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SYNTHESIS AND SPECTRAL PROPERTIES OF 3-FURYL-4-HYDROXYCOUMARINS

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3-(5-Ethoxycarbonyl-2-furyl)-4-hydroxy-6-R-7-methoxycoumarins and their 4-acyl derivatives have been synthesized. Their ^1H and ^{13}C NMR and IR spectra, and also their absorption and fluorescence spectra, have been studied. It has been shown that some of the substances synthesized can be used as fluorescent probes in the study of membranes and of protein preparations.

Together with various chromone derivatives, their structural isomers — coumarins — are widely distributed in the plant world. Great interest in the study of coumarin compounds appeared with the detection of an anticoagulant action of 4-hydroxycoumarin and it increased in the following decades in connection with the discovery of many coumarin derivatives having hypothermal, anthelmintic, hypotensive, antimicrobial, diuretic, insecticidal, and vasodilatory actions and the possibility of their use as effective drugs (dicoumarol, novobiocin, nafarin, etilendikumarin, propenan, nitrofarin, ksilokumarol, and many others) in various diseases.

Coumarin compounds with aryl substituents in position 3 are rarely encountered among substances of plant origin. Coumarins containing heterocyclic residues in this position are not found in nature. They can be obtained synthetically.

Recently, intensive investigations on the synthesis and study of the chemical and biological properties of heterocyclic coumarin derivatives have been pursued in many laboratories [1], and compounds with interesting biological activities have already been found among them.

In order to study their chemical and biological properties and to find new highly effective drugs among heterocyclic coumarin derivatives, we have synthesized derivatives of 4-hydroxycoumarin by the scheme given below. The synthesis was achieved under the conditions of the Claisen condensation with sodium *tert*-butanolate as catalyst. The 3-furyl-4-hydroxycoumarins obtained consisted of yellow crystalline substances readily soluble in the majority of organic solvents. They possessed a powerful characteristic fluorescence both in solutions and in the crystalline state.

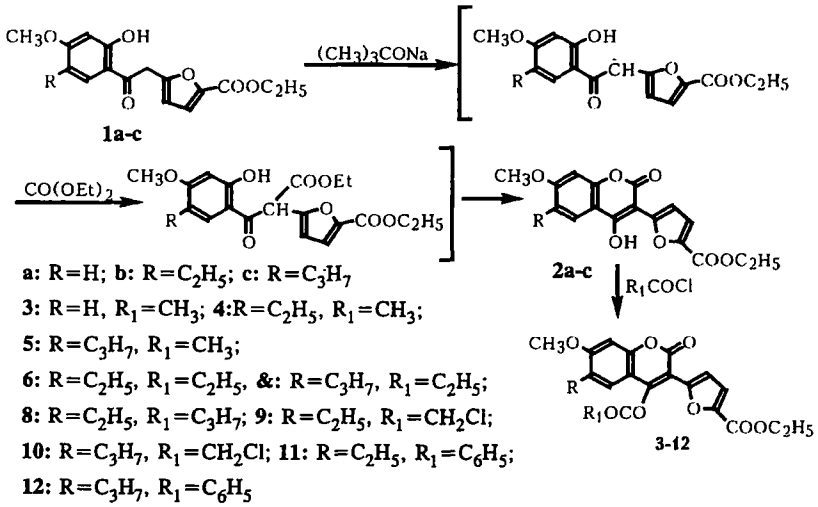

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TABLE 1. Chemical Shifts in the PMR Spectra of Acyl Derivatives of 3-Furyl-4-hydroxycoumarins (**3-12**)

Compound	mp, °C	Yield, %	Coumarin protons					Furan protons		
			OR-4	H-5	R_1-6	CH_3O-7	H-8	H-3	H-4	$COOC_2H_5$-5
3	202	95	2.74	7.46	6.86	3.91	6.79	7.18	7.43	4.42; 1.44
4	203	87	2.78	7.30	2.75; 1.26	3.98	6.83	7.27	7.51	4.47; 1.45
5	107	98	2.77	7.32	2.68; 1.67; 0.98	1.98	6.85	7.27	7.53	4.46; 1.44
6	108	90	3.18; 1.40	7.31	2.75; 1.26	3.99	6.87	7.27	7.50	4.47; 1.45
7	151	87	3.20; 1.41	7.30	2.71; 1.68; 1.00	4.00	6.86	7.27	7.51	4.48; 1.45
8	171	91	3.10; 1.91; 1.13	7.24	2.70; 1.24	3.95	6.76	7.21	7.44	4.42; 1.43
9	200	70	4.97	7.34	2.74; 1.27	3.99	6.82	7.23	7.51	4.47; 1.47
10	185	65	4.95	7.31	2.70; 1.65; 1.00	3.99	6.81	7.19	7.48	4.45; 1.46
11	137	69	8.37; 7.66	7.33	2.69; 1.03	3.98	6.88	7.27	7.48	3.98; 1.16
12	178	67	8.32; 7.71	7.27	2.64; 1.47; 0.92	3.98	6.87	7.22	7.43	3.99; 1.03

TABLE 2. Spectral Characteristics of 3-Furyl-4-hydroxycoumarins and Their Acyl Derivatives

Compound	UV spectrum, λ_{max}, nm (log ε)	IR spectrum, ν, cm^{-1}			
		coumarin $\nu_{C=O}$	ester $\nu_{C=O}$	Skeletal vibrations of the heterocycle	ν_{OH}
2a	357 (4.23)	1700	1715	1620, 1610, 1560, 1520	3390
2b	362 (4.36)	1710	1725	1625, 1570, 1515	3410
2c	362 (4.33)	1700	1720	1620, 1595, 1575, 1520	3400
3	362 (4.47)	1710	1770, 1730	1630, 1610, 1560, 1520	–
4	383 (4.47)	1715	1770, 1730	1625, 1585, 1560, 1510	–
5	383 (4.46)	1710	1770, 1725	1625, 1585, 1665, 1510	–
6	383 (4.47)	1710	1765, 1730	1625, 1560, 1505	–
7	382 (4.48)	1710	1770, 1722	1622, 1562, 1505	–
8	383 (4.47)	1710	1780, 1725	1622, 1582, 1565, 1510	–
9	377 (4.37)	1710	1775, 1730	1620, 1565, 1505	–
10	382 (4.38)	1715	1780, 1730	1625, 1560, 1510	–
11	384 (4.46)	1705	1760, 1722	1620, 1610, 1570, 1505	–
12	384 (4.38)	1700	1765, 1725	1630, 1610, 1485, 1510	–

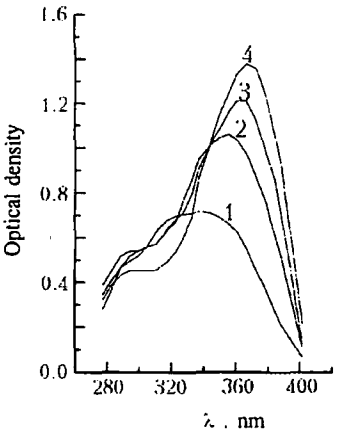

Fig. 1. Absorption spectra of the 3-furylcoumarin (**2b**) in various solvents: *1*) water; *2*) methanol; *3*) ethanol; *4*) isopropanol; ε is the optical density.

The most typical property of products (**2a-c**) is their capacity for undergoing acylation. The acyl derivatives (**3-12**) were formed in good yields by brief boiling with anhydrides and halides of aliphatic and aromatic acids. We confirmed the structures of the 3-(2-furyl)coumarins and their acyl derivatives that had been synthesized not only by elementary analysis but also by their NMR spectra on ^1H nuclei (Table 1) and on ^{13}C nuclei (see the Experimental part) and by their UV and IR spectra (Table 2).

The main absorption band in the UV spectra of the 3-(2-furyl)coumarins was present in the narrow range of 357-363 nm. In the IR spectra there were characteristic absorption bands corresponding to the stretching vibrations of a hydroxy group (3390-3460 cm^{-1}), of the carbonyl group of a coumarin (1660-1720 cm^{-1}), of the ester group of the furan fragment (1720-1730 cm^{-1}), and of an allyl group (1765-1780 cm^{-1}). In the regions of the PMR spectra characteristic for them there were signals corresponding to all the protons of the compounds studied.

In the carbon spectra the most characteristic were the chemical shifts of the carbon atoms of the γ-pyrone ring, which differed in absolute magnitude from the corresponding chemical shifts of the isomeric 3-(2-furyl-2-hydroxychromones [2]. The 2-C and 4-C carbon atoms absorbed in the narrow intervals of 160.8-162.5 and 152.3-154.4 ppm, respectively. The C-3 carbon atom in the 3-furylcoumarins absorbed at 92.5-93.7 ppm, in contrast to that in unsubstituted coumarin (115.6 ppm). The chemical shifts of the other carbon atoms of the coumarin nucleus absorbed in the same regions as in the natural 7-hydroxycoumarins [2].

At the present time intense searches are being made for compounds capable of acting as fluorescent probes in the investigation of the structure and conformational rearrangements of proteins and membranes [3-5]. In this connection, great interest is presented by analogs of natural biologically active substances possessing characteristic fluorescence that are sensitive

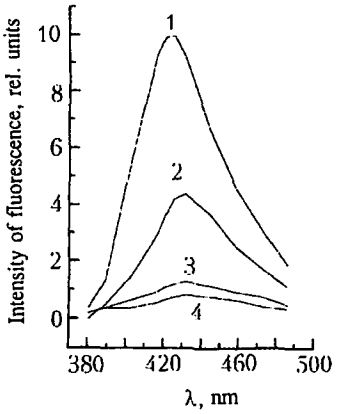

Fig. 2. Fluorescence spectra of the 3-furylcoumarin (**2b**) in various solvents: *1*) water; *2*) methanol; *3*) ethanol; *4*) isopropanol. Concentration of the 3-furylcoumarin 10^{-5} M; λ_{exc} = 365 nm.

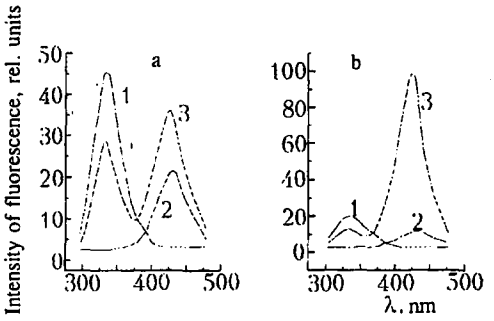

Fig. 3. Fluorescence spectra: *a*: *1*) myosin, 0.8 mg/ml; *2*) the 3-furylcoumarin (**2b**), $c = 10^{-5}$ M; *3*) the complex of myosin with the 3-furylcoumarin (**2b**); *b*: *1*) HSA, 0.69 mg/ml; *2*) the 3-furylcoumarin (**2b**), $c = 10^{-5}$ M; *3*) the complex of HSA with (**2b**). Experimental conditions: pH 7.5, t = 20°C, λ_{exc} = 296 nm.

to a change in the conditions of the external medium. Coumarins form one of the most promising classes of compounds possessing such properties. Among the coumarin derivatives that we have synthesized, the best luminescent properties are possessed by compound (**2b**). We have therefore studied the dependence of the spectral properties of this compound on the nature of the microenvironment.

In a UV spectrum the absorption maximum and the molar extinction coefficient ε depend on the polarity of the medium. It can be seen from Fig. 1 that with an increase in the hydrophobicity of the environment the optical density of the 3-furylcoumarin (**2b**) increased and its absorption maximum shifted in the long-wave direction. The absorption maxima (λ, nm, and ε, $mole^{-1}cm^{-1}$) in various solvents were as follows: water: λ 337.6, ε $1.38 \cdot 10^4$; methanol: λ 349.5, ε $2.09 \cdot 10^4$; ethanol: λ 358.6, ε $2.42 \cdot 10^4$; and isopropanol: λ 362.8, ε $2.78 \cdot 10^4$. In isopropyl alcohol the absorption maximum shifted in the long-wave direction by more than 20 nm as compared with the spectrum in water, while ε doubled. In the light of such a pronounced difference in the absorption spectra according to the hydrophobicity of the environment it may be assumed that a study of UV spectra on the binding of compound (**2b**) with proteins could give useful information on the structure of these complexes.

Measurements of the fluorescence spectra showed that their maximum shifted in the short-wave direction with an increase in the hydrophobicity of the environment (Fig. 2). An increase in the intensity of fluorescence was observed at the same time. The quantum yield, φ, was determined by the method of Parker and Rees [6]. As the standard we chose 1-anilinonaphthalene-8-sulfonate (ANS) for which the quantum yields in various solvents are known. The fluorescence parameters of compound (**2b**) are as follows: in water: λ_{max} 440 nm, φ 0.10; methanol: λ_{max} 432 nm, φ 0.16; ethanol: λ_{max} 429 nm, φ

0.38; isopropanol, λ_{max} 425 nm. These results indicate that the fluorescent parameters are fairly sensitive to the microenvironment, which will permit compound (**2b**) to be used as a fluorescent probe for studying proteins and membranes.

We have studied the interaction of compound (**2b**) with myosin and human serum albumin (HSA) (Fig. 3). The fluorescence maximum of the complex of HSA with the 3-furylcoumarin was found in a shorter-wave region than for the analogous complex with myosin. The intensity of fluorescence in the HSA complex is twice as high as in the myosin complex. It can be seen from Fig. 3 that on excitation with λ_{exc} 296 nm a decrease in the intensity of the ultraviolet fluorescence of proteins and an increase in the intensity of fluorescence of the 3-furylcoumarin were observed. This shows a transfer of excitation energy from the tryptophan residues to the 3-furylcoumarin bound to a protein.

EXPERIMENTAL

The absorption and fluorescence spectra were measured on a Beckman V-25 spectrophotometer, the NMR spectra on WP 100 SY and CXP-200 spectrometers with working frequencies of 100 and 200 MHz, respectively, and the IR spectra on a UR-20 spectrometer.

The course of the reactions and the purity of the compounds obtained were monitored by TLC on Silufol UV-254 plates in the benzene–ethanol (9:1) system. The elementary analyses of all the compounds corresponded to the calculated figures.

3-(5-Ethoxycarbonyl-2-furyl)-6-ethyl-4-hydroxy-7-methoxycoumarin (2b). With stirring, at room temperature in an atmosphere of argon, 62.98 g (656 mmole) of sodium *tert*-butanolate was added to a solution of 21.8 g (65.6 mmole) of α-(5-ethoxycarbonyl-2-furyl)-5-ethyl-2-hydroxy-4-methoxyacetophenone [7] in 197 ml of dry diethyl carbonate. The reaction mixture was stirred at 125-135°C for 8 h and then the solvent was distilled off in vacuum. The dry residue was dissolved in 100 ml of iced water, the solution was acidified with dilute hydrochloric acid to pH 2-3, and the precipitate was filtered off and was carefully washed with water to neutrality. The yield was 22.6 g (96%). After crystallization from ethanol, 18.4 g (78%) of pure product was obtained. Colorless needles with mp 175-176°C. Compounds (**2a**) and (**2c**) were obtained analogously.

PMR (100 MHz $CDCl_3$): 9.71 (1H, s, H-4), 7.71 (1H, s, H-5), 2.66 (2H, q, CH_2-6), 1.23 (3H, t, CH_3-6). 3.90 (3H, s, OCH_3-7), 6.73 (1H, s, H-8), 7.30 (2H, m, H-3′, 4′), 4.39 (2H, q, $COO\underline{CH_2}CH_3$-5′), 1.41 (3H, t, $COOCH_2\underline{CH_3}$-5′). Here and below, underlining indicates the atoms of the furan nucleus.

^{13}C NMR (50 MHz, $CDCl_3$): 161.08 (C-2), 93.56 (C-3), 152.90 (C-4), 122.87 (C-5), 128.73 (C-6), 26.06 (C_6-$\underline{CH_2}CH_3$), 13.50 (C_6-$CH_2\underline{CH_3}$), 163.23 (C-7), 56.05 (C_7-OCH_3), 98.78 (C-8), 161.3 (C-9), 107.81 (C-10), 142.5 (C′-2), 112.8 (C′-3), 119.06 (C′-4), 150.56 (C′-5), 157.8 (C'_5-$\underline{C}OOCH_2CH_3$), 60.14 (C'_5-$COO\underline{CH_2}CH_3$), 14.05 (C'_5-$COOCH_2\underline{CH_3}$).

3-(5-Ethoxycarbonyl-2-furyl)-4-hydroxy-7-methoxycoumarin (2a). Yield 89%, mp 193°C.

PMR (100 MHz, $CDCl_3$): 9.52 (1H, s, H-4), 7.93 (1H, s, H-5), 6.92 (1H, s, H-6), 3.90 (3H, s, OCH_3-7), 6.82 (1H, s, H-8), 7.31 (1H, d, H-3′), 7.34 (1H, d, H-4′), 4.40 (2H, q, -$COO\underline{CH_2}CH_3$-5′), 1.41 (3H, t, $COOCH_2\underline{CH_3}$-5′).

^{13}C NMR (50 MHz, $CDCl_3$): 160.79 (C-2), 92.46 (C-3), 154.40 (C-4), 125.66 (C-5), 111.44 (C-6), 166.56 (C-7), 55.77 (C_7-OCH_3), 100.08 (C-8), 162.85 (C-9), 111.35 (C-10), 141.10 (C′-2), 111.11 (C′-3), 119.40 (C′-4), 153.50 (C′-5), 158.26 (C'_5-$\underline{C}OOCH_2CH_3$), 60.16 (C'_5-$COO\underline{CH_2}CH_3$), 14.24 (C'_5-$COOCH_2\underline{CH_3}$).

3-(5-Ethoxycarbonyl-2-furyl)-4-hydroxy-7-methoxy-6-propylcoumarin (2c). Yield 61%, mp 158°C.

PMR (100 MHz, $CDCl_3$): 9.71 (1H, s, H-4), 7.69 (1H, s, H-5), 2.61 (2H, t, $\underline{CH_2}CH_2CH_3$-C_6), 1.63 (2H, q, $CH_2\underline{CH_2}CH_3$-C_6), 0.96 (3H, t, $CH_2CH_2\underline{CH_3}$-C_6), 3.89 (3H, s, OCH_3-7), 6.73 (1H, s, H-8), 7.30 (2H, m, H-3′, 4′), 4.39 (2H, q, -$COO\underline{CH_2}CH_3$-5′), 1.41 (3H, t, $COOCH_2\underline{CH_3}$-5′).

^{13}C NMR (50 MHz, $CDCl_3$): 161.03 (C-2), 93.76 (C-3), 153.06 (C-4), 123.84 (C-5), 127.34 (C-6), 31.24 (C_6-$\underline{CH_2}CH_2CH_3$), 22.12 (C_6-$CH_2\underline{CH_2}CH_3$), 13.73 (C_6-$CH_2CH_2\underline{CH_3}$), 163.42 (C-7), 56.19 (C_7-OCH_3), 98.45 (C-8), 161.51 (C-9), 107.97 (C-10), 142.65 (C′-2), 112.90 (C′-3), 119.27 (C′-4), 150.96 (C′-5), 158.02 (C'_5-$\underline{C}OOCH_2CH_3$), 60.36 (C'_5-$COOCH_2CH_3$), 14.30 (C'_5-$COOCH_2\underline{CH_3}$).

4-Acetoxy-3-(5-ethoxycarbonyl-2-furyl)-6-ethyl-7-methoxycoumarin (4). A solution of 0.716 g (2 mmole) of the coumarin (**2b**) in 12 ml of acetic anhydride was boiled for 10 min and it was then left for several hours at room temperature and for 2 h in a refrigerator. The resulting precipitate was filtered off and was washed on the filter with cold alcohol. Yield 0.7 g (87%). Light yellow needles with mp 203°C (from a mixture of ethyl acetate and hexane).

Compounds (**7-12**) were obtained analogously. The yields of the acyl derivatives amounted to 65-95%.

REFERENCES

1. R. Subba, Khim. Geterotsikl. Soedin., No. 3, 291 (1977).
2. E. Breitmeyer and W. Voelter, ^{13}C NMR Spectroscopy: Methods and Applications, 2nd ed., Verlag Chemie, Weinheim–New York (1978).
3. G. Weber and L. B. Young, J. Biol. Chem., **239**, No. 5, 1415 (1964).
4. L. Stryer, Science, **162**, No. 3853, 526 (1968).
5. Yu. A. Vladimirov and G. E. Dobrentsov, Fluorescent Probes in the Investigation of Biological Membranes [in Russian], Nauka, Moscow (1980).
6. C. Parker, The Photoluminescence of Solutions, Elsevier, New York (1968)
7. V. P. Khilya, K. Uteniyazov, K. Ismailova, and A. Aitmambetov, Vestn. Karakalpak. Fil. Akad. Nauk RUz, No. 4, 58 (1984).