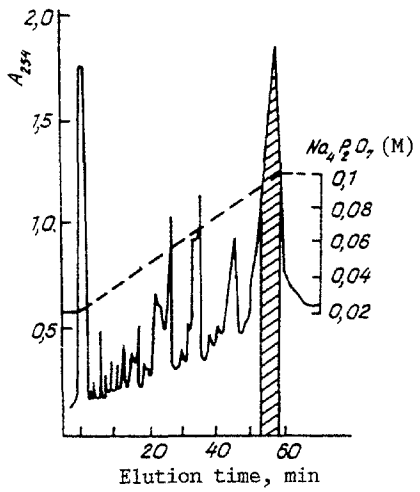


Fig. 3. Elution profile of the isolation by anion-exchange chromatography of a 70-membered oligodeoxyribonucleotide from a reaction mixture. Eluent: 0.02-0.1 M concentration gradient of $Na_4P_2O_7$ in 0.02 M KH_2PO_4, pH 6.5, in 30% acetonitrile, t = 55°C, rate of flow 1.5 ml/min.

The authors thank V. A. Ryabinin and N. N. Karpyshev for providing the synthetic oligonucleotides.

LITERATURE CITED

1. B. Karger, in: Modern Practice of Liquid Chromatography, J. J. Kirkland (ed.), Wiley-Interscience (1974).

SYNTHESIS OF VERONGIAQUINOL AND RELATED COMPOUNDS AND STUDY OF THEIR INHIBITING ACTION ON RAT BRAIN Na^+,K^+-ATPase

B. A. Gorshkov, O. P. Shestak, I. A. Gorshkova, T. N. Makar'eva, V. L. Novikov, and V. A. Stonik

UDC 577.352.45

The 4-acetamido-2,6-dibromo-4-hydroxycyclohexa-2,5-dienone (VII) (verongiaquinol) isolated from sponges of the family Aplysinidae is a powerful inhibitor of Na^+,K^+-ATPase, interacting specifically with the sulfhydryl groups of the enzyme [1]. In order to establish a structure-activity relationship, we have effected a total synthesis of verongiaquinol (VII) and a number of compounds related to it, (I-VI) and (VII-X), starting from commercial p-hydroxyphenylacetic acid, and, by the method described in [2], have studied their action in relation to Na^+,K^+-ATPase isolated from rat brain by the method of [3]. The bromination and chlorination (Br_2/HOAc and $SOCl_2$, respectively) of this acid led to the 3,5-dibromo- and 3,5-dichloro derivatives the esterification of which (CH_3OH and C_2H_5OH) gave the corresponding Me and Et esters. The corresponding amides were obtained by the aminolysis of the esters (NH_3, MeOH, NaCN). Oxidation of the 3,5-dibromo- and 3,5-dichlorophenylacetic acids and their esters and amides with concentrated HNO_3 gave the known compounds (I), (V), (VII) [4],

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 149-151, January-February, 1992. Original article submitted February 27, 1991.

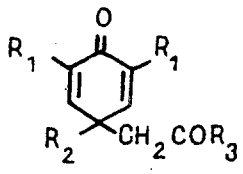
 0009-3130/92/2801-0128$12.50

TABLE 1

Compound	I_{50}*	Compound	I_{50}*
I	$3{,}8\times10^{-5}$M	VI	4.2×10^{-7}M
II	$9{,}0\times10^{-5}$M	VII	$4{,}5\times10^{-7}$M
III	$2{,}8\times10^{-7}$M	VIII	8.0×10^{-7}M
IV	$2{,}0\times10^{-7}$M	IX	$1{,}3\times10^{-4}$M
V	$2{,}7\times10^{-7}$M	X	$1{,}3\times10^{-5}$M

*Concentration of inhibitor causing 50% inhibition of enzymatic activity.

and (VIII) [5] and the previously undescribed (II)-(IV) and (VI) with overall yields of 31, 25, 67, 78, 68, 74, 20, and 35%, respectively. Oxidation of the ethyl ester and the amide of 3,5-dibromophenylacetic acid with $Pb(OAc)_4$ in HOAc gave the previously undescribed (IX) and the known (X) [6] with overall yields of 22 and 34%, respectively.



I. R_1=Br, R_2=R_3=OH; II. R_1=Cl, R_2=R_3=OH; III. R_1=Br, R_2=OH, R_3=OMe; IV. R_1=Cl, R_2=OH, R_3=OMe; V. R_1=Br, R_2=OH, R_3=OEt; VI. R_1=Cl, R_2=OH, R_3=OEt; VII. R_1=Br; R_2=OH, R_3=NH_2; VIII. R_1=Cl, R_2=OH, R_3=NH_2; IX. R_1=Br, R_2=OAc, R_3=OEt; X. R_1=Br, R_2=OAc, R_3=NH_2

The results of a study of the relationship between the structures of the compounds and their biological activities are given in Table 1. The table shows that in the series of compounds studied the nature of the radicals R_2 and R_3 exerts a powerful influence on the manifestation of inhibiting activity. Thus, among compounds (I-VIII), where R_2 = OH, the least active were (I) and (II), in which R_3 = OH, while in the corresponding pairs of compounds (III-VIII) differing by the values of R_1 no great difference in activity was observed, although the bromine- and chlorine-containing Me esters (III) and (IV) proved to be the most active. The synthetic (VII) did not differ in activity from the natural compound. When R_2 = OAc, in (IX) and (X), there was a sharp fall in activity as compared with the corresponding analogues (V) and (VII). A study of the features of the inhibiting effects of (III-VIII) showed that they were similar to that of the natural dienone (VII) [1]. Thus, inhibition was irreversible (the activity of the enzyme that had been treated with the inhibitors was not restored on 10- to 50-fold dilution) and depended on the concentration of the inhibitors (complete inhibition of enzymatic activity was observed at an inhibitor concentration of $1.0\cdot10^{-4}$ M). Inhibition also depended on the time of preincubation of the enzyme with the inhibitors: The activity of the Na^+,K^+-ATPase fell sharply in the course of 10 min, but a constant level of the inhibition was reached after 50-60 min. The Na^+,K^+-ATPase substrate (ATP) and thiol compounds (cysteine and dithiothreitol) protected the enzyme from the action of the inhibitors. Moreover, the synthetic (VII), like the natural verongiaquinol [1], caused a concentration-dependent decrease in the number of free reactive sulfhydryl groups of the enzyme, with a simultaneous fall in enzymatic activity. The results of the investigation show that verongiaquinol analogues are of interest as highly effective inhibitors of Na^+,K^+-ATPase.

LITERATURE CITED

1. I. A. Gorshkova, B. A. Gorshkov, T. N. Makar'eva, V. A. Stonik, and M. V. Zamaraevsa, Izv. Akad. Nauk SSSR, Ser. Biol., No. 5, 676 (1988).
2. V. A. Gorshkov, I. A. Gorshkova, T. N. Makarieva, and V. A. Stonik, Toxicon, 20, 1092 (1982).
3. I. Klodos, P. Ottolenghi, and A. Boldyrev, Anal. Biochem., 67, 397 (1975).
4. G. M. Sharma, B. Vig, and P. R. Burkholder, J. Org. Chem., 35, 2823 (1970).
5. M. D'Ambroisio, A. Guerriero, and F. Pietra, Helv. Chim. Acta, 67, 1484 (1984).
6. G. M. Sharma and P. R. Burkholder, Tetrahedron Lett., No. 42, 4147 (1967).