Phosphorylation of Polyprenols via Their Trichloroacetimidates

Leonid L. DANILOV*, Vladimir N. SHIBAEV, Nikolai K. KOCHETKOV

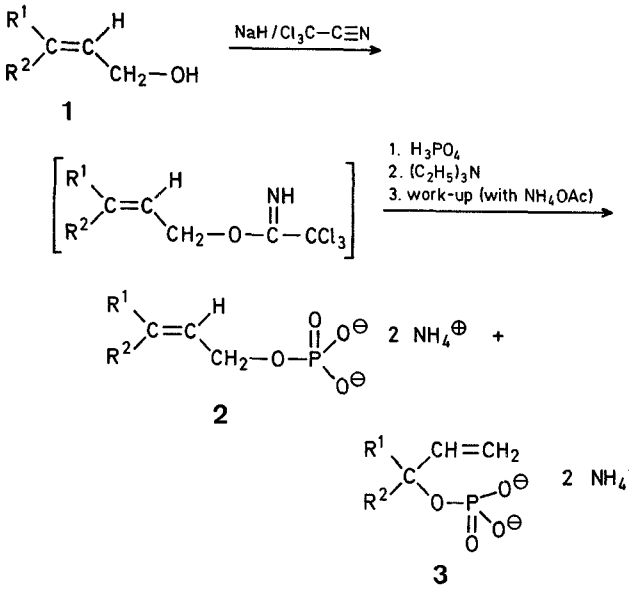
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.

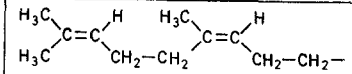
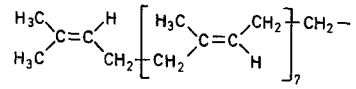
Polyprenyl phosphates with unsaturated α-isoprene units are intermediate acceptors for sugar residues in the biosynthesis of glycosyl-containing biopolymers in bacteria[1]. There are two methods for the chemical phosphorylation of polyprenols having an allylic hydroxy group:

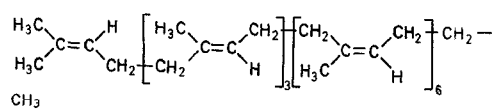
- treatment of polyprenols with bis[triethylammonium] phosphate[2,3,4], in the presence of trichloroacetonitrile;
- reaction with *o*-phenylene phosphorochloridate[4,5].

Both approaches are based on the activation of the phosphate component. The first method is simple but gives relatively low yields (up to 20%) of the desired polyprenyl phosphates because of competing pyrophosphate formation. The second approach results in higher yields of phosphates (usually 30–60%) but requires deblocking of the intermediate phosphodiesters with lead(IV) acetate which gives rise to the formation of coloured by-products.

We propose here a new alternative approach to the chemical phosphorylation of allylic polyprenols via activation of the polyprenyl component. For this purpose, (*E,Z*)-farnesol (**1a**), moraprenol[5] [**1b**,(Z_7E_3)-undecaprenol from *Morus alba* leaves], and solanesol [**1c**, (Z_8)-nonaprenol from tobacco leaves] were converted into their trichloroacetimidates by reaction[6,7,8] with trichloroacetonitrile in the presence of sodium hydride under modified conditions (use of only small amounts of starting alcohols). The polyprenyl trichloroacetimidates were treated without previous purification with anhydrous orthophosphoric acid. Phosphorylation was completed within few minutes (T. L. C.). After neutralization with excess triethylamine, polyprenyl phosphates (**2**) may be readily separated from rearragement products (**3**) and purified by ion-exchange chromatography[5,9] on DEAE-cellulose with a linear gradient of ammonium acetate in methanol and isolated as ammonium salts.



1,2,3	R^1
a	
b	CH_3
c	

1,2,3	R^2
a	CH_3
b	
c	CH_3

Compounds **2** are eluted at higher salt concentration than compounds **3**. Separation of the two products is monitored by T. L. C. and by determination of acid-labile phosphate (P_{AL}) in the eluate fractions.

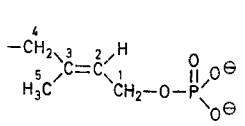
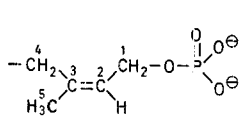
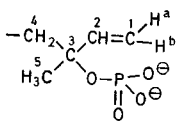
The N. M. R. spectra of the polyprenyl phosphates **2** are consistent with the proposed structures. In the spectra of compounds **3**, the signals ascribed to the atoms of the α-isoprene unit differ from the corresponding signals of compounds **2** but they correspond to the signals in the spectra of linalool and nerolidol[10,11] when the influence of the phosphate group is taken into account. The N. M. R. signals of the α-isoprene unit of the polyprenyl phosphates **2a**, **2b**, and **3a** listed in the Table are also characteristic of derivatives **2** and **3** with the same type of α-unit. The presence of a terminal vinyl group in **3** and its absence in **2** was also shown by I. R. spectrometry (1630, 1415, and 920 cm^{-1} bands are characteristic). Similar data were reported for (*E,E*)-farnesyl, linaloyl, and nerolidyl phosphates[2]. Polyprenyl phosphates **2** as well as compounds **3** have a ratio P_{AL}/polyprenol close to the calculated ratio 1 : 1.

The suggested method is fast and simple and gives satisfactory yields of polyprenyl phosphates without formation of coloured by-products or pyrophosphates. This procedure seems to be specific for allylic alcohols. When saturated alcohols such as citronellol or decanol were treated under similar conditions phosphorylated products were not detected.

Tetrahydrofuran and benzene were distilled from metallic potassium. The sodium hydride suspension (50% in oil) was washed twice anhydrous hexane before use. P_{AL} and unsaturated substances were determined as described in Ref.[9]. All polyprenyl phosphates after ion-exchange chromatography were stored as the methanolic eluate containing ammonium acetate (pH 7.0) at 5 °C and were desalted for analytical purposes and for recording of spectra on a column with Sephadex LH-20 as described in Ref.[9]. T. L. C. analyses were performed on Kieselgel H (Merck) in chloroform (system A) or chloroform/methanol/water 60/25/4 (system B). I. R. spectra were obtained with Perkin-Elmer 167 instrument. ^{1}H- and ^{13}C-N. M. R. spectra were recorded on Bruker WM-250 spectrometers (250 and 62.89 MHz, respectively) in CCl_4/CD_3OD (3/1).

Phosphorylation of (*E,Z*)-Farnesol (1a); Typical Procedure:
A solution of (*E,Z*)-farnesol (**1a**; 160 mg, 0.72 mmol) in dry benzene (2 ml) is mixed with a sodium hydride suspension (50 mg, 1.04 mmol) and trichloroacetonitrile (0.3 ml, 3.0 mmol) is added with stirring and with exclusion of atmospheric moisture. After 15–20 min at 20 °C, T. L. C. shows the absence of starting material (R_f 0.30, A) and a new broad zone forming near the top of the plate. The reaction mixture is evaporated to dryness on the oil pump, mixed with anhydrous tetrahydrofuran (2 ml), and added to a stirred solution of crystalline orthophosphoric acid (280 mg, 2.8 mmol, Merck) in tetrahydrofuran (3 ml). Stirring is continued at 20 °C for 15 min, dry triethylamine is added to pH 9, and the mixture is evaporated with toluene (3 ml) 3 times. The residue is mixed with toluene (5 ml), the precipitate is removed by filtration, and washed with toluene (3 ×). The resultant solution and the washings are evaporated, the residue is dissolved in chloroform/methanol (2/1, 50 ml), and applied to a column (25 × 1.5 cm) of Whatman DE-52 ($OAc^{\ominus}$ form) equilibrated with chloroform/methanol (2/1). The column is washed with chloroform/methanol (2/1, 50 ml) and methanol (50 ml) and eluted (60 ml/h) with a linear gradient (0 → 30 mmol/l, 150 ml in each vessel) of ammonium acetate in methanol (7 ml fractions). Fractions 5–15 and 16–35 containing ammonium salts of **3a** and **2a**, respectively, (individual spots on T. L. C.) are collected. Ammonium acetate is removed by chromatography on a column (70 × 2 cm) of Sephadex LH-20 in chloroform/methanol (2/1).

Table. N.M.R. Data ($CCl_4/CD_3OD/TMS_{int}$) of the α-Isoprene Units of Polyprenyl Phosphates **2a,b** and **3a**; δ [ppm]

Atom No.	 **2a**		 **2b**		 **3a**	
	^{1}H-N.M.R.	^{13}C-N.M.R.	^{1}H-N.M.R.	^{13}C-N.M.R.	^{1}H-N.M.R.	^{13}C-N.M.R.
1	4.40 (dd, 2H, $J_{HH} = J_{HP} = 6.0$ Hz)	62.1 (br.s)	4.38 (br., 2H)	61.75 (d, $J = 6.5$ Hz)	5.07 (dd, 1H, 1-H^b, $J_{1^b,2} = 10.5$ Hz, $J_{1^b,1^a} = 1.5$ Hz); 5.20 (dd, 1H, 1-H^a, $J_{1^a,2} = 17.5$ Hz, $J_{1^a,1^b} = 1.5$ Hz)	112.3
2	5.37 (t, 1H, $J = 6.0$ Hz)	121.15 (d, $J = 7.4$ Hz)	5.37 (t, 1H, $J = 7.0$ Hz)	122.45 (d, $J = 7.4$ Hz)	6.03 (dd, 1H, $J_{2,1^a} = 17.5$ Hz, $J_{2,1^b} = 10.5$ Hz)	143.15 (d, $J = 4.0$ Hz)
3	–	139.45	–	138.9	–	81.05 (d, $J = 6.0$ Hz)
4	in group of signals 1.95–2.10	39.8	in group of signals 1.90–2.10	31.95	in group of signals 1.57–1.71	42.15 (d, $J = 3.5$ Hz)
5	1.69 (br. s)	15.9	1.73 (s, 3H)	23.15	1.27 (s, 3H)	24.3

Phosphate **2a**: yield: 115 mg (0.34 mmol, 47%); R_f: 0.33(B); P_{AL}/farnesol: 1/1.10.

Phosphate **3a**: yield: 18 mg (0.053 mmol, 7.4%); R_f: 0.35(B); P_{AL}/farnesol: 1/0.91.

$C_{15}H_{33}N_2O_4P$ calc. P 9.23
(336.4) found 9.10 (**2a**); 9.05 (**3a**)

Analogously, the reaction of moraprenol (**1b**; 46 mg, 60 μmol) with sodium hydride suspension (3 mg, 63 μmol), trichloroacetonitrile (20 μl, 200 μmol), and orthophosphoric acid (20 mg, 205 μmol) gives **2b** and **3b**. Fractionation on DE-52 allows to obtain individual **2b** and a mixture of **3b** and **2b** (~ 1/1 by T.L.C. and ^{1}H-N.M.R.).

Moraprenyl phosphate **2b** thus obtained was identical with an authentic sample prepared from *o*-phenylene phosphorochloridate[5] as regards T.L.C., ^{1}H- and ^{13}C-N.M.R. spectra, and the ability to serve as acceptor for galactosyl phosphate in the enzymatic preparation from *Salmonella*.

Phosphate **2b**; yield: 21 mg (24 μmol, 40%); R_f: 0.50(B); P_{AL}/moraprenol: 1/0.97.

Phosphates **3b** + **2b**; yield: 5.3 mg (6.6 μmol, 11%); R_f: 0.50 (**2b**) and 0.55 (**3b**)(B); P_{AL}/moraprenol: 1/1.00.

$C_{55}H_{97}N_2O_4P$ calc. P 3.52
(881.3) found 3.44 (**2b**); 3.40 (**2b** + **3b**)

Solanesol (**1c**; 63 mg, 100 μmol) upon reaction with sodium hydride suspension (10 mg, 210 μmol) and trichloroacetonitrile (30 μl, 300 μmol), followed by treatment with orthophosphoric acid (40 mg, 410 μmol) and ion-exchange chromatography gives the isolated products **2c** and **3c**.

Phosphate **2c**; yield: 15 mg (20 μmol, 20%); R_f: 0.45(B); P_{AL}/solanesol: 1/0.98.

Phosphate **3c**; yield: 5.6 mg (7.5 μmol, 7.5%); R_f: 0.49(B); P_{AL}/solanesol: 1/0.96.

$C_{45}H_{81}N_2O_4P$ calc. P 4.17
(745.1) found 3.88 (**2c**); 3.89 (**3c**)

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* Address for correspondence.

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