Synthesis of Mono-, Di-, and Tripeptidyl Amides of Dihydrosphingosine

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Amino acid amides of dihydrosphingosine were prepared by condensation of the long chain base with the product from the reaction of the appropriate *N*-carbobenzoxy-protected amino acid, dipeptide, or tripeptide with *N*-ethyl-5-phenylisoxazolium-3′-sulfonate in the presence of triethylamine. The amino acid amide of the base was obtained after hydrogenolysis of the protective group. Over-all yields of product ranged from 20 to 55%. Some physical and chemical characteristics of these compounds are reported.

THE synthesis of fatty acid amides of long-chain bases, the primary structural units of sphingolipids (*2, 4-7, 10*), was reported (*9, 12*). Amino acid amides of long-chain bases have never been prepared and their existence in biological materials has never been demonstrated. The authors were interested in synthesizing a variety of these compounds to determine their physical and chemical characteristics. Such studies might provide the basis for an investigation of the occurrence of similar compounds in natural sources. In addition, the authors hoped that with the synthetic procedures radioactive substrates could be prepared to test for the presence in mammalian tissues of enzymes active on these compounds. Similar efforts were made following the synthesis of amino acid esters of glycerol (*1, 3*).

The preparation of amino acid amides of long-chain bases was accomplished by use of the isoxazolium salt procedure of Woodward *et al.* (*13, 14*). Dihydrosphingosine was the base chosen for these syntheses because of purity and ease of preparation. The base was condensed with the product from the reaction of the appropriate *N*-carbobenzoxy-protected amino acid, dipeptide, or tripeptide with *N*-ethyl-5-phenylisoxazolium-3′-sulfonate in the presence of triethylamine. The amino acid amide of the base was obtained after hydrogenolysis of the protective group. Several *tert*-butyloxycarbonyl-protected amino acid amides of the unsaturated base, sphingosine, were prepared; however, treatment of these compounds with 5% HCl gas in glacial acetic acid (*8*) to remove the protective group failed to yield the desired product.

The over-all yields of products ranged from about 20 to 55% (Tables I and II). The compounds had broad melting points and were soluble in chloroform and methanol but insoluble in acetonitrile and nitromethane. The dipeptidyl and tripeptidyl amides of the bases, in contrast to the monopeptidyl compounds, were soluble in hot water (approximately 10 mg. per ml.) and formed gelatinous solutions on cooling. The infrared spectra, obtained from KBr disks, showed the characteristic absorption bands for amide (1650 $cm.^{-1}$, 1550 $cm.^{-1}$), amino (1650 $cm.^{-1}$), and hydroxyl (1075 $cm.^{-1}$, 1050 $cm.^{-1}$) groups.

The compounds were analyzed for purity as trimethylsilyl derivatives (*11*) on a Perkin-Elmer Gas Chromatograph with a hydrogen flame ionization detector. A glass column, 6 feet × ⅛ inch, was packed with 2.5% SE-30 (Applied Science Laboratories, College Station, Pa.) on 100- to 120-mesh, silanized Gas Crom Q; the column was maintained at either 200° or 260° C. and the flow rate of nitrogen was 37 ml. per minute.

EXPERIMENTAL

Preparation of *N*-Carbobenzoxy Mono-, Di-, and Tripeptidyl Amides of Dihydrosphingosine (A). To 2.53 grams (10 mmoles) of *N*-ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's Reagent K, Pilot Chemicals, Watertown,

Table I. Physical and Analytical Values on *N*-Carbobenzoxy Amino Acid Amides of Dihydrosphingosine

N-Carbobenzoxy Amino Acid Amide of Dihydrosphingosine	Yield, %	M.P., °C.	Formula	Mol. Wt.	Carbon, %		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
L-Alanyl	58	109–113	$C_{29}H_{50}O_5N_2$	506.4	66.72	66.55	9.95	10.01	5.52	5.45
L-Asparaginyl	46	208–212	$C_{30}H_{51}O_6N_3$	549.4	65.53	65.88	9.36	9.30	7.64	7.71
Glycyl	73	68–72	$C_{28}H_{48}O_5N_2$	492.4	68.24	68.20	9.83	9.66	5.69	5.70
L-Phenylalanyl	35	127–130	$C_{35}H_{54}O_5N_2$	582.4	72.11	72.06	9.35	9.26	4.81	4.89
L-Prolyl	49	54–56	$C_{31}H_{52}O_5N_2$	532.4	69.87	69.80	9.84	5.26	5.23	
DL-Seryl	46	173–177	$C_{29}H_{50}O_6N_2$	522.4	66.62	66.31	9.65	9.81	5.26	5.23
L-Tyrosyl	44	112–116	$C_{35}H_{54}O_6N_2$	598.4	70.18	69.76	9.10	9.73	5.36	5.40
								9.08	4.68	4.73
N-Carbobenzoxy Dipeptidyl amide of dihydrosphingosine										
Glycyl-L-alanyl	41	145–149	$C_{31}H_{53}O_6N_3$	563.4	66.02	66.21	9.48	9.58	7.45	7.36
Glycyl-L-phenylalanyl	35	112–116	$C_{37}H_{57}O_6N_3$	639.5	69.43	69.48	8.99	9.09	6.57	6.58
Glycyl-L-prolyl	36	73–77	$C_{33}H_{55}O_6N_3$	589.4	67.18	67.29	9.41	9.52	7.13	7.10
N-Carbobenzoxy tripeptidyl amide of dihydrosphingosine										
L-Alanylglycylglycyl	27	123–127	$C_{33}H_{56}O_7N_4$	620.4	63.82	63.85	9.98	9.78	9.03	9.05
L-Leucylglycylglycyl	39	83–88	$C_{36}H_{62}O_7N_4$	662.5	65.21	65.14	9.43	9.63	8.45	8.48
L-Valyglycylglycyl	34	124–129	$C_{35}H_{60}O_7N_4$	648.5	64.77	64.85	9.33	9.64	8.64	8.59

Table II. Physical and Analytical Values on Amino Acid Amides of Dihydrosphingosine

Amino Acid Amide of Dihydrosphingosine	Yield, %	M.P., °C.	Formula	Mol. Wt.	Carbon, %		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
L-Alanyl	70	117–121	$C_{21}H_{44}O_3N_2$	372.4	67.68	67.11	11.91	11.75	7.52	7.44
L-Asparaginyl	68	138–142	$C_{22}H_{45}O_4N_3$	415.4	63.56	63.66	10.92	11.01	10.11	10.20
Glycyl	80	113–117	$C_{20}H_{42}O_3N_2$	358.3	66.98	67.17	11.81	11.71	7.81	7.75
L-Phenylalanyl	75	114–118	$C_{27}H_{48}O_3N_2$	448.4	72.26	72.00	10.79	10.67	6.24	6.34
L-Prolyl	83	84–88	$C_{23}H_{46}O_3N_2$	398.4	69.28	69.40	11.64	11.47	7.03	7.06
DL-Seryl	78	110–114	$C_{21}H_{44}O_4N_2$	388.4	64.89	64.49	11.42	11.29	7.21	7.16
L-Tyrosyl	69	79–83	$C_{27}H_{48}O_4N_2$	464.4	69.77	69.34	10.42	10.68	6.03	6.07
Dipeptidyl amide of dihydrosphingosine										
Glycyl-L-alanyl	74	140–144	$C_{23}H_{47}O_4N_3$	429.4	64.28	64.95	11.03	10.93	9.78	9.72
Glycyl-L-phenylalanyl	64	156–160	$C_{29}H_{51}O_4N_3$	505.4	68.86	68.75	10.17	10.06	8.31	8.41
Glycyl-L-prolyl	73	92–96	$C_{25}H_{49}O_4N_3$	455.4	65.88	65.97	10.85	10.95	9.22	9.12
Tripeptidyl amide of dihydrosphingosine										
L-Alanylglycylglycyl	74	132–137	$C_{25}H_{50}O_5N_4$	486.4	61.68	61.60	10.36	10.72	11.51	11.40
L-Leucylglycylglycyl	49	122–127	$C_{28}H_{56}O_5N_4$	528.4	63.58	63.87	10.68	10.99	10.60	10.39
L-Valylglycylglycyl	65	111–116	$C_{27}H_{54}O_5N_4$	514.4	62.98	63.43	10.58	10.75	10.89	10.81

Mass.) in 45 ml. of nitromethane (Spectro grade) were added 10 mmoles of the appropriate *N*-carbobenzoxy substituted amino acid, dipeptide, or tripeptide (obtained from Mann Research, N. Y.; International Chemical and Nuclear, City of Industry, Calif.; and Fluka, Buchs, Switzerland, respectively) followed by 1.4 ml. (10 mmoles) of freshly distilled triethylamine; the reaction mixture was magnetically stirred at room temperature. When the suspension cleared (about 30 minutes.), 3.0 grams (10 mmoles) of dihydrosphingosine prepared according to the procedure of Carter *et al.* (*6*) in 50 ml. of dry benzene were added; stirring was continued for 4 hours. After addition of 300 ml. of ethyl acetate and 75 ml. of water, the organic layer was successively washed with two 25-ml. portions of water, 5% $NaHCO_3$, water, 1*N* HCl, and water until neutral; methanol was added to the solution when turbidity developed. After filtration of the organic layer, the filtrate was concentrated under reduced pressure. The product, dried in vacuo over P_2O_5, was crystallized successively from 75-ml. portions of nitromethane and acetonitrile; in those instances where the compound was slightly soluble in hot acetonitrile, ethanol was added to about 10% concentration. The compounds gave negative ninhydrin reactions (Table I).

Preparation of Mono-, Di-, and Tripeptidyl Amides of Dihydrosphingosine. The *N*-carbobenzoxy compound (*A*) was hydrogenated over 50 mg. each of PtO_2 and PdO_2 in 30 ml. of glacial acetic acid for 18 hours with magnetic stirring. The reaction mixture was warmed and filtered, and the catalyst was washed with a small amount of warm glacial acetic acid. The combined acid filtrates were concentrated under reduced pressure. After the addition of 50 ml. each of 95% ethanol and water, sufficient solid $NaHCO_3$ to neutralize the residual acidity, and 300 ml. of ethyl acetate, the organic layer was washed with water until neutral and concentrated with the frequent addition of ethanol; vigorous foaming occurred during the removal of water. The dried residue was crystallized successively from nitromethane containing 10 to 20% ethanol and acetonitrile. All compounds gave positive ninhydrin reactions (Table II). Gas chromatography revealed the presence of 2 to 6% impurity; the retention times of some of the compounds overlapped (Table III).

Table III. Gas Chromatography of Amino Acid Amides of Dihydrosphingosine as Trimethylsilyl Derivatives

Compound	Retention Time, Minutes
L-Alanyl	1.5
L-Asparaginyl	6.2
Glycyl	1.6
L-Phenylalanyl	7.8
L-Prolyl	3.4
DL-Seryl	3.2
L-Tyrosyl	14.0
Glycyl-L-alanyl	6.8
Glycyl-L-phenylalanyl	20.4
Glycyl-L-prolyl	9.4
L-Alanylglycylglycyl	40.0[a]
L-Leucylglycylglycyl	39.3[a]
L-Valylglycylglycyl	42.0[a]

[a] Except for the tripeptidyl compounds which were chromatographed at 200° C., all of the other derivatives were examined at 260° C. See text for details.

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