Formation of Phenyl- β -D-glucoside by γ -Irradiation of *p*-Bromophenyl- β -D-glucoside in Aqueous Solution

Takashi KOMIYA, Tetsuya YAMADA,
Shozo NARA and Mitsuo NAMIKI*

Department of Agricultural Chemistry, Mie
University, Tsu 514, Japan

*Department of Food Science and Technology,
Nagoya University, Nagoya, Japan

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It was reported by Namiki *et al.*^{1,2)} that the bactericidal activity was enhanced when an aqueous solution of *p*-bromophenol, a typical halogenophenol, was irradiated by γ -ray. When *p*-bromophenol is exchanged for *p*-bromophenyl- β -D-glucoside (BPG), it will be considered that the change of permeability of cell membrane and the protection of phenolic hydroxyl group may affect its bactericidal action. In connection with the investigation on the above effect, the radiation chemistry of BPG in aqueous solution has been studied. Isolation and identification of phenyl- β -D-glucoside (PG) as a main radiolysis product of BPG and some speculations on its formation pathways were discussed in this paper.

BPG and PG were prepared by the Koenigs-Knorr method.³⁾ The aqueous BPG solutions (5 mM) were irradiated at the total dose of 1.5×10^6 rads by γ -ray of ⁶⁰Co. In order to examine the reaction of hydrated electron,⁴⁾ the aqueous BPG solution (5 mM) containing potassium ferrocyanide (25 mM) was irradiated in a quartz test tube with a 60 W low pressure mercury lamp (2537 Å) at room temperature for 3 hr. Both analytical and preparative paper chromatography were developed on Toyo filter paper No. 50 with *n*-butanol: acetic acid: water (4:1:2, v/v) as solvent system. After hydrolysis of the aryl glucosides on the filter paper with hydrochloric acid at 110°C, a phenolic moiety was detected by spraying the alkaline solution of diazonium salt of sulfanilic acid. Gas chromatography was performed with a Gas Chromatograph model JGC 1100 equipped with FID and a stainless steel column (0.3 $\times$ 100 cm, 3% OV-17 on Gaschrom Q; column temp., 150~250°C programed at 3°C/min). The samples were dissolved in dry pyridine and silylated with hexamethyldisilazane and trimethylchlorosilane. High-speed liquid chromatography was carried out with a Hitachi Chromatograph 634 under the following conditions; column: 2.2 $\times$ 500 mm, Hitachi Gel #3010, solvent: 50% aqueous methanol solution, pressure: 40 kg/cm², flow rate: 0.7 ml/min, temp.:

25°C, detection: UV (absorption at 260 nm). Authentic samples of BPG and PG were eluted at the retention time of 15 and 6 min, respectively by the chromatography.

After extraction of the γ -irradiated BPG solution with ether, the residual aqueous solution was extracted with *n*-butanol. The *n*-butanol extract was subjected to the paper chromatography after concentration. It gave two main spots of F₁ and F₂ having *R_f* values of 0.91 and 0.82, respectively. The *R_f* value and color reaction of F₁ were identical with those of authentic BPG. The crude F₂ was isolated from the *n*-butanol extract by preparative paper chromatography and then purified by high-speed liquid chromatography and F₂ was obtained as a crystalline form from water (mp 178°C). As shown in Fig. 1, the silylated irradiation products of BPG subjected to gas chromatography showed two large peaks, P₃ and P₄, which corresponded to the silyl derivatives of F₂ and authentic BPG, respectively. Also, it was considered that F₂ did not contain bromine atom in the molecule, because it was negative with Beilstein test. It was suggested that F₂ did not have free aromatic hydroxyl group, hence it did not react with diazosulfanylic acid on filter paper before hydrolysis. It was proved that the peak of P₃, silylated F₂ having 23.3 min of *t_R* was identical with that of silylated PG by comparison of the retention time of them on gas chromatogram. F₂ was identical with authentic PG in all respects including the UV spectrum, *R_f* value and color reaction on PPC and a melting point (178°C). Both P₁ and P₂ were proved to be glucose by

FIG. 1. Gas Chromatogram of Silylated γ -Irradiation Products of *p*-Bromophenyl- β -D-glucoside in Aqueous Solution.

comparison of retention time on gas chromatogram (Fig. 1). This may suggest the occurrence of bond scission on glucosidic linkage.

In order to elucidate the formation pathway of PG by γ -irradiation of BPG in aqueous solution, the effect of addition of N_2O , a scavenger of hydrated electron, on the formation of PG was examined and then the addition of N_2O markedly decreased the yield of PG in comparison with that in the absence, as shown in Fig. 1. Furthermore, to examine the reaction of BPG with hydrated electron, the *n*-butanol extract of the UV-irradiated BPG solution above-mentioned was concentrated and subjected to paper chromatography to give a main spot of F_2' identical with that of F_2 in respect to *R_f* value. The isolation of F_2' was performed with the same treatment as that of F_2 . As the results, F_2' was found to be identical with PG in all respects including *t_R* on gas chromatogram, UV spectrum and melting point (178°C).

From the above results, we consider that the formation process of PG by γ -irradiation of BPG in aqueous solution is as follows.

Namely, the reaction of BPG with hydrated electron may bring about C-Br bond cleavage and produce bromide-ion and an intermediate, PG radical (I), which may successively abstract hydrogen atom from

another BPG molecule and/or react with hydrogen radical produced by radiolysis of water molecule to give PG (II). This radiolysis process of BPG may be significantly different from that of *p*-bromophenol. Because, in the latter case, the asymmetrical biphenyls and terphenyls are predominantly formed by reaction of another *p*-bromophenol or of the dimeric products with the aryl radical intermediate, resulting from the debromination of *p*-bromophenol by hydrated electron, and in addition, hydroquinone is slightly formed but phenol could not be detected.⁶⁾ This radiolysis of BPG is not similar to that of 4-nitrophenyl- β -D-glucoside.⁶⁾ In the latter case, the predominant pathway is the scission of glycosidic linkage by OH radical, while in the former, the formation of PG, resulting from debromination of BPG by hydrated electron is predominant. Further detail of the radiolysis mechanism of BPG in aqueous solution is in progress.

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