained will be employed in further immunological and medical studies. This procedure opens a new way to cationic lipids that are useful as antivirotics and potential transfection mediators.

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