Notes

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The Structure of Iodine[^{131}I] Labeled 3,3'-(Tetrabromophthalidylidene)-bis[6-hydroxybenzenesulfonic Acid]

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3,3'-(Tetrabromophthalidylidene)bis[6-hydroxybenzenesulfonic acid](BSP:I) is well known as a valuable dye for liver function testing. However, it sometimes produces undesirable effects, such as, pain following intravenous injection, or anaphylaxis. The BSP-test requires colorimetry determinations on samples and therefore it cannot be used for jaundiced patients. Because of these problems, the use of the BSP-test has been decreasing in recent years.

On the other hand, Tubis, *et al.*[2] reported the utilization of the radioactive dye for this purpose. This method provides several advantages. For examples, administration of the labeled dye in a very small quantities will not produce undesirable effects, the measurement of radioactivity is more simple and accurate than colorimetric determinations and the method can also be applied in jaundiced patients. Tubis, *et al.* prepared ^{131}I-labeled BSP[3] and confirmed its value as a clinical agent but chemical composition and structure of the dye was not described. Jirsa, *et al.*[4] iodinated BSP, using an excess of ICl. From the iodine content (22.5%) of the material obtained, they assumed that it was BSP-diiodide (III) in which two iodine atoms were attached in *o*-position to the phenolic OH radicals.

The purpose of this study was to identify the structure of iodinated BSP[^{131}I] which is valuable for clinical test.

Iodination of BSP(I) with ICl involves two steps as expected and the ratio of the products and the starting material, which were isolated from the reaction mixtures utilizing various amounts of ICl, is summarized in Fig. 1. Monoiodide(II) is formed as the main product in the presence of a small quantity of ICl in the reaction and diiodide(III) is furnished with increasing amounts of ICl.

To isolate monoiodide (II), molar equivalent amount of ICl was used and the reaction product was purified *via* column chromatography utilizing Dowex (OH-form), Sephadex LH-20 and then Dowex (H-form), followed by recrystallization. Diiodide (III), obtained by iodination with over two molar equivalent amounts of ICl, was purified by Dowex (H-form and OH-form) column chromatography and by recrystallization.

Both II and III, thus purified, gave the satisfactory results of elemental analysis (*cf.* Table I). The ultraviolet (UV) absorption maxima and *Rf* values by thin-layer chromatography (TLC) of these compounds are summarized in Table II.

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[1] Location: *a) 344, Minoridai, Matsudo-shi, Chiba-ken; b) 1-1-1, Yayoi, Bunkyo-ku, Tokyo.*

[2] M. Tubis, R. Nordyke, E. Posnick and W.H. Blahd, *J. Nuc. Med.*, **2**, 282 (1961); R. Suwanik and M. Tubis, *J. Appl. Rad. Iso.*, **19**, 883 (1968); R. Suwanik, M. Tubis, V. Viranuvatti and K. Limwongse, *Nuclear Medizine*, **9**, 268 (1970).

[3] We assume that it contains mainly BSP itself and a very small amount of BSP-monoiodide[^{131}I].

[4] M. Jirsa and P. Hykes, *Nature*, **5049**, 645 (1966).

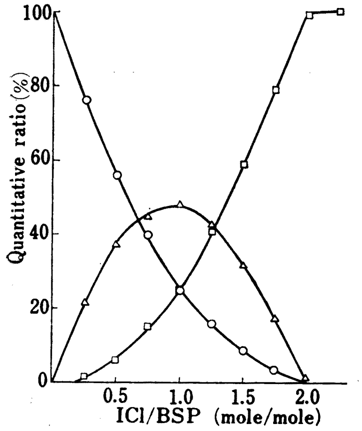

Fig. 1. Quantitative Ratio of Reaction Products in Iodination of BSP with Various Amounts of ICl

—○—: BSP (I), —△—: BSP-monoiodide (II), —□—: BSP-diiodide (III)

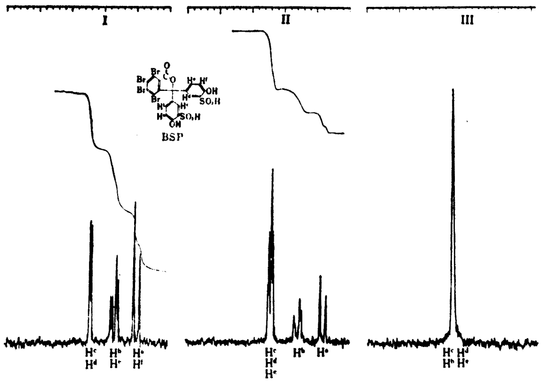

Fig. 2. NMR Spectra of I, II and III

Measured by Nichiden-Varian 100 MHz, in D_2O at 90°. Sodium 4,4-dimethylsilapentane sulfonate was used as a standard substance. The signals in NMR spectra of these compounds are summarized as follows.

I: $\delta=7.67$ (1H, doublet, $J_{bc}=3$ cps, H_c), $\delta=7.31$ (1H, quartet, $J_{bc}=3$ cps, $J_{ab}=9$ cps, H_b), $\delta=6.96$ (1H, doublet, $J_{ab}=9$ cps, H_a)

II: $\delta=7.69$ (3H, sextet, J is obscure, H_c, H_d and H_e), $\delta=7.29$ (1H, quartet, $J_{bc}=3$ cps, $J_{ab}=9$ cps, H_b), $\delta=6.90$ (1H, doublet, $J_{ab}=9$ cps, H_a)

III: $\delta=7.69$ (singlet, H_b, H_c, H_d and H_e)

Table I. Elemental Analysis

		C	H	S	Br	I(%)
II[a]	Calcd.	25.13	1.37	6.71	33.44	13.27
	Found	25.05	1.03	6.47	33.65	13.15
III[b]	Calcd.	22.04	0.55	5.88	29.33	23.29
	Found	—	—	5.79	28.95	23.66

a) BSP-monoiodide: $C_{20}H_{13}O_{12}S_2Br_4I\cdot 2H_2O$ M.W.=955.990

b) BSP-diiodide: $C_{20}H_6O_{10}S_2Br_4I_2Na_2$ M.W.=1089.810

Table II. Physicochemical Properties

	I[a]	II[b]	III[c]
λmax. in 0.1N NaOH (mμ)	577	589	600
Discoloration point (pH)	6.9	6.3	5.5
Rf values by TLC[d]			
n-BuOH : AcOH : H_2O 74 19 50	0.50	0.58	0.64
MeOH : EtAc : AcOH : H_2O 5 20 1 4	0.55	0.61	0.67

a) BSP; b) BSP-monoiodide; c) BSP-diiodide; d) Eastman Chromatogram Sheet 6061 silica gel

Although the analytical data may clarify the structures of II and III, the nuclear magnetic resonance (NMR) spectra of these compounds gave the further definite proofs for their structures proposed above, as seen in Fig. 2.

It is rather interesting that one sharp singlet is only observed in NMR spectrum of III indicating the equivalency of H_b and H_c. The NMR spectrum of II is reasonably expected to be the hybrid of those of I and III. The signals due to H_a, H_b and H_c are quite similar to those of I. The signals corresponding to H_d and H_e appear as a quartet contrary to the case of III. This difference may be rationalized as follows. In the case of III, the mutual anisotropic interaction of two phenyl function results in the equivalency of the two type protons H_b and H_c. On the other ahnd, H_d and H_e of II cannot be equivalent.

Recently Iio, *et al.*[5] have reported that BSP-monoiodide [^{131}I][6] is cleared from the blood and excreted through the liver into the intestine more rapidly than is BSP-diiodide [^{131}I].[6] Therefore, BSP-monoiodide[^{131}I] is a very useful dye for testing liver function while BSP-diiodide[^{131}I] may be adequate for liver scanning.

Experimental

Iodination of I——2 ml of I solution (0.03 mmole/ml) and 1mCi of Na^{131}I solution were pipetted to nine vials. To each vial, ICl solution (0.015 mmole/ml: prepared as 99.6 mg of KI, 66 mg of KIO_3 and 1 ml of conc. HCl in 60 ml of water) were pipetted as 1 ml, 2 ml,——9 ml aliquots respectively. After standing at room temperature for 24 hours, the reaction mixtures in each vial were spotted on thin layer films (Eastman Chromatogram Sheet 6061 silica gel). The thin-layer films were then developed with a mixture of *n*-butanol: acetic acid: water (74: 18.5: 50, v/v) for 8 hours, and spots were identified with ammonia vapor. Each spot was cut from the film and the radioactivity was assayed in a well-type scintillation counter. The quantitative ratio of each spot was then calculated.

Isolation of II——Disodium salt of BSP (250 mg, 0.3 mmole) was dissolved in 30 ml of water. To this solution, KI (33.2 mg, 0.2 mmole), KIO_3 (22 mg, 0.1 mmole) in 20 ml of water and 1 ml of 3N HCl were added. After allowed to stand at room temperature for 24 hours, the reaction mixture was adjusted to pH 7 with 1N NaOH, and then passed through a Dowex 2X 8 (OH-form) column. The eluate was dried and left overnight in a desicator. The dried reaction products were dissolved in approximately 30 ml of methanol, and loaded on a Sephadex LH-20 (100 g) column which had been previously treated with methanol. The column was eluted with methanol and each fraction was examined by TLC in order to isolate the fraction of II which showed lower *Rf* values than that of III. After removing the solvent, the residue was recrystallized from water-ethanol, and 110 mg of crystalline material was obtained. This recrystallized product was dissolved in water, and passed through a Dowex 50W X 8 (H-form) column. The eluate was dried and repeatedly recrystallized from methanol-ethanol. Seventy three mg of refined crystal was thus obtained.

Isolation of III——Disodium salt of BSP (210 mg, 0.25 mmole) was dissolved in 30 ml of water. To this solution, KI (83 mg, 0.5 mmole), KIO_3 (56 mg, 0.26 mmole) in 30 ml of water and 1 ml of 3N HCl were added. After allowing to stand at room temperature for 24 hours, the reaction mixture was passed through a Dowex 50W X 8 (H-form) column. The eluate, which had been adjusted to pH 5.5 with NaOH, was passed through a Dowex 2 X 8 (OH-form) column, and was then dried. The residue was repeatedly recrystallized from water-ethanol, and 137 mg of pure III which showed an increased *Rf* value by TLC was obtained.

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5) M. Iio, H. Yamada, H. Kameda and H. Ueda, *The Korean Journal of Nuclear Medicine*, (submitted).

6) These compounds were purified by our methods.