# SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF 3-THIABIS(CYCLOHEXANECARBOXYLIC) ACID DERIVATIVES

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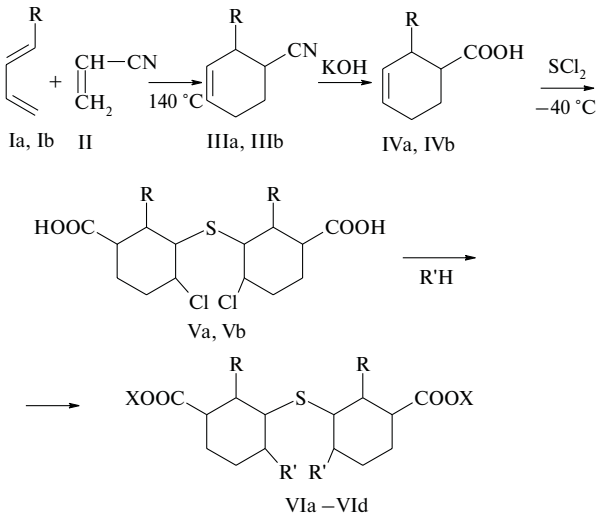
The class of antiinflammatory drugs includes a group of sulfur-containing organic compounds such as metiazinic acid, tinoridine (kenfamin), tiaramide (solantol), etc. However, all these drugs give rise to side effects, including gastrointestinal tract disorders. A pronounced antiinflammatory activity was reported for patented cyclic hydroxysulfones [1], the most active of which was 3-hydroxytetrahydrothiophene-1,1-dioxide (3-hydroxysulfolan).

The results of investigations carried out in the Laboratory of New Drugs at the Institute of Organic Chemistry (Ufa) showed that most of the synthetic sulfones possess a more or less pronounced antiinflammatory activity. The maximum activity was observed for dihydroxysulfolan, producing, in contrast to most of the known antiinflammatory sulfur-containing compounds, no ulcerogenic action.

The purpose of this work was to synthesize and characterize a series of new 3-thiabis(cyclohexanecarboxylic) acid derivatives.

The condensation of piperylene (Ia) with acrylic acid nitrile (II) was conducted by the method described in [2 – 4] to obtain 2-methyl-1,2,5,6-tetrahydrobenzonitrile (IIIa) with a yield of 90%. As was previously established by A. A. Petrov and A. F. Sapozhnikova for an initial mixture containing *cis* and *trans* forms of piperylene in a 50 : 50 ratio, the condensation with acrylonitrile at 130 – 135°C involves only the *trans* form, whereas the *cis* form does not enter the reaction at this temperature. However, a complete condensation takes place at 180°C, when the *cis* to *trans* conversion obviously takes place. We conducted the condensation process with a *cis*– *trans* mixture for 12 h at 140°C; the unreacted *cis*-piperylene was distilled off.

The synthesis was conducted according to the following scheme.




I – V: R = Me (a), H (b);
VI: R = Me (a – c), H (d); $R^1 = C_4H_8NO$ (a, b, d), $C_5H_{10}N$ (c);
X = H (a), Na (b – d).

The saponification of 2-methyl-1,2,5,6-tetrahydrobenzonitrile (IIIa) with a 10% KOH solution, conducted for 2 h at 180°C in an autoclave and followed by acidification with concentrated sulfuric acid, led to the formation of tetrahydrotoluic acid (IVa) with an 88% yield. The treatment of acid IVa and 1,2,5,6-tetrahydrobenzoic acid (IVb) with sulfur dichloride was carried out at –40°C [5] in a methylene chloride medium. These reactions led to 3-thiabis(2-methyl-4-chlorocyclohexanecarboxylic) acid (Va) and 3-thiabis-(4-chlorocyclohexanecarboxylic) acid (Vb). The reaction of $SCl_2$ with tetrahydrotoluic acid may lead to the formation of solid and liquid isomers. Based on the available data, it is difficult to assign the structures. However, judging by the fact that absorption bands in the region characteristic of the C–Cl bond vibrations are confined within a 710 – 770 $cm^{-1}$ fre-

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quency interval, the synthesized compounds occur in the 1-ee, 2-ee molecular configuration.

The subsequent reactions of acids Va and Vb with morpholine or piperidine were carried out according to a procedure described in [6]. These interactions led to 3-thiabis(2-methyl-4-morpholinocyclohexanecarboxylic) acid (VIa) with a 90% yield. Additional treatment of the reaction mixture with water and alkali gave a disodium salt VIb of this acid with a yield of 80%. The other disodium salts were obtained using analogous procedures [7].

Purity of the reaction products was checked and the proposed structures of compounds were confirmed by data of the IR absorption spectroscopy and elemental analyses. The IR spectra of compound VI exhibit absorption bands in the regions of 625 – 670 and 710 – 770 $cm^{-1}$ assigned to stretching vibrations of the C–S bonds and tertiary nitrogen atoms, respectively. The IR spectra of compounds V and VI contain additional bands at 1710 – 1740 $cm^{-1}$ belonging to CO groups.

## EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on a UR-20 spectrophotometer (Carl Zeiss – Jena, Germany) equipped with NaCl and LiF prisms. The samples were prepared as thin liquid films of nujol mulls. The melting temperatures were determined with the aid of a Boetius heating table. The data of elemental analyses (C, H, Cl, N, S) agree with the results of analytical calculations according to the empirical formulas.

**Tetrahydrotoluic acid (IVa).** A 2000-ml autoclave vessel was sequentially charged in an argon atmosphere with 369 g (5.45 mole) of piperylene (Ia) preliminarily dried over $CuCl_2$ and distilled over $CaH_2$, 177 g (3.3 mole) of acrylonitrile (II) distilled at atmospheric pressure, and 2 g of hydroquinone. Then the autoclave was closed and heated to 140°C. The reaction mixture was stirred at this temperature for 12 h, cooled, and discharged (530 g). The light fractions were distilled off at atmospheric pressure (b.p., 40 – 60°C) to obtain 100 g of *cis*-piperylene ($n_D^{20}$, 1.4339). The residue was distilled in vacuum to obtain 352 g (90%) of 2-methyl-1,2,5,6-tetrahydrobenzonitrile (IIIa); b.p., 72 – 75°C/5 Torr; $n_D^{20}$, 1.4708.

The autoclave vessel was sequentially charged with 100 g (0.63 mole) of nitrile and 1100 ml of a 10% KOH solution. Then the autoclave was closed, heated to 180°C, kept at this temperature for 2 h, and cooled. The reaction mixture was discharged into a flask, acidified with concentrated sulfuric acid (until obtaining an acid test response), and extracted with benzene. Finally, the solvent was distilled off and the residue was distilled in vacuum to obtain 102.3 g (88%) of 2-methyl-1,2,5,6-tetrahydrobenzoic acid (IVa); b.p., 162°C/1 Torr; $n_D^{20}$, 1.4722. On standing, acid IVa exhibits crystallization.

**3-Thiabis(2-methyl-4-chlorocyclohexanecarboxylic) acid (Va)**. To a mixture of 280 g (2 mole) acid IVa and 300 ml $CH_2Cl_2$ in a flask cooled down to – 40°C were added dropwise so as to maintain this temperature 103 g (1 mole) of $SCl_2$. The reaction mixture was stirred at this temperature for 30 min and allowed to heat up to room temperature, after which the stirring was continued for 2 h. Then $CH_2Cl_2$ was distilled off at a reduced pressure (water-jet pump) and a water bath temperature not exceeding 40°C to obtain 380 g (100%) of crystalline acid Va; m.p., 65 – 70°C. The rough product was recrystallized from an ethyl ether – petroleum ether mixture (40 – 70°C) to obtain diacid Va; yield, 80%; m.p., 70 – 71°C; $C_{16}H_{24}Cl_2O_4S$ (mol. weight, 383.33); IR spectrum (ν, C–S): 625 – 670 (C–S), 710 – 770 (CCl), 1710 – 1740 (COCH), 1080 – 1180.

**3-Thiabis(2-methyl-4-morpholinocyclohexanecarboxylic) acid (VIa)**. To 115 g (0.3 mole) of acid Va in a flask was gradually (over 1 h) added 261 g (3 mole) of morpholine. The reaction mixture was heated to 135°C, stirred at this temperature for 6 – 8 h, and allowed to stand cooling overnight. The precipitated crystals of $C_4H_{10}NO \cdot HCl$ were filtered and washed with toluene. Finally, excess morpholine and toluene were distilled off to leave 130 g (90%) of acid VIa. The rough product was purified by washing with water and extracting with toluene. Acid VIa: $C_{24}H_{40}N_2O_6S$ (mol. weight, 484.65); IR spectrum (ν, C–S): 1710 – 1740 (C–O), 1070 – 1170 (C–O), 625 – 670 (C–S).

**3-Thiabis(2-methyl-4-morpholinocyclohexanecarboxylic) acid disodium salt (VIb)**. To 115 g (0.3 mole) of acid Va in a flask was gradually (over 1 h) added 261 g (3 mole) of morpholine. The reaction mixture was heated to 135°C, stirred at this temperature for 6 – 8 h, and allowed to stand cooling overnight. On the next day, 400 ml of water was added and the solution was saturated with solid NaOH until separation into two layers. The organic layer was separated and the excess morpholine was distilled off on a rotor evaporator. The residue was subjected to azeotrope drying with benzene and washed with ether to obtain 126 g (80%) of a crystalline disodium salt VIb. The residual NaOH was removed by repeated washing with water (50 ml), followed by azeotrope drying with benzene. Disodium salt VIb: m.p., 200 – 202°C; $C_{24}H_{38}Na_2N_2O_6S$; IR spectrum (ν, C–S): 1580, 1410 ($RCOO^-$), 1070 – 1120 (C–O–C).

**3-Thiabis(2-methyl-4-piperidinocyclohexanecarboxylic) acid disodium salt (VIc)**. To 115 g (0.3 mole) of acid Va in a flask was gradually (over 1 h) added 255 g (3 mole) of piperidine. The reaction mixture was gradually heated to 120°C, stirred at this temperature for 6 – 8 h, and allowed to stand cooling overnight. On the next day, 400 ml of water were added to dissolve the precipitated crystals and the resulting homogeneous solution was saturated with solid NaOH until separation into two layers. The upper organic layer was separated and the excess piperidine was distilled off on a rotor evaporator. The residue was subjected to

**TABLE 1.** Acute Toxicity of Compounds VIa – VId upon Single Intraperitoneal Injection in Mice

| Compound | $LD_{50}$, mg/kg |
|---|---|
| VIa | 1350 |
| VIb | 4800 |
| VIc | 1200 |
| VId | 1750 |
| Phenylbutazone | 250 |
| Acetysalicylic acid | 3000 |
| Brufen | 370 |
| Indomethacin | 30 |

azeotrope drying with benzene and washed with ether to obtain 95 g (60%) of disodium salt VIc – a crystalline yellow powder melting at 105 – 108°C. For the analysis, the product was purified by additional washing with water, followed by azeotrope drying with benzene. Disodium salt VIc: m.p., 108 – 110°C; $C_{26}H_{42}Na_2N_2O_4S$; IR spectrum (ν, C–S): 1570, 1410 ($RCOO^-$).

**3-Thiabis(4-chlorocyclohexanecarboxylic) acid (Vb)**. To a mixture of 378 g (3 mole) 1,2,5,6-tetrahydrobenzoic acid (IVb) and 40 ml $CH_2Cl_2$ in a flask cooled down to –(20 – 40)°C was added dropwise so as to hold the temperature in this interval 154 g (1.5 mole) of sulfur dichloride in 200 ml of methylene chloride. Upon completely adding the $SCl_2$, the reaction mixture was stirred at this temperature for 1 h and allowed to heat up to room temperature, after which the stirring was continued for 2 h. Then methylene chloride was distilled off at a reduced pressure (water-jet pump) and a water bath temperature not exceeding 40°C to obtain 512 g (96%) of acid Vb in the form of a highly viscous ropy liquid (mol.weight, 355.28).

**3-Thiabis(4-morpholinocyclohexanecarboxylic) acid disodium salt (VId)**. Compound VId was obtained from diacid Vb using a procedure analogous to that described above for disodium salt VIb. Disodium salt VId: yield, 80%; m.p., 160°C (with sublim.); $C_{22}H_{34}Na_2N_2O_6S$; IR spectrum (ν, $cm^{-1}$): 1580, 1410 ($COO^-$).

**TABLE 2.** Antiinflammatory Activity of Compounds VIa – VId with Respect to Formalin and Carrageenan Edema Models in Rats

| Compound | Formalin edema[1] | | | Carrageenan edema[2] | | |
|---|---|---|---|---|---|---|
| | Dose, mg/kg | Foot edema volume gain, % of initial | *P* | Dose, mg/kg | Foot edema volume gain, % of initial | *P* |
| VIa | 135 | 40.0 ± 3.0 | < 0.002 | 135 | 54.5 ± 5.4 | < 0.01 |
| | 67 | 43.6 ± 1.0 | < 0.001 | | | |
| VIb | 480 | 39.6 ± 3.6 | < 0.002 | 480 | 55.8 ± 2.7 | < 0.01 |
| | 240 | 40.0 ± 2.1 | < 0.001 | | | |
| VIc | 120 | 39.3 ± 2.6 | < 0.001 | 120 | 47.3 ± 4.2 | < 0.02 |
| | 60 | 46.4 ± 2.6 | < 0.001 | | | |
| VId | 175 | 47.3 ± 2.9 | < 0.02 | 175 | 40.6 ± 2.7 | < 0.001 |
| | 87 | 45.0 ± 2.6 | < 0.01 | | | |
| Brufen | 80 | 46.8 ± 4.3 | < 0.05 | 80 | 47.3 ± 3.5 | < 0.001 |
| Control | – | 60.4 ± 3.5 | – | – | 79.8 ± 6.0 | |

**Notes**: [1,2] Each compound was studied (in certain doses) on test groups containing seven (for formalin model) and six (carrageenan model) animals.

## EXPERIMENTAL PHARMACOLOGICAL PART

### Experimental Methods

The experiments were performed on 320 white male and female mongrel mice weighing 18 – 20 g obtained from the Rappolovo nursery (Russian Academy of Medical Sciences). The acute toxicity was studied on a group of 160 mice, to which the synthesized compounds were introduced by single intraperitoneal injections in a dose range from 50 to 1600 mg/kg. The $LD_{50}$ values were determined by a conventional method on the 10th day after drug injection [8].

The antiinflammatory activity was studied on mice and rats with inflammation models induced by 1% carrageenan or 3% formalin solutions injected under one foot aponeurosis in each animal of the test group. The thermal burn model was induced by a conventional method [9], whereby a mice paw below the knee is exposed for 30 sec in hot (55°C) water.

The inflammation process development and the antiinflammatory activity in rats were determined by the oncometric techniques 1, 2, 3, 6, and 24 h after the inflammation model induction. The inflammation process in test mice divided into three groups was monitored by cutting paws and determining percentage weight gain for the damaged feet relative to intact ones. The experimental data presented in Tables 2 and 3 refer to the volume or weight gain determined 3 h after the inflammation model induction (this time corresponds to the maximum degree of edema development). The synthesized compounds were introduced into the test animals (1 h before and 3 and 6 h after the inflammation

**TABLE 3.** Antiinflammatory Activity of Compounds VIa – VId with Respect to Thermal Burn Models in Mice

| Compound | Dose, mg/kg | Foot weight gain, % of intact | *P* |
|---|---|---|---|
| VIa | 135.0 | 74.7 ± 6.3 | > 0.25 |
| VIb | 480.0 | 68.9 ± 3.3 | < 0.05 |
| VIc | 120.0 | 70.8 ± 3.9 | > 0.1 |
| VId | 175.0 | 60.0 ± 4.2 | < 0.05 |
| Brufen | 80.0 | 64.4 ± 4.4 | |
| Control | – | 83.3 ± 3.9 | – |

model induction) via intragastric tube in a dose of 1/10 or 1/20 $LD_{50}$.

**Results and Discussion**

It was established that, according to the standard classification scheme [10 – 12], all the synthesized compounds belong to the group of low-toxicity substances (Table 1).

It was found that all compounds decrease the extent of the carrageenan-induced inflammatory edema, the drug effect in the 1/10 $LD_{50}$ dose being comparable with that of the reference drug brufen taken in a dose of 1/5 $LD_{50}$ (Table 2). Similar results were observed for the formalin induced inflammation model, where a positive effect (edema volume decrease) was produced by the drugs administered in a dose of both 1/10 and 1/20 $LD_{50}$.

Compounds VIb and VId (but not VIa and VIc) produced an antiinflammatory action with respect to the thermal burn model as well (Table 3). Thus, the synthesized compounds possess antiinflammatory properties comparable with those of brufen, but their therapeutic breadth exceeds that of the reference drug.

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