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Thiol의 친핵성 첨가물의 합성 (VIII). β,β-Diethoxycarbonylstyrene에 대한 L-Glutathione의 첨가

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Synthesis of Nucleophilic Adducts of Thiols (VIII). Addition of L-Glutathione to β,β-Diethoxycarbonylstyrene Derivatives

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요 약. 물-메탄올(9 : 1) 용매 속에서 β,β-diethoxycarbonylstyrene과 L-gluthathione을 반응시켜 좋은 수득율로 다음과 같은 화합물을 합성하였다. 즉 S-(2, 2-diethoxycarbonyl-1-phenyl)-*L*-glutathione, S-[2, 2-diethoxycarbonyl-1(3′, 4′-methyleneoxy)phenylethyl]-L-glutathione, S-[2, 2-diethoxycarbonyl-1-(3′, 4′, 5′-thimethoxy)phenylethyl]-*L*-glutathione, S-[2, 2-diethoxycarbonyl-1-(4′-hydroxy)phenylethyl]-L-glutathione, S-[2, 2-diethoxycarbonyl-1-(4′-methoxy) phenylethyl]-*L*-glutathione. 위 화합물들의 구조는 원소분석과 분광학적 방법으로 확인하였고 수득율에 미치는 pH와 용매의 영향을 실험한 결과 pH는 4.0에서 8.0사이가 용매는 물-메탄올이 가장 적합하다는 것을 알았다. 한편 이 화합물의 Gram (+) 박테리아에 대한 항균성을 실험한 결과 약함을 알았다.

ABSTRACT. A series of S-(2, 2-diethoxycarbonyl-1-phenylethy)-*L*-glutathione derivatives(11a-e) were synthesized from the reaction of β, β-diethoxycarbonylstyrene with *L*-glutathione in 9 : 1 aqueous methanol. Thus, S-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-glutathione(11a), *S*-2, 2-diethoxycarbonyl-1-(3′, 4′-methylenedioxy)phenylethy-*L*-glutathione(11b), *S*-2, 2-diethoxycarbonyl-1-(3′, 4′, 5′-trimethoxy)phenylethyl-*L*-glutathione(11c), *S*-2, 2-diethoxycarbonyl-1-(4′-hydroxy)phenylethyl-*L*-glutathione(11d), *S*-2, 2-diethoxycarbonyl-1-(4′-methoxy) phenylethyl-*L*-glutathione(11e) were obtained in good yields. The structure of the adducts was characterized by analytical and spectral data. The effects of pH and solvents upon the yields were also briefly examined. In the range of pH from 4.0 to 8.0, the aqueous methanol were found to be the best solvent for the addition reaction and the antibacterial activities of the adducts to Gram(+) bacteria were found to be weak.

1. INTRODUCTION

There have been growing interests in the synthesis of glutathionyl peptide derivatives with biological activities.[1-6] We reported the synthesis of *S*-(2-nitro-1-phenylethyl)-*L*-cysteine,[7] S-(2-nitro-1-phenylethyl)-*L*-glutathione[8] and *S*-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-cysteine derivatives.[9] In each case, the products were obtained in excellent yields by the reaction of

β-nitrostyrene[10,11] with cysteine or glutathione and β, β-diethoxycarbonylstyrene with glutathione under mild conditions.[12~16] The major advantage of this synthesis is that biologically important products can be obtained in good yields by simple addition reactions without protecting the functional groups. In this work, we have synthesized a series of *S*-(2, 2-diethoxycarbonyl-1phenylethyl)-*L*-glutathione derivatives from the reaction of β, β-diethoxycarbonylstyrene derivatives with *L*-glutathione. The effects of pH and solvents upon the yields werealso briefly examined and the antibacterial activities of the adducts to Gram(+) bacteria were tested.

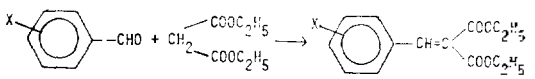
2. EXPERIMENTAL

General. Melting points were determined on a Fisher Johns melting point apparatus. Infrared spectra were obtained with a JASCO IRA-2 spectrophotometer. UV spectra were recorded on a JASCO UNIDEC-430B. Proton nmr spectra were obtained with a Varian Model EM360A (60MHZ) spectrometer in DMSO-d_6. Elemental analyses were conducted with MOO-1106 Model Carlo Erba, Italy. All of the reagents were commercially available and used without purification except 4-hydroxybenzaldehyde.

Synthesis of β, β-Diethoxycarbonylstyrenes. β, β-Diethoxycarbonylstyrene derivatives were prepared by the substituted benzalde hydes and diethylmalonate according to the known method.[17]

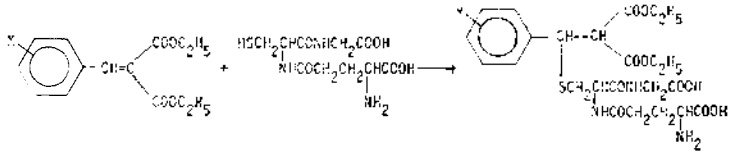
Synthesis of *S*-(2, 2-Diethoxycarbonyl-1-phenylethyl)-*L*-glutathione Derivatives. *L*-glutathione (3.07g, 0.01mole) and N-methylmorpholine (1.01g, 0.01mole) were dissolved

Table 1. Yields, Melting Points and Analytical Data of Elements of β, β-Diethoxycarbonylstyrene Derivatives(9a-e)



X	Yield (%)	mp(°C)	Analytical data of elements (%)			
			Calcd.		Found	
			C	H	C	H
H(9a)	85.1	31	67.74	6.45	68.10	6.62
3, 4-methylene-dioxy (9b)	86.0	53-55	62.06	4.83	61.45	4.90
3, 4, 5-trimethoxy (90)	88.7	70~70.5	60.35	6.51	60.54	9.30
4-hydroxy (9d)	72.4	92~93	63.63	6.06	62.78	6.41
4-methoxy (9e)	86.0	36.5	64.74	6.47	63.60	6.15

Table 2. Yields, Melting Points, and Analytical Data of S-[2, 2-Diethoxycarbony]-1-(substituted) phenylethyl] -*L*-glutathione Derivatives(11a-e)



X	Yielld (%)	mp (°C)	Analytical data (%)							
			Calcd				Found			
			C	H	N	S	C	H	N	S
H(11a)	82.9	152~153	51.89	5.99	7.56	5.77	52.21	5.75	7.85	5.60
3, 4-methylene-dioxy(11b)	51.8	152~153	50.08	5.55	7.01	5.35	50.62	5.46	7.20	5.26
3, 4, 5-trimethoxy (11C)	79.1	151~152	50.23	6.09	6.51	4.97	50.41	5.79	6.21	5.01
4-hydroxy (11d)	87.6	169.5~170.5	50.43	5.82	7.35	5.61	50.84	5.69	7.27	5.69
4-methoxy (11e)	53.8	180~181	51.28	6.02	7.18	5.48	51.95	5.89	7.25	5.39

Table 3. Characteristic UV and IR absorptions of β, β-diethoxycarbonylstyrenes(9a-e) and S-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-glutathione derivatives (11a-e)

Compds.	UV absorptions nm(ε)	IR-bands (cm^{-1}) (KBr pellet)					
9a	217(15400)[a]	2975.	2930.	2890.	1720.	1630.	
	279(7700)	1575.	1495.	1365.	1200~690.		
9b	218(17100)[a]	2950.	2900.	1720.	1625.	1600.	
	295(550)	1490.	13651	1220.	810~750.		
	311(7000)						
9c	229(16300)[a]	2980.	2960.	2900.	2840.	1725.	1690.
	311(7000)	1625.	1580.	1505.	1220.	830.	
9d	217(17700)[a]	3300.	2980.	2840.	2900.	1720.	1690.
	314(12000)	1625.	1590.	1525.	1220.	770~735	
9e	215(15000)[a]	2980.	2940.	2840.	1720.	1675.	
	313(9500)	1605.	1570.	1515.	1210.	760.	
11a		3350.	3050.	2975.	1730.	1660~1640.	
		1510.	1303.	1215.	1030.	700.	
11b	203(16900)	3370.	3280.	2960.	1730.	1640.	1500.
	286(2200)	1445.	1370.	1240.	1035.		
11c	204(14000)	3450~3350.		2960.	2840.	1730.	1650.
		1590.	1510.	1455.	1423.	1255.	1125.
		845.					
11d	228(5100)	3370.	3280.	2975.	1730.	1640.	1510.
		1460.	1400.	1250.	1177.	1030.	835.
11e	227(5000)	3300.	2980.	1735.	1650.	1510.	1460.
		1423.	1252.	1030.	870.		

[a] in Methanol.

Table 4. Proton nmr Spectra of S-(2, 2-Diethoxycarbonyl-1-phenylethyl)-*L*-glutathione Derivatives(11a-e)

Compds.	Chemical shifts in ppm (DMSO-d_6)		
11a	0.9(*t*, 3H, CH_3), 2.4(m, 1H, CH), 7.4(s, 5H, phenyl),	1.2(*s*, 3H, CH_3) 2.5(*m*, 2H, SCH_2), 8.3(*s*, 2H, MH_2)	2.1(*m*, 2H, CH_2) 3.7(*m*, 2H, CH_2)
11b	1.0(*t*, 6H, CH_3), 2.5(*m*, 2H, SCH_2), 6.0(*s*, 2H, CH_2),	2.1(*m*, 2H, CH_2), 3.7(*m*, 2H, CH_2), 7.0(*s*, 3H, phenyl),	2.3(*m*, 1H, CH) 4.0~4.2(*m*, 3H, CH_2, MH) 8.3(*s*, 2H, NH_2)
11c	1.0(*t*, 3H, CH_3), 2.3(*m*, 1H, CH), 3.7(*s*, 3H, OCH_3) 8.5(*s*, 2H, NH_2)	1.3(*t*, 3H, CH_3), 2.6(*m*, 2H, SCH_2), 3.9(*s*, 6H, OCH_3),	2.1(*m*, 2H, CH_2) 4.3(*m*, 4H, CH_2) 6.8(*s*, 2H, phenyl)
11d	0.9~1.2(*m*, 6H, CH_3), 2.5(*m*, 2H, SCH_2), 6, 9, 7.2(d, 4H, phenyl)	2.0(*m*, 2H, CH_2), 3.7(*m*, 2H, CH_2),	2.3(*m*, 1H, CH) 5.8(*m*, 1H, CH)
11e	0.9~1.2(*t*, 6H, CH_3), 2.5(*m*, 2H, SCH_2) 8.3(*s*, 2H, NH_2)	2.0(*m*, 2H, CH_2), 3.7(*m*, 5H, OCH_3, CH_2),	2.3(*m*, 1H, CH) 6.8, 7.2(*d*, 4H, phenyl)

Table 5. Optical rotations, Rf values, and molecular weight of *S*-(2, 2-Diethoxycarbonyl-1-phenylethyl)-*L*-glutathione derivatives

Compds.	$[\alpha]_D^{20a}$	Rf	Amine Content (%)	Molecular Weight	
				Caled.	found
Glutathione	− 7.0°	0.13	100.5	307.33	305.80
11a	−12.4°	0.78	99.1	550.60	560.65
11b	− 0.8°	0.77	100.9	599.61	594.26
11c	−48.8°	0.77	99.8	645.68	646.97
11d	− 8.8°	0.77	99.2	571.60	576.20
11e	− 4.8°	0.75	100.5	585.63	582.72

* Determinec in 1. ON HCl.

Table 6. The yields of *S*-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-glutathione derivatives (11a, 11c) at various pH

Adducts	Base (equivalent amount)	pH	Yield (%)
11a	N-mm 0.0	2.9	34.6
	N-mm 0.5	3.9	72.9
	N-mm 1.0	7.5	82.9
	TEA 1.0	8.0	54.9
	N-mm 1.0+TEA 0.5	8.3	42.2
	N-mm 1.0+TEA 1.0	8.6	7.6
	N-mm 1.0+TEA 2.0	9.2	NO PPT
	N-mm 1.0+TEA 3.0	9.6	No PPT
11c	N-mm 0.0	3.0	31.5
	N-mm 0.5	4.0	53.9
	N-mm 1.0	7.4	79.1
	TEA 1.0	7.9	75.3
	N-mm 1.0+TEA 0.5	8.2	67.7
	N-mm 1.0+TEA 1.0	8.6	65.4
	N-mm 1.0+TEA 2.0	9.2	8.7
	N-mm 1.0+TEA 3.0	9.6	Small

N-mm : N-methylmorpholine, TEA : triethylamine.

in 55m*l* of 10% aqueous methanol β, β-Diethoxycarbonstyrene(9A : 3.72g, 0.015mole) was added to the solution and the mixture was heated to 55 °C and reacted for 1hr. The solution was cooled to room temperature and neutralized by 10% methanolic sulfuric acid to pH 2.9. Methanol was evaporated and the product was precipitatedin acetonitrile. The product was collected by filtration and puripied. Yields, melting points and the elemental analyses data are summarized in *Table* 2. UV, IR and nmr spectral data are summarized in *Table* 6 and 7.

Table 7. The yields of *S*-(2, 2-diethoxycarbonyl-1-pheylethyl)-*L*-glutathione derivatives(11c, 11d) with varions solvents

Compds.	Solvents	Yield (%)
11c	CH_3OH	79.1
	2-Propanol	72.0
	CH_3CN	12.8
	Acetone	Small
	Dioxane	Small
11d	CH_3OH	87.6
	2-Propanol	44.2
	CH_3CN	56.7
	Acetone	Small
	Dioxane	13.4

Determination of Optical Rotations, Rf values, and Molecular Weight of *S*-(2, 2-Diethoxycarbonyl-1-phenylethyl)-*L*-glutathione Derivatives.

Optical rotations of the adducts were determined in 1.0 N HCl(aq). The Rf values of the products were determined on a TLC plate(silica gel) using a mixture of ethyl acetate/acetic acid/water (v/v : 3/1/1) as a developing solvent. The molecular weieght of the adducts were determined by nonaqueous amine titration. Since 1.0m*l* of 0.1N $HClO_4$ is equvalent to 0.030733g of *S*-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-glutathione, the molecular weight of the adduct was calculated from the volume of the $HClO_4$ solution added to reach the end point. The optical rotations, Rf values, and molecular weight of the adducts are recorded in *Table* 3.

3. RESULUS AND DISCUSSION

A series of *S*-(2, 2-diethoxycarbonyl-1-phenylethyl)-*L*-glutathione derivatives were obtained in moderate to very good yields by the reactions of the β, β-diethoxycarbonylstyrene with *L*-glutathione under mild conditions. The struc-

Table 8. Antibacterial activities of the adducts

Compds.	Concentration	Inhibition zone to sarcina lutea 9341	Inhibition zone to staphylococcus aureus 6538 P
11a	1mg/ml	—	—
11b	1mg/ml	7.2mm	6.9mm
11c	1mg/ml	11.1mm	10.3mm
11d	1mg/ml	21.7mm	20.7mm
11e	1mg/ml	9.7mm	12.3mm
Ampicllin Trihydrate	0.4μg/ml	24/2mm	23.8mm

Amicillin Trihydrate : Potency 86.7%.

tures of the adducts were characterized by the analytical and spectral data. The results of elemental analyses(*Table* 2) and molecular weight determination(*Table* 5) are consistent with those expected from the adducts. The infrared spectra (*Table* 3) show characteristic peaks corresponding to NH stretching vibration at 3370~3300 cm^{-1}, ester carbonyl at 1735~1730cm^{-1}, assym. bending $^{+}NH_3$ and assym. stretching vibration of COO^{-1} at 1660~1640cm^{-1}, S-CH_2 at 1423~1400cm^{-1}, aryl O-CH_2 at 1252~1240cm^{-1}. The stretching vibration of conjugated C=C at 1570~1600cm^{-1} disappeared. The UV spectra in 67% aqueous methanol (Table 3) show marked decrease in absorptions at max of the corresponding β, β-diethoxycarbonylstyrene derivatives, indicating again the absence of C=C bond in the adduct. The nmr spectra also agree well with the proposed structure(*Table* 4) The yields of the reactions between β, β-diethoxycarbonylstyrenes and *L*-glutathion at various pH are summarized in *Table* 6. In each case an appropriate amount of *N*-methylmorpholine and triethylamine was added to control the pH. Yields are always higher at neutral pH than those at acidic or basic region. The low yields observed at low pH may be ascribed to the low concentration of the reactive thiolate anion. At high pH, the competing hydrolysis of β, β-diethoxycarbonylstyrenes may become predominant, decreasing the yields. In an attempt to optimize the yields, the reaction was conducted in several solvents. As shown in *Table* 7, aqueous methanol gave the best results for the reactions. Antiabcterial activity of S-[2, 2-diethoxycarbonyl-1-(4-hydroxy)phenylethyl]-*L*-glutathione (11d) was also 1/2500 compared to that of Ampicillin Trihydrate.

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