SYNTHESIS OF METHYL ESTERS OF N^{α}-ARYLSULFONYL DERIVATIVES OF l-ARGININE — SUBSTRATES OF TRYPSIN AND THROMBIN

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Currently, synthetic substrates are widely used for the study of the specificity and the mechanism of the action of various enzymes. For example, this method is used for investigating trypsin and thrombin.

Thrombin is a trypsin-like protease which induces the transformation of fibrinogen into fibrin monomer by the specific hydrolysis of the Arg—Gly bond of fibrinogen. In order to investigate thrombin, we have synthesized various substrates, which are oligopeptides [1] or methyl esters of arginine [2]. The kinetics of the hydrolysis of these substrates have not enabled us to determine the nature of the high specificity of thrombin.

It appeared of interest to synthesize a number of substrates containing substituents of different electronic natures and volumes in the immediate proximity to the bond undergoing hydrolysis.

A process of obtaining a number of methyl esters of N^{α}-arylsulfonyl derivatives of l-arginine — substrates of trypsin and thrombin — has been described previously [3, 4]. The compounds synthesized were used for studying the specificity of the action of trypsin [4, 5].

The present communication gives the results of the synthesis of other representatives of this class of compounds; in particular, esters with such substituents as chlorine and carboxymethyl and sec-butyl groups are described.

The reaction of arylsulfonyl chlorides and l-arginine in an alkaline medium at room temperature led to the formation of N^{α}-arylsulfonyl derivatives of l-arginine. The transformation of these compounds into the corresponding methyl esters was readily performed by the method of Brenner and Huber in methanol with thionyl chloride [6]. Table 1 gives the formulas and some properties of the compounds synthesized, including those which had been synthesized previously [3, 4]. Judging from the results of chromatography and electrophoresis at pH 6.5 on paper, we obtained these compounds in a purer form.

The UV spectra of the compounds synthesized were investigated, these being necessary for studying the kinetics of their hydrolysis by thrombin. As can be seen from Fig. 1, the UV absorption curve of the methyl ester of tosylarginine has three maxima: at 232, 268, and 275 nm. Compounds containing a 4-methyl, 4-ethyl, or 4-chloro substituent in the aryl nucleus possess similar spectra. When a methoxy group was introduced into position 4 of the benzene ring, the peak at 268 nm disappeared, and in place of the maximum at 275 nm there was only an inflection; at the same time, the short-wave peak underwent a bathochromic shift by 12 nm. The 2,4-dinitro derivative showed a completely different spectrum.

Figure 2 shows the optical rotatory dispersion curves of some of the arylsulfonylarginines and their methyl esters recorded so that the optical activities of these compounds can be used in the study of their hydrolysis by enzymes. However, the small difference in the ORD curves of the arylsulfonylarginines and the corresponding methyl esters interferes with the possibility of their utilization.

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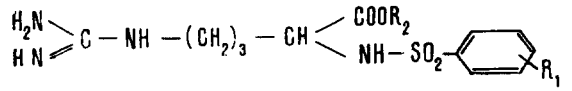
TABLE 1. N^{α}-Arylsulfonyl Derivatives of l-Arginine and Their Methyl Esters



No.	R_1	R_2	Yield, %	mp, °C	Solvent for crystallization	Optical activity $[\alpha]_D^{25}$, deg	Optical activity solvent
1	4-CH_3	H	68	256—257	Water	−15,5	6 N HCl
2	4-C_2H_5	H	65	228	Water	−13,1	6 N HCl
3	4-iso-C_3H_7	H	39	223—225	Ethanol	—	6 N HCl
4	4-sec-C_4H_9	H	43	148—151	Ethanol	−4,0	6 N HCl
5	4-OCH_3	H	70	228—230	Water	−13,7	6 N HCl
6	4-Cl	H	60	240	Water	−10,2	6 N HCl
7	2-NO_2	H	35	169	Methanol	−31,3	6 N HCl
8	2,4-di-NO_2	H	45	242	Water	−72,0	6 N HCl
9	3-COOH	H	60	237—239	Water	−4,5	6 N HCl
10	4-CH_3	CH_3	86	147—149	Cyclohexanone	−14,0	Water
11	4-C_2H_5	CH_3	75	138—141	Cyclohexanone	−11,0	Water
12	4-iso-C_3H_7	CH_3	70	137—139	Methanol + ether	—	Water
13	4-sec-C_4H_9	CH_3	88	124—127	Methyl ethyl ketone	−4,0	Water
14	4-OCH_3	CH_3	87	146	Cyclohexanone	−12,5	Water
15	4-Cl	CH_3	85	139—142	Methyl ethyl ketone	−12,0	Water
16	2-NO_2	CH_3	65	133	Methanol + ether	−28,1	Acetic acid
17	2,4-di-NO_2	CH_3	86	191—192	Methanol + ether	−58,0	Acetic acid
18	3-$COOCH_3$	CH_3	84	95—98	Methyl ethyl ketone	−9,0	Water

Note: The specific rotations were determined at a concentration of 1% in a 1-cm cell in all cases. Compounds (10-18) were obtained in the form of hydrochlorides.

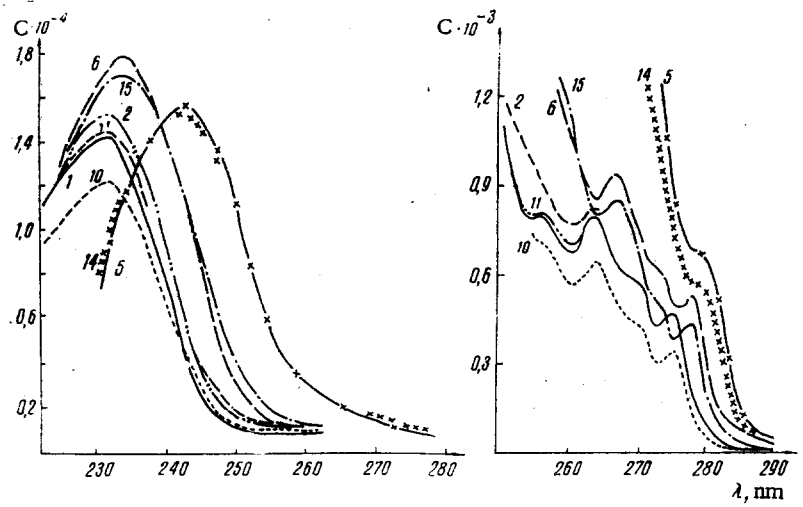


Fig. 1. UV spectra of N^{α}-arylsulfonyl derivatives of l-arginine and their methyl esters (the numbers on the curves correspond to the numbers of the compounds in Table 1).

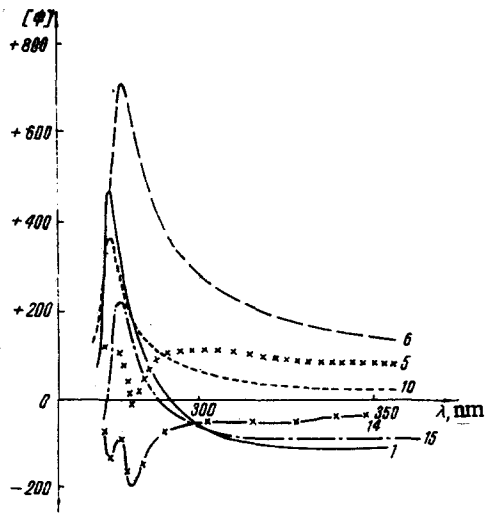


Fig. 2. ORD spectra of the N^{α}-arylsulfonyl derivatives of l-arginine and their methyl esters (the numbers on the curves correspond to the numbers of the compounds in Table 1).

EXPERIMENTAL

The melting points were determined in open capillaries.

Chromatography and electrophoresis were performed on paper from the Voikov Leningrad Mill. The buffer solution for electrophoresis at pH 6.5 was prepared by diluting a mixture of 100 ml of pyridine and 4 ml of acetic acid to 1000 ml with water. For paper chromatography, the butanol–acetic acid–water (4:1:5) system was used. The analyses of all the compounds corresponded to the calculated figures.

The UV spectra were recorded on a Specord UV-Vis recording spectrophotometer (C. Zeiss, Jena). The specific rotations and ORD curves were determined on a Spektropol-1 spectropolarimeter (Fika, Paris).

The arylsulfonyl chlorides were synthesized by the method of Huntress and Autenrieth [7]. An exception was 2,4-dinitrobenzenesulfonyl chloride, which was obtained by Lunt's method [8], and 3-carboxybenzenesulfonyl chloride [9]. The sulfonyl chlorides that were solids were purified in the following way: 10 g of a sulfonyl chloride was dissolved in the minimum amount of chloroform, five volumes of n-hexane was added to precipitate the impurities, and the solution was filtered. Then it was treated with activated carbon, filtered again, evaporated in a rotary evaporator under reduced pressure to small volume, and left overnight in the refrigerator. The crystals that deposited were filtered off, washed with n-hexane, and dried in a vacuum desiccator. Yield 70%. The sulfonyl chlorides that were liquids were used without additional purification.

Synthesis of the N^{α}-Arylsulfonyl Derivatives of l-Arginine. With vigorous stirring, a solution of 35 mmole of an arylsulfonyl chloride in 40 ml of ether was added dropwise to a solution of 30 mmole of l-arginine in 40 ml of water, the pH of the solution being kept between 9 and 10 by the addition of a 30% solution of caustic soda. After 2-3 h, stirring was stopped, and the precipitate was filtered off, washed with water and ether, and dried in a vacuum desiccator over phosphorus pentoxide. After recrystallization from a suitable solvent (see Table 1), compounds were obtained which were analytically pure, and were homogeneous according to electrophoresis and chromatography.

Hydrochlorides of the Methyl Esters of N^{α}-Arylsulfonyl Derivatives of l-Arginine. At –5 to –10°C with vigorous stirring, 6.6-10.1 mmole of thionyl chloride was added dropwise to a suspension of 6-10 mmole of an arylsulfonylarginine in 5-10 ml of absolute methanol. Then the temperature was slowly raised to 40°C and the solution was kept at 2 h, after which it was filtered and was left in the refrigerator for 12 h. The subsequent working up depended on the solubility of the product in methanol. If crystals deposited, they were filtered off, washed with cold methanol and ether, and dried in a vacuum desiccator over alkali. If no precipitate was formed, the solvent was evaporated in a rotary evaporator, and the residue was triturated with ether, dried over alkali, and then recrystallized from a suitable solvent (see Table 1). According to electrophoresis and chromatography, the substrates obtained were homogeneous.

SUMMARY

1. For a comparative study of the esterase activities of trypsin and thrombin on synthetic substrates we have obtained a number of N^{α}-arylsulfonyl derivatives of l-arginine and their methyl esters containing substituents with different electronic natures and volumes in the benzene ring.

2. The UV spectra and the ORD spectra of the compounds obtained have been studied.

LITERATURE CITED

1. R. H. Andreatta, R. K. H. Liem, and H. A. Scheraga, Proc. Nat. Acad. Sci., 68, 253 (1971).
2. R. K. H. Liem, R. H. Andreatta, and H. A. Scheraga, Arch. Biochem. Biophys., 147, 201 (1971).
3. S. B. Serebryanyi, L. G. Yurganova, and V. M. Neplyuev, Ukr. Khim. Zh., 27, 365 (1961).
4. S. B. Serebryanyi, L. G. Yurganova, and N. E. Fedorova, Biokhimiya, 33, 3 (1968).
5. L. G. Yurganova and N. E. Fedorova, Biokhimiya, 35, 493 (1970).
6. M. Brenner and W. Huber, Helv. Chim. Acta, 36, 1109 (1953).
7. E. H. Huntress and J. S. Autenrieth, J. Amer. Chem. Soc., 63, 3446 (1941).
8. E. Lunt, J. Appl. Chem., 7, 446 (1957).
9. S. Smiles and J. Stewart, J. Chem. Soc., 1792 (1921).