# SYNTHESIS OF COMPLEX ESTERS OF MYOINOSITOL AND ANALYSIS OF THEIR ANTIISCHEMIC ACTIVITY

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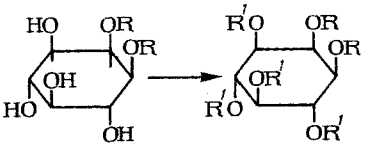
Derivatives of myoinositol (I) have a wide spectrum of pharmacological activity. A mixture of butanol and myoinositol is used for the treatment of post-traumatic shock [3]. The preparations Phytin, Hephytin, and Phytoferrolactol are mixtures of the calcium—magnesium salts of myoinositol hexaphosphate, and stimulate hematopoiesis, accelerate the growth and development of bone tissue, and improve nervous system function; they are used in the treatment of vascular hypotonia, neurasthenia, and other diseases [1]. There have recently been many reports on the synthesis and study of the biological activity of myoinositol 1,4,5-triphosphate, which plays an important role in the cascade of reactions involved in the transmission and amplification of extracellular signals [11]. Thus, the number and positions of identical functional groups in the myoinositol molecule have significant effects on the biological activity of the derivatives concerned. There is, therefore, potential to be found in searching for pharmacologically active substances among myoinositol derivatives with different numbers of residues in different positions of the molecule. In addition, it would be interesting to prepare myoinositol derivatives containing residues of different substances with different pharmacological activities in a single molecule. In creating this type of complex compound, it is important to consider the presence of transport-mediating substances in the body, as well as their membranotropic properties. Thus, the possibility of creating new pharmacologically active compounds from myoinositol with improved spectra of therapeutic actions is far from having been exhausted.

We report here the synthesis and antiischemic activity of complex esters of myoinositol and ω-chlorocarbonic acids and, in addition, of myoinositol tetra- and hexanicotinates.

The hydroxyl groups in positions 1 and 2 of myoinositol were temporarily protected with isopropylidene (IP) and cyclohexylidene (CH) derivatives (II) and (III) [10, 13].



I-III IV-XI

R=H (I, VII, IX), NC (VIII); R+R= IP (II, IV, VI, X), CH (III, V, XI); $R^1$=$COCH_2Cl$ (IV, V), $CO(CH_2)_2CH_2Cl$ (VI, VII), NC (VIII—XI).

Ketals (II) and (III) were reacted with the chloroanhydride of chloroacetic acid [9] at 0-5°C in chloroform in the presence of pyridine, to yield 1,2-O,O-isopropylidene (IP)- or 1,2-O,O-cyclohexylidene (CH)-3,4,5,6-tetrachloroacetylmyoinositol (IV, V). A similar method was used to react ketal III with the chloroanhydride of γ-chlorobutyric acid [12], to form 1,2-O,O-IP-3,4,5,6-tetra-3-chlorobutyroylmyoinositol (VI).

Acid hydrolysis of chloroacetyl derivatives IV and V using 80% acetic acid or concentrated HCl in methanol (1:10) was used to produce a complex mixture of products, from which we were unable to isolate sufficient quantities of 1,3,4,5-

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TABLE 1. Comparative Evaluation of the Antiischemic Activities of Myoinositol Derivatives and Reference Antianginal Preparations

| Compound | Total shift in ST segment, μV | Creatine phosphokinase | Locate dehydrogenase | Arbitrary index of efficacy, % |
|---|---|---|---|---|
| | | μmol/liter/h | | |
| Intact group | 0±0 | 0,05±0,01 | 0,22±0,05 | — |
| Control group | 154±7 | 0,19±0,01 | 2,06±0,23 | |
| V, 50 mg/kg | 67±16 | 0,14±0,04 | 1,50±0,26 | 110,0 |
| | —56,5 | —28,3 | —27,2 | |
| IV, 50 mg/kg | 54±18 | 0,14±0,04 | 0,91±0,11 | 108,7 |
| | —64,9 | —26,3 | —17,5 | |
| VIII, 50 mg/kg | 39±11 | 0,07±0,01 | 1,03±0,07 | 187,9 |
| | —74,7 | —63,2 | —50,0 | |
| VIII: | | | | |
| 100 mg/kg | 54±12 | 0,10±0,02 | 1,19±0,08 | 154,5 |
| | —64,9 | —47,4 | —42,2 | |
| 200 mg/kg | 31±15 | 0,16±0,07 | 1,70±0,45 | 113,2 |
| Control group | 216,39 | 0,17±0,01 | 1,52±0,12 | |
| Nitrong, 10 mg/kg | 144±9 | 0,12±0,02 | 0,87±0,02 | 105,5 |
| | —233,3 | —29,4 | —42,8 | |
| Obsidan, 10 mg/kg | 59±25 | 0,09±0,02 | 0,80±0,05 | 167,2 |
| | —72,7 | —46,1 | —47,7 | |
| Isoptin, 20 mg/kg | 71±14 | 0,07±0,02 | 0,74±0,05 | 174,2 |
| | —64,1 | —51,3 | —58,8 | |

tetrachloroacetylmyoinositol. Similar acid hydrolysis of 1,2-O,O-CH- or 1,2-O,O-IP-3,4,5,6-tetraacetylmyoinositol is known to proceed smoothly, and to produce 1,4,5,6-tetraacetylmyoinositol at high yield [10]. In the case of the chloroacetyl analog, not only is the ketal protector group split off, but it also appears that the complex ester bond is broken, because of the electron-accepting effect of the chlorine atom. Ketal VI was more resistant to acid hydrolysis. Acid hydrolysis of this ketal produced, along with 1,3,4,5-tetrachlorobutyroylmyoinositol (VII), products of its partial degradation and γ-chlorobutyric acid. Derivatives of myoinositol with ω-chlorocarbonic acids may find use as intermediate compounds for introducing biologically active substances into the myoinositol molecule by alkylation, and are also of interest in their own right as pharmacologically active compounds (see below).

The next stage of this work consisted of the preparation of nicotinylated derivatives of myoinositol, containing different quantities of nicotinic acid residues. 1,2,3,4,5,6-Hexanicotinoyl (NC)-myoinositol (VIII) (Hexopal, Hexanicit) is used abroad as a vasodilator in the treatment of ischemic heart disease [15]. The therapeutic effect of Hexopal is greater than those of Nitrong, Isoptin, and Obsidan, which are used in Russia [14]. Most work on the synthesis of hexanicotinoyl-myoinositol VIII has been described in the patent literature. Acylation of myoinositol with the chloroanhydride of nicotinic acid, which can be prepared by the reaction of nicotinic acid with thionyl chloride [2, 4], benzosulfochloride [5], *p*-toluolsulfochloride [6], or phosphorus oxychloride [7], occurs with low yield. The most effective reagent for preparing the chloroanhydride is phosphorus pentoxide [8]. The chloroanhydride, without purification, was added to the reaction with myoinositol at 60-65°C in the presence of pyridine. With the aim of increasing the yield of hexanicotinate VIII, the reaction mixture was poured into phosphate buffer solution (pH 7), and the yield of final product reached 96%.

A nicotinoyl derivative of myoinositol with new properties was prepared by making 1,4,5,6-tetra(NC)-myoinositol (IX). In comparison with the hexanicotinate VIII, this compound was more soluble in water, and the presence of two free hydroxyl groups allows us, at least in principle, to insert residues of other biologically active compounds into the molecule, thus obtaining myoinositol derivatives with complex pharmacological activity. For this purpose, the reaction of ketals II and III with the chloroanhydride of nicotinic acid was used to prepare 1,2-O,O-IP- (X) and 1,2-O,O-CH-3,4,5,6-tetra(NC)-myoinositol (XI); boiling of these with concentrated HCl in methanol yielded tetranicotinate IX.

The structures of the compounds synthesized were confirmed by elemental analysis, IR spectroscopy, and PMR spectroscopy. The compounds were tested for cardioprotector activity in vivo (see Pharmacological Experimental section).

## EXPERIMENTAL (CHEMICAL)

IR spectra were recorded on a Unicam SP-1000 apparatus in KCl tablets; PMR spectra were taken on a Varian XL 100 A-12 in $CDCl_3$ (using TMS as the internal standard). Thin-layer chromatography was carried out using Silufol UV-254 plates in chloroform—methanol (system A), acetonitrile—25% $NH_3$ (9:1) (system B). Column chromatography was carried out on silica gel L 40.100.

**1,2-O,O-Isopropylidene-3,4,5,6-tetrachloroacetylmyoinositol (IV).** Dry pyridine (4 ml) was added to a solution of compound II (1.3 g, 0.005 mol) in 50 ml of anhydrous chloroform at 0-5°C; after mixing for 4 h, the chloroanhydride of chloroacetic acid (3.4 ml, 0.023 mol) was added. The reaction mixture was then poured over ice and extracted with chloroform, and the extract was washed with saturated NaCl solution and dried with $Na_2SO_2$; the solvent was removed by evaporation, and the yield of tetrachloroacetate IV was 1.07 g (35%). The melting point was 131-133°C (ethanol), and $R_f$ was 0.4 in system A (6:1). The IR spectrum had $\nu_{max}$ 1775 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was 2.2, 2.3 s (6H, 2-$CH_3$), 4.25-4.45 m (8H, 4-$CH_2$), 4.5, 5.2-5.61 m (6H, 6-CH). The elemental formula was $C_{15}H_{20}Cl_4O_{10}$.

**1,2-O,O-Cyclohexylidene-3,4,5,6-tetrachloroacetylmyoinositol (V).** This was prepared as for compound IV, but starting with ketal III. The yield was 35%. The melting point was 105-107°C (ethanol), and $R_f$ was 0.6 in system A (6:1). The IR spectrum had $\nu_{max}$ 1775 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was 1.3-1.7 m (10H, 5-$CH_2$), 4.2-4.3 m (8H, 4-$CH_2$), 4.5, 5.1-5.7 m (6H, 6-CH). The elemental formula was $C_{20}H_{24}Cl_4O_{10}$.

**1,2-O,O-Isopropylidene-3,4,5,6-tetra(3-chlorobutyroyl)myoinositol (VI).** Pyridine (8 ml) was added to a solution of compound II (2.2 g, 0.01 mol) in 1 ml of dry chloroform, and the mixture was stirred at 0-4°C for 3 h, during which 12 ml (0.06 mol) of the chloroanhydride of $\gamma$-chlorobutyric acid was added. The mixture was poured onto ice and was extracted with chloroform, and the extract was washed with water and dried with $Na_2SO_4$ and evaporated. The residue was chromatographed on a silica gel column (chloroform with an increasing gradient of methanol), which yielded 2.4 g (86%) of derivative VI. The melting point was 58-59°C, and the $R_f$ was 0.9 (system A, 14:1). The IR spectrum had $\nu_{max}$ 1750 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was 1.15-1.25 m, 1.6 s (6H, 2-$CH_3$), 2.0-2.7 m (24H, 12-$CH_2$), 4.1-4.4 m (6H, 6-CH). The elemental formula was $C_{23}H_{36}Cl_4O_{10}$.

**1,4,5,6-Tetra(3-chlorobutyroyl)myoinositol (VII).** A solution of compound VI (1 g, 0.16 mol) in 20 ml of methanol was boiled for 20 min in the presence of 2 ml of concentrated HCl. The mixture was cooled, and 10 ml of water and 50 ml of chloroform were added, and the organic layer was separated, washed with water, dried with $Na_2SO_4$, evaporated, and chromatographed on a silica gel column, which was eluted with chloroform containing an increasing gradient of methanol. This yielded 0.13 g (15%) of tetrachlorobutyroylmyoinositol VII, which was an oil with $R_f$ of 0.4 (system A, 30:1). The reaction mixture also contained products of the partial deacylation of VII, with $R_f$ of 0.5 and 0.9. The IR spectrum had $\nu_{max}$ 1760 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was: 2.05-2.5 m (24H, 12-$CH_2$), 5-5.61 m (6H, 6-CH). The elemental formula was $C_{22}H_{30}Cl_4O_8$.

**Myoinositol hexanicotinate (VIII).** Nicotinic acid (3.68 g, 0.03 mol) was added to a mixture of 2.4 g (0.01 mol) of $PCl_5$ and 9 ml of dry pyridine at 60-65°C, and the mixture was heated to 70-75°C for 1 h; myoinositol (0.9 g, 0.005 mol) was added to the resulting chloroanhydride, and the mixture was heated for 2 h at 90-95°C. The mixture was cooled, poured into 100 ml of phosphate buffer solution (pH 7). The precipitate was collected by filtration and dissolved at 0-4°C in 50 ml of 0.1 M HCl, and was stirred for 30 min with 0.5 g of activated charcoal; neutralization was then carried out with 25% $NH_4OH$, and the precipitate was separated. The yield was 8.9 g (96%), and the melting point was 253-254°C (ethanol). The IR spectrum had $\nu_{max}$ 1780 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was: 5.93-6.4 m (6H, 6-CH), 8.12-9.1 m (24H, 24-CH). The elemental formula was $C_{42}H_{30}N_6O_{12}$.

**1,2-O,O-Isopropylidene-3,4,5,6-tetranicotinoylmyoinositol (X).** $PCl_5$ (4.8 g, 0.02 mol) and 20 ml of dry pyridine were added to 7.75 g (0.058 mol) of nicotinic acid, and the mixture was heated to 100°C for 1 h and cooled to 20°C; compound IV (2.2 g, 0.01 mol) was then added and the mixture was heated at 100°C for 1.5 h and poured into saturated NaCl solution, extracted with chloroform, dried with $Na_2SO_4$ and evaporated to yield 4.5 g (70%) of X. The melting point was 240-242°C (ethanol), $R_f$ was 0.5 (system B). The IR spectrum had $\nu_{max}$ 1790 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was: 1.25, 1.55 s (6H, 2-$CH_3$), 6.0 m (6H, 6-CH), 7.5, 8.2, 8.8 m (16H, 16-CH). The elemental formula was $C_{33}H_{28}N_4O_{10}$.

**1,2-O,O-Cyclohexylidene-3,4,5,6-tetranicotinoylmyoinositol (XI).** This was prepared by a similar method. The yield was 60%. The melting point was 207-210°C (ethanol). The $R_f$ was 0.5 (system B). The IR spectrum had $\nu_{max}$ 1790 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was: 5.8, 6.1, 6.3 m (6H, 6-CH), 7.3 m, 8.8-9.3 m (16H, 16-CH), 1.4-1.7 m (10H, 5-$CH_2$). The elemental formula was $C_{36}H_{32}N_4O_{10}$.

**1,4,5,6-Tetranicotinoylmyoinositol (IX).** Mixtures of 0.5 mol of recrystallized compounds X or XI were boiled for 25 min with 2 ml of concentrated HCl in 20 ml of methanol; the mixtures were cooled, neutralized with $NaHCO_3$ to pH 7, and precipitates were collected by filtration, dissolved in chloroform, washed with saturated NaCl solutions and then with water, and were dried with $Na_2SO_4$ and evaporated. The yield was 2.4 g (85%) of tetranicotinate IX; the melting point was 227-229°C, $R_f$ was 0.35 (system B). The IR spectrum had $\nu_{max}$ 1730 $cm^{-1}$ (OCO). The PMR spectrum ($\delta$, ppm) was: 5.8 m (6H, 6-CH), 7.1, 8.2, 8.9 m (16H, 16-CH). The elemental formula was $C_{30}H_{24}N_4O_{10}$.

## EXPERIMENTAL (PHARMACOLOGICAL)

Experiments were carried out using white Wistar rats of both sexes and body weights of 240-280 g. Experimental coronary-metabolic myocardial infarcts were induced using pituitrin and isadrine. Pituitrin (0.5 U/kg) was given i.p. 20 min before s.c. dosage with isadrine (100 mg/kg); a second dose of isadrine was given after 6 h, and 24 h later rats received repeat doses of both substances. Acute myocardial ischemia appeared 3 h later, as shown by electrocardiography (total shift in the ST segment relative to the baseline, taken in three standard leads and from 21 chest electrodes) and biochemical measurements (elevated activities of the cardiospecific isoforms of creatine phosphokinase and lactate dehydrogenase). Compounds IV, V, and VIII were given enterally (using a gastric tube) as suspensions; doses were given three times daily in a therapeutic-prophylactic regimen, i.e., in parallel with formation of pathology. Reference agents were used in the same conditions in average effective doses. Antiischemic effects were evaluated in terms of the reduction in severity of the electrocardiographic and biochemical parameters of acute myocardial ischemia mentioned above, relative to an untreated control group, using an arbitrary index of efficacy consisting of the total reduction of all parameters expressed as a percentage.

These studies revealed a sharp increase in creatine phosphokinase and lactate dehydrogenase isoform activities in controls (3.8- and 9.4-fold, respectively), and a shift in the ST segment of 154 $\mu$V in comparison with the intact group, demonstrating the presence of pronounced myocardial ischemia. Test substances reduces these changes to different levels, i.e., they had antiischemic activity (Table 1).

A comparative evaluation of the antiischemic activities of the test substances showed that the efficacies of compounds IV and V were virtually equal (arbitrary indexes of 108.7 and 110, respectively), and were comparable with that of Nitrong (105.5), though the $\beta$-blocker Obsidan and the calcium antagonist Isoptin were more effective (167.2 and 174.2, respectively). Hexopal VIII was more effective than Nitrong at a dose of 50 mg/kg, and its efficacy approached those of Obsidan and Isoptin, which are the major substances used in the therapy of ischemic heart disease. Preliminary studies of tetranicotinate XI showed it to be as active as VIII.

These results show that there is potential in searching for cardioprotective agents among myoinositol derivatives.

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