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Esterification of 7-theophyllineacetic acid with diethylene glycol monomethyl ether

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The kinetics of esterification of 7-theophyllineacetic acid with diethylene glycol monomethyl ether in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine as catalyst was studied. According to the known mechanism, besides the main process, the side-reaction of intramolecular rearrangement with formation of pharmacologically active *N*-acylurea occurs. The course of the main and the side-process was monitored by RP-HPLC with UV-detection. For that purpose, quantification of both ester and *N*-acylurea in the reaction mixture was performed. Influence of the concentration of the reactants (acid, alcohol and catalyst) on the progress of esterification and preparation of the by-product was investigated. Based on the obtained results, the reaction conditions leading to maximal yield of the ester and *N*-acylurea are proposed. The possibility of turning esterification to the synthesis of the side-product was also found. Reactions of the preparation of both the ester and *N*-acylurea were found to follow first-order kinetics. The rate constants of both processes were estimated.

Keywords: 7-theophyllineacetic acid ester, *N*-acylurea, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, esterification, kinetics

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It is well known that 7-theophyllineacetic acid (1,2,3,6-tetrahydro-1,3-dimethyl-2,6--dioxopurine-7-acetic acid) is a pharmacologically active compound prescribed as an antiasthmatic agent, cardiac stimulant or diuretic (1). Its esters are also interesting because of their improved absorption and reduced toxicity and side effects. A widely used and promising method for esterification is the carbodiimide coupling approach, involving interaction between the acid and alcohol in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP) as catalyst (2, 3). In previous studies, several 7-theophyllineacetic acid esters were described, which were synthesized by means of this method at the molar ratio of the reactants acid/alcohol/catalyst 0.01:0.011:0.0025 (4–6). According to the known mechanism, the side-reaction during DCC/4-DMAP me-

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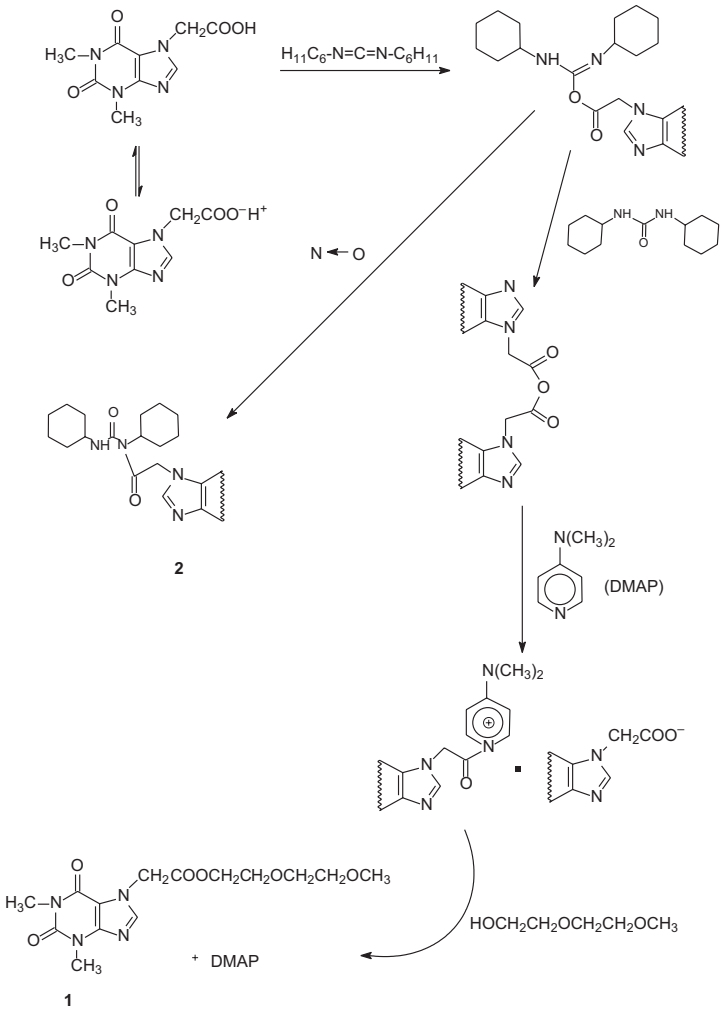
diated esterification is the formation of *N*-acylurea by an intramolecular rearrangement of *O*-acylurea (Scheme 1) (7). The side-product belongs to the group of acylureas. Some structurally similar compounds are used in therapy as antiepileptic and sedative drugs. Pharmacological and toxicological tests of the side product demonstrated extremely reduced acute toxicity of this substance (LD_{50} *i.p.* in mice > 7.88 mmol kg^{-1}). Lack of stimulating influence on the CNS was also established (5). The above mentioned pharmacological and toxicological properties of *N*-acylurea are a serious prerequisite for selection of appropriate reaction conditions ensuring satisfactory high yields of the ester as well as the side-product. Development of suitable reaction conditions for optimization of es-



Scheme 1

ter synthesis is very important. In cases of processes involving side-reactions, like this one, it is of interest to find conditions under which the by-product is obtained in a high yield compared to the ester.

The aim of this study is kinetic investigation of the esterification of 7-theophyllineacetic acid with diethylene glycol monomethyl ether [2-(2-methoxyethoxy)-ethanol)] and the choice of optimal reaction conditions for preparation of the ester (1,3-dimethyl-2,6--dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetic acid-2-(2-methoxy-ethoxy)-ethyl ester (**1**) and the by-product 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7--yl)-acetyl]-urea (**2**) (8), as well as for the side-process (Fig. 1).

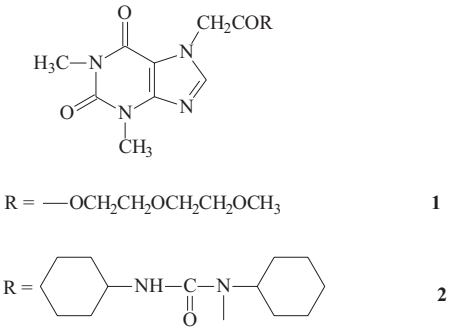


Fig. 1. Structures of the ester **1** and urea by-product **2**.

EXPERIMENTAL

Reagents

The mobile phase was prepared from HPLC grade methanol. All other reagents (*p. a.*) were used without further purification.

Instrumentation and chromatographic conditions

Chromatography was carried out isocratically, on a modular HPLC system LC-10A Shimadzu (Japan) provided with a LC-10A pump, Rheodyne injector with 20 μL loop, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved on a reversed phase analytical column LiChrosorb RP-8, 250 x 4 mm, 10 μm (Merck, Germany). The mobile phase consisting of methanol and water mixture (50:50, *V/V*) was pumped at a flow rate of 1 mL min^{-1}. The column effluent was monitored at 220 nm.

Sample preparation

The reaction was initiated by complete dissolution of 7-theophyllineacetic acid, diethylene glycol monomethyl ether and 4-DMAP in 150 mL dimethylformamide (DMF), after which 2.7 g DCC dissolved in 10 mL DMF was added. Esterification was carried

Table I. Molar ratio of reactants

Variant	Molar ratio acid/alcohol/catalyst
A	0.01:0.011:0.0025
B	0.01:0.011:0.0050
C	0.01:0.022:0.0050
D	0.01:0.022:0.0025

out at different molar ratios of acid/alcohol/catalyst, as indicated in Table I. The reaction mixture was stirred at room temperature until a white precipitate of dicyclohexylurea (DCU) was formed, which was filtered off. After concentration of the filtrate on the rotary evaporator to 1/3 volume, 10 mL anhydrous ethanol was added and the solution was kept for 24 h at –5 °C. The crystals (DCU and free 7-theophyllineacetic acid) were filtered off and the filtrate was evaporated. The samples were incubated for various time intervals up to 8 h.

At the end of incubation, the samples were immediately treated as follows: the residue was dissolved in 50.0 mL methanol and then stepwise diluted. The RP-HPLC procedure, described above, was adopted to monitor the appearance of the ester (**1**) and 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea (**2**) (Scheme 1). The analytical method proposed was specific for determination of the ester and *N*-acylurea because no other peaks were co-eluted and the retention times of the compounds investigated coincided with those of reference substances. On the other hand, peaks of 4-dimethylaminopyridine and dicyclohexylcarbodiimide that usually accompany analyzed samples were not detectable under the chosen chromatographic conditions. Quantification of both compounds was achieved with reference to suitably prepared calibration curves, using peak areas.

RESULTS AND DISCUSSION

According to the mechanism of esterification (Scheme 1) suggested by Wiener (7), the initial formation of an *O*-acylurea occurs by the reaction of carboxylic acid and DCC. Intramolecular migration of *O* to *N* produces *N*-acylurea. Nucleophilic attack of the carboxylate forms a symmetrical anhydride and DCU. The symmetrical anhydride then reacts with the catalyst 4-DMAP to generate an acylpyridinium carboxylate ion pair, which upon reaction with alcohol gives the ester, liberates the catalyst and carboxylate. The latter is recycled by DCC to form more anhydride.

In our previously reported investigations, the TLC method was used for monitoring the progress of esterification and formation of 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea (4–6). This method is semi-quantitative and was applied only for preliminary estimation of the progress of reactions. In the present study, the course of reactions was followed by means of the more appropriate RP-HPLC procedure, which enabled separation and simultaneous determination of 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea and the ester in the presence of DCU, DCC and 4-DMAP.

The basic reaction variant, variant A, included the molar ratio acid/alcohol/catalyst = 0.01:0.011:0.0025. Analytical yields for both compounds were determined varying the molar ratios of the reactants – alcohol and catalyst (Table I). It was found that a change in the molar ratio leads to variation in the yields of the ester and 1,3-dicyclohexyl-1-[2--(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea. Influence of an excess of alcohol and catalyst was studied and the following results were obtained (Fig. 2): (*i*) Doubling the alcohol concentration leads to a three-fold increase in the quantity of synthesized ester and to a reduction of the yield of 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6--dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea by about a half (variant D). (*ii*) Utilizing a double excess of catalyst results in a four-fold rise in the ester quantity, and no significant decrease of *N*-acylurea yield (variant B). This enhancement of the ester yield can be explained by the action of 4-DMAP as a base that accelerates the acylation of al-

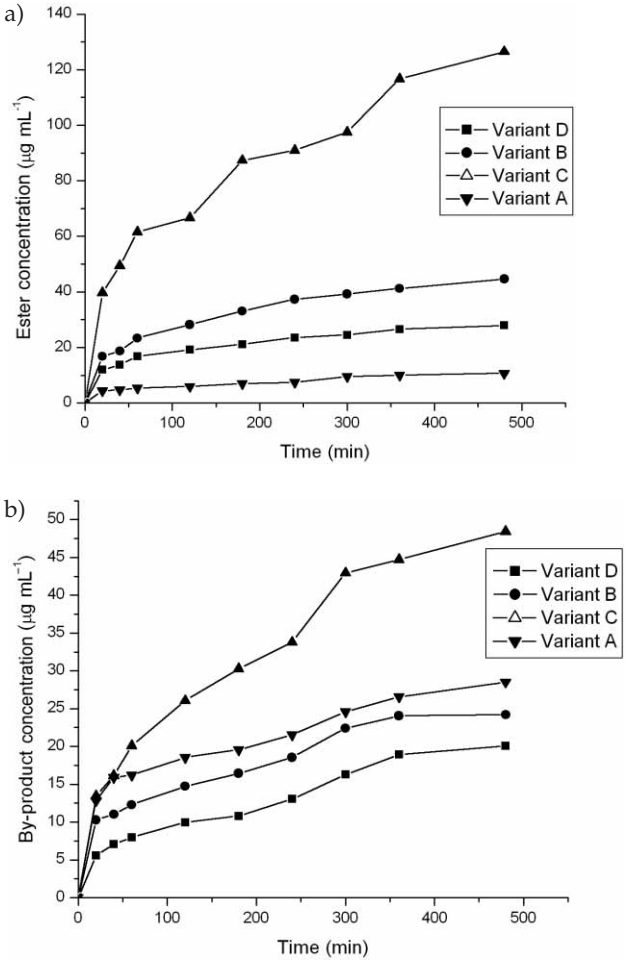


Fig. 2. Time course of appearance of: a) ester and b) urea by-product under different reaction conditions.

Table II. Rate constants of the formation of the ester and urea products

Variant	$K \times 10^{-4}$ (min^{-1})	
	Ester	Urea
A	42.2	54.5
B	105.5	50.3
C	95.5	52.5
D	96.0	74.1

cohol by the acylpyridinium salt. On the other hand, when excess 4-DMAP is used, a direct attack of the latter on the *O*-acylurea occurs, resulting in the formation of the acylpyridinium-dicyclohexyl uronium ion pair, which reacts with alcohol to give the ester, DCU and liberates 4-DMAP. Next, the catalyst is recycled to react with *O*-acylurea. (*iii*) Appreciable increase in the yields of both compounds was observed – about 2 times for 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea and about 10 times for the ester, when the concentrations of alcohol and catalyst were enhanced simultaneously (variant C). This can be attributed to additive effects of both factors.

Among the reaction conditions investigated, those of variant A appeared to be optimal for the preparation of 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea (yield of *N*-acylurea was found to be 3 times higher than that of the ester). It was proved that simultaneous enhancement of both alcohol and catalyst concentrations stimulated the formation of the ester in the highest yield.

It was found that the interdependence of ester and *N*-acylurea concentrations as a function of time is linear, indicating good correlation with the model of first-order reaction kinetics (regression coefficients were higher than 0.98). The rate constants of esterification and *N*-acylurea formation were calculated in accordance with the relationships typical of parallel first-order kinetics processes. The rate constants of both reactions were estimated from the slopes of the semilogarithmic plots of c_∞-c/t curves, where c_∞ represents the concentration of the compound at the end of reaction time and c is the concentration of the product at each of the time intervals. The results obtained are summarized in Table II.

CONCLUSIONS

It was found that the molar ratio acid/alcohol/catalyst = 0.01:0.022:0.0050 is optimal for preparation of the ester. At the molar ratio of the mentioned reactants 0.01:0.011:0.0025 the by-product 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7--yl)-acetyl]-urea was obtained in predominantly high yield compared to the ester. The possibility of turning esterification to the synthesis of pharmacologically active 1,3-dicyclohexyl-1-[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)-acetyl]-urea was also found, which is of great importance. Further studies are needed to clarify the influence of different alcohol substituents on the kinetics of the observed processes.

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S A Ž E T A K

Esterifikacija teofilin octene kiseline s dietilenglikol monometil eterom

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U radu je proučavana kinetika esterifikacije 7-teofiln octene kiseline s dietilenglikol monometileterom u prisutnosti diciklohekslkarbodiimida i 4-dimetilaminopiridina kao katalizatora. Prema poznatom mehanizmu, pored glavne reakcije esterifikacije, zbiva se i sporedna reakcija u kojoj intramolekularnom pregradnjom nastaje farmakološki aktivna *N*-acilurea. Tijek glavne i sporedne reakcije praćen je pomoću RP-HPLC s UV detekcijom, uz kvantifikaciju estera i uree. Praćen je utjecaj koncentracije reaktanata (kiseline, alkohola i katalizatora) na reakciju esterifikacije i sporedne reakcije te su pronađeni reakcijski uvjeti za usmjeravanje sinteze prema esteru ili ureii. Obje reakcije slijede kinetiku prvog reda. U radu su određene njihove konstante brzine.

Ključne riječi: ester 7-teofilin octene kiseline, *N*-acilurea, diciklohekslkarbodiimid, 4-dimetilaminopiridin, esterifikacija, kinetika

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