SYNTHESIS AND CHARACTERIZATION OF A NEW STARCH ESTER WITH *N*-[(*N'*-TIAZOLYL)-*p*'-(BENZENESULPHONE)] AMIDE OF *N*-(*o*-NITROBENZOYL)-*D,L*-ASPARAGIC ACID

MIHAELA HOLBAN, VALERIU SUNEL[*], MARCEL POPA[*] and CATALINA LIONTE[***]

"Alexandru Ioan Cuza" University, Department of Organic Chemistry, Faculty of Chemistry, Iasi, Romania
[*]*"Gheorghe Asachi" Polytechnic University, Department of Natural Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, Iasi, Romania*
[***]*"Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Medicine, Iasi, Romania*

Received October 25, 2010

The paper studies the dicyclohexylcarbodiimide-activated coupling reaction through ester-type covalent bonds of a sulfonamide derived from N-(*m*-nitrobenzoyl)-L-asparagic acid on starch. The amount of sulfonamide chemically-bonded to the support is dependent on the reaction parameters, the maximum coupling efficiency being attained at the highest values of the reaction parameters (DCC/sulfonamide molar ratio, sulfonamide/starch molar ratio and reaction time). The kinetics of drug release from the conjugates is studied under alkaline hydrolysis conditions. The conjugates present low toxicity and medium level antimicrobial activity against *E. coli* and *S. aureus* strains.

Keywords: starch, sulfonamide, polymer-drug system, controlled release

INTRODUCTION

Most amino acid derivatives present biological activity. Their antibacterial,[1-4] antituberculosis,[5,6] antihypertensive[7,8] and antitumor properties[9,10] are remarkable. Moreover, some acyl-amino acids also have hepatoprotecting[11] and anti-inflammatory activity.[12] Literature data show acceptable biological activity for L-asparagic acid and its acylated derivatives, especially with its *m*– or *p*–substituted benzoyl radicals,[6,13-17] the compounds participating in various animal metabolic processes.[10,18-28]

Generally, sulfonamides present a low to medium antibacterial activity, their cell toxicity limiting the usage of such substances. Their chemo-therapeutical indices can be improved by coupling to macromolecular supports (especially polysaccharides, which are known to present high biocompatibility or biodegradability). The drug toxicity of sulfonamides and their derivatives may be reduced by a reaction with organism-friendly substances, such as hormones, metabolites, or amino acids.

The attachment of biologically active compounds to synthetic or natural polymers has been increasingly often preferred for drugs in free form, as it improves the efficiency of drug control/release and prolongs the release of bioactive compounds with minimum side effects. The controlled release of the bioactive compounds, which are covalently coupled to a polymeric carrier, can be achieved *via* hydrolytic or enzymatic cleavage of the linking bonds. One of the main challenges in designing drug delivery systems is to synthesize a biocompatible carrier, which is easily metabolized and eliminated, does not give adverse affects and is quite inexpensive. Polysaccharides are well-accepted candidates for drug carriers, as they are abundant and cheap. Moreover,

starch presents good biocompatibility and biodegradability.

The paper presents the synthesis of starch esters by covalent coupling of a polysaccharide with a sulfonamide derived from *N*-*m*-nitro-benzoyl-*L* asparagic acid, and the examination of the conjugates capacity of being control-hydrolyzed under digestive tract conditions, as to their toxicity and antimicrobial activity. The influence of certain reaction parameters on the efficiency of the esterification reaction is also analyzed by evaluating the percentage of immobilized drug into the conjugate.

EXPERIMENTAL

2-(o-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 (I) synthesis

0.018 moles of N-(o-nitrobenzoyl)-L-asparagic acid (II) were treated with 16 mL acetic anhydride and then refluxed for 60 min. After cooling, the solution was added to a mixture of 30 mL anhydrous ethyl ether and 50 mL anhydrous petroleum ether, a light yellow precipitate being thus formed. The product was vacuum-filtered and then vacuum-dried for 3-4 h at 45-60 °C. Oxazolone, which was purified from anhydrous benzene, was obtained in a 72% yield; its melting point was found at 156-157 °C and it was soluble in dimethylformamide (DMF) and dimethylsulphoxide (DMSO).

N-[(N'-tiazolyl)-p'-(benzenesulphone)] amide of N-(o-nitrobenzoyl)-D,L-asparagic acid (III) synthesis

A mixture of 0.01 moles Δ^2-oxazolinone-5 (I) dissolved in 30 mL anhydrous dioxane and 0.01 moles sulfathiazole was heated to 115-120 °C for 6 h in a glass reactor equipped with a condenser. Then, the excess solvent was removed by vacuum-distillation, until reaching a volume of 10 mL, then transferred into a glass crystallizer and allowed to cool. The addition of bidistilled water determined the formation of a yellow voluminous precipitate, which was subsequently filtered, washed with hot water and dried under vacuum at 50-60 °C for 5-6 h. The product was purified by recrystallization from a mixture of ethanol and water (1/2; v/v). The purified sulfonamide was synthesized in a 62% yield; its melting point was found at 125-126 °C and it was soluble in DMSO and DMF.

Conjugate synthesis and characterization

A dicyclohexyl carbodiimide (DCC) solution in DMSO was added to a solution of sulfonamide in DMSO, and the mixture was left to react for 24 h at 16-18 °C. Then, 0.2 g starch swollen in 20 mL DMSO was added (a moment considered as the beginning of the esterification reaction) and the mixture was stirred for various durations at 16-18 °C. The reaction mixture was precipitated with 15 mL acetone and the suspension centrifuged. Finally, the product was washed 3-4 times with acetone and water, then purified by Soxhlet acetone extraction for 24 h and finally dried under vacuum.

Sulfonamide release from conjugates

0.12 g of conjugate were suspended under stirring in 20 mL HCl or NaOH aqueous solution (pH = 3 or 12) at 37 ± 0.5 °C. The pH variations were registered with a Labcor Consort pH-meter, and corrected by considering the pH variation in time for NaOH solution and starch suspended in the NaOH solution.

Toxicity tests

Toxicity of the ester conjugates was assessed by determining the LD_{50} on male *Wistar* white mice of approximately 20 ± 2 g. The substance was dissolved in Tween80 and administered intraperitoneally to groups of four mice; the mortality after 7 days was noted.

Antibacterial activity of N-[(N'-tiazolyl)-p'-(benzenesulphone)] amide of N-(o-nitrobenzoyl)-D,L-asparagic acid

Sulfonamide was tested for its possible antibacterial activity against the growth of standard bacteria strains, such as *Staphylococcus aureus* ATCC 25932, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 17933, and hospital bacteria strains, such as *S. aureus, E. coli* and *P. aeruginosa*.

The Kirby-Bauer diffusion method was used, the tests being performed on *Mueller–Hinton agar*, with 48 h incubation, at 37° C.

RESULTS AND DISCUSSION

2-(o-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 and sulfonamide synthesis and characterization

The synthesis of sulfonamide (**III**) was based on the cyclization of *N*-(*o*-nitrobenzoyl)-*D,L*-asparagic acid (**II**) with acetic anhydride, followed by opening of the oxazolinone ring of 2-(*o*-nitrophenyl)-4-(β-carboxymethyl)-Δ2-oxazolinone-5 (**I**) through the reaction with sulfathiazole (Fig. 1).

The spectroscopic data summarized below proved the assumed chemical structure of synthesized 2-(*o*-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 (I). FT-IR (solid state): 1112 cm^{-1} (C-O-C); 1360 cm^{-1} and 1520 cm^{-1} (symmetrical/ asymme-

trical–NO_2); 1620 cm^{-1} (C=N); 720 cm^{-1} (C-H Ar). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 3-3.1 (2H, CH_2); 5.1 (1H, CH); 7.8 (1H, CH Ar); 8.4 (2H, CH Ar); 8.7 (1H, CH Ar).

Also, the structure of synthesized sulfonamide (III) was determined by spectral analysis.

FT-IR (solid state): 1311 cm^{-1} (–CO-O-); 1340 cm^{-1} and 1596 cm^{-1} (symmetrical/ asymmetrical–NO_2); 785 cm^{-1} (disubstituted aromatic ring –C-H); 1174 cm^{-1} (–SO_2-N-); 3224 cm^{-1} (amide) and 1719 cm^{-1} (-NH-). The assignment and chemical shifts of ^{1}H-RMN (DMSO d_6, 400MHz) δ_{ppm}: 2.95 (2H, CH_2); 10.3 (1H, COOH); 4.6 (1H, CH-); 7-7.8 (8H, CH Ar); 9 (1H, NH).

Sulfonamide immobilization onto starch and ester characterization

The coupling of sulfonamide to starch was based on the dicyclohexylcarbodiimide activated esterification reaction of the carboxylic groups from the active principle, with the polysaccharide hydroxyl groups (Fig. 2). The coupling reaction occurred in two distinctive steps: first, the addition of the sulfonamide carboxylic group to DCC, followed by the reaction of the intermediate with starch, with the formation of starch-sulfonamide conjugate and dicyclohexyl-urea (Fig. 2).

FT-IR data on the starch-sulfonamide conjugates showed the esterification between the active principle and starch by the presence of absorbance peaks at 1750 cm^{-1} (ester groups), 1596 cm^{-1} (-NO_2), 780-900 cm^{-1} (aromatic ring), 1174 cm^{-1} (–SO_2-N-).

Three varying parameters were considered for the esterification reaction: the DCC/sulfonamide molar ratio, the sulfonamide/starch molar ratio and the reaction time. As 1 mole sulfonamide contains 5 atoms of nitrogen and 1 atom of sulphur, while the starch molecule contains none of those, elemental analysis (nitrogen content assessment) was used for determining the sulfonamide content immobilized onto starch.

Table 1 shows the influence of the varying reaction parameters on the efficiency of sulfonamide conjugation onto starch.

Obviously, the percentage of sulfonamide immobilized in the final conjugate highly depended on only two of the considered reaction parameters: sulfonamide/starch molar ratio and reaction time.

As expected, the increase of sulfonamide content in the reaction mixture determined an increased percentage of immobilized sulfonamide in conjugates. The great number of hydroxyl groups contained by 1 mole of starch presented enough sites for esterification; however, the immobilization of the active principle in the conjugates had only relative efficiency, due to the steric hindrance created by the bulky molecule of sulfonamide. If calculated, the immobilized sulfonamide/starch molar ratio appears subunitary, suggesting that less than 1 hydroxyl group (primary -OH) of the starch structural unit participated at esterification.

The reaction time was also an important parameter of esterification, at least 24 h being necessary for attaining medium immobilization efficiency.

Table 1
Experimental program for the reaction of sulfonamide with starch

Sulfonamide/starch molar ratio	DCC/sulfonamide molar ratio	Reaction time (h)	% sulfonamide in conjugates
10	1.3	24	38
5	1.3	24	27
3	1.3	24	18.6
2	1.3	24	10.8
5	1.1	24	26.1
5	1.6	24	28.2
5	2	24	29
5	1.3	4	7.8
5	1.3	10	16.9
5	1.3	35	30

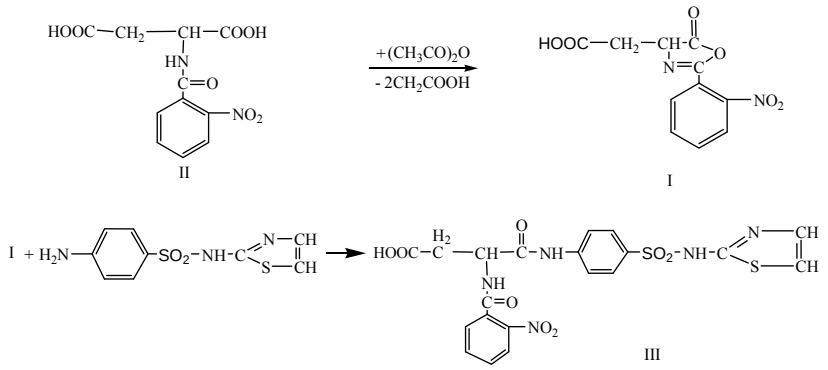

Figure 1: Synthesis of *N*-[(*N*'-tiazolyl)-*p*'-(benzenesulphone)] amide of *N*-(*o*-nitrobenzoyl)-*D,L*-asparagic acid

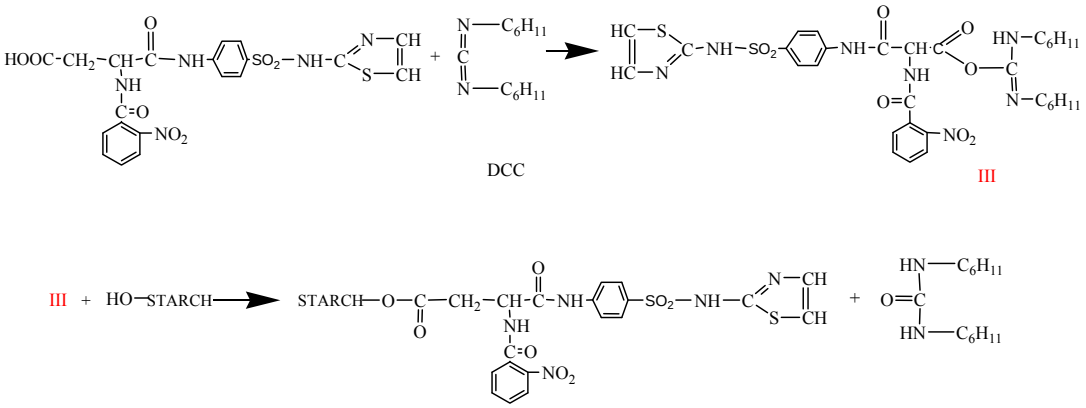

Figure 2: Sulfonamide covalent coupling with starch

Sulfonamide release from conjugates

Although the drug release kinetics from drug delivery systems is frequently monitored by spectral or chromatographic methods, drug release from the conjugates can be also done by recording the pH variation in time, due to acid or alkaline hydrolysis.

In this study, alkaline hydrolysis was considered, as acid hydrolysis proved to occur in a very rapid manner, being practically impossible to quantify.

Under the applied experimental conditions, pH variation in time was due to partial consumption of NaOH for the hydrolysis of ester bonds, thus determining the drug release.

The variation in time of the quantity of sulfonamide released from the conjugates (Fig. 3) was calculated from the variation of pH in an alkaline environment. Initially, a rather fast sulfonamide release was noticed (Fig. 3), when up to 50% of the total immobilized sulfonamide was released in the first 4-5 h of hydrolysis, followed by a much slower drug release. In the end of the release study (48 h), 65-80% of the chemically bonded drug was released. Such an evolution of released sulfonamide amount in time permitted the conclusion that the starch-sulfonamide conjugates can be viewed as a controlled-release drug system.

Ester toxicity

The obtained starch-sulfonamide conjugates were tested for toxicity by determining the lethal dose (Table 2). The values for LD_{50} ranged between 3010 and 5563 mg/kg body, indicating a rather low toxicity. Conjugates toxicity appeared to decrease at lower amounts of sulfonamide immobilized onto starch (Table 2). The high

values of LD_{50} allowed conjugate testing on microbial cultures.

Antibacterial activity

N-[(*N*'-tiazolyl)-*p*'-(benzenesulphone)] amide of N-(*o*-nitrobenzoyl)-*D,L*-asparagic acid was tested for its antibacterial activity against the development of *S. aureus*, *E. coli* and *P. aeruginosa* strains. The active principle inhibited the growth of bacterial cultures of *S. aureus* and *E. coli* (Table 3).

Generally, classical sulfonamides are considered to have antibacterial effect, if a growth inhibition area of at least 16 mm diameter appears in the bacterial culture (Table 3).

Table 2
Toxicity level of starch-sulfonamide esters

% sulfonamide in conjugates	LD_{50} mg/ kg body
7.8	5563
10.8	5010
16.9	4850
18.6	4850
26.1	4300
27	4020
28.2	3850
29	3600
30	3630
38	3010

Table 3
Antibacterial effect of *N-[(N'*-tiazolyl)-p'-(benzenesulphone)] amide of *N-(*o-nitrobenzoyl)-*D,L*-asparagic acid

	Diameter of growth inhibion area (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
Standard strains	18	20	7
Antibacterial activity	+	+	-
Hospital strains	17	17	6
Antibacterial activity	+	+	-

+ antibacterial effect; - no antibacterial effect

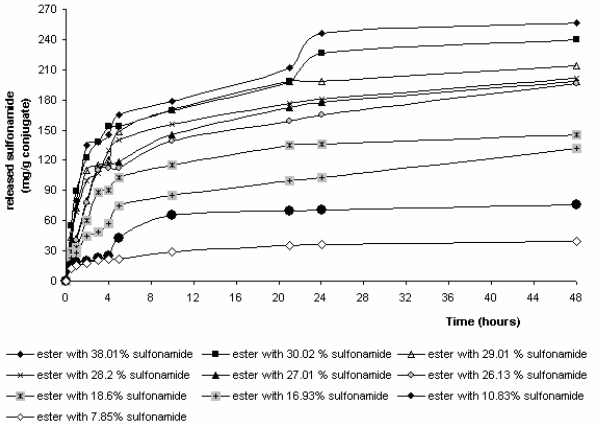

Figure 3: Sulfonamide release from starch-sulfonamide conjugates

CONCLUSIONS

Two new derivatives of the *N*-(*o*-nitrobenzoyl)-*D,L*-asparagic acid: *2*-(*o*-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 and *N*-[(*N*'-tiazolyl)-*p*'-(benzenesulphone)] amide of *N*-(*o*-nitrobenzoyl)-*D,L*-asparagic acid were synthesized and characterized. New

conjugates were synthesized by covalent coupling of sulfonamide onto starch. The content of chemically bonded sulfonamide increased with the increase of the covalent immobilization varying parameters (DCC/sulfonamide molar ratio, sulfonamide/starch molar ratio and reaction time). The esters presented low toxicity and medium antibacterial activity. Sulfonamide release in an alkaline environment indicated a classical drug delivery system-like profile, with an efficiency of up to 80%.

ACKNOWLEDGEMENT: This work was carried out with the support of the POSDRU/89/1.5/S/49944 program entitled "Developing the Innovation Capacity and Improving the Impact of Research through Post-doctoral Programs", financed by the European Social Found and the Romanian Government.

REFERENCES

[1] M. Wjec, M. Pitucha, M. Dobosz and A. Malm, *Acta Pharm*., **54**, 251 (2004).
[2] O. Pintilie, L. Profire, V. Sunel, M. Popa and A. Pui, *Molecules*, **12**, 103 (2007).
[3] V. Sunel, O. Pintilie, M. Popa, L. Profire and A. Popa, *Cellulose Chem*. *Technol*., **40**, 791 (2006).
[4] O. Pintilie, M. Moise, L. Profire and V. Şunel, *Farmacia*, **5**, 61 (2006).
[5] F. Clerici, D. Pocar, M. Guido, A. Loche, V. Perlini and M. Brufani, *J. Med. Chem*., **44**, 931 (2000).
[6] V. Şunel, C. Băsu, C. Oniscu and C. Şoldea, *Roum*. *Biotechnol*. *Lett*., **5**, 393 (2000).
[7] V. Şunel, C. Băsu, C. Oniscu and G. Drochioiu, *Roum*. *Biotechnol*. *Lett*., **6**, 469 (2001).
[8] O. Pintilie, V. Sunel, L. Profire and M. Popa, *An*. *St*. *Univ*. *"Al*. *I*. *Cuza" Iasi*, **15**, 29 (2006).
[9] M. Popa, V. Sunel, N. Dulea, A. Popa, R. Ottenbrite and C. Uglea, *J. Bioact. Polym*., **22**, 651 (2007).
[10] V. Şunel, M. Popa, J. Desbrieres, L. Profire, O. Pintilie and C. Lionte, *Molecules*, **13**, 177 (2008).
[11] V. Şunel, C. Lionte, M. Popa, O. Pintilie, P. Mungiu and S. Telemans, *Eurasian Chem*. *Tech*. *J*., **4**, 201 (2002).
[12] M. Moise, V. Sunel, L. Profire, M. Popa, J. Desbrieres and C. Peptu, *Molecules*, **14**, 2621 (2009).
[13] V. Şunel and C. Şoldea, *Comp*. *Rend*. *Acad*. *Bulg*. *Sci*., **46**, 61 (1993).
[14] V. Şunel, C. Ciugureanu and M. Ungureanu, *Rev*. *Med*. *Chir*. *Soc*. *Med*. *Nat*., **98**, 273 (1993).
[15] V. Şunel, S. Ciovică, N. Asandei and C. Şoldea, *Cellulose Chem*. *Technol*., **29**, 411 (1995).
[16] V. Şunel, M. Popa, A. Popa and C. Şoldea, *Cellulose Chem*. *Technol*., **34**, 269 (2000).
[17] Opinion of the Scientific Panel on Food Additives, Flavourings, *The EFSA Journal*, **22**, 1 (2003).
[18] V. Şunel and C. Şoldea, *Indian J. Chem*., **34B**, 227 (1995).
[19] C. Şoldea and V. Şunel, *Comp*. *Rend*. *Acad*. *Bulg*. *Sci*., **48**, 67 (1995).
[20] V. Şunel, C. Ciugureanu and M. Ungureanu, *Rev*. *Med*. *Chir*. *Soc*. *Med*. *Nat*., *Iaşi*, **100**, 163 (1996).
[21] V. Şunel and C. Băsu, *An*. *St*. *Univ*. *"Al*. *I*. *Cuza" Iasi*, **7**, 111 (1999).
[22] V. Şunel, C. Băsu, G. Drochinoiu and M. Filimon, *Rev*. *Med*. *Chir*. *Soc*. *Med*. *Nat*., *Iaşi*, **105**, 288 (2001).
[23] V. Şunel, C. Băsu, D. Sîrbu, M. Popa, E. Diaconu and C. Şoldea, *Acta Pharm*., **51**, 35 (2001).
[24] M. Popa, V. Şunel, L. Bălăiţă-Rusu and C. Băsu, *Eurasian Chem*. *Tech*. *J*., **5**, 121 (2003).
[25] M. Popa, V. Şunel and L. Bălăiţă-Rusu, *Molecules*, **8**, 297 (2003).
[26] O. Pintilie, V. Şunel, L. Profire and A. Pui, *Farmacia*, **55**, 345 (2007).
[27] M. Moise, V. Şunel, L. Profire, M. Popa and C. Lionte, *Farmacia*, **56**, 283 (2008).
[28] V. Şunel, C. Lionte, C. Băsu and C. Cheptea, *Chem*. *Indian J*., **2**, 1 (2005).