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Synthesis of phosphatidylated-monoterpene alcohols catalyzed by phospholipase D and their antiproliferative effects on human cancer cells

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ABSTRACT

In order to prepare functional phospholipids in the medical and pharmaceutical fields, perillyl alcohol, myrtenol, and nerol were transphosphatidylated via phospholipase D in an aqueous system. The yields of phosphatidyl-perillyl alcohol, -myrtenol, and -nerol were 79 mol %, 87 mol %, and 91 mol %, respectively. The synthetic phosphatidylated monoterpenes showed a markedly antiproliferative effect on human prostate PC-3 and human leukemia HL-60 cells at 100 μM, while the free monoterpene alcohols had no effect at 400 μM.

Terpenes are functional compounds with an isoprenoid structure, found in the essential oils of plants such as lemon grass, cherry, mandarin, and citronella. Perillyl alcohol and geraniol have good pharmacological or chemopreventive effects in vitro and in vivo.[1–7] Perillyl alcohol, a naturally occurring cyclic monoterpene, exhibits apoptotic effects on prostate cancer cell lines and has an angiogenesis inhibitory effect.[1,2] Several clinical trials, including phase I and II trials of perillyl alcohol, have recently been conducted.[3,4] Geraniol also exhibits antitumor activity against various cancer cells and enhances sensitivities of 5-fluorouracil toward colon cancer cells.[5–7]

To utilize functional terpenes in various fields, phospholipid-derivatization of terpene alcohols presents a novel strategy (Fig. 1). Because phosphatidylation adds amphiphilic properties, phosphatidyl derivatives of terpene alcohols could be emulsified in water to make a liposome which could be used as a drug delivery system.[8,9]

Phospholipase D (PLD) (EC 3.1.4.4) is a lipolytic enzyme that hydrolyzes terminal phosphodiester bonds of phospholipids. Due to its ability to transfer the phosphatidyl moiety of phospholipids to various alcohols, PLD has been used to synthesize phosphatidylglycerol, phosphatidylserine, and phosphatidylethanolamine on laboratory and industrial scales.[10] Furthermore, novel phospholipids containing various functional molecules which are poorly accessible via chemical routes have been synthesized via PLD-mediated transphosphatidylation. The cytotoxity of phosphatidyl-genipin on cancer cell lines was stronger than that of genipin and enhanced efficient genipin penetration.[11] 5-Fluorouridine, a known antitumor reagent, was phosphatidylated and the resulting compound exhibited high antitumor activity against both P388 and Meth A fibrosarcoma compared to 5-fluorouridine.[12] Thus, phospholipid derivatives with functional compounds are excellent candidates for drugs or fine chemicals.[13,14]

We have recently reported that novel types of phosphatidylated terpenes alcohols, for example, phosphatidyl-geraniol (**2**), phosphatidyl-farnesol (**4**), phosphatidyl-geranylgeraniol (**6**), and phosphatidyl-phytol (**8**), were synthesized via PLD-mediated transphosphatidylation in organic and aqueous systems.[15] In this study, the first synthesis of three phosphatidylated terpene alcohols (PTs, **10**, **12**, and **14**) including cyclic structures was performed via PLD-mediated transphosphatidylation in the aqueous system. Furthermore, the antiproliferative effects of the synthesized PTs on human prostate PC-3 and human leukemia HL-60 cells were examined.

PTs, phosphatidyl-perillyl alcohol (**10**), phosphatidyl-myrtenol (**12**), and phosphatidyl-nerol (**14**), were synthesized using dioleoyl-phosphatidylcholine (DOPC) and terpene alcohols, perillyl alcohol (**9**), myrtenol (**11**), and nerol (**13**), respectively, by PLD (Fig. 1). A new spot was detected between DOPC and terpene alcohols on silica gel TLC with an Rf value of 0.46 in chloroform–methanol–water (65:25:4, v/v/v). The PTs were isolated by using preparation TLC. Data from negative high resolution APCI-MS of the synthesized phospholipids, **10**, **12**, and **14**, coincided with the

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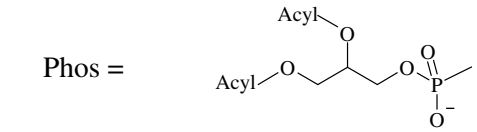
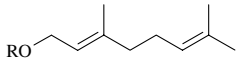
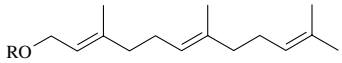
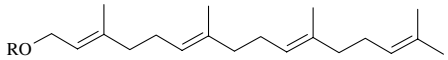




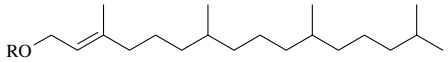
R = H; Geraniol (**1**)
R = Phos; Phosphatidyl-geraniol (**2**)



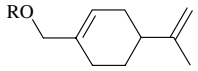
R = H; Farnesol (**3**)
R = Phos; Phosphatidyl-farnesol (**4**)



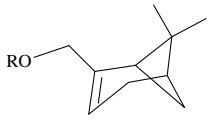
R = H; Geranylgeraniol (**5**)
R = Phos; Phosphatidyl-geranylgeraniol (**6**)



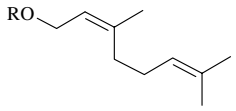
R = H; Phytol (**7**)
R = Phos; Phosphatidyl-phytol (**8**)



R = H; Perillyl alcohol (**9**)
R = Phos; Phosphatidyl-perillyl alcohol (**10**)



R = H; Myrtenol (**11**)
R = Phos; Phosphatidyl-myrtenol (**12**)



R = H; Nerol (**13**)
R = Phos; Phosphatidyl-nerol (**14**)

Figure 1. Terpene alcohols and their phosphatidyl derivatives.

predicted molecular formulas.[16] The ^{1}H and ^{13}C NMR data for **10**, **12**, and **14** were also supported their structures.[16]

We compared the yields of PTs synthesized via PLD-mediated transphosphatidylation of phosphatidylcholine from soy been (soy PC) with terpene alcohols under reaction conditions previously reported.[15] Cyclic terpene alcohols **9** and **11** could be acceptors for transphosphatidylation in the aqueous reaction system. The yields of **10** and **12** were 79 and 87 mol % which is a little lower than that of **2** (90 mol %),[15] but higher than those of **6** (54 mol %)[15] and **8** (17 mol %)[15] (Table 1). In addition, the yield of **14** was 91 mol % which is comparable to that of **2**. This latter result shows that PLD from *Streptomyces* sp. does not recognize the *Z*/*E*-configuration of terpene alcohols in the transphosphatidylation reaction in the aqueous system.

The antiproliferative effect of PTs on human prostate cancer PC-3 cells and human leukemia HL-60 cells was examined by the WST-1 assay.[17] Among the seven PTs, **2**, **10**, **12**, and **14**, showed a markedly antiproliferative effect on PC-3 after 72-h incubation at 100 μM, while substrate soy PC showed no antiproliferative effect at 100 μM (Fig. 2A). Compounds **10** and **12** especially showed a reduced viability of PC-3 cells to 10% of control. On the other hand, free monoterpene alcohols, geraniol (**1**), **9**, **11**, and **13**, did not show any reduction of viability of PC-3 cells even at 400 μM (Fig. 2B). Furthermore, each PT reduced the viability of PC-3 cells in a time-dependent manner, and cell viability decreased up to 10–30% of control after 72-h incubation (Fig. 3). These four PTs also showed an antiproliferative effect on human leukemia HL-60 cells in a time-dependent manner (Fig. 4). Compound **10** in particular showed the strongest antiproliferative effect among the four PTs at an early incubation time, although monoterpene alcohols showed no antiproliferative effect on HL-60 cells even at 400 μM (data not shown). These data show that phosphatidylation is effective in enhancing the antiproliferative effects of terpenes on PC-3 and HL-60 cells.

Table 1
Yield of phosphatidylated terpene alcohols synthesized by PLD-mediated transphosphatidylation in aqueous system

Phosphatidylated terpene	Yield (mol %)
Phosphatidyl-perillyl alcohol (**10**)	79
Phosphatidyl-myrtenol (**12**)	87
Phosphatidyl-nerol (**14**)	91

[a]Reaction mixture: 50 μmol soyPC, 2 mmol alcohol, 1.6 U PLD and 0.8 ml of 0.2 M sodium acetate buffer (pH 5.6). The reaction was conducted at 37 °C for 24 h.

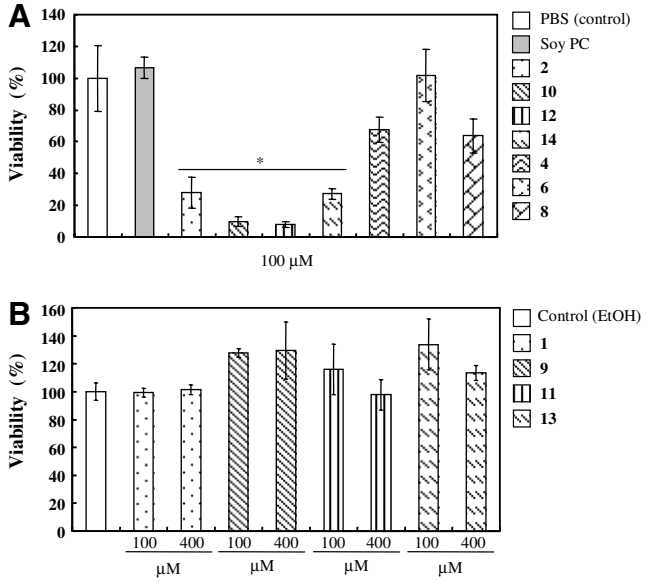


Figure 2. Viability of PC-3 cells treated with phosphatidylated terpene alcohols. (A) PC-3 cells were incubated for 72 h in culture media containing phosphatidylated terpene alcohols. (B) PC-3 cells were incubated for 72 h in the culture media containing terpene alcohols. $^{*}p < 0.01$ versus control.

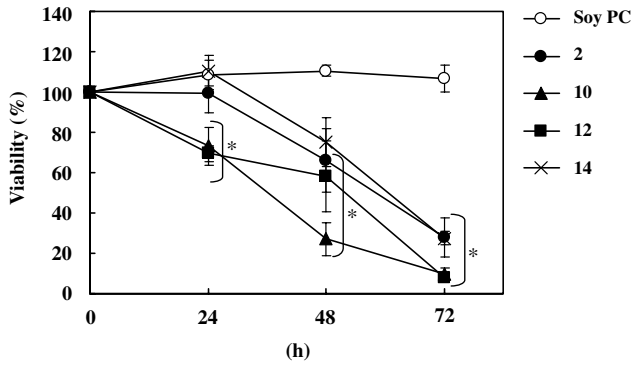


Figure 3. Time course of viability of PC-3 cells treated with phosphatidylated terpene alcohols (100 μM). $^{*}p < 0.01$ versus control (PBS).

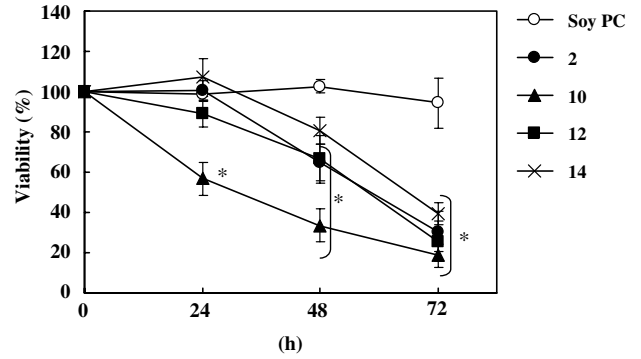


Figure 4. Time course of viability of HL-60 cells treated with phosphatidylated terpene alcohols (100 μM). $^{*}p < 0.01$ versus control (PBS).

In conclusion, novel PTs were synthesized via transphosphatidylation catalyzed by PLD. Compounds **10**, **12**, **14**, and **2** showed a remarkably antiproliferative effect on PC-3 and HL-60 cells.

Acknowledgments

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16. Phosphatidyl-perillyl alcohol (**10**): APCIHRMS: *m*/*z* 833.6060 $(M-H)^{-}$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.71 (m, 1H), 5.33 (m, 4H), 5.23 (m, 1H), 4.70 (m, 1H), 4.68 (m, 1H), 4.39 (dd, J = 12 Hz and 2.5 Hz, 1H), 4.21 (br s, 2H), 4.18 (dd, J = 12 Hz and 7 Hz, 1H), 3.93 (m, 2H), 2.26 (t, J = 6.5 Hz, 4H), 2.12 (m, 1H), 2.09 (m, 2H), 2.07 (m, 1H), 2.00 (m, 8H), 1.71 (s, 3H), 1.57 (m, 4H), 1.29 (m, 40H), 1.18 (m, 2H), 0.88 (t, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 173.6, 149.6, 134.3, 130.0, 123.7, 108.7, 70.8, 69.6, 63.7, 62.9, 41.0, 34.1, 31.27 (several C's), 30.4, 27.4, 27.1, 26.1, 24.9, 20.8, 14.1. Phosphatidyl-myrtenol (**12**): APCIHRMS: *m*/*z* 833.6060 $(M-H)^{-}$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.52 (m, 1H), 5.33 (m, 4H), 5.23 (m, 1H), 4.48 (dd, J = 12 Hz and 2.5 Hz, 1H), 4.19 (br s, 2H), 4.18 (dd, J = 12 Hz and 7 Hz, 1H), 3.93 (m, 2H), 2.35 (m, 1H), 2.31 (m, 1H), 2.28 (m, 1H), 2.26 (t, J = 6.5 Hz, 4H), 2.11 (t, J = 5.5 Hz, 1H), 2.07 (m, 2H), 2.00 (m, 8H), 1.58 (m, 4H), 1.29 (m, 40H), 1.29 (s, 3H), 1.15 (d, J = 9 Hz, 1H), 0.88 (t, J = 6.5 Hz, 6H), 0.81 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 173.6, 144.8, 130.0, 118.9, 70.8, 68.2, 63.8, 62.9, 43.1, 40.8, 37.9, 34.1, 31.27 (several C's), 31.5, 31.1, 27.1, 26.1, 24.9, 21.0, 14.1. Phosphatidyl-nerol (**14**): APCIHRMS: *m*/*z* 835.6217 $(M-H)^{-}$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.33 (m, 5H), 5.23 (m, 1H), 5.07 (m, 1H), 4.40 (dd, J = 12 Hz and 2.5 Hz, 1H), 4.35 (br s, 2H), 4.19 (dd, J = 12 Hz and 7 Hz, 1H), 3.95 (m, 2H), 2.27 (t, J = 6.5 Hz, 4H), 2.01 (m, 4H), 2.00 (m, 8H), 1.70 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H), 1.57 (m, 4H), 1.29 (m, 40H), 0.88 (t, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 173.5, 139.3, 131.7, 130.0, 123.9, 122.3, 70.5, 63.5, 63.0, 62.3, 34.3, 31.9, 31.27 (several C's), 27.2, 24.9, 24.8, 23.4, 23.4, 17.7, 14.1.
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