PREPARATION AND CHARACTERIZATION OF PHOSPHOLANES AND PHOSPHA SUGARS AS NOVEL ANTI-CANCER AGENTS

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Abstract: Diastereo isomeric *erythro* and *threo* forms of 2,3-epoxy-1-phenylphospholane 1-oxides were synthesized from *threo* and *erythro* forms of 2-bromo-3-hydroxy-1-phenylphospholane 1-oxides being prepared from 1-phenyl-2-phospholene 1-oxide. Alternatively, the epoxides were also prepared by the epoxidation of the 2-phospholene with peroxides such as sodium peroxide and hydrogen peroxide. The reactivity and regioselectivity for the reaction of *erythro* and *threo* forms of the 2,3-epoxides with nucleophiles were investigated by using amines, and the reaction afforded 2-amino-3-hydroxy-1-phenylphospholane 1-oxides, which correspond to phospha sugar *N*-glycosides. 2,3-Dibromo-3-methyl-1-phenylphospholane 1-oxides were first prepared from 3-methyl-1-phenyl-2-phospholene 1-oxide. The prepared phospholanes or phospha sugars were biologically qualified by MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) *in vitro* method to find that some of these phosphorus heterocycles or phospha sugars have quite efficient anti-cancer activity for leukemia cells in manners of (i) wide spectra, (ii) high activities, and (iii) high specificities.

Keywords: Heterocyclic compounds, Epoxyphospholane, Tumors, Leukemia cells, MTT method

Introduction

Phospha sugars have a phosphorus atom in place of the ring oxygen atom of normal sugars, and are classified into the category of pseudo sugars. Many nucleoside

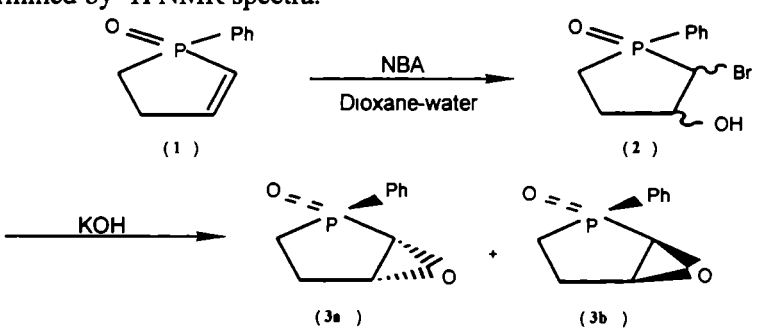
derivatives of sugars, e.g., AZT (1) and Ribavirin (2), are prepared and used as antivirus agents (1,2). In addition to the nucleoside of normal sugars, sugar modified nucleoside derivatives of pseudo sugars or hetero sugars such as aza- (3), carba- (4), and thia-sugars (5) have been synthesized and reported.

Substitution reaction of the bromo group of 2-bromophospholane derivatives, which corresponds to 2-bromo-2-deoxyphospha sugar derivatives, with amine nucleophiles gave N-glycosides of phospha sugar derivatives. Further, nucleic acid bases such as uracil were formed by the cyclization reaction of acrylamide intermediates to prepare phospha sugar nucleosides (6). We are continuously searching biologically active phosphorus heterocycles, phospha sugars and phospholanes, for a couple of ten years.

In this paper, we will deal with the successful preparation of epoxy- and bromophospholane derivatives from 1-phenyl-2- phospholene 1-oxide. The evaluation of the biological activity for leukemia cells by using 3-(4,5-Dimethyl-2-thiazolyl)-2,5- diphenyltetrazolium bromide (MTT) *in vitro* methods for these phosphorus heterocycles or phospha sugars is first reported in this paper.

Results and Discussion

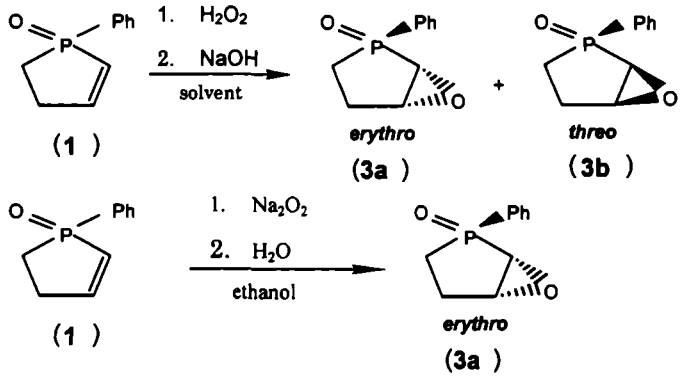
The reaction of 1-phenyl-2-phospholene 1-oxide **1** with bromine (7) in aqueous media followed by treatment with 0.5 *N* KOH aq. solution gave 2,3-epoxy-1-phenylphospholane 1-oxide **3** via bromohydrin **2**. Diastereomers of *erythro* and *threo* epoxides **3a** and **3b**, respectively, were isolated in a ratio of 5:3 (Scheme 1) by column chromatography on silica gel and the each structure was determined by ^{1}H NMR spectra.



Scheme 1: Epoxyphospholanes **3a** and **3b** from 2-phospholene **1** via bromohydrin **2**.

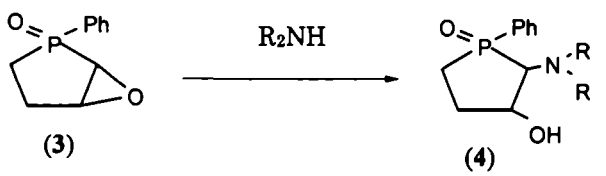
Epoxidation of **1** with sodium peroxide afforded only *erythro* **3a** under limited reaction condition (8), however, the epoxidation of **1** with one of the most popular oxidizing reagent, hydrogen peroxide, produced *erythro* **3a** and *threo* **3b** in a variable

ratio depending on the conditions (Scheme 2). On the other hand, the epoxidation with *m*-CPBA was not successful at all, because 2-phospholene 1-oxide **1** has a strong electron withdrawing phosphoryl group at the adjacent to the C=C bond, and then the C=C bond becomes electron deficient.



Scheme 2. Epoxidation of 2-phospholene **1** with peroxides to give **3**.

2,3-Epoxyphospholanes of *erythro* **3a** and *threo* **3b** were allowed to react with amines, e.g., ammonia, diethylamine, and diisopropylamine, to give 2-amino-3-hydroxy-1-phenylphospholane 1-oxide **4**, which corresponds to *N*-glycosides of phospha sugar derivatives (Scheme 3 and Table 1).



Scheme 3. Nucleophilic substitution reaction of the epoxide **3** with amines.

From the results shown in Table 1, the nucleophilic substitution reaction of 2,3-epoxyphospholanes **3a** and **3b** with amines occurred at the C-1 position and the difference of reactivity between *threo* and *erythro* diastereomers of epoxides **3a** and **3b**, respectively, was large. The substitution reaction of epoxides with amines suffers from substituent effects of amines, especially

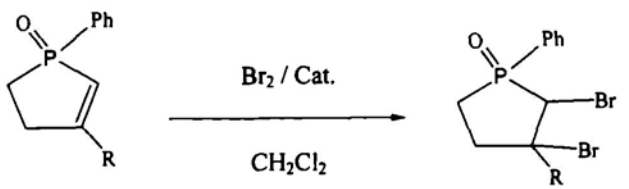
Table 1. Nucleophilic substitution reaction of the epoxide **3a** (*erythro*) and **3b** (*threo*) with

Entry	Starting material: Epoxide Diastereomer	Amine	Reaction Condition: Solv.	Temp.	Time	Product: R	R'	Compound No	Yield (%)	Structure
1	erythro	NH_3	water	r.t	2days	H	H	4a,	20	
2	erythro	Et_2NH	CH_3OH	40°C	1week	Et	Et	4a,	trace	
3	erythro	i-Pr NH_2	CH_3OH	40°C	1week	Pr	Pr	4a,	N.R a)	
4	threo	NH_3	water	r.t	2days	H	H	4b,	quant	
5	threo	Et_2NH	CH_3OH	40°C	2days	Et	Et	4b,	80	
6	threo	i-PrNH_2	CH_3OH	40°C	2days	Pr	H	4b,	96	
7	threo	t-BuNH_2	CH_3OH	40°C	4days	Bu	H	4b,	71	
8	threo	i-Pr_2NH	EtOH	80°C	2days	Pr	Pr	4b,	13	

a) N R : No reaction

reactivity of *erythro* epoxide **3a** is very low by the steric hindrance of the phenyl group of phospholane and the alkyl group of amines. Thus, the smaller amine nucleophile, i.e., ammonia, reacts with 2,3-epoxyphospholane **3** even at room temperature, however, the larger nucleophile, i.e., diisopropylamine, is much less reactive than ammonia with the epoxide **3**.

Addition reaction of bromine with 3-methyl-1-phenyl-2-phospholene 1-oxide **1** (R=Me) with bromine in dichloromethane in the presence of catalyst, i.e., manganese dioxide, gave 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide **5** (R=Me).



1 (R=Me) **5** (R=Me)

Scheme 4. Preparation of 2,3-dibromophospholane derivative **5** (R=Me).

The prepared phospholane or phospha sugar derivatives were bio-assayed by MTT *in vitro* method for leukemia cells of K562 and U937 cell lines for the first time. Some of the results of the *in vitro* bio-assay for leukemia cell of K562 cell line with the bromohydrin **2**, epoxide **3**, and dibromide **5** of phospholanes are shown in Figures 1-3.

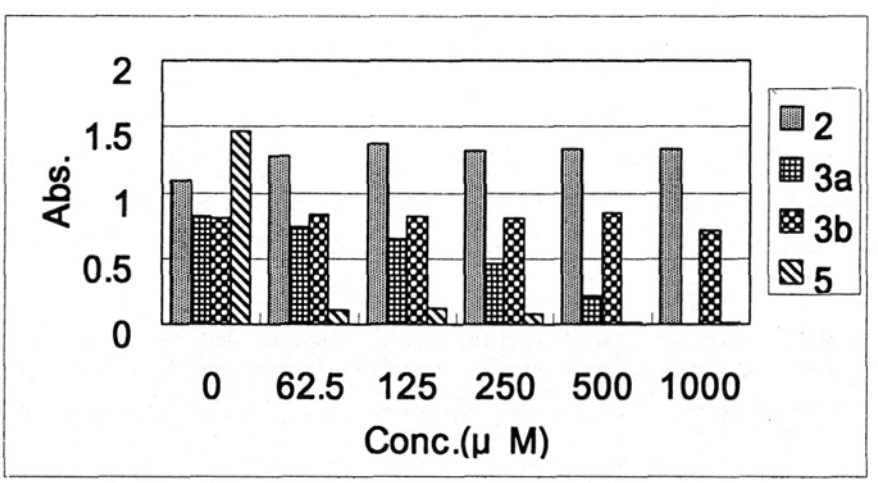


Figure 1. MTT *in vitro* evaluation results for K562 leukemia cell by bromohydrin **2**, epoxide **3**, and dibromide **5**.

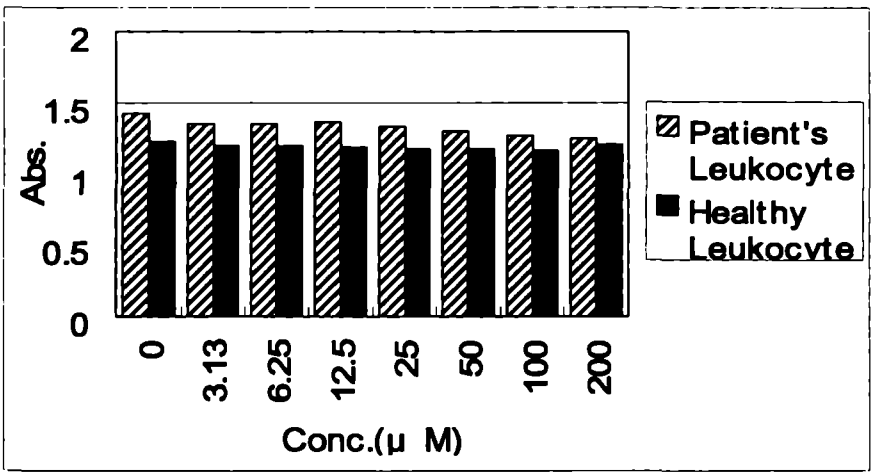


Figure 2. MTT *in vitro* evaluation results for patient's and healthy leukocyte by dibromide

Figure 1 shows that among the four samples epoxide **3a** and dibromide **5** killed effectively K562 leukemia cell, however, the other samples **2** and **3b** did not kill the leukemia cell at all just as the control (DMSO). Figure 2 shows that even the active sample **5** did not kill the healthy leukocyte (blast 0 %) at all but partially kill the patient's leukocyte (blast 40 %) selectively. Figure 3 shows that the separated four diastereomeric dibromide (retention times for each diastereomer are shown in Figure 3) have the higher activity for K562 leukemia cell. Unfortunately all 2-amino-3-hydroxy-1-phenylphospholane 1-oxide derivatives **4a** and **4b** in Table 1 did not kill the leukemia cell shown by *in vivo* evaluation of MTT method. The results of *in vivo* evaluation experiments shown in Figures

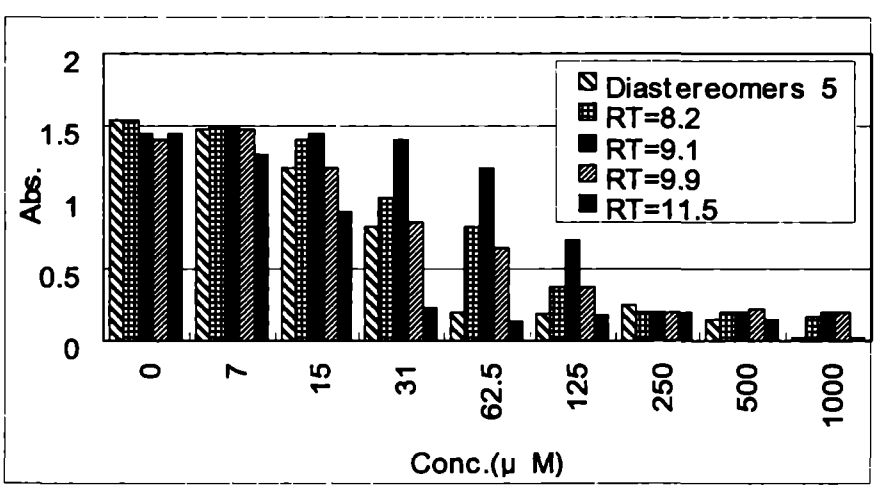


Figure 3. MTT *in vitro* evaluation results for K652 leukemia cell by the four separated diastereomers of 2,3-dibromide **5**. (RT means the retention time (min.) of the diastereomers recorded by HPLC on silica gel column with eluent ($CHCl_3$: MeOH = 20 : 1))

1-3 show that the prepared phospholanes or phospha sugars of epoxide and dibromide have quite efficient anti-cancer activities for leukemia cells in manners of (i) wide spectra, (ii) high activities, and (iii) high specificities. The further research on the structure-activity and the mechanism of the phospha sugars as the anti-tumor agents will be disclosed separately.

Experimental Section:

General Procedures and Methods:

NMR spectra were collected on JEOL EX300 (300MHz) and HITACHI H-90 (90MHz) instruments. TLC plates of "Chromato Sheet" of Wako Pure Chemical Industries were used. HPLC instruments of JASCO Corporation equipped with silica gel column (Wakopak; 4.6 mm in diameter × 250 mm in length) and UV detector were used. Silica gel of "Wakogel C-200" (75 – 150 μm) was used for column chromatography. The eluents used for TLC, HPLC, and column chromatography are shown in parenthesis.

2,3-Epoxy-1-phenylphospholane 1-Oxide 3:

2-Bromo-3-hydroxyphospholane **2** (2.5 g, 9.7 mmol) was dissolved in 50 ml of potassium hydroxide solution (0.5 *N*) and the solution was stirred at 40 □ for 1 hr. After completion of the reaction the solvent was evaporated and the product was extracted with $CHCl_3$ (30 ml × 3 times). The $CHCl_3$ layer was separated and evaporated under diminished pressure. The residue was separated by column chromatography on silica gel (EtOAc : MeOH = 20 : 1) to afford 0.83 g (44 % yield) of **3a** and 0.49 g (26 % yield) of **3b**.

Compound **3a**; ^{1}H NMR ($CDCl_3$), δ (ppm):1.94-2.17 (m, 2H, H-4),1.94-2.68 (m, 2H, H-5), 3.51 (dd, 1H, J_{HCP}=28.7Hz J=3.0Hz, H-2), 3.93-3.96 (m, 1H, H-3), and 7.51-8.00 (m, 5H, Ph-H); TLC (EtOAc : MeOH = 20 : 1), Rf: 0.55.

Compound **3b**; ^{1}H NMR ($CDCl_3$), δ (ppm): 1.94-2.17 (m, 2H, H-4), 1.94-2.68 (m, 2H, H-5), 3.45 (dd, 1H, J_{HPC}=29.1 Hz, J=3.0Hz, H-2), 3.82-3.86 (m, 1H, H-3), and 7.76-7.51 (m, 5H, Ph-H); TLC (EtOAc : MeOH = 20 : 1), Rf: 0.39.

Typical Procedure for 2-Amino-3-hydroxy-1-phenylphospholane 1-Oxide 4 (2-Diethylamino Derivative 4b$_1$):

A mixture of 2,3-epoxy-1-phenylphospholane 1-oxide **3b** (0.050 g, 0.26 mmol) with 20 ml of aqueous ammonia was stirred at room temperature for 2 days. After evaporation of volatile materials reaction products were separated by silica gel column chromatography ($CHCl_3$: CH_3OH = 10:1) to give 0.065 g (0.26 mmol; in aquantitative yield) of **4b$_1$**.

Compound **4b**$_1$; ^{1}H NMR ($CDCl_3$), δ (ppm): 1.63-2.43 (m, 4H, H-4,4',5,5'), 2.95 (dd, 1H, *J*=2.2 Hz, *J*=8.0 Hz, H-2), 4.01-4.11 (m, 1H, H-3), 7.33-7.75 (m, 5H, Ph-H), TLC (EtOAc : MeOH = 20 : 1), Rf: 0.18.

Synthesis of 2,3-Dibromo-3-methyl-1-phenylphospholane 1-Oxide 5 (R=Me)

To a mixture of 3-methyl-1-phenyl-2-phospholene 1-oxide **1** (R=Me) 0.266 g (1.38 mmol) and manganese dioxide 0.239 g (2.38 mmol; 2.0 eq.) in dichloromethane (5 ml) was added dichloromethane (5 ml) solution of bromine 0.400 ml (7.81 mmol; 5.7 eq.), and then stirred for 12 h under Ar atmosphere. Reduction of excess bromine with sodium sulfite solution, and then the reaction mixture was extracted by chloroform (10 ml x 3 times). The organic layer was neutralized with saturated sodium hydrogencarbonate, washed with saturated sodium chloride solution, and then dried over sodium sulfate. Removal of the solvent under reduced pressure followed by separation from the residue gave 0.376 g (yield 78%) of **5** (R=Me). RT value of each diastereomer is shown in Figure 3.

5 (R=Me); m.p. 189.20 °C; b.p. 280.24 °C; TLC ($CHCl_3$: MeOH = 20 : 1), Rf = 0.42; MS (m/z), 353.20 (MH^+); IR (KBr) 1126 cm^{-1} (P=O), 748 cm^{-1}, 1396 cm^{-1} (C-Br); ^{1}H-NMR ($CDCl_3$, 300 MHz), δ(ppm); 1.67 (s, 3H, CH_3), 2.36-2.46 (m, 2H, H-4), 2.97-3.02 (m, 2H, H-5) 4.28-4.31 (m, 1H, C-2), 7.51-7.70 (m, 5H, Ph-H).

Conclusion:

The stereospecific epoxidation of 1-phenyl-2-phospholene 1-oxide **1** to form *erythro* epoxides **3a** was performed by using sodium peroxide. Epoxidation of 2-phospholene derivatives **1** with hydrogen peroxide as well as epoxide formation via bromohydrin route from 2-phospholene **1** afforded a mixture of the *erythro* and *threo* diastereomers **3**. The nucleophilic substitution reaction of 2,3-epoxyphospholane 1-oxides **3a** and **3b** occurred at C-1 position with amines to give the *N*-glycoside derivative **4** of phospha sugars. 2,3-Dibromophospholane derivatives **5** (R=Me) were prepared as a mixture of four diastereoisomers. MTT *in vitro* bio-assay method revealed that the prepared phospholanes or phospha sugars have quite efficient anti-cancer activities for leukemia cells in manners of (i) wide spectra, (ii) high activities, and (iii) high specificities and selectivities.

Acknowledgment:

Financial supports by Shizuoka Prefectural Government, the Ministry of Health, Labor and Welfare, and the Ministry of Education, Culture, Sports, Science and

Technology, Japan, as well as by some collaborated companies and Shizuoka University are deeply acknowledged.

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Received on October 4,2008.