Chemistry of Natural Compounds, Vol. 35, No. 4, 1999

SYNTHESIS OF AMINO ACID DERIVATIVES OF 7-METHOXYCARBONYLNEOFLAVONES

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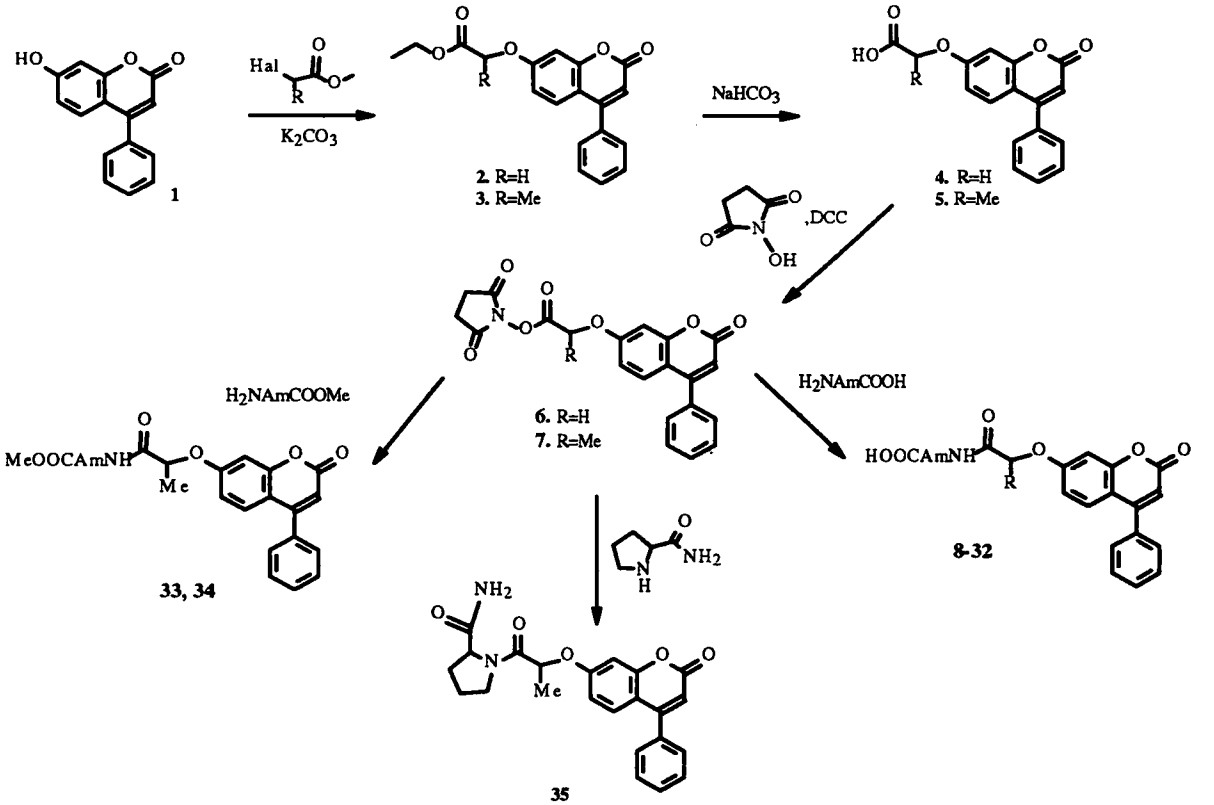
UDC 547.814.5

A number of aminoacyl derivatives of 4-phenylcoumarin have been obtained by the condensation of N-hydroxysuccinimide esters of 7-(1-carboxyalkoxy)-4-phenylcoumarins with L-amino acids and their derivatives.

Derivatives of 4-phenylcoumarin (known as neoflavones) are widely distributed in the plant world, particularly among plants of the families Dalbergia, Guttiferae, and Rubiaceae. Up to the present time, more than 60 compounds based on the skeleton of 4-phenylcoumarin have been isolated from natural raw material. Both natural and synthetic 4-phenylcoumarins possess a broad spectrum of biological action. Thus the oil of *Mesua ferrea* seeds has yielded mesuol, which possesses antibiotic properties, and mesuagin, which exhibits an antibacterial action against *Staphylococcus aureus* [1]. Mammein, which possesses insecticidal properties, has been isolated from the oil of the seeds and the fruit flesh of *Mammea americana* [2]. Synthetic derivatives of 4-phenylcoumarin possess vasodilating [3], analeptic [4], antiatherosclerotic [5], and antibacterial [6, 7] actions. Therefore, modification of the structure of 4-phenylcoumarin by introducing into its molecule a fragment containing an amino acid residue will possibly enable compounds with useful biological properties to be obtained.



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0009-3130/99/3504-0415$22.00

TABLE 1. Physicochemical Properties of Amino Acid Derivatives of 7-(1-Carboxyalkoxy)-4-phenylcoumarins

Empirical formula	Yield, %	mp, °C	Details of the PMR spectra, δ, ppm; solvent: DMSO-d_6							
			Signals of the coumarin ring						NHCO	Signals of the amino acid fragment
			H-3	Ph-4	H-5	H-6	COCHRO-7	H-8		
8. $C_{19}H_{15}NO_6$	70.9	222	6.25	7.55	7.38	6.97	4.68 (R=H)	7.06	8.51	CH_2COOH 3.83; 12.70
9. $C_{20}H_{17}NO_6$	51.0	214	6.24	7.54	7.34	6.95	4.68 (R=H)	7.04	8.47	$CH(CH_3)COOH$ 4.33; 1.35; 12.60
10. $C_{20}H_{17}NO_6$	68.0	235	6.25	7.55	7.35	6.95	4.62 (R=H)	7.08	8.25	CH_2CH_2COOH 3.34; 2.44; 12.30
11. $C_{21}H_{19}NO_6$	65.8	205	6.25	7.55	7.38	6.98	4.62 (R=H)	7.05	8.22	$CH_2CH_2CH_2COOH$ 3.14; 1.65; 2.21; 12.0
12. $C_{22}H_{21}NO_6$	36.1	194	6.25	7.55	7.35	6.95	4.71 (R=H)	7.02	8.45	$CH(CH_2CH_2CH_3)COOH$ 4.26;1.67;1.32;0.85;12.30
13. $C_{23}H_{23}NO_6$	71.3	168	6.25	7.55	7.38	6.95	4.71 (R=H)	7.02	8.42	$CH(CH_2CH(CH_3)_2)COOH$ 4.27;1.58;1.58;0.86;12.67
14. $C_{23}H_{23}NO_6$	74.6	147	6.25	7.56	7.38	6.98	4.72 (R=H)	7.02	8.40	$CH(CH_2CH_2CH_2CH_3)COOH$ 4.25;1.71;1.25;1.25;0.84;12.72
15. $C_{23}H_{23}NO_6$	71.5	128	6.25	7.55	7.38	7.00	4.61 (R=H)	7.05	8.18	$CH_2CH_2CH_2CH_2CH_2COOH$ 3.12;1.40;1.40;1.40;2.15;12.10
16. $C_{22}H_{21}NO_6S$	68.0	174	6.25	7.55	7.36	7.00	4.73 (R=H)	7.08	8.47	$CH(CH_2CH_2SCH_3)COOH$ 4.40;1.95;2.48;2.02;12.80
17. $C_{26}H_{20}NO_6$	60.2	185	6.26	7.57	7.32	6.85	4.64 (R=H)	6.98	8.42	$CH(CH_2Ph)COOH$ 4.40; 3.06; 7.20; 12.70
18. $C_{20}H_{17}NO_6$	88.4	208	6.25	7.55	7.35	6.95	1.49; 4.96 ($R=CH_3$)	7.04	8.54	CH_2COOH 3.79; 12.70
19. $C_{21}H_{19}NO_6$	51.0	195	6.25	7.55	7.35	6.81	1.51: 4.98 ($R=CH_3$)	6.98	8.58	$CH(CH_3)COOH$ 4.30; 1.33; 12.60
20. $C_{21}H_{19}NO_6$	91.2	214	6.25	7.55	7.38	6.91	1.44; 4.87 ($R=CH_3$)	6.96	8.28	CH_2CH_2COOH 3.29; 2.37; 12.30
21. $C_{22}H_{21}NO_6$	84.7	185	6.24	7.55	7.36	6.94	1.48; 4.87 ($R=CH_3$)	6.98	8.22	$CH_2CH_2CH_2COOH$ 3.11; 1.63; 2.13; 12.08
22. $C_{23}H_{23}NO_6$	76.4	173	6.24	7.55	7.35	6.90	1.52; 5.09 ($R=CH_3$)	6.98	8.45	$CH(CH(CH_3)_2)COOH$ 4.16; 1.52; 0.90; 12.30
23. $C_{23}H_{23}NO_6$	90.9	182	6.25	7.55	7.36	6.92	1.47; 4.94 ($R=CH_3$)	6.98	8.51	$CH(CH_2CH_2CH_3)COOH$ 4.21;1.67;1.32;0.84;12.40
24. $C_{24}H_{25}NO_6$	78.7	156	6.25	7.54	7.35	6.90	1.51; 4.95 ($R=CH_3$)	6.98	8.51	$CH(CH_2CH(CH_3)_2)COOH$ 4.26;1.58;1.58;0.79;12.75
25. $C_{24}H_{25}NO_6$	70.3	143	6.23	7.54	7.36	6.90	1.52; 5.04 ($R=CH_3$)	6.98	8.42	$CH(CH(CH_3)CH_2CH_3)COOH$ 4.20;1.88;0.85;1.20;0.85;12.70
26. $C_{24}H_{25}NO_6$	78.7	132	6.25	7.55	7.34	6.91	1.51; 4.96 ($R=CH_3$)	7.02	8.51	$CH(CH_2CH_2CH_2CH_3)COOH$ 4.20;1.71;1.19;1.19;0.77;12.72
27. $C_{23}H_{23}NO_6S$	87.9	169	6.25	7.55	7.35	6.95	1.52; 4.98 ($R=CH_3$)	7.00	8.55	$CH(CH_2CH_2SCH_3)COOH$ 4.34;2.10;2.48;1.96;12.80
28. $C_{24}H_{25}NO_6$	92.1	119	6.25	7.55	7.38	6.92	1.47; 4.87 ($R=CH_3$)	6.98	8.20	$CH_2CH_2CH_2CH_2CH_2COOH$ 3.05;1.40;1.40;1.40;2.11;12.10
29. $C_{22}H_{19}NO_8$	78.7	195	6.25	7.55	7.35	6.92	1.51; 5.02 ($R=CH_3$)	7.00	8.55	$CH(CH_2COOH)COOH$ 4.58; 2.73; 12.70; 12.70

TABLE 1. (Continued)

Empirical formula	Yield, %	mp, °C	Details of the PMR spectra, δ, ppm; solvent: DMSO-d_6							
			Signals of the coumarin ring						NHCO	Signals of the amino acid fragment
			H-3	Ph-4	H-5	H-6	COCHRO-7	H-8		
30. $C_{23}H_{21}NO_8$	75.7	167	6.24	7.55	7.35	6.91	1.51; 5.00 (R=CH_3)	7.00	8.50	$CH(CH_2CH_2COOH)COOH$ 4.20;2.35;2.25;12.70;12.70
31. $C_{26}H_{21}NO_6$	52.6	190	6.24	7.55	7.32	6.88	1.48; 5.08 (R=CH_3)	7.01	9.00	$CH(Ph)COOH$ 5.35; 7.38; 13.10
32. $C_{27}H_{23}NO_6$	84.7	171	6.24	7.55	7.35	6.94	1.44; 4.88 (R=CH_3)	7.05	8.45	$CH(CH_2Ph)COOH$ 4.40; 3.06; 7.20; 12.70
33.* $C_{25}H_{27}NO_6$	62.5	74	6.27	7.50	7.32	6.84	1.65; 4.81 (R=CH_3)	6.95	6.75	$CH(CH(CH_3)CH_2CH_3)COOCH_3$ 4.62;1.88;0.92;1.20;0.72;3.71
34.* $C_{28}H_{25}NO_6$	70.9	89	6.29	7.50	7.32	6.81	1.56; 4.80 (R=CH_3)	7.09	6.86	$CH(CH_2Ph)COOCH_3$ 4.80;3.07;7.27;3.75
35. $C_{23}H_{22}N_2O_5$	42.7	174	6.23	7.55	7.30	6.91	1.49; 5.14 (R=CH_3)	7.00	-	$-NCH_2CH_2CH_2CHCONH_2$ 3.48;1.14;2.00;4.25;6.88

*Spectrum measured in deuterochloroform.

The initial 7-hydroxy-4-phenylcoumarin (**1**) was obtained by the Pechmann condensation of resorcinol and ethyl benzoylacetate in the presence of trifluoroacetic acid [8]. By alkylating this compound in acetone in the presence of potash with ethyl chloroacetate and with ethyl 2-bromopropionate we obtained 7-(ethoxycarbonylmethoxy)-4-phenylcoumarin (**2**) and 7-[1-(ethoxycarbonyl)ethoxy]-4-phenylcoumarin (**3**), respectively. The corresponding acids (**4**) and (**5**) were obtained by saponifying the esters (**2**) and (**3**) with 5% sodium hydrogen carbonate.

To obtain the amino acid derivatives (**8—35**) we made use of the method of activated esters that is widely employed in peptide synthesis. As the activated esters we took the N-hydroxysuccinimide esters, which are characterized by high reactivity and the use of which does not lead to racemization [9]. The N-hydroxysuccinimide esters (**6**) and (**7**) of the substituted hydroxy acids were obtained with high yields by the interaction of the corresponding acids (**4**) and (**5**) with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide as condensing agent (DCC) [10, 11].

The N-[2-(4-phenylcoumar-7-yloxy)alkanoyl]-(*L*-amino acid)s (**8—32**) were obtained by condensing the activated esters (**6**) and (**7**) with the sodium salts of *L*-amino acids in water—THF at room temperature with the subsequent acidolysis of the salts formed. The syntheses were performed with glycine (**8, 18**), alanine (**9, 19**), β-alanine (**10, 20**), γ-aminobutyric acid (**11, 21**), ε-aminocaproic acid (**15, 28**), aspartic acid (**29**), glutamic acid (**30**), valine (**22**), norvaline (**12, 23**), leucine (**13, 24**), isoleucine (**25**), norleucine (**14, 26**), methionine (**16, 27**), phenylalanine (**17, 32**), and phenylglycine (**31**).

The methyl esters of the N-[2-(4-phenylcoumar-7-yloxy)propionyl]-*L*-amino acids (**33, 34**) were obtained by condensing the N-hydroxysuccinimide ester of the acid (**7**) and the methyl esters of isoleucine (**33**) and of phenylalanine (**34**) in absolute THF at 0°C. N-[2-(4-phenylcoumar-7-yloxy)propionyl]-*L*-prolinamide (**35**) was obtained by condensing the ester (**7**) and *L*-prolinamide in dioxane.

The structures of the aminoacyl derivatives of 7-(1-carboxyalkoxy)-4-phenylcoumarins that had been obtained were shown by quantitative analysis and PMR spectroscopy. The following differences from the spectra of the initial acids (**4, 5**) were observed in the PMR spectra of compounds (**8—35**): the signal of the proton of the amide bond appeared in the 8.20—8.50 ppm region, and the signals of the protons of the amino acid residue were also present. The physicochemical constants of the compounds obtained, (**8—35**), are given in Table 1. Thus, the reaction of amino acids with activated esters of certain carboxylic acid derivatives of neoflavones is a convenient way of modifying their molecules.

EXPERIMENTAL

The course of the reactions and the purity of the compounds obtained were monitored by TLC on Silufol UV-254 plates (Czech Republic) in the chloroform—methanol (9:1) and benzene—ethanol (9:1) systems. PMR spectra were measured on Bruker WP 100SY and Varian VXR-300 instruments in DMSO-d_6 and $CDCl_3$ relative to TMS (internal standard).

7-Hydroxy-4-phenylcoumarin (1) was obtained as in [8].

7-(Ethoxycarbonylmethoxy)-4-phenylcoumarin (2) and 7-[1-(Ethoxycarbonyl)ethoxy]-4-phenylcoumarin (3). A hot solution of 12.7 g (50 mmole) of coumarin (1) in 100 ml of absolute ethanol was treated with 20.7 g (150 mmole) of freshly calcined potash and then, with vigorous stirring and heating (50—56°C), 4.75 ml (55 mmole) of ethyl chloroacetate and 1 g of anhydrous potassium iodide or 6.90 ml (55 mmol) of ethyl 2-bromopropionate, respectively, were added. The reaction mixture was kept at 50—56°C for 1—2 h with vigorous stirring (the end of the reaction was determined by TLC). The inorganic deposit was filtered off, the acetone was evaporated off in vacuum, and the residue was crystallized from aqueous ethanol.

7-(Ethoxycarbonylmethoxy)-4-phenylcoumarin (2); yield 91%, mp 105°C. PMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 1.32 (3H, t, CH_3); 4.30 (2H, q, CH_2); 4.70 (2H, s, $OOCH_2O$-7); 6.24 (1H, s, H-3); 6.82 (1H, dd, J = 2.0, 8.0 Hz, H-6); 6.88 (1H, d, J = 2.0 Hz, H-8); 7.32 (1H, d, J = 8.0 Hz, H-5); 7.50 (5H, m, Ph-4).

7-[1-(Ethoxycarbonylethoxy)-4-phenylcoumarin (3): yield 85%, mp 82°C. PMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 1.32 (3H, t, CH_3); 1.86 (3H, d, CH_3); 4.30 (2H, q, CH_2); 5.15 (1H, q, OOCCHO-7); 6.25 (1H, s, H-3); 6.83 (1H, dd, J = 2.0, 8.0 Hz, H-6); 7.02 (1H, d, J = 2.0 Hz, H-8); 7.35 (1H, d, J = 8.0 Hz, H-5); 7.50 (5H, m, Ph-4).

7-Carboxymethoxy-4-phenylcoumarin (4) and 7-(1-Carboxyethoxy)-4-phenylcoumarin (5). A solution of 20 mole of the ethyl ester of the appropriate acid (2) or (3) in 70 ml of ethanol was treated with 70 ml of a 5% solution of sodium hydrogen carbonate (40 mmole). The reaction mixture was boiled for 3—4 h (the end of the reaction being determined by TLC), and it was then diluted with 300 ml of water and acidified to pH 4. The resulting precipitate was filtered off and recrystallized from aqueous ethanol.

7-Carboxymethoxy-4-phenylcoumarin (4), yield 95%, mp 159°C. PMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 4.62 (2H, s, $OOCCH_2O$-7); 6.25 (1H, s, H-3); 6.95 (1H, dd, J = 2.0, 8.0 Hz, H-6); 7.08 (1H, d, J = 2.0 Hz, H-8); 7.38 (1H, d, J = 8.0 Hz, H-5); 7.55 (5H, s, Ph-4); 13.15 (1H, br. s, COOH).

7-(1-Carboxyethoxy)-4-phenylcoumarin (5): yield 90%, mp 148°C. PMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 1.53 (3H, d, CH_3); 5.06 (1H, q, OOCCHO-7); 6.24 (1H, s, H-3); 6.90 (1H, dd, J = 2.0, 8.0 Hz, H-6); 6.98 (1H, d, J = 2.0 Hz, H-8); 7.35 (1H, d, J = 8.0 Hz, H-5); 7.55 (5H, s, Ph-4); 13.10 (1H, br. s, COOH).

The N-Hydroxysuccinimide Esters (6) and (7). A cooled solution of 10 mmole of the appropriate acid (4) or (5) and 1.27 g (11 mmole) of N-hydroxysuccinimide in absolute THF was treated with 2.06 g (10 mmole) of dicyclohexylcarbodiimide. The reaction mixture was kept at 0°C for 1 h, after which the dicylohexylurea formed was filtered off, the THF was evaporated off in vacuum, and the residue was crystallized from propan-2-ol.

N-Hydroxysuccinimide Ester of 7-Carboxymethoxy-4-phenylcoumarin (6): yield 79%, mp 168°C. PMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 2.85 (4H, s, CH_2CH_2-groups of N-hydroxysuccinimide); 4.70 (2H, s, $OOCCH_2O$-7); 6.24 (1H, s, H-3); 6.82 (1H, dd, J = 2.0, 8.0 Hz, H-6); 6.88 (1H, d, J = 2.0 Hz, H-8); 7.32 (1H, d, J = 8.0 Hz, H-5); 7.50 (5H, m, Ph-4).

N-Hydroxysuccinimide Ester of 7-(1-Carboxyethoxy)-4-phenylcoumarin (7): yield 86%, mp 152°C. PMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 1.86 (3H, d, CH_3); 2.85 (4H, s, CH_2CH_2 groups of N-hydroxysuccinimide); 5.15 (1H, q, OOCCHO-7); 6.25 (1H, s, H-3); 6.83 (1H, dd, J = 2.0, 8.0 Hz, H-6); 7.02 (1H, d, J = 2.0 Hz, H-8); 7.35 (1H, d, J = 8.0 Hz, H-5); 7.50 (5H, s, Ph-4).

N-[2-(4-Phenylcoumar-7-yloxy)alkanoyl]-*L*-Amino Acids (8—32). A solution 3 mmole of the appropriate activated ester (6) or (7) in 10 ml of THF was added to a solution of 3.5 mmole of the appropriate *L*-amino acid and 0.30 g (3.5 mmole) of sodium hydrogen carbonate in 20 ml of water. The resulting mixture was vigorously stirred for 1 h and, after the addition of 100 ml of water, it was acidified to pH 4. The precipitate that deposited was filtered off and crystallized from propan-2-ol. Yields and constants are given in Table 1.

Methyl Esters of N-[1-(4-Phenylcoumar-7-yloxy)propionyl]-L-Amino Acids (33) and (34). With cooling to 0°C, 0.42 ml (3.5 mmole) of triethylamine was added dropwise to a suspension of 3.5 mmole of the appropriate *L*-amino acid methyl ester hydrochloride in 20 ml of THF. The mixture was subjected to vigorous stirring and cooling (0°C) for 30 min, after which the precipitate of triethylamine hydrochloride was filtered off. A solution of 3 mmole of the activated ester (7) in 10 ml of THF

was added to the filtrate and the resulting mixture was stirred vigorously at 0°C for 2 h. after which it was left overnight at room temperature. The solvent was evaporated off in vacuum, and the residue was dissolved in ethyl acetate. The ethyl acetate solution (50 ml) was treated successively with 1 N sulfuric acid (30 ml), water (30 ml), 5% sodium hydrogen carbonate solution (30 ml), water (30 ml), and saturated sodium chloride solution (30 ml). The organic layer was dried on anhydrous $MgSO_4$ and then the solvent was evaporated off in vacuum. The oily residue crystallized on treatment with diethyl ether. The yields and constants of compounds (33) and (34) are given in Table 1.

N-[1-(4-phenylcoumar-7-yloxy)propionyl]-*L*-prolinamide (35). A solution of 1.17 g (3 mmole) of compound (7) and 0.342 g (3 mmole) of *L*-prolinamide was kept in dioxane at 90—100°C for 1 h. After this the dioxane was evaporated off in vacuum and he residue was crystallized from propan-2-ol. The yield and constants are given in Table 1.

REFERENCES

1. D. P. Chakraborty and D. Chatterji, *J. Org. Chem.*,, **34**, No. 12, 3784 (1969).
2. R. A. Finnegan, M. P. Morris, and C. Djerassi, *J. Org. Chem.*, **26**, 1180 (1961).
3. Cassella Farbwerke Mainkur A.-G., *Ethers of 7-Hydroxycoumarins*, Belgian Patent No. 621,327, 11 Feb., 1963; Ger. Appl. 12 Aug. and 9. Nov., 1961, and 26 Jan., 1952 [Chem. Abstr., **59**, 11438c (1963)].
4. D. Holho and E. Boschetti, *LIPHA, Fr. Pat. 1,310,535*, 30 Nov., 1962; Appl 28 July, 1961. [*Chem. Abstr.*, **58**, 12517f (1963)].
5. K. Meguro and H. Tawada (Takeda Chemical Industries, Ltd), *PCT Int. Appl. WO 91 12,239* (Cl. C 07 D 311/08) Aug. 22, 1991; Jpn Pat. Appl. 90/29,240, Feb. 10, 1990 (*Chem. Abstr.*, **115**, 279815f (1991).
6. S. Shah, R. Vyas, and R. H. Mehta, *J. Indian Chem. Soc.*, **68**, No. 7, 411 (1991).
7. P. Desai and R. Mehta, *Indian J. Heterocycl. Chem.*, **5**, No. 4, 319 (1996).
8. L. L. Woods and J. Sapp, *J. Org. Chem.*, **27**, No. 10, 3703 (1962).
9. G. W. Anderson, J. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **85**, No. 19, 3039 (1963).
10. A. A. Gershkovich and V. K. Kibirev, *The Chemical Synthesis of Peptides* [in Russian], Naukova Dumka, Kiev (1992), p. 71.
11. T. V. Shokol, A. S. Ogorodniichuk, V. V. Shilin, V. B. Milevskaya, and V. P. Khilya, *Khim. Geterotsikl. Soedin.*, No. 4, 482 (1998).