Synthesis of Nucleophilic Adducts of Thiols (IV). Addition of Glutathione to β-Nitrostyrene Derivatives

Tae-Rin Kim† and Sung-Yong Choi

Department of Chemistry, College of Sciences, Korea University, Seoul 132, Korea.

Won-Sik Choi

Department of Chemistry, Kangreung National University, Kangreung 210 , Korea *(Received August 26, 1982)*

The addition products of glutathione to β-nitrostyrene derivatives were synthesized. β-Nitrostyrene (1a), *p*-methyl-β-nitrostyrene (1b), 3,4,5-trimethoxy-β-nitrostyrene (1c), *o*-, *m*-and *p*-chloro-β-nitrostyrene (1e, 1f, 1g) and *o*-,*m*-and *p*-methoxy-β-nitrostyrene (1h, 1i, 1j) undergo addition reactions with glutathione to form S-(2-nitro-1-phenylethyl)-L-glutathione (5a), S-[2-nitro-1-(*p*-methyl) phenylethyl]-L-glutatione (5b), S-[2-nitro-1-(3', 4', 5'-trimethoxy) phenylethyl]-L-glutathione (5c), S-[2-nitro-1-(*o*-chloro) phenylethyl]-L-glutathione (5e), S-[2-nitro-1-(*m*-chloro) phenylethyl]-L-glutathione (5f), S-[2-nitro-1-(*p*-chloro) phenylethyl]-L-glutathione (5g), S-[2-nitro-x-(*o*-methoxy)-phenylethyl]-L-glutathione (5h), S-[2-nitro-x-(*m*-methoxy) phenylethyl]-L-gltuathione (5i), and S-[2-nitro-1-(*p*-methoxy) phenylethyl)-L-gluatahione (5j), respectively. The structre of adducts were identified by UV and IR-spectra, molecular weight measurement, and elemental analysis.

1. Introduction

In recent years, there has been a growing interest in the synthesis of cysteinyl peptides in conjunction with the studies of many other peptides and proteins of biological importance.[1-5] One of such cysteinyl peptides is glutathione. It is an important constituent of living cells. The biological function and the chemistry of glutathione has been widely studied and was the subject of a symposium.[6] Glutathione was shown by Harington and Mead[7] to be γ-L-glutamyl-L-cysteinylglycine by synthesis, after it had been isolated and investigated by others, notably by Hopkins[8] and by Kendall, Mackenzie, and Mason.[9] Du vigneaud and Miller[10] improved Harington and Mead's synthesis[7] by protecting the thiol group with benzyl group. The syntheses described by later investigators differ only in the methods of preparing the final intermediates, benzyloxy car bonyl-S-benzylgultathione. The new synthetic method with thiazolidine intermediates gave glutathione with a satisfactorily high specific rotation and in a favourable yield. Acetone was initially chosen to protect the thiol group of L-cysteine because of the ease of fission of 2, 2-dimethylthiazolidines[11] to aminothiols. Contrary to the vast efforts made in this area, there has been little attempts to synthesize glutathione derivatives. It is known that the addition reaction of active methylene and thiol groups to β-nitrostyrene derivatives leads to the formation of their adducts[12,13] and base-catalyzed Michael addition.[14-17] The adducts often show sterilizing, insecticiding and vermiciding effects. Thus we have dicided to synthesize the glutathione derivatives by reacting glutathione with β-nitrostyrene derivatives.[18-20] The adducts may find usefulness as biochemical or pharmaceutical products.

2. Experimental

2.1. General

Melting points (uncorrected) were determined by the Fisher Johne's melting point apparatus. Ultraviolet spectra were obtained on a Beckmann Model 26 spectrophotometer. Infrared spectra were taken with JASCO IRA-2 spectrophotometer, and elemental analysis was conducted with a MOO-1106 Model carlo Erba, Italy. Optical ratation was measured with a JASCO DIP-181 (Japan) polarimeter. Thin-layer was conducted with a MOO-1106 Model carlo Erba, Italy. Thin-layer chromatography were run on Merck HF-254 silica gel plates, and spots on chromatograms were detected by their ultraviolet absorption or by spraying ninhydrin reagent. L-glutathione was purchased from Aldrich Chemicals, Co. Ltd., USA. Various substituted benzal dehydes, nitromethane, N-methylmorpholine and triethylamine were obtained from Merck and Kyoei Chemical Cooperation and used without further purification. Unless otherwise indicated, all other reagents were used as received.

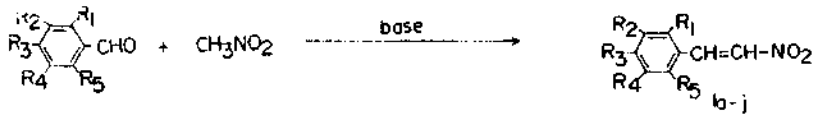
2.2. Preparation of β-nitrostyrene derivatives.

β-Nitrostyrene derivatives were prepared according to David E. Worrall method.[21] The yields, melting points and elemental analysis results are recorded in Table 1.

2.3. Preparation of Adducts

S-(2-nitro-1-L-glutathione (5a). L-glutathione (3.10 g, 0.01 mole) was dissolved in 30 ml of distilled water and 60m*l* of isopropyl alcohol. β-Nitrostyrene (1a) 1.5 g, 0.01 mol was added to the soluiion. The reaction mixture was stirred at room temperature for 24 hours. The resulting precipitates were collected by filtration and washed with methanol. The adduct was recrystallized from ethyl acetate. Other β-nitrostyrene derivatives are reacted with L-glutathione similarly in aqueous methanol (65 %, 90 m*l*) or in aqueous acetonitrile (65 %, 90 m*l*). The yields, melting points, and

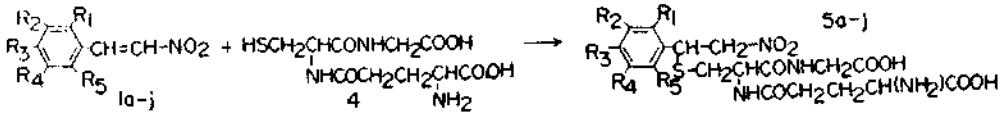
TABLE 1: Analytical Data Of β-Nitrostyrene Derivatives



Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	mp (°C dec)	Analytical data of elements (%) Calculated C	H	N	Found C	H	N
1a	H	H	H	H	H	80.5	56–58[a]	64.42	4.73	9.39	64.70	4.60	9.50
1b	H	H	CH_3	H	H	57.0	99–100[b]	66.25	5.52	8.59	66.10	5.90	8.20
1c	H	OCH_3	OCH_3	OCH_3	H	67.3	123–124[c]	55 23	5.44	5.90	55.40	5.66	6.10
1d	H		$-OCH_2O-$		H	57.0	162–163	55.97	3.62	7.25	55.40	3.40	7.40
1e	Cl	H	H	H	H	53.0	46–48	52.34	3.30	7.63	52.10	3.10	7.43
1f	H	Cl	H	H	H	51.0	48–49				52.63	3.45	7.75
1g	H	H	Cl	H	H	68.0	111–112[d]				52.26	3.60	7.41
1h	OCH_3	H	H	H	H	70.0	47–48	60.33	5.06	7.82	60.15	4.81	7.60
1i	H	OCH_3	H	H	H	72.2	93–95				60.55	5.23	7.95
1j	H	H	OCH_3	H	H	59.0	120–121[e]				60.47	5.21	7.74

[a] 57–58 °C (Ref. 21); [b] 102 °C (22); [c] 123 °C (23); [d] 113–114 °C (24); [e] 120–121 °C (24)

TABLE 2: Analytical Data of S-[2-Nitro-1-(substiuted) pheny(ethyl]-L-Glutathione Derivatives



Comp.	R_1	R_2	R_3	R_4	R_5	Yield (%)	mp (°C.)	Analytical data of elements (%) Calculated C	H	N	S	Found C	H	N	S	
5a	H	H	H	H	H	73.9	166–168	47.36	5.30	12.27	7.02	48.10	5.41	12.57	6.88	
5b	H	H	CH_3	H	H	87.2	167–169	48.50	5.57	11.91	6.81	48.30	5.71	12.30	6.68	
5c	H	OCH_3	OCH_3	OCH_3	H	89.7	155–157	46.15	5.53	10.25	5.86	45.90	5.75	10.43	5.75	
5d	H		$-OCH_2O-$	H	H		not isolated									
5e	Cl	H	H	H	H	80.9	164–166	44.04	4.72	11.41	6.53	44.34	4.98	12.00	6.45	7.10[a]
5f	H	Cl	H	H	H	74.7	155–157	44.04	4.72	11.41	6.53	43.87	4.58	11.70	6.48	7.37
5g	H	H	Cl	H	H	92.3	165–167	44.04	4.72	11.41	6.53	44.40	4.98	11.23	6.70	7.37
5h	OCH_3	H	H	H	H	80.0	164–166	46.90	5.39	11.52	6.59	46.63	5.48	11.97	6.37	
5i	H	OCH_3	H	H	H	87.4	163–165	46.90	5.39	11.52	6.59	47.30	5.65	11.37	6.46	
5j	H	H	OCH_3	H	H	86.0	165–167	46.90	5.39	11.52	6.59	47.30	5.55	11.87	6.30	

[a] Calculated content of Cl; 7.22 %

TABLE 3: Spectral Data of Glutathione Adducts of β-Nitrostyrene Derivatives

Comp.	λ_{max} (nm): (extinction coef.)	Characteristic absorption peak of IR-spectrum (cm^{-1})
5a	241(12300)	3350, 2950, 1730, 1655, 1565, 1450, 1415, 1385
5b	241(12100)	3400, 3050–2950, 1730, 1650, 1555, 1457, 1420, 1380, 650
5c	242(11900), 349(300)	3400, 3100–2980, 2880, 1730, 1650, 1605, 1565, 1470, 1435, 1385, 785, 660
5d	not isolated	
5e	245(9400), 300(100)	3400, 3100–2960, 1730, 1650, 1565, 1480, 1383, 1320, 1085, 780, 660, 600–500
5f	244(8200), 295(180)	3460, 3100–2960, 1730, 1645, 1565, 1480, 1385, 1045, 965, 665, 600–500
5g	244(12600), 313(600)	3440, 3100–2980, 1730, 1660, 1570, 1490, 1395, 1316, 1035, 670, 600–500
5h	241(11150), 302(1400), 350(1300)	3460, 3100–2960, 2860, 1710, 1660, 1555, 1470, 1385, 755, 670
5i	241(11180), 307(13300)	3440, 3100–2960, 2870, 1720, 1660, 1560, 1390, 1465, 745, 650
5j	226(13200), 284(10800), 351(40)	3440, 3100–2960, 2880, 1720, 1650, 1565, 1470, 1385, 275, 650

elemental analysis results and the spectral data are recorded in Table 2 and 3, respectively.

2.4. Determination of molecular weight, optical rotation, and purity.

Nonaqueous amine titrations were used to determine the molecular weight of the glutathione derivatives. One m*l* of 0.1*N*-$HClO_4$ is equivalent to 0.045647 g of S- (2-nitro-1-phenylethyl)-L-glutathione. Thus the molecular weight was

TABLE 4: Analytical Data of Glutathione Adducts of β-Nitrostyrene Derivatives

Compound	$[\alpha]_D^{20}$[a]	Rf-value[b]	Amine content (%)	Molecular Weight	
				Calculated	Found
5a	−57.5	0.89	99.90	456.47	456.92
5b	−14.8	0.92	100.50	470.50	468.16
5c	−11.7	0.88	99.80	546,45	547.63
5d	not isolated				
5e	−10.0	0.91	99.80	490.91	491.98
5f	−18.1	0.92	100.70		487,50
5g	−12.3	0.92	101.80		482,22
5h	−13.5	0.92	101.85	486.50	477,66
5i	−36.7	0.93	101.10		481.20
5j	−36.7	0.92	99.00		491.41

[a] Determined in DMSO; [b] R_f-value of glutathione: 0.58 (Ethyl acetate: Acetic acid: Water, 3: 1: 1)

calculated from the volume of 0.1*N*-$HClO_4$ solution used to reach the end point. Specific rotation of adducts were determined in dimethyl sulfoxide. The purity of the adducts was checked by TLC. $[\alpha]_D$, R_f and molecular weight are recorded in Table 4.

3. Results and Discussions

3.1. Identification of adducts

As shown in Table 2, L-glutathione reacts with β-nitrostyrene derivatives to afford the adducts in excellent yields. The structure of the adducts were identified by IR and UV spectra, elemental analysis and molecular weight determination. The IR show characteristic peaks corresponding to NH streching vibration at 3350–3460 cm^{-1}, intra H-bond chelation at 3160cm^{-1}, $-NH^+_3$, $-CH_2$, and $-CH_3$ streching vibration of glutathione portion at 3100–2960cm^{-1}, $-OCH_3$ at 2830cm^{-1}, amide carbonyl; C(=O) NH-at 1730cm^{-1}, asymmeteric bending of $-NH^+_3$ and asymmetric streching vibration of $-COO^-$ at 1640cm^{-1} 1660cm^{-1}, $-CH_2NO_2$ at 1555–1565cm^{-1}, $-S-CH_2-$at 1455–1475cm^{-1}, CH bending vibration at 1385cm^{-1}, phenyl at 780cm^{-1} and sulfide at 600–660cm^{-1}. The characteristic $-CH=CH-NO_2$ streching at 1510cm^{-1} was abscent. The formation of adducts was also confirmed by the change of UV-absorptions in methanol solution at $10^{-4}M$ concentration. The absorption characteristics of β-nitrostyrene derivatives containing chromophoric nitro groups conjugated with double bond absorbed strongly in the region of 295 nm and 350 nm. The glutathione adducts of β-nitrostyrene derivatives shift the absorption to shorter wavelength with disapsppearance of conjugated double bond. When glutathione was added to the solution the maximum absorption peak of β-nitrostyrene at 311 nm decreased with time and a new peak appeared at 241 nm.

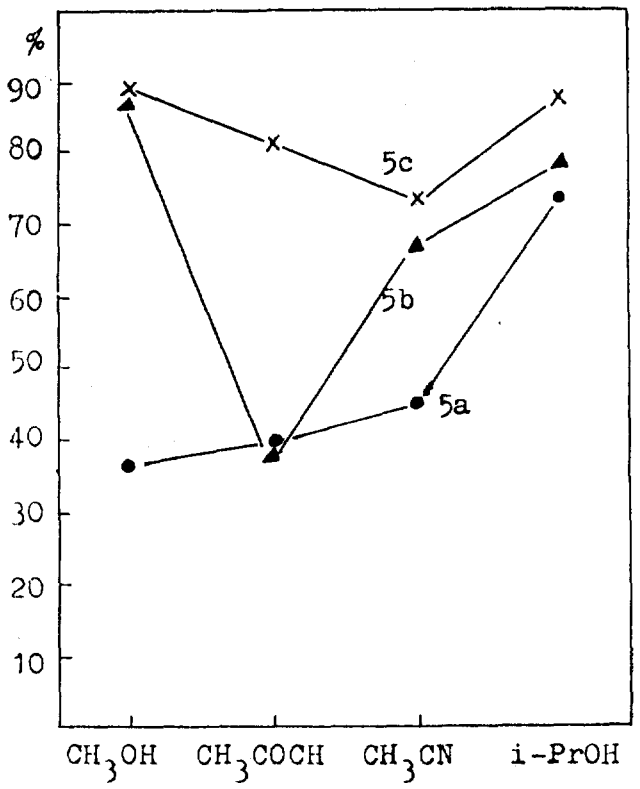


Figure 1. The Changes of yield of adducts with solvents

TABLE 5: The Data of NMR Spectrum of Adducts (DMSO-d6)

Adducts	δ-Value
glutathione	1.3 (SH), 2.0(–CH_2CH (NH_2)COOH), 2.4(CH_2SH), 3.6(–$COCH_2$), 4.4(CONH), 6.0(CH–NH), 8,3(–NH_2), 10.0(–COOH)
5a	2.3($CHCH_2NO$), 2.5(–S–CH_2–), 4.4(–$CHCH_2NO_2$), 7,3(phenyl)
5b	2.1(–CH_3 *p*), 2.3(–$CHCH_2NO$), 2.5(–SCH_2–), 4.4(–$CHCH_2NO_2$)
5c	2.3(–$CHCH_2NO_2$), 2.5(–SCH_2–), 3.8(–OCH_3), 4.4(–$CHCH_2NO_2$) 6.7(phenyl)
5e	2.3(–CH–CH_2NO_2), 2.5(–SCH_2–), 4.4(–$CHCH_2NO_2$), 7.3(phenyl)
5f	2.3(–$CHCH_2NO_2$), 2.5(–SCH_2–), 4.4(–$CHCH_2NO_2$), 7.4(phenyl)
5g	2.3(–$CHCH_2NO_2$), 2.5(–SCH_2–), 4.4(–$CHCH_2NO_2$), 7.5(phenyl)
5h	2.3(–$CHCH_2NO_2$), 2.5(–SCH_2–), 3.9(–OCH_3,*o*), 4.4(–$CHCH_2NO_2$), 7.2(phenyl)
5i	2.3(–$CHCH_2NO_2$), 2.5(–SCH_3–), 3.7(–OCH_2,*m*), 4.4(–$CHCH_2NO_2$), 6.9(phenyl)
5j	2.3(–$CHCH_2NO_2$), 2.5(–SCH_2–), 3.8/–OCH_3,*p*), 4.4(–$CHCH_2NO_2$), 7.0(phenyl)

The λ_{max} of the glutathione adducts og β-nitrostyrene derivatives are listed in Table 4. In no case, is the a bsorption characterictics of β-nitrostyrene derivatives observed. The abscence of glutathione in the product is also determined by TLC. The Rf values of the adducts were 0.81-0.93, whereas that of glutathione is 0.58. The molecular weight determined by nonaqueous amine titration and elemental analysis results are also consistent with the proposed structure. The structure of glutathione adducts were also confirmed by NMR spectrum comparing with that of glutathione. The results are recorded in Table 5.

3-2. The effects of solvents on the addition of glutathione to β-nitrostyrene derivatives.

In an attempt to optimize the product yields, the reaction was conducted in several solvents. As shown in Figure 1, methanol gave the best results for the synthesis of 5b and 5c whereas isopropyl alcohol was the best solvent for 5a. The yield of adducts depend the solvent used and the substituted group of phenyl ring of β-nitrostyrene derivatives.

Acknowledgement. We thank the A-San Foundation for support of this work.

References

(1) J. C. Sheehan and D. H. Yang, *J. Org. Chem.*, **80**, 1158 (1985).
(2) G. E. Foley, E. F. Barell, R. A. Adams and H. Lazarus, *Exp. Cell Res.*, **57**, 129(1969).
(3) K. A. Harrap and D. E. M. Speed, *Br. J. Cancer Res.*, **18**, 809(1964).
(4) K. Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, **13**, 414(1970).
(5) K. Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, **15**, 13(1972).
(6) "Glutathione, Proceedings of the symposium held at Ridgefield, Connecticu Academid Press, New York, 1954.
(7) Harington and Mead, *Biochem. J.*, **29**, 1602(1935).
(8) Hopkins, *ibid.*, 1921, **15**, 286; *J. Biol. Chem.*, **84**, 269. (1929).
(9) Kendall, Mackenzie, and Mason, *ibid.*, p.657.
(10) Du Vigneaud and Miller, (a) *J. Biol. Chem.*, **116**, 469; (1936). (b) *Biochemical preparations*, **2**, 74. (1952).
(11) Cook and Heilbron, "Chenistry of Penicillin," Princeton Univ. Press, 1949, Chap, 25.
(12) M. J. Kamlet, *J. Amer. Chem. Soc.*, **77**, 4896(1955).
(13) M. J. Kamlet and D. J. Glover, *ibid.*, **78**, 4556(1956).
(14) I. Belsky, *J. C. S. Chem. Comm.*, 237(1977).
(15) H. Hiemstra and H. Wynberg, *ibid*, 238(1978).
(16) S. Colonna and A. R. Cenro, *J, C. S. Perkin* **1**, 547(1981).
(17) Ali. Mohamed I. Abou-State, M. Amine, Hassan, N. N., El-Behairy, M. A., *Egypt. J. Chem.*, Vol. **18** (1) 55-61 (Eng.) (1976).
(18) N. Runsch, *et al., FEBS Letters*, **30**, 286(1976).
(19) M. Esterbauer, *Carbohydrate Res.*, **43**, 779(1975).
(20) I. H. Hall ,K. H. Lee, E. C. Mar and C. O. Starness, *J. Med. Chem.*, **20**, 3, 333(1977).
(21) D. E. Worral "Organic Synthesis", Col. Vol. **1**. 413, John Wiley and Sons, Inc. (1958).
(22) D. E. Wrral, *J. Amer. Soc.*, **60**, 2841(1938).
(23) E. Spath, *Monatsch.*, **40**, 129(1919).
(24) N. Cawbell, *et al. J. Chem. Soc.*, 446(1940).

Photoreaction of 8-Methoxypsoralen with Thymine

Sang Chul Shim† and Yong Zu Kim

Department of Chemistry, Korea Advanced Intitute of Science and Technology, P. O. Box 150, Chongyangni, Seoul 131, Korea *(Received February 2, 1983)*

Photoreaction of 8-methoxypsoralen (8-MOP) with thymine ($\geq$ 300 nm) was carried out in the dioxane-water frozen state. One major and two minor monoaddition products between 8-MOP and thymine were isolated by various chromatographic methods. Major monoadduct was characterized to be a C_4 cycloaddition product formed between 5,6-double bond of thymine and 3,4-double bond of 8-MOP with *cis-anti* stereochemistry. Two minor adducts were proved to be stereoisomers of this major adduct.

Introduction

Furocoumarins, naturally occurring coumarin derivatives, are known to photoreact with pyrimidine bases, free or in DNA, upon irradiation with long wavelength UV light (320-380 nm). Various physiological actions such as skin erythema on human and guinea pig skin, mutagenic and lethal effect in bacteria, inactivation of DNA viruses, inhibition of tumor transmitting capacity of various tumor cells have been attributed to this photoreaction.[1,2] Extensive study on the skin