

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

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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-glutathione (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-glutathione (5i), and S-[2-nitro-1-(*p*-methoxy) phenylethyl]-L-glutathione (5j), respectively. The structure 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 Mead⁷ to be γ -L-glutamyl-L-cysteinylglycine by synthesis, after it had been isolated and investigated by others, notably by Hopkins⁸ and by Kendall, Mackenzie, and Mason.⁹ Du vigneaud and Miller¹⁰ improved Harington and Mead's synthesis⁷ 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 carbonyl-S-benzylglutathione. 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-dimethylthiazolidines¹¹ 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 decided 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

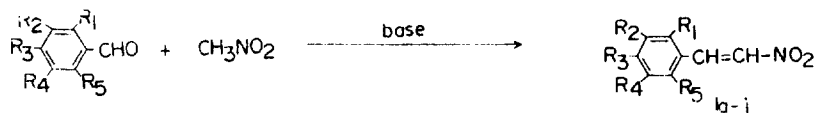
Melting points (uncorrected) were determined by the Fisher Johnes melting point apparatus. Ultraviolet spectra were obtained on a Beckmann Model 26 spectrophotometer. Infrared spectra were taken with JASCO IRA-2 spectrophotometer, and elemental analysis was conducted with a MOO-1106 Model carlo Erba, Italy. Optical rotation was measured with a JASCO DIP-181 (Japan) polarimeter. Thin-layer chromatography 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 benzaldehydes, 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

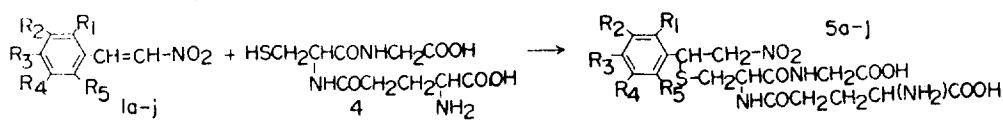
S-(2-nitro-1-L-glutathione (5a). L-glutathione (3.10 g, 0.01 mole) was dissolved in 30 ml of distilled water and 60 ml of isopropyl alcohol. β -Nitrostyrene (1a) 1.5 g, 0.01 mol was added to the solution. The reaction mixture was stirred at room temperature for 24 hours. The resulting precipitates were 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 ml) or in aqueous acetonitrile (65 %, 90 ml). 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 | | | Found | | |
| | | | | | | | | C | H | N | 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 ₂ O- | H | 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-(substituted) phenylethyl]-L-Glutathione Derivatives



| Comp. | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Yield (%) | mp (°C.) | Analytical data of elements (%) | | | | | | | | |
|-------|------------------|------------------|----------------------|------------------|----------------|-----------|--------------|---------------------------------|------|-------|------|-------|------|-------|------|-------------------|
| | | | | | | | | Calculated | | | | Found | | | | |
| | | | | | | | | C | H | N | S | 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 ₂ O- | 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(13200) | 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 ml of 0.1N-HClO₄ 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 | $[\alpha]_D^{25}$ | R _f -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 0.1N-HClO₄ 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 stretching vibration at 3350-3460 cm⁻¹, intra H-bond chelation at 3160cm⁻¹, -NH⁺₃, -CH₂, and -CH₃ stretching vibration of glutathione portion at 3100-2960cm⁻¹, -OCH₃ at 2830cm⁻¹, amide carbonyl; C(=O)NH- at 1730cm⁻¹, asymmetric bending of -NH⁺₃ and asymmetric stretching vibration of -COO⁻ at 1640cm⁻¹ 1660cm⁻¹, -CH₂NO₂ at 1555-1565cm⁻¹, -S-CH₂- at 1455-1475cm⁻¹, CH bending vibration at 1385cm⁻¹, phenyl at 780cm⁻¹ and sulfide at 600-660cm⁻¹. The characteristic -CH=CH-NO₂ stretching at 1510cm⁻¹ was absent. The formation of adducts was also confirmed by the change of UV-absorptions in methanol solution at 10⁻⁴M 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

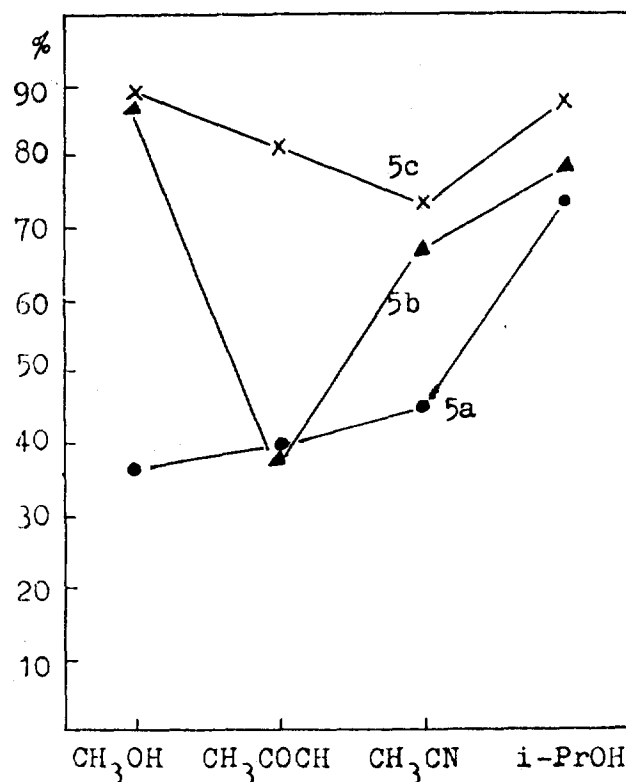


Figure 1. The Changes of yield of adducts with solvents

to shorter wavelength with disappearance 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.

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

| Adducts | δ -Value |
|-------------|--|
| glutathione | 1.3 (SH), 2.0(-CH ₂ CH (NH ₂)COOH), 2.4(CH ₂ SH), 3.6(-COCH ₂), 4.4(CONH), 6.0(CH-NH), 8,3(-NH ₂), 10.0(-COOH) |
| 5a | 2.3(CHCH ₂ NO), 2.5(-S-CH ₂ -), 4.4(-CHCH ₂ NO ₂), 7,3(phenyl) |
| 5b | 2.1(-CH ₃ p), 2.3(-CHCH ₂ NO), 2.5(-SCH ₂ -), 4.4(-CHCH ₂ NO ₂) |
| 5c | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₂ -), 3.8(-OCH ₃), 4.4(-CHCH ₂ NO ₂) 6.7(phenyl) |
| 5e | 2.3(-CH-CH ₂ NO ₂), 2.5(-SCH ₂ -), 4.4(-CHCH ₂ NO ₂), 7,3(phenyl) |
| 5f | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₂ -), 4.4(-CHCH ₂ NO ₂), 7.4(phenyl) |
| 5g | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₂ -), 4.4(-CHCH ₂ NO ₂), 7.5(phenyl) |
| 5h | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₂ -), 3.9(-OCH ₃ , o), 4.4(-CHCH ₂ NO ₂), 7.2(phenyl) |
| 5i | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₃ -), 3.7(-OCH ₂ , m), 4.4(-CHCH ₂ NO ₂), 6.9(phenyl) |
| 5j | 2.3(-CHCH ₂ NO ₂), 2.5(-SCH ₂ -), 3.8(-OCH ₃ , p), 4.4(-CHCH ₂ NO ₂), 7.0(phenyl) |

The λ_{\max} of the glutathione adducts of β -nitrostyrene derivatives are listed in Table 4. In no case, is the absorption characteristics of β -nitrostyrene derivatives observed. The absence of glutathione in the product is also determined by TLC. The R_f 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.

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Photoreaction of 8-Methoxypsoralen with Thymine

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Photoreaction of 8-methoxypsoralen (8-MOP) with thymine (≥ 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 C₄-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