Investigation of Synthesis of Functionally Substituted Endiines and Their Chemical and Microbiological Conversion

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ABSTRACT

The methods of preparation of endiine and endiallene diols by interaction of cis-1,4-dibrombutene and cis-1,4-dichlorbutene with monosubstituted acetylene alcohols in presence of the catalytic systems consisting of one-iodide copper, triethylamine and K2CO3 in a medium of dimethylformamide have been developed. It has been shown that unlike 1,4-dibrombutene, the nucleophilic substitution reaction with 1,4-dichlorbutene proceeds by acetylene-allene isomerization with formation of endiallene diols. It has been established that the endiine diols can be used in thin organic synthesis (in the reactions of oxidation, splitting, dehydration, epoxidation, hydrolysis, 1,2-cycloaddition and hypochlorination) with the aim of preparation of practically useful substances. It has been revealed during hydrolysis of epoxide compounds by the chemical and microbiological methods that in the course of microbiological hydrolysis (Aspergillus niger), the optically active trans-structured diols are formed.

Keywords: Substitution Reaction; Endiine Diols; Chemical Methods; Oxidation of Diols; Hydrolysis of Epoxide; Microbiological Hydrolysis; Micromycetes

1. Introduction

The chemistry of high unsaturated compounds, in particular, endiine having various functional groups in molecule, is of large scientific and practical interest [1-10]. It was found out that the endiine derivatives isolated from plants and microorganisms possessed strong fungicide, antimicrobial and insecticide actions [11-19]. Therefore, the attention of researchers to the development of new approaches to synthesis of such compounds increases. Therefore, as a continuation of the investigation in this direction [20-25], in this study, the results of the investigations on synthesis of functionally substituted unconjugated endiines being analog of natural compounds and on study of some of their chemical and microbiological conversions have been presented.

2 Results and Discussion

The investigations showed that in interaction of cis-1,4-dibrombutene with monosubstituted acetylene alcohols (dimethylethynylcarbinol and propargyl alcohol) in the presence of catalytic system consisting of one-iodide copper, triethylamine and K2CO3 in a medium of dimethylformamide, by the substitution reaction at 55°C - 60°C the unconjugated endiine diols (I, II) with yields of 65.6% and 74.2% are respectively formed: it has been revealed that unlike 1,4-dibrombutene the nucleophilic substitution reaction proceeds by acetylene-allene isomerization with formation of endiallene diols. It has been established that the endiine diols can be used in thin organic synthesis (in the reactions of oxidation, splitting, dehydration, epoxidation, hydrolysis, 1,2-cycloaddition and hypochlorination) with the aim of preparation of practically useful substances. It has been revealed during hydrolysis of epoxide compounds by chemical and microbiological methods that in the course of microbiological hydrolysis (Aspergillus niger), the optically active trans-structured diols are formed.

The structure of the synthesized diols (I, II) has been confirmed by the data of the IR- and PMR-spectra. In the IR-spectra of diols (I, II) the absorption bands in the field of 1640 - 1665, 2260 - 2200 and 3350 - 3450 cm⁻¹, characteristic for C=С, C≡С and OH bonds have been respectively detected. Along with this the absorption bands in the field of 680 - 730 cm⁻¹, characteristic for unconjugated cis-ethylene and acetylene bonds have been also detected. In the NMR ¹H spectra of diols (I, II) the signals of protons of double bond (2H, HC=CH) as a singlet with chemical shifts δ = 6.08 and 6.10 ppm have
been detected. Along with this the signals of protons with the following chemical shifts: $\delta$ (ppm.); (I), 6.08 (2H, s, HC=CH), 2.85 - 3.00 (2H, m, CH$_2$), 4.13 (2H, s, CH$_2$O), 3.45 (1H, s, OH); (II), 6.10 (2H, s, HC=CH), 2.75 - 3.00 (2H, m, CH$_2$), 1.30 [6 H, s, (CH$_3$)$_2$], 3.15 (1H, s, OH) have been also detected [26,27].

In the IR-spectra of the compounds (III, IV) along with the absorption bands characteristic for C=\(C\) and O=H bonds, there are also the bands in the field of 1940 - 1960 cm$^{-1}$, characteristic for >C=C=C< fragment. In the PMR-spectrum of the compounds (III, IV) the protons of CH=C=CH bonds are appeared as a multiplet with chemical shift $\delta$ = 5.10 - 5.55 ppm.

It has been established that the synthesized diols are very reactive compounds and can undergo the reactions of oxidation, splitting, dehydration, epoxidation, hydrolysis, 1,2-cycloaddition and hypochlorination with formation of new derivatives of diines and endiines.

During carrying out of the oxidation reaction of endiine diol (I) at 35˚C - 40˚C by means of oxidative mixture consisting of aqueous solution of chromium anhydride and sulphuric acid the corresponding unsaturated dicarboxylic acid (V) with yield 76.6% is formed (Scheme 2).

In the IR-spectrum of the synthesized compound (V) with presence of strong absorption band at 1725 cm$^{-1}$ (C=O), the intensive wide band in the field of 2600 - 3000 cm$^{-1}$, belonging to hydroxyl group has been also detected.

The endiine diol (II) in the presence of powdered potassium hydroxide is subjected to Favorsky reaction accompanying by acetone detachment and is easily converted into endiine (VI) with two terminal acetylene bonds. The dehydration of diol (II) by interaction of potassium bisulphate realized by heating of mixture of reagents in a medium of toluene at 70˚C for 6 h leads to the formation of polyunsaturated compounds (VI, VII) with yields 78.6% and 65.4% respectively (Scheme 3).

The structure of the synthesized compounds (VI, VII) has been established on the basis of data of the IR- and PMR-spectra.

In the IR-spectrum of the compound (VI) along with presence of the absorption band at 685 and 1655 cm$^{-1}$, characteristic for double HC=CH bond, there is also a band at 2125, 3295 cm$^{-1}$, characteristic for terminal acetylene bond. In the IR-spectrum of the compound (VII) along with the absorption bands characteristic for C=C bonds the band at 2240 cm$^{-1}$, characteristic for non-end acetylene bond has been also detected. In this case the absorption bands characteristic for OH group are absent.

In the NMR $^1$H-spectrum of the compound (VI) along with the signals characteristic for cis-HC=CH fragment it has been also identified the signal of proton (2H, C=CH) as a triplet at $\delta$ = 2.43 ppm.

It has been established that the synthesized diol (II) undergoes the epoxidation reaction due to double bond by means of acetyl hydroperoxide (peracetic acid) forming a diol of diine series with oxirane ring (VIII) with yield 76.5%. Dichlorocyclopropanation of diol (II) proceeds in conditions of interphase catalysis (triethylbenzylammonium chloride, 50% NaOH, chloroform, 20˚C - 25˚C) with formation of the corresponding dichlorocyclopropane (IX) on Scheme 4.

In the IR-spectra of the prepared compounds (VII-X) the absorption bands characteristic for C=C=CH fragments are absent. In the IR-spectra of the compounds (VIII, IX) along with the absorption bands characteristic for C=C=CH and O-H bonds the absorption bands at 3065, 1250 and 955 cm$^{-1}$, characteristic for oxirane ring are also present. In the IR-spectrum of the compound (X) the absorption bands at 765, 3085 and 2235 cm$^{-1}$, characteristic for valence vibrations of bonds of C-Cl and C-H methylene groups of three-membered carbon-carbon ring are present.

It has been shown (Scheme 5) that the endiine (IX) undergoes the oxidation reaction with peracetic acid at 20˚C - 25˚C on double bond forming an epoxide with two terminal acetylene bonds (XI). The diine epoxide (XI) has been also synthesized by counter synthesis-splitting of epoxydiindol (IX) on Favorsky reverse reaction: the reaction proceeds (yield 68.6%) in the presence of potas-
sium hydroxide in a medium of toluene at 105°C - 110°C.

The structure of the synthesized epoxydiine (XI) has been confirmed by data of the IR- and PMR-spectra.

It has been established that the endiine diols (I, II) react at 35°C - 40°C with HOCl in situ, leading to the formation of the corresponding chlorohydrins (XII, XIII). The synthesized chlorohydrins (XII, XIII) are easily dehydrochlorinated in the presence of powdered potassium hydroxide. In this case the oxirane with two terminal acetylene bonds (VIII, XI) with yields 85% - 90% is formed (Scheme 6).

The structure of the synthesized chlorohydrins (XII, XIII) has been established by determination of elemental analysis, physical-chemical constants, IR- and NMR ¹H spectral data. In the IR-spectra of the synthesized chlorohydrins (XII, XIII) the absorption bands in the field of 3360 - 3450, 2225 - 2245 and 750-600 cm⁻¹, characteristic for bonds of O-H, C≡C and C-Cl respectively have been detected. In this case the absorption bands of valence vibrations of C≡C bonds in the field of 1630 - 1650 cm⁻¹ are absent.

A hydrolysis of epoxydiine (XI) by the chemical and microbiological methods is of also interest.

The hydrolysis reaction (XI) on oxirane ring in the presence of acidic catalysts has been carried out. It has been established that in the presence of 10% aqueous solution of sulphuric acid the epoxydiine (XI) is subjected to the hydrolysis on oxirane ring and in this case the corresponding diol (XIV) with high yield (86.7%) is formed (Scheme 7).

In the IR-spectrum of the compounds there are the bands characteristic for terminal acetylene bond (2140, 3300 cm⁻¹) and hydroxyl group (3450 cm⁻¹). In this case the band characteristic for oxirane ring is absent.

As it was known a leading role in the processes of biodeterioration of the chemical nature construction materials belongs to microscopic fungi (micromycetes) [2]. This property of fungi is related to their ability for quick growth and capacity of their enzymatic apparatus. A result of growth of micromycetes on surface of construction materials is decreasing of physical-mechanical and operational characteristics of materials and deterioration of their external view. The microbiological damage of lacquer coating is one of the frequently occurring cases of biodeteriorations. Among microorganisms damaging lacquer coatings the fungi of following genera: Aspergillus, Penicillium, Fusarium, Trioderma, Alternaria, Cephalosporium, and bacteria—Pseudomonas, Flavobacterium are frequently occurred. The damages of coatings by fungi occur either due to components entering in compositions of coating or due to substances polluting surface of coating, under action of metabolites isolated by mycelium which grows due to substances polluting coating [28]. A fungus-resistance of these coatings is decreased in the following series: epoxide, polyurethane, melamine-alkide, organosilicon, pentaphthalic. It has been shown that the isolated strains Aspergillus and Fusarium carried out the biohydrolysis of the epoxide compounds of diacetylene series for preparation of products of thin synthesis with the aim of their practical application and prevention of biodeterioration process.

The maximum degree of biohydrolysis observed in strain Aspergillus niger 15. The analysis of biohydrolysis products of epoxides of acetylene series by the chroma-
hromatography and spectral methods showed that during microbiological hydrolysis of epoxide compounds by strains Aspergillus niger unlike chemical one of the reaction proceeds with formation of optically active diol with trans-structure (XIV) as it was presented at the Scheme 8.

The physical-chemical and spectral data of diol (IX) obtained by microbiological hydrolysis correspond to data prepared by chemical method. It has been established during implementation of the investigations by the chemical method of hydrolysis by microbiological method the optically active diols with diterminal acetylene bond are formed (XIV).

It has been established that the biological oxidation of diol (XIV) using Fusarium flociferum mycelium proceeds with formation of the corresponding diacetylene ketone (XV) and presented on the following Scheme 7.

The results of the mycological investigations showed that the micromycetes can be used as the chemical reagents for preparation of practically useful compounds and the investigation of enzymes causing oxidation will facilitate the development of methods of prevention of biodeteriorations’ processes.

Thus, it has been established that based on the high reactivity the prepared functionally substituted endiines can be widely used as the syntones in different syntheses for preparation of practically useful products and intermediate products.

3. Experimental Material

The IR-spectra of the synthesized compounds were taken on spectrophotometer UK-20 in thin layer. The PMR-

Scheme 7. Hydrolysis of epoxydiyne by the chemical method.

Scheme 8. Hydrolysis of epoxydiyne by the microbiological method.

Scheme 9. Microbiological oxidation of diols.

spectra were recorded on spectrophotometer Tesla BS-487B (80 MHz), as the internal standard was used hexamethyldisiloxane, solvent-CCl4.

The investigations were carried out with use of the basic chemical reagents such as cis-1,4-dibrombutene, cis-1,4-dichlorbutene and monosubstituted acetylene alcohols) propargyl alcohol and dimethylenecarbinol).

The microbiological experiments were conducted using microscopic fungi isolated from oil polluted soil of Absheron. For study of ability of the micromycetes to degrade selected chemicals of the most active strains-degrader from the following genera: Aspergillus, Fusarium, Mucor, Penicillium was used.

4. Experimental Methods

4.1. Chemical Method

Synthesis of dec-5-en-2,8-diine-1,10-diol (I). 100 ml DMF, 1.5 g K2CO3, 9.5 g (0.05 mol) CuJ, 0.5 ml TEA was placed in a three-necked flask and was stirred at heating in a nitrogen current at 50°C - 55°C. In 15 - 20 min. 5.6 g (0.1 mol) of propargyl alcohol was added to a mixture. After two hours’ stirring on dropwise 10.7 g (0.05 mol) of 1,4-dibrombut-2-ene was added and was continued to stir still for 6 h at 50°C - 55°C. Then the cooled mixture was washed by water and extracted with ether. The ether layer was dried over MgSO4. After evaporation of a solvent the residue was crystallized in petroleum-ether as the needles (I) with m. p. 115.5°C - 116.5°C, yield 65.6%. Found, %: C 73.26, H 7.22. C10H12O2. Calculated, %: C 73.14, H 7.37.

Analogously from dimethylethenecarbinol and 1,4-dibrombut-2-ene it has been prepared the compound (II) with m. p. 119°C, yield 74.2%. Found, %: C 76.20, H 9.28, C12H20O2. Calculated, %: C 76.32, H 9.15.

Synthesis of diol of diacene series (III, IV). Analogously from propargyl alcohol (or dimethylethenecarbinol) and 1,4-dichlorbut-2-ene it has been prepared diol (III) with m. p. 115°C - 120°C, yield 74.2%. Found, %: C 73.29, H 7.28. C10H12O2. Calculated, %: C 73.14, H 7.37; (IV), M. p. 12.5°C - 13.5°C, yield 70.1%. Found, %: C 76.21, H 9.27. C12H20O2. Calculated, %: C 76.32, H 9.15.

Synthesis of dicarboxylic acid of endiine series (V). 8.2 g (0.05mol) dec-5-en-2,8-diine-1,10-diol (I) and 20 ml acetone was placed in a three-necked flask, then in mixing was gradually added the oxidative mixture consisting of 15 g chromium anhydride, 8.5 g concentrated sulphuric acid and 20 ml water. The temperature of reaction mixture was kept in the range of 35°C - 40°C. The contents of flask was mixed still for 6 h at room temperature, then was diluted by water, was extracted by ether and dried over calcined magnesium sulphate. After
distillation of ether the residue was distilled in vacuum. At precipitation from aqueous solution it is prepared an amorphous powder (V) with m. p. 142°C, yield 80.7%. Found, %: C 62.19, H 4.08. C₁₀H₁₆O₄. Calculated, %: C 62.05, H 4.19.

**Oct-4-ene-2,7-diene (VI).** In nitrogen current (110°C - 115°C) it has been distilled 5.5 g (0.025 mol) compound (II) in the presence of 1g powdered potassium hydroxide. The heating was carried out so that the splitting products were slowly distilled to coil receptor. It has been prepared the equimolar mixture of acetone and compound (VI) (in ratio 52:48 on GLC) with m. p. 98°C - 99°C, n_d^20 1.4450, d_4^20 0.8735, yield 78.6%. Found, %: C 92.37, H 7.61. C₅H₈. Calculated, %: C 92.26, H 7.74.

**2.11-Dimethyldodeca-1,6,11-trien-3,9-diine (VII).** 3.5 g (0.016 mol) of the compound (II), 4 g KHSO₄, 0.03 g of hydroquinone and 10 ml of toluene was placed in a two-necked flask equipped with mechanical mixer, reflux condenser and thermometer. The mixture was stirred at temperature 70°C - 80°C for 6 h. Isolated 65.4% of substance (VII) with m. p. 55°C - 56°C (20 mm), n_d^20 1.4625, d_4^20 0.8993. Found, %: C 91.70, H 8.89. C₁₄H₁₆. Calculated, %: C 91.85, H 8.75.

**Epoxidation of the compound (I).** To the mixed solution 8.2 g (0.05mol) of the compound (I) in 30 ml abs. diethyl ether at 15°C - 20°C 8.1 ml of 45% peracetic acid was added. In 4 h 19 ml of 5% aqueous solution of sodium bicarbonate was added to a reaction mixture, was washed by water, dried sodium sulphate and distilled off a solvent. After recrystallization the compound (VIII) was isolated with m. p. 80°C - 81.5°C, yield 76.5%. Found, %: C 66.52, H 6.29, C₁₀H₁₆O₃. Calculated, %: C 66.65, H 6.17.

Analogously from compounds (II) the epoxide (IX) with m. p. 86°C - 87°C, yield 70.4% has been synthesized. Found, %: C 71.09, H 8.43. C₁₁H₂₀O₃. Calculated, %: C 71.21, H 8.54.

**Dichlorocyclopropanation of the compound (II).** To a mixture of 50 ml of 50% aqueous solution of sodium hydroxide and 0.4 g triethylbenzylammonium chloride in mixing for 4 h the solution of 6.6 g (0.03 mol) of the compound (II) in 50ml chloroform was added. The reaction temperature was kept equal to 22°C - 25°C. The mixture was mixed still for 1h at 40°C, then was added 200 ml water and extracted by ether. The organic layer was separated, dried over MgSO₄. After recrystallization the compound (X) was isolated with m. p. 90°C - 91°C, yield 28.5%. Found, %: C 59.58, H 6.52, Cl 23.22. C₁₁H₂₀Cl₂O₂. Calculated, %: C 59.41, H 6.65, Cl 23.39.

**Epoxidation of endiene (VI).** 1) Analogously to a method of preparation of the compound (VIII) from endiene (VI) and peracetic acid the epoxide (XI) with m.p. 119°C - 120°C, n_d^20 1.4565, d_4^20 0.8910, yield 78.3% has been synthesized. Found, %: C 79.81, H 66.47. C₅H₈O. Calculated, %: C 79.97, H 66.39.

2) **Counter synthesis (XI).** Analogously to a method of preparation of the compound (VI) from 6,7-epoxydeca-3,9-diene-2,11-diol (IX) the compound (XI) with yield 68.6% with identical data has been prepared.

**Synthesis of chlorohydrins of diine series (XII, XIII).** **General technique:** To a mixture of 0.05 mol of 10% aqueous solution of HCl for preparation of chlorohydrin and 0.05 mol of the compound (I or II) in mixing through dropping funnel 0.06 mol of 26% - 30% aqueous solution of hydrogen peroxide (feeding rate 10 g/h) was introduced and was continued the mixing for 5 - 6 h at 35°C - 40°C. After neutralization and drying of organic layer by distillation in vacuum the compounds (XII, XIII) with the following physical-chemical characteristics were isolated: (XII), m. p. 126°C - 127°C, yield 69.2%. Found, %: C 55.36, H 6.18, Cl 16.23. C₁₃H₁₇ClO₃. Calculated, %: C 55.44, H 6.05, Cl 16.36; (XIII), m. p. 131.5°C - 132.5°C, yield 73.1%. Found, %: C 61.52, H 7.89, Cl 13.12. C₁₄H₂₁ClO₃. Calculated, %: C 61.64, H 7.76, Cl 13.

**Counter synthesis of epoxide (XI).** To a mixture 13.6 g (0.05 mol) chlorhydrin (XIII) and 25 ml toluene in mixing 10 g potassium hydroxide keeping temperature of reaction mass in the ranges of 40°C - 50°C was gradually added, then in mixing was heated to 95°C - 100°C and was ended at this temperature for 8 h. After cooling the organic layer was separated and dried by sodium sulphate. By distillation in the presence of hydroquinone the epoxide (XI) with yield 86.7% with identical data was isolated.

By analogous method from chlorhydrin (XII) the epoxide (VIII) with yield 89.2% with identical data has been prepared.

**Chemical hydrolysis of epoxide (XI).** 6 g (0.05 mol) of 4,5-epoxyocta-1,7-diine (XII) was gradually added to 20 ml of 10% aqueous solution of sulphuric acid. In view of considerable isolation of heat the reaction flask was cooled and in the course of reaction by ice water. After half-hourly mixing the reaction finished. The aqueous solution was saturated by common salt and was repeatedly extracted by ether and then chloroform. After distillation of solvent it was isolated a glycol (XIV) with m. p. 85°C - 86°C, yield 86.7% by recrystallization. Found, %: C 69.40, H 7.49. C₁₀H₁₀O₂. Calculated, %: C 69.54, H 7.30.

**4.2. Microbiological method**

**Microbiological hydrolysis of epoxide (XI).**

1) **Preparation of biomass.** For preparation of biomass of fungi 3 liter fermenter which filled by 1 litre liquid nutrient medium was used. 10ml of liquid paraffin and 0.005 ml of silicon antifoamer for prevention of mass outflow was added to a medium. The incubation
was carried out under 25°C - 27°C. Then the suspension of mycelium of the investigated strains of fungi was added. After two days the incubation of mycelium was filtered, washed with sterile water and placed back in fermenter which already filled by 11 pH 7 phosphate buffer (0.1 M) solution and also medium of enriched nitrogen and air. The yield of product provided by step-wise treatment of samples: for each sample the mycelium was filtered and the obtained biomass of fungi was used in further experiments for realization of biohydrolysis. After decantation the liquid phase was treated by solution NaCl and then was extracted twice by ether. The organic layer was dried (MgSO4), then was steam-er in vacuum. The crystalline diol (XIV) crushed to powdered mass which was added to flask (2%) containing Czapek's medium without sugar (100 ml) was used for process of oxidation. The biomass was prepared according to the above-mentioned method and in a quantity 71.63, yield 78.9%. After incubation the prepared biomass was filtered, and the remaining liquid was filtered and the obtained biomass of fungi was used in this experiment. The chosen epoxides of acetylene series (XI), (0.1 - 1 g) in EtOH (1 ml), the mixture was poured to flask and was incubated at temperature 25°C - 26°C for 1 - 4 days. After decantation the liquid phases were treated by solution of NaCl, and then were twice extracted by ether. The organic layer was dried (MgSO4), then was steam-er in vacuum. The crystalline diol (XIV) crushed to powdered mass which was added to flask (2%) containing Czapek's medium without sugar (100 ml) was used for process of oxidation. The biomass was prepared according to the above-mentioned method and in a quantity 71.63, yield 78.9%. After incubation the prepared biomass was filtered, and the remaining liquid was extracted in petroleum-ether after recrystallization the glycol (XIV) with m. p. 85.5°C - 86.5°C, yield 86.7% was isolated. Found, %: C 69.38, H 7.40. C9H10O2. Calculated, %: C 69.54, H 7.30.

2) Biohydrolysis. Biohydrolysis was carried out in Erlemeyer flask (0.5l) containing phosphate buffer (0.1l, 0.1 M, pH 8) and 10% of biomass of fungi prepared from previous experiment. The chosen epoxides of acetylene series (XI), (0.1 - 1 g) in EtOH (1 ml), the mixture was poured to flask and was incubated at temperature 25°C - 26°C for 1 - 4 days. After decantation the liquid phases were treated by solution NaCl, and then were twice extracted by ether. The organic layer was dried (MgSO4), then was steam-er in vacuum. After removal of ether by recrystallization the glycol (XIV) with m. p. 85.5°C - 86.5°C, yield 86.7% was isolated. Found, %: C 69.38, H 7.40. C9H10O2. Calculated, %: C 69.54, H 7.30.

5. Conclusions

On the basis of splitting reaction (Favorsky reverse reaction) and dehydration of endiine tertiary alcohol, the endiine hydrocarbons being of interest for preparation of analogs of the natural compounds have been synthesized. The synthesized epoxide derivatives of diacetylene series can be used as the chemically active reagents for polymer materials.

The prepared diketones with two terminal acetylene bonds by the microbiological oxidation from the corresponding diols being potential biologically active substances can be used in organic chemistry with the aim to study theoretical and practical problems. It has been revealed during hydrolysis of epoxide compounds by the chemical and microbiological methods that during microbiological hydrolysis, the optically active trans-structured diols are formed which are practically useful substances.

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