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Reaction of Saligenin Cyclic Phosphorus Esters with Some Amino Acids and Purine and Pyrimidine Bases

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2-Phenoxy-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (K-2), a kind of saligenin cyclic phosphorus esters, reacts with some nucleophiles to form the 2-hydoxybenzyl derivatives. The reactivity of K-2 with some biologically important components of organisms, such as amino acids, purine bases and pyrimidine bases, were investigated. Under neutral conditions, the reactivity of K-2 with them was not observable. Although serine and tyrosine did not react with K-2 under alkaline conditions, cysteine, histidine and arginine did and gave the corresponding 2-hydroxybenzyl derivatives. With purine and pyrimidine bases, no observable reaction occurred under any employed conditions.

INTRODUCTION

Saligenin cyclic phosphorus esters (SCPs, Fig. 1) have been reported to be unique enzyme inhibitors (Shiotsuki, 1991; Eto, 1992). SCPs have a phosphoryl phosphorus atom, Pearson's hard acid, and a benzyl carbon, the soft acid (Pearson, 1963; Edwards and Pearson, 1961). The former is activated by the ring strain and the latter is activated by the ring opening at the P-O (aryl) bond. Thus serine enzymes, such as chymotrypsin and acetylcholinesterase are phosphorylated, whereas cysteine enzymes such as papain and alcohol dehydrogenase are alkylated by SCPs (Eto, 1983).

2-Phenoxy-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (K-2) is known to synergize some organophosphorus (OP) insecticides against OP-resistant arthropods, as malathion in house flies via inhibition of carboxyesterase (Ohkawa et al., 1968) and malaoxon and paraoxon in green peach aphid by inhibiting hydrolytic degradation (Oppenoorth and Voerman, 1975). K-2 is an effective synergist for fenitroxon against OP-resistant rice stem borers by inhibiting the degradation of fenitroxon (Konno and Shishido, 1985). K-2 was also synergistic with fenitrothion against OP-resistant house flies because of suppression of glutathione (GSH)-dependent detoxification (Shiotsuki et al., 1989; Shiotsuki et ***al.,*** 1992). The inhibition of glutathione-S-transferase by K-2 was found to be partially caused by *S*-(2-hydroxybenzyl)glutathione, which is an active conjugate of GSH (Shiotsuki and Eto, 1987). K-2 reacts with nucleophiles to form the corresponding 2-hydroxybenzyl derivertives. Therefore, we have investigated the reactivity of K-2 with amino acids, purine bases and pyrimidine bases, which are important components for maintaining life.

MATERIALS AND METHODS

Chemicals

K-2 was prepared from saligenin and phenyl phosphrodichloridate as reported (Eto and Ohshima, 1962).

S-(2-Hydroxybenzyl)cysteine was nonenzymatically obtained from cysteine and K-2. To a solution of cysteine (lmmol) in the mixture of ethanol (1.0ml) and 2N sodium hydroxide (1.2ml), K-2 (lmmol) was added at room temperature. After stirring for 2 hours, the reaction mixture was submitted to gel-filtration on Sephadex G-10 and Cosmosil C_{18}750 OPN to give the product in 30% yield: m.p. 213-214 (dec.), ^{1}H-NMR (DMSO-d,) δ: 2.26-2.98 (2H, m), 3.36-3.52 (1H, m), 3.70 (2H, s), 6.60-7.30 (4H, m).

All other chemicals were of available reagent grade.

Analytical Methods

A Shimazu LC-5A high performance liquid chromatograph (HPLC) fitted with an SPD-1 UV detector and the following conditions were employed for HPLC analyses : column, LiChrospher RP-18 (5 X 300 mm) ; mobile phase, methanol / water / acetic acid = 50 / 50 / 1; flow rate, 1ml/min ; pressure, 100kg/cm²; column temperature, 40" C; UV, 280nm. TLC analyses were performed with precoated silica gel plate (60 F_{254}) supplied by E. Merck. A mixture of n-butanol, acetic acid and water (4:1:2) was used as a developing solvent system. Chromatograms were visualized with UV light and a ninhydrin reagent for amino acids, an acid molybdate reagent for phosphate, or a diazotized sulfanilic acid reagent for phenols. ^{1}H-NMR spectra were measured with a Fourier transformation JEOL JNM-FX 100 spectrometer at 100 MHz by using tetramethyl silane as an internal standard.

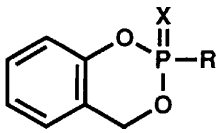
Reaction of K-2 with amino acids, purine bases and pyrimidine bases

Amino acids, purine bases and pyrimidine bases (lmmol) were dissolved in M/15 phosphate buffer, pH 7.0 or 2N sodium hydroxide and were preincubated at 37°C for 5 min. An ethanol solution of K-2 was added to the solution and incubated 37°C. After 1 and 2 hours, the reaction mixture was submitted to HPLC analysis. Determination of standard retention times of K-2 and the test compounds was duplicated (Table 1).

Table 1 Standard Retention Time of K-2 and Amino Acids, Purine Bases and Pyrimidine Bases.

compounds	retention time (min)	compounds	retention time (min)
K-2	3.16		
cysteine	2.68	adenine	3.97
serine	2.89	cytosine	3.67
tyrosine	2.15	guanine	2.91
histidine	2.10	thymine	3.39
arginine	2.11	uracil	2.80

*For HPLC analyses, 10μl of samples (lmmol/ml) were used



compound	X	R
salithion	S	OMe
salioxon	0	OMe
K-1	0	Ph
K-2	0	OPh
MTBO	0	SMe

Fig. 1. Structures of Saligenin Cyclic Phosphorus Esters.

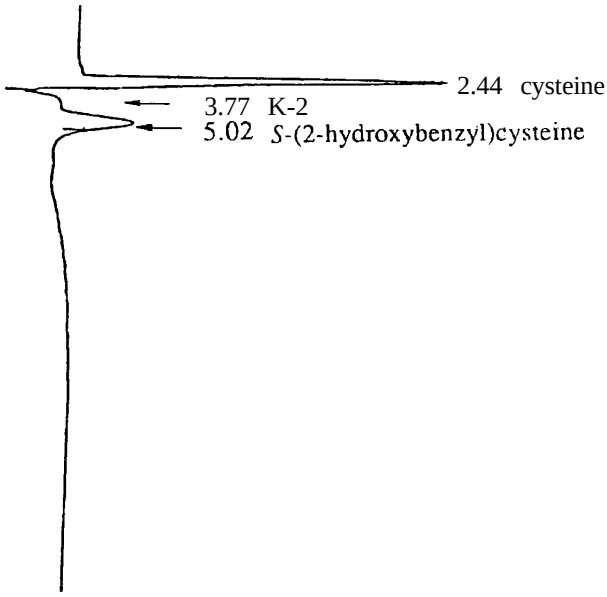


Fig. 2. High Performance Chromatogram of the Reaction Mixture of K-2 with Cysteine under Alkaline Condition.

RESULTS AND DISCUSSION

Reaction of K-2 with Amino Acids

Under the neutral conditions, the reactivity of K-2 with cysteine was not observable. But under the basic conditions, the reaction product with a retention time of 5.02 min was detected by HPLC (Fig. 2). By comparing with authentic sample, this product was proved to be S-(2-hydroxybenzyl)cysteine.

In the cases of serine and tyrosine, any reaction products were detected neither under neutral nor alkaline conditions. Thus, it was concluded that K-2 was unreactive to serine and tyrosine.

No reaction of K-2 with histidine and arginine was observed under the neutral conditions. But histidine and arginine gave reaction products with K-2 under the alkaline conditions at the retention time of 4.72, 5.59 min and 4.93, 5.88 min, respectively.

In order to investigate the reaction products of K-2 with histidine under the basic conditions, the following experiments were carried out. To a solution of histidine monohydrochloride (lmmol) in a mixture in ethanol (1.0mmol) and 2N sodium hydroxide (1.0ml), K-2(1mmol) was added. After stirring overnight at room temperature, the reaction mixture was neutralized with 1N hydrochloric acid, and filtered. The filtrate

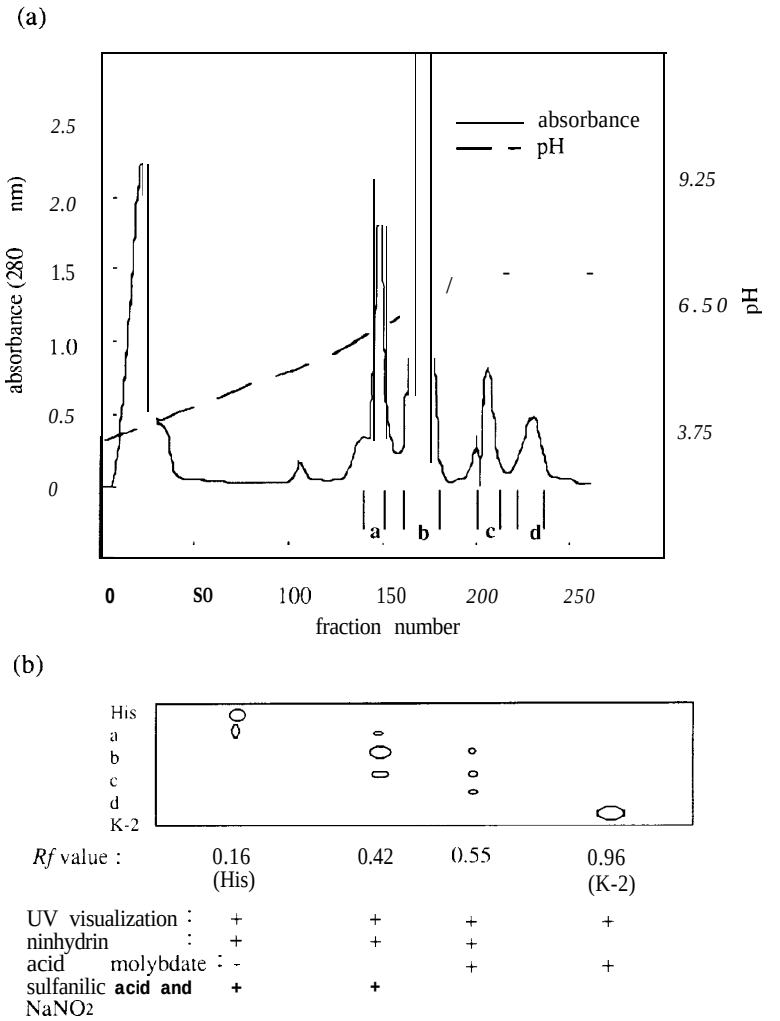


Fig. 3. (a) Ion Exchange Chromatography of the Reaction Mixture of K-Z and Histidine. Conditions: column, CM-Sephadex C-25 (15 x 210mm); eluent, acetate-ammonium acetate buffer with a linear gradient (0.01-0.2M, pH 3.55-7.20). Ion exchange chromatography was done at room temperature. Solid line, absorbance at 280 nm; dashed line, pH.
(b) TLC analysis of the Reaction Mixture of K-2 with Histidine.

was concentrated under reduced pressure. The residue was submitted to a CM-Sephadex C-25 column and eluted with a linear gradient from 0.01-0.2 M acetate-ammonium acetate buffer (Fig. 3a), giving four fractions **(a,** tube number 141-158; b, 160-179; c, 200-212; **d** 218-239) that absorb UV light at 280 nm. They were analyzed by TLC using a mixture of 1-butanol, acetic acid and water (4:1:2) as a developing solvent (Fig. 3b). A product ($Rf = 0.42$) absorbing UV light was positive to ninhydrin and diazotized reagents, but negative to the acid molybdate reagent. A similar procedure was applied to the products from arginine (Fig. 4a). As a result of TLC analyses, one of these products (Rf = 0.41) absorbing UV light was positive to ninhydrin and diazotized reagents, but negative to the acid molybdate reagent (Fig. 4b). These results support that the products of K-2 with histidine and arginine were N^{im}-(2-

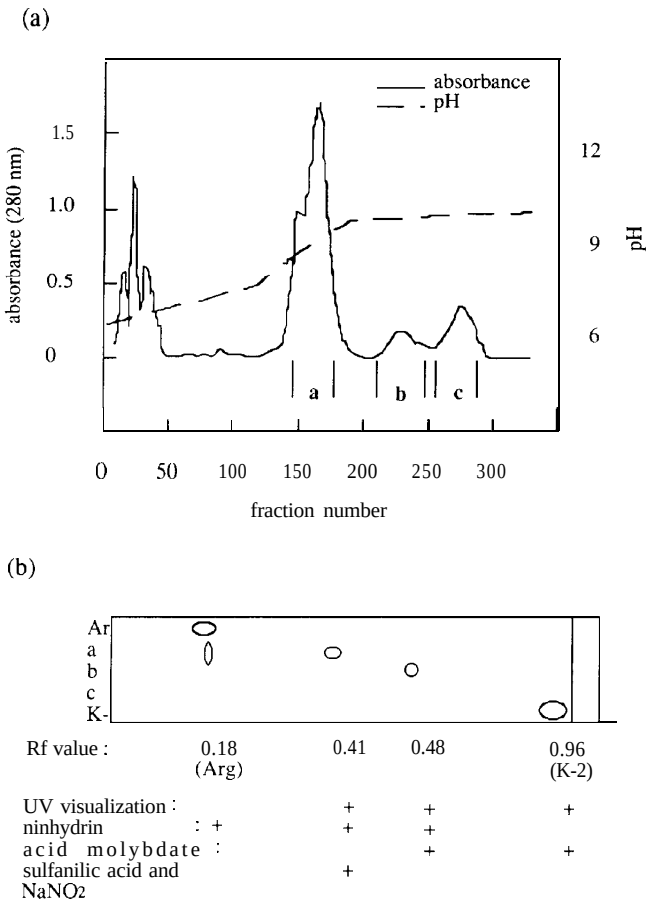


Fig. 4. (a) Ion Exchange Chromatography of the Reaction Mixture of K-Z and Arginine. Ion exchange chromatography was done with same conditions described as Fig. 5a. Solid line, absorbance at 280 nm; dashed line, pH.
(b) TLC analysis of the Reaction Mixture of K-2 with Arginine.

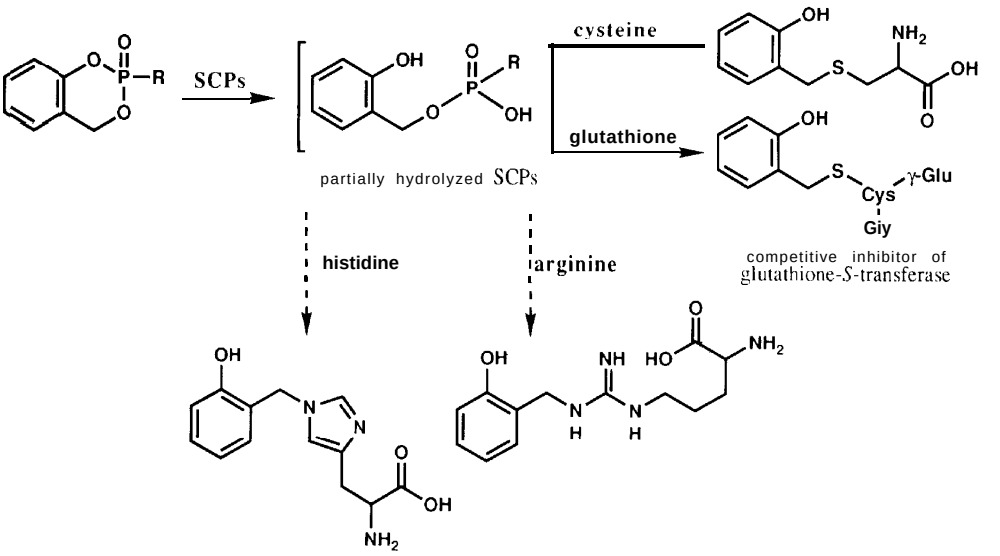


Fig. 5. Proposed Scheme for 2-Hydroxybenzylamino Acid Formation.

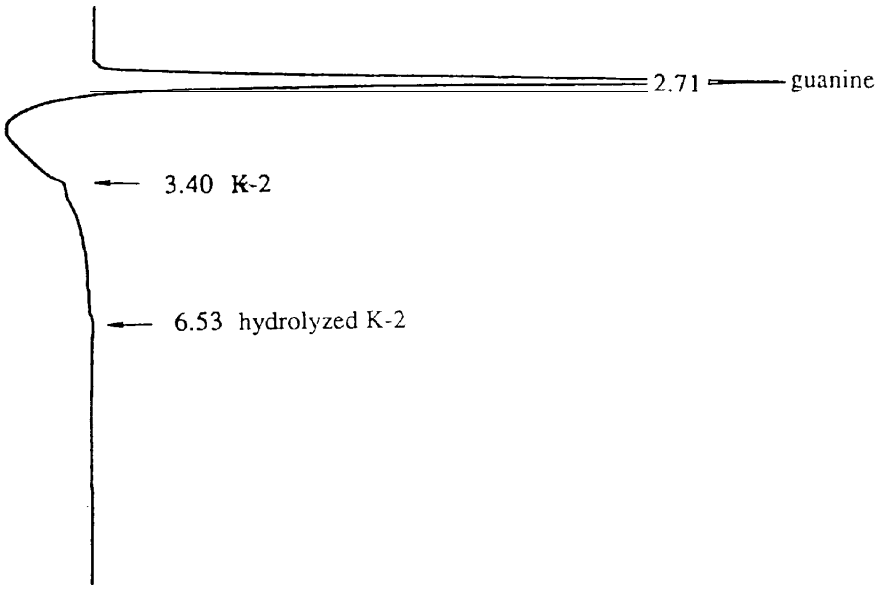


Fig. 6. High Performance Chromatogram of the Reaction Mixture of K-2 with Guanine under Alkaline Conditions.

hydroxybenzyl)histidine and N^g-(2-hydroxybenzyl)arginine, respectively.

The scheme of (2-hydroxybenzyl)amino acid formation is proposed in Fig. 5. K-2 has a benzyl carbon, a soft electrophile. Thus, the partially hydrolyzed intermediate which is activated by the o-hydroxyl group reacts with soft nucleophiles and gives 2-hydroxybenzylation products.

Reaction of K-Z with Bases of Nucleic Acids

For the purpose of investigating the reactivity of K-2 with purine and pyrimidine bases, the reaction mixture of K-2 with these bases (adenine, guanine, cytosine, thymine and uracil) was analyzed by HPLC. Adenine, cytosine and thymine gave no reaction products under the neutral conditions. Guanine and uracil were not tested under the neutral conditions. No products were detected from the reaction mixture of K-2 and guanine under the alkaline conditions for 2 hours (Fig. 6). In the cases of four other bases, the same results were obtained. Thus, it was concluded that K-2 was unreactive to purine and pyrimidine bases under the employed conditions.

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