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SYNTHESIS OF HYDROBROMIDE OF 2-(GLYCYLCYSTINYLAMINO) – 2-(4-METHOXYPHENYL)-1,3-INDANDIONE AND INVESTIGATION OF ITS EFFECT ON SOME FUNCTIONS OF ISOLATED LIVER MITOCHONDRIA

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ABSTRACT

2-(N-benzyloxycarbonylcystinylamino)-2 -(4-methoxyphenyl)-1,3-indandione (Z-Ⓓys-NH-AID)$_2$ (compound **1**) has been obtained as a result of the aminolysis of the activated ester 3-0-(N-benzyloxycarbonylcystinyl)-hydroxy-2-phenylindenone (Z-Ⓓys-OA)$_2$ with 2-amino-2-(4-methoxyphenyl)-1,3-indandione H_2N-AID. By analogy, the interaction of the activated ester 3-0-(N-benzyloxycarbonylglycyl)-hydroxy-2-phenylindenone (Z-Gly-OA) with (H-Ⓓys-NH-AID)$_2$ leads to the synthesis of amide of the dipeptide 2-(N-benzyloxycarbonylglycylcystinyl-amino)-2-(4-methoxyphenyl)-1,3-indandione (Z-Gly-Ⓓys-NH-AID)$_2$ (compound **3**). The hydrobromide of 2-(glycylcystinylamino)-2-(4-methoxyphenyl)-1,3-indandione (HBr.H-Gly-Ⓓys-NH-AID)$_2$ (compound **4**) has been found to show a stimulatory effect on functions of respiratory chain in isolated rat liver mitochondria.

Key words: 2-phenyl-1,3-indandione and its isomeric form 3-hydroxy-2-phenylindenone, activated ester, aminolysis, 2-amino-2-(4-methoxyphenyl)-1,3-indandione, adenosine tri-phosphatase activity.

INTRODUCTION

Indane compounds exhibit different physiological action. Derivatives of 1,3-indandiones have been investigated in detail (Wanag, 1960). Many of these compounds are used as blood anticoagulants (Ozol, Germane, et al., 1969) or as compounds with pronounced influence on the central nervous system (Belenkii, Germane, et al., 1960; Germane, Kamenov, et al., 1978; Kabat, Stohlman, et al., 1944). According to their relation to the blood, 2-aryl-1,3-indadiones are classified as antagonists of K group vitamins (Quick, Gollentine, 1951; Miloshev, Aleksiev, et al., 1972). Other indandione derivatives decrease adenosine tri-phosphatase (ATF-ase) activity of the myosin (Bogdanov, 1970) and infringe upon the bioenergetical functions of mitochondria (Martins, 1966; Perlick, 1964). The large spectrum of physiological activities of 1,3-indandiones, as well as the ability of the internal membrane of mitochondria to prevent passing of many metabolites including most of the aminoacids, determined the aim of the present work – to investigate the influence of amides of natural aminoacids and peptides with 2-amino-2-(4-methoxyphenyl)-1,3-indandione on mitochondria functions.

Among the investigated hydrobromides of natural aminoacids and dipeptides with 2-amino-2-aryl-1,3-indandiones having generalized formulas I and II, the hydrobromide of 2-(glycylcystinylamino)-2-(4-methoxy-phenyl)-1,3-indandione **4** showed pronounced influence on the mitochondria functions.

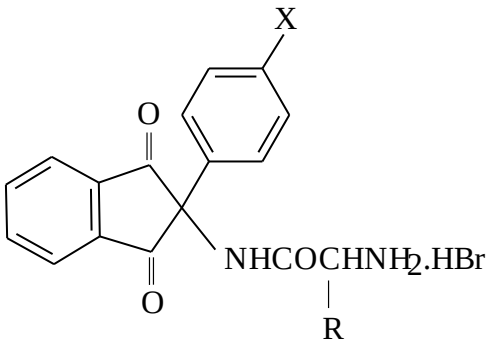
The compound enumerated as **4** can be synthesized in good yield and high purity according to the scheme presented below.

All abbreviations non-described in the paper follow the IUPAC-IUB system (IUPAC-IUB Commission of Biochemical Nomenclature, 1967).

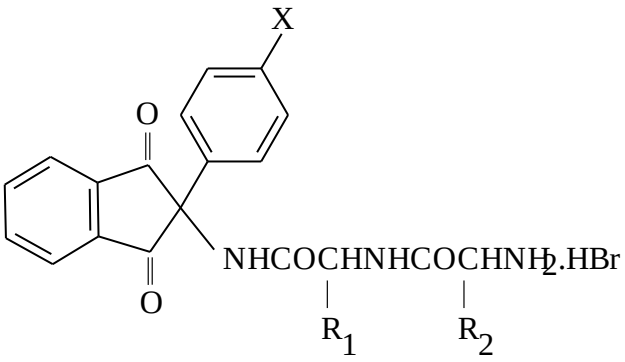
Compounds enumerated as **1**, **3**, and **4** are crystalline, and the hydrobromide **2** is amorphous substance. All data on newly synthesized compounds are presented in "Results and discussion" section.

MATERIALS AND METHODS

The melting points were determined in an opened capillary without correction. The infrared spectra were taken in a nuoil suspension on an IKS-22 spectrophotometer. The optical rotation $[\alpha]_D$ was determined with a CarlZeiss Polarimeter. The homogeneity of the synthesized compounds was checked by TLC on Silufol plates using the following developing systems: B, ethyl acetate – petroleum ether (1:1); C, pyridine – n-butanol – acetic acid – water (10:15:3:12); D, benzene – methanol – acetic acid (15:2:3); E, n-butanol – methanol – water (4:1:1).

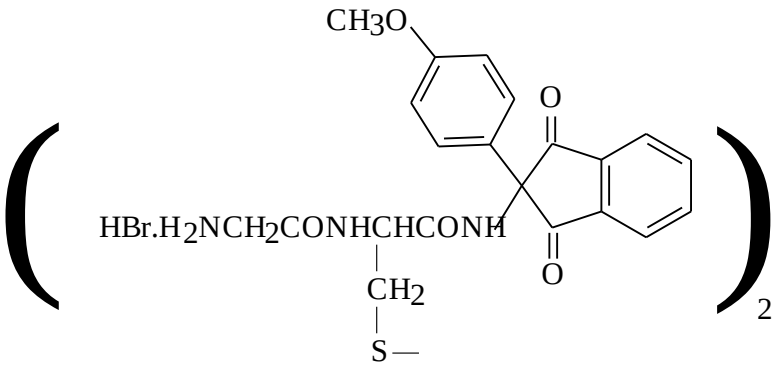


I



II

X = H, OCH_3



4

The following scheme has been used for synthesis of compound **4**:

$$(\text{Z-Cys-OA})_2 + 2\text{H}_2\text{N-AID} \longrightarrow (\text{Z-Cys-NH-AID})_2 \xrightarrow{\text{HBr/CH}_3\text{COOH}}$$

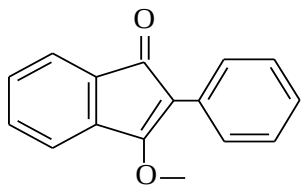
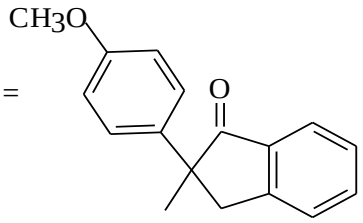
1

$$(\text{HBr.H-Cys-NH-AID})_2 \xrightarrow{\text{NH}_3/\text{CHCl}_3} (\text{H-Cys-NH-AID})_2 \xrightarrow{\text{2Z-Gly-OA}}$$

2

$$(\text{Z-Gly-Cys-NHAID})_2 \xrightarrow{\text{HBr/CH}_3\text{COOH}} (\text{HBr.H-Gly-Cys-NH-AID})_2$$

3 4

A =  ; AID = 

The initial compounds were synthesized by known methods: 2-amino-2(4-methoxyphenyl)-1,3-indandione (H_2N-AID) (Aren, Wanag, 1959), 3-0-(N-benzyloxycarbonylglycyl)-hydroxy-2-phenylindenone (Z-Gly-OA) and 3-0-(N-benzyloxycarbonylcystinyl)-hydroxy-2-phenylindenone (Z-Ⓣys-OA)$_2$ (Minchev, Derdowska, et al., 1980).

Intact rat liver mitochondria were isolated with insignificant modifications using Johnson and Lardi method (Johnson, Lardi, 1967). Following Sholts, Ostrovskii (1975) mitochondria respiration was registered with a Universal polarograph OH-105 supplemented with covered Pt electrode combined with chloride-silver electrode as reference electrode. Following Lowry, Lopes (1946) conclusions on the ATF-ase activity were drawn based on the increase of inorganic phosphate.

RESULTS AND DISCUSSION

1. Preparation of 2 - (N-benzyloxycarbonylcystinyl-amino) - 2 - (4-methoxyphenyl) - 1, 3 - indandione (Z-Ⓣys-NH-AID)$_2$ 1.

0.003 M (2.75 g) of activated ester (Z-Ⓣys-OA)$_2$ dissolved in 30 ml of dry ethyl acetate were added to 0.006 M (1.6) g of H_2N-AID dissolved in 25 ml of dry ethyl acetate. The obtained solution was left for 24 hours at room temperature. 2-Phenyl-1,3-indandione released in the aminolysis course was separated by washing the ethyl acetate layer several times with 10 % sodium bicarbonate solution, 2 N HCl, and water. Then the organic phase was dried over anhydrous Na_2SO_4. The ethyl acetate was evaporated in vacuo to dryness. The oily residue was re-crystallized from chloroform/petroleum ether and a light-green crystalline compound was obtained. Yield 2.31 g (76.5 %); m. p. 141-142 °C; $[\alpha]_D^{21}$ = +88.9° (c=1, ethyl acetate); $C_{54}H_{46}N_4O_{12}S_2$ (1007.1); calculated N 5.56; found N5.56; calculated S 6.37; found S 6.11; R_{fB} = 0,43; R_{fE} = 0.90; IR:$\nu_{(C=0)}$ of indandione 1750, 1720; $\nu_{(C=0)}$ amide I 1650; $\delta_{(N-H)}$ + $\nu_{(C-N)}$ 1510; $\nu_{(N-H)}$ 3300-3100; $\nu_{(C-O-C)}$ 1250, 1060 / cm.

2. Preparation of hydrobromide of 2-cystinylamino-2-(4-methoxyphenyl) - 1, 3 - indandione (HBr.H-Ⓣys-NH-AID)$_2$ 2.

2g of hydrogen bromide saturated in glacial acetic acid were added to 0.002 M (2.01 g) of (Z-Ⓣys-NH-AID)$_2$. The reaction flask was preserved from the atmospheric humidity by use of tubule filled with calcium chloride. After the mixture had stayed about 30-40 min the excess hydrogen bromide and glacial acetic acid were removed in vacuo at temperature of 40 °C. 50 ml of dry ethyl ether were added to the oily residue, and the obtained mixture was left for several hours at 0°C. Then the separated amorphous hydrobromide was filtered. The obtained hydrobromide was purified by reprecipitation from absolute methanol/ethyl ether. Yield 1.1 g (61.1 %); R_{fC} = 0.67; R_{fE} = 0.51.

Dehydrobromation of the compound **2** was made by addition of chloroform saturated with gaseous ammonia for an hour at temperature of 5 °C to 0.001 M (0.9 g) of (HBr.H-Ⓣys-NH-AID)$_2$. Having the ammonium bromide separated, the filtrate was evaporated in vacuo. The obtained oily product (H-Ⓣys-NH-AID)$_2$ was used immediately for synthesis of dipeptide **3**.

3. Preparation of 2-(N-benzyloxycarbonylglycylcystinyl-amino)-2-(4-methoxyphenyl)-1,3-indandione (Z-Gly-Ⓣys-NH-AID)$_2$ 3.

0.002 M (0.83 g) of activated ester Z-Gly-OA dissolved in 15 ml of dry ethyl acetate were added to 0.001 M solution of (H-Ⓣys-NH-AID)$_2$ in 15 ml dry ethyl acetate. (The (H-Ⓣys-NH-AID)$_2$ was obtained by dehydro-bromation of 0.001 M (0.9 g) (HBrH-Ⓣys-NH-AID)$_2$ with chloroform saturated with ammonia.) The solution was left for 24 hours at room temperature. The ethyl acetate layer was washed several times with 10 % solution of sodium bicarbonate, 2 N HCl, and water. Then it was treated with activated carbon, dried over anhydrous Na_2SO_4, and evaporated in vacuo to dryness. The oily residue was recrystallized from ethylacetate/petroleum ether and a light-green substance was obtained under the form of fine needles. Yield 0.7 g (62.4 %); m. p. 150-151 °C; $[\alpha]_D^{18}$ = -57.9° (c=1, dimethylformamide); $C_{58}H_{52}N_6O_{14}S_2$ (1121.2); calculated N 7.49; found N 7.44; calculated S 5.72; found S 5.83, R_{fC} = 0.80; R_{fD} = 0.30; IR:$\nu_{(C=0)}$ of indandione 1720, 1710; $\nu_{(C=0)}$ amide I 1650; $\delta_{(N-H)}$ + $\nu_{(C-H)}$ 1510; $\nu_{(N-H)}$ 3300-3000; $\nu_{(C-O-C)}$ 1250, 1040 / cm.

4. Preparation of hydrobromide of 2-(glycylcystinyl-amino) – 2-(4-methoxyphenyl)-1,3-indandione (HBr.H-Gly-Ⓣys-NH- AID)$_2$ 4.

0.0005 M (0.56 g) of (Z-Gly-Ⓣys-NH-AID)$_2$ **3** were treated with 0.5 g of hydrogen bromide dissolved in glacial acetic acid. Having the mixture stayed for 30-40 min, the glacial acetic acid was distilled in vacuo at 40 °C. 30 ml of dry ethyl ether were added to the oily residue obtained. The mixture was kept for several hours at 0 °C and the separated amorphous hydrobromide was crystallized from methanol/diethyl ether. Yield 89.4 % (0.45 g); m. p. 207-209 °C; $[\alpha]_D^{20}$ = -13.4° (c=1, methanol); $C_{42}H_{42}N_6O_{10}S_2Br_2$ (1014.8); calculated N 8.28; found N 8.34; calculated S 6.32; found S 6.36; calculated Br 15.75; found Br 15.93; R_{fC} = 0.70; R_{fE} = 0.40.

5. Determination of ATF-ase activity of rat liver mitochondria

The hypothesis that investigated compound possesses activity similar to the activity of dinitrophenol (DNF) was studied by determination of latent mitochondria ATF-ase. When applied in concentrations doubling the 2,4-dinitrophenol (DNF) concentration, compound **4** stimulates the hydrolysis of ATF-ase – Fig. 1.

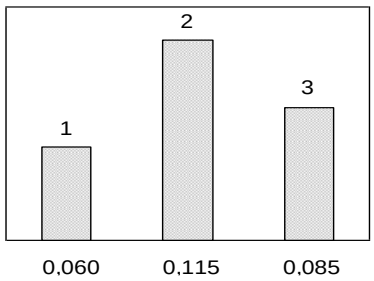


Figure 1. ATF-ase activity of rat liver mitochondria:
*1 – standard; 2 – in the presence of DNF (80 μg); 3 – in the presence of compound **4** (160 μg); reaction medium: 0.2 M saccharose, 0.02 M tris –HCl (pH 7.5), 50 mM KCl, 2mM ATF, mitochondria - 3.72 mg protein; final volume of 4 ml; room temperature.*
Numbers denote the rates of ATF-ase activity in μM of inorganic phosphate for 10 min.

Bearing in mind the results from similar experiments with other indandione modifiers, where physiological effect has not been registered, it could be supposed that the activity of compound **4** is due to its molecule as a whole. That is why the investigation of mitochondria processes in presence of the compound **4** and similar compounds represents an interesting scientific task.

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