Digest Journal of Nanomaterials and Biostructures Vol. 5, No 2, April 2010, p. 347 – 354

BIOLOGICAL ACTIVITIES OF NANOMATERIALS (BUFADIENOLIDES, PEPTIDES AND ALKOLOIDS) IN THE SKIN OF AMPHIBIAN ON *GAMMARUS PULEX L.*

VAHDETTİN BAYAZİT*
Muş Alparslan University, Faculty of Arts and Sciences, Department of Biology, 49100, Muş, Turkey, Phone

The purpose of this sudy was to examine the toxic effects of bufadienolides of antimicrobial peptides and alkoloids on *Gammarus pulex L.(Crustacea:Amphipoda:Gammaridae)* Bufadienolides (Arenobufagin, Arenobufagin hemisuberate, Arinobufagin 3- sulfate, Bufalin, Bufalin hemisuberate, Bufoatlin 3-sulfate, Bufotalinin, Bufotalone, Cinobufagin, Cinobugagilon, Cinobufotalin, Desacetylcinobufagin, Gamabufotalin hemisuberoate, Gamabufotalin 3-sulfate, 15-hydroxybufalin, 19-hydroxybufalin, Marinobufagin, Marinoic acid, Marinosin and Resibufaginol), Amphibian peptides (Caerin 1.1, Caerin 1.9, Caerin 4.1, Dahlein 5.6, Dermaseptin, Esculentin-1ARb, Esculentin-2P, Maculatin 1.1, Magainin II, MRP, Palustrin-3AR, Ranatuerin-6, Ranatuerin-2P, Uperin 3.6, RCCP) and Alkoloids (Samandarine, Batrachotoxin, Histrionictoxin, Pumiliotoxin, Allopumiliotoxin, Homopumiliotoxin, Decahydroquinoline, Epibatidine) killed significantly the animals in the experimental groups ($p < 0.01$).

(Received March 2, 2010; accepted April 21, 2010)

Keywords: Bufadienolides,Amphibian peptides and alkoloids *Gammarus pulex*,

1. Introductıon

Amphibian skin secretions are considered a rich source of biologically active compounds and are known to be rich in peptides, bufadienolides and alkaloids. Bufadienolides are cardioactive steroids from animals and plants that have also been reported to possess antimicrobial activities. Amphibian skin contains a remarkable spectrum of biologically active compounds, including biogenic amines, peptides, proteins, bufadienolides, tetrodotoxins and lipophilic alkaloids[1-7]. The lipophilic alkaloids include the samandarines and an incredible array of piperidine-based, pyrrolidine-based, and steroidal alkaloids. Such an array of over four hundred new alkaloids has been detected in skin extracts from four genera of dendrobatid frogs of New World tropics, the bufonid genus *Melanophryniscus* of Southeastern South America, the mantelline genus *Mantella* of Madagascar, and the myobatrachid genus *Pseudophryne* of Australia. Many frogs contain mild toxins that make them unpalatable to potential predators. For example, all toads have large poison glands (the parotoid glands) located behind the eyes, on the top of the head. Some frogs, such as some poison dart frogs, are especially toxic. The chemical makeup of toxins in frogs varies from irritants to hallucinogens, convulsants, nerve poisons, and vasoconstrictors. Many predators of frogs have adapted to tolerate high levels of these poisons. Others, including humans, may be severely affected. *Oophaga pumilio*, a poison dart frog, contains numerous alkaloids which deter predators Some frogs obtain poisons from the ants and other arthropods they eat; others, such as the Australian Corroboree Frogs (*Pseudophryne corroboree* and *Pseudophryne pengilleyi*), can manufacture an alkaloid not derived from their diet. Poisonous frogs tend to advertise their toxicity with bright colours, an adaptive strategy known as aposematism. There are at least two non-

*Corresponding author: bvahdettin@yahoo.com; v.bayazit@alparslan.edu.tr

poisonous species of frogs in tropical America (*Eleutherodactylus gaigei* and *Lithodytes lineatus*) that mimic the colouration of dart poison frogs' coloration for self-protection (Batesian mimicry). Because frog toxins are extraordinarily diverse, they have raised the interest of biochemists as a "natural pharmacy". The alkaloid epibatidine, a painkiller 200 times more potent than morphine, is found in some species of poison dart frogs. Other chemicals isolated from the skin of frogs may offer resistance to HIV infection. Arrow and dart poisons are under active investigation for their potential as therapeutic drugs. Bufadienolides and cardenolides are described as cardiacglycosides owing to the similarity in their biological activity, the increase in the contractile force of the heart by inhibiting the enzyme Na+, K+-ATPase. The enzyme is the only receptor for the cardiac glycosides and is responsible for the active extrusion of intercellular Na+ in exchange for extracellular K+. Cardiac glycosides contain a perhydrophenanthrene nucleus substituted at C-17 with a pentadienolide and butenolide for the bufadienolides (*e.g.* bufalin 1) and cardenolides (*e.g.* digitoxigenin 2), respectively. Over 20 major structural alkaloid classes, several of which may co-occur in a single frog, have been detected in anuran skin. Such alkaloids include the batrachotoxins (sodium channel activators), the histrionicotoxins (noncompetitive blockers of nicotinic channels), the pumiliotoxin, allopumiliotoxin, and homopumiliotoxin group decahydroquinolines, various izidines, epibatidine (a potent nicotinic agonist), the tricyclic coccinellines, the pseudophrynamines and spiropyrrolizidines (potent noncompetitive blockers of nicotinic channels). Structures of some alkaloids from amphibian skin are shown in Fig. 1. In this study, the toxicological effects of amphibian bufadienolides and peptides was evaluated[1-19].

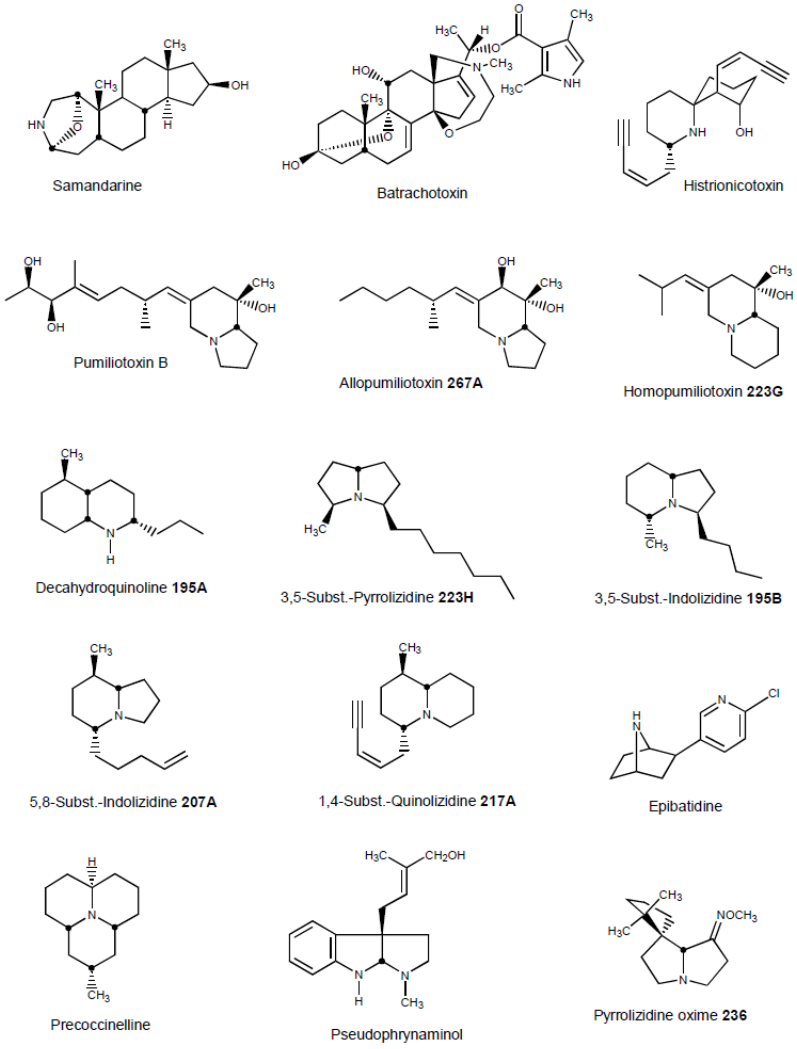

Fig.1. Representative Alkaloids of Amphibian Skin

2. Materıals and methods

Animals were collected while living from cold spring sources at approximately 20 km away from Muş, Turkey.One hundred live animals (*Gammarus pulex*) were used for each experiment. Experimental groups were arranged as 15 groups for bufadienolides and 20 groups for Amphibia peptides. Chemicals concerning with Amphibian bufadienolides and peptides purchased were purchased commercially (Sigma,Merck). Percentage of deaths within one hour of animals were recorded. Completely stopped the movement of animals dying.The concentrations 30% of these agents were tested on animals. Results were considered statistically and analysis of varians was performed (4-19].

3. Results

Amphibian bufadienolides and peptides killed the experimental animals in different percentage according to the findings. The results of studies were given in Figs. 1-3.

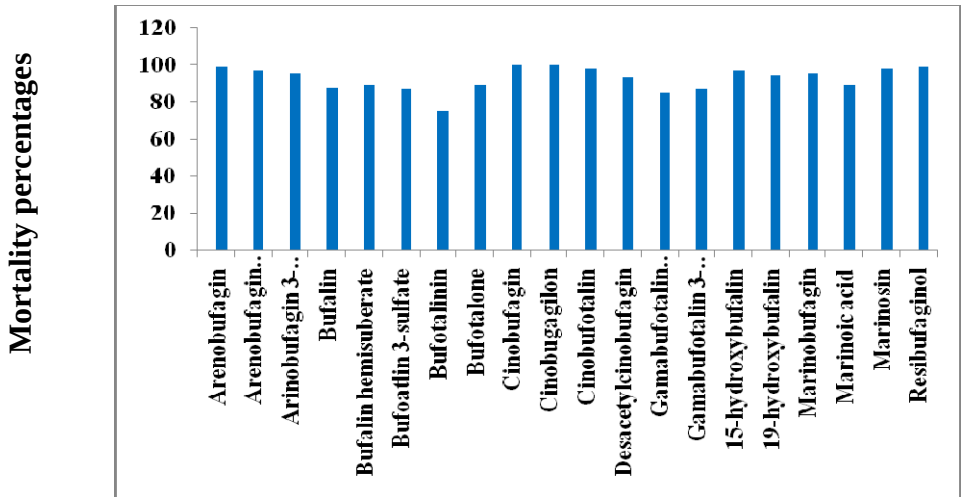

Fig. 2. Mortality percentages at a concentration of 30 percent of some bufadienolides on Gammarus pulex (significantly degree for all of them is p<0.001).

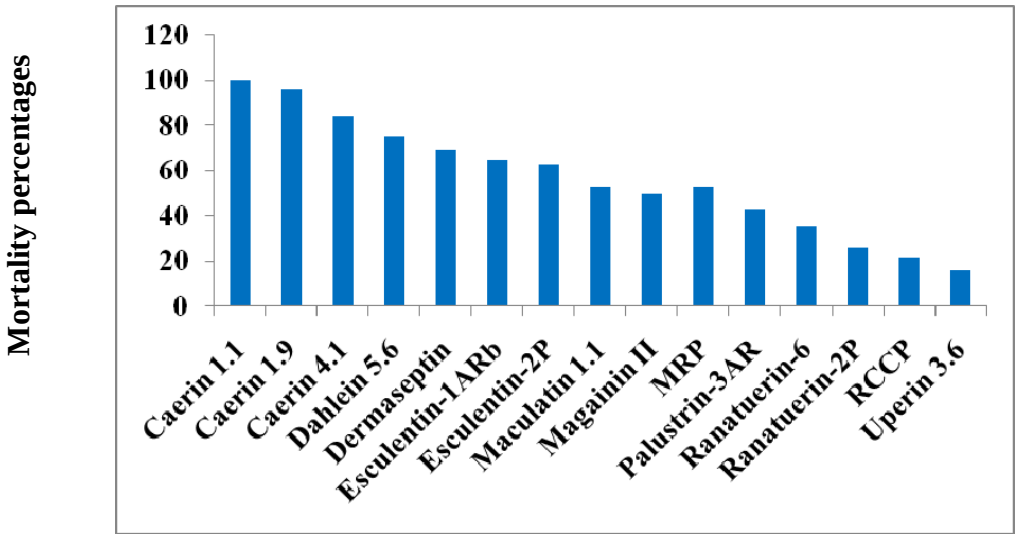

Fig. 3. Mortality percentages at a concentration of 30 percent of some Amphibian peptides on Gammarus pulex(significantly degree for all of them is p<0.001).

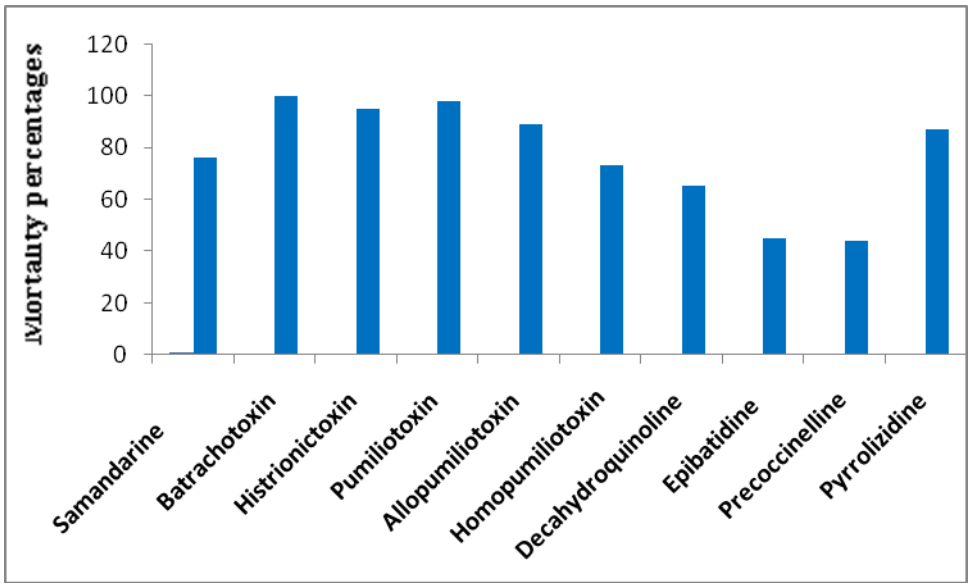

Fig 4. Mortality percentages at a concentration of 30 percent of Amphibian some alkaloids on Gammarus pulex(significantly degree for all of them is $p<0.001$).

All of bufadienolides, peptides and alkoloids the Amphibian used in the mortality experiments have toxic effects. Generally, symptoms of death have occured with the stopping of breathing and movements.

4. Discussion

We could not find a big numbers the frogs in the ecological areas of these animals.This situation is auspiciousness for Gammarus species. On the other hand, water birds eating frogs are not effected, suc as storks, ducks,herons,cranes. Probably,it is possibly that animals eating the frogs have strong immune system than invertebrata. Toad secretions of both mucous and granular glands can sometimes be poisonous however granular glands produce more toxic secretions than mucous glands. Granular gland secretions in toads contain chemicals that can be broadly classified into four categories: (1) Biogenic amines, (2) Bufadienolides, (3) alkaloids and steroids and (4) peptides and proteins. Chemically, other than biogenic amines and peptides, granular gland secretions of toads may contain nearly 86 types of Bufadienolides along with other components like Bufotoxin, Bufagin and Bufotenine. Bufalin, Bufogenin, Bufotalin, Cinobufagin, Marinobufagin, Resibufagin are some of the most important bufadienolides. Bufadienolides related to cardiac glycosides are normally grouped with the cardenolides, and only occupy a small subsection. However, drugs prepared from bufadienolide containing plants and toads are widely used in traditional medicine, whilst, on the other hand, bufadienolide-containing plants create a problem in agriculture in On the other hand, the antineoplastic and cell growth inhibitory properties as well as the effect on the central nervous system of several bufadienolides are also well documented. Mammalian bufadienolides will be briefly mentioned. Bufadienolide is a type of steroid with a characteristic α-pyrone ring at C-17, and show significant cardiotonic, blood-pressure-stimulating, anesthetic, and antitumor activities (Figures 5-6)[1,2,4,6,7,8,12,13,18].

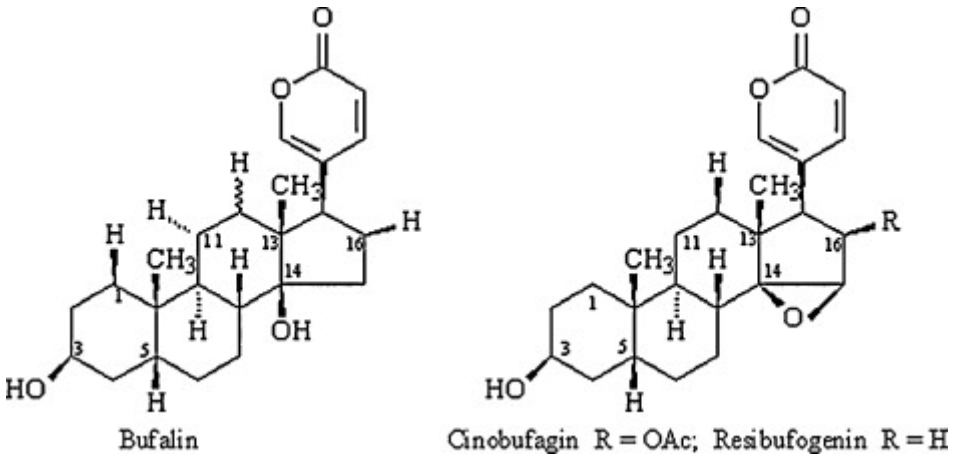

Fig. 5. Structures of three bufadienolides

Most of the clinical attention was directed to the cardenolides owing to their therapeutic use. Digoxin 3 and digitoxin 4 are the two most widely used digitalis inotropes; there are an estimated two million patients receiving these cardenolides (Fig. 1). Bufadienolides and the more polar conjugates, the bufotoxins, are present in the bodies of toads of the genus *Bufo*. The toad bufadienolides occur not only in the unconjugated form, but several C-3 conjugates are also known: sulfates, dicarboxylic esters and amino acid – dicarboxylic acid esters. The arginine–suberoyl esters, *e.g.* bufalitoxin , are known as the bufotoxins[1,7,10,17,18,19].

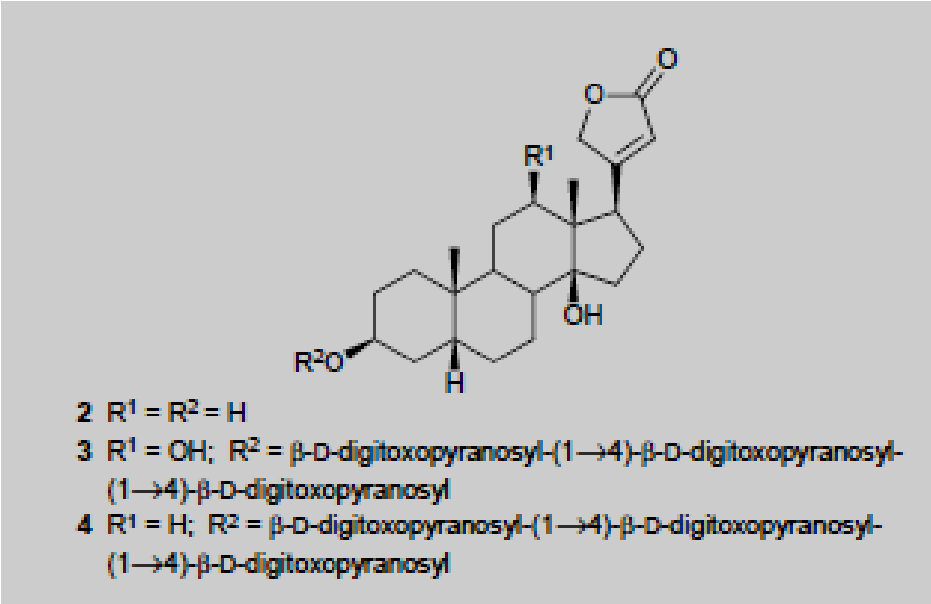

Fig. 6. General structure bufadienolides

The bufadienolides isolated from *Bufo* species are listed in Table 1. Some amphibian peptides was shown in Table 2.

Table 1. Bufadienolides isolated from Bufo species [1-19]

Arenobufagin
Arenobufagin hemisuberate
Arenobufagin 3- suberoyl L-arginine ester
Arenobufagin 3-sulfate
Argentinogenin
Bufalin
Bufalin 3-adipoyl L-arginine ester
Bufalin hemisuberate
Bufalin 3-pimeloyl L-arginine ester
Bufalin 3-suberoyl L-arginine ester (bufalitoxin)
Bufalin 3-succinoyl L-arginine ester
Bufalin 3-sulfate
Bufalin 3-hemisuberate
Bufalin 3-suberoyl L-1 methylhistidine ester
Bufatalinin
Bufatalone
Cinobufagin
Cinobufagin 3-adipoyl L-arginine ester
Cinobufagin 3-glutaryl L-arginine ester
Cinobufagin 3-pimeloyl L-arginine ester
Cinobufagin 3-suberoyl L-arginine ester
Cinobufagin 3-succinoyl L-arginine ester
Cinobufagin 3-sulfate
Cinobufaginol
Cinobufatalin
Cinobufatalin 3-suberoyl L-arginine ester
Deacetylciobufagin 3-hemisuccinate
Deacetylciobufagin 3-succinylarginine
16-Deacetylcinobufaginol
16-Deacetylcinobufaginol
Deacetylcinobufatalin
Deacetylcinobufaginol
Gamabufotalin
Gamabufotalin 3-adipoyl L-arginine ester
Gamabufotalin hemisuberota
Gamabufotalin 3-pimeloyl L-arginine ester
Gamabufotalin 3-suberoyl L-arginine ester (gamabufatalitoxin)
Gamabufotalin 3-sulfate
Gamabufotaliniol
Hellebrigenin
15-Hydroxybufalin
19-Hydroxybufalin
19-Hydroxycinobufalin
Marinobufagin
Marinoic acid
Marinobufagin 3-suberoyl L-arginine ester
Marinobufagin 3-pimeloyl L-arginine ester
Marinosin
Resibufagin
Resibufagin 3-sulfate
Resibufaginol
Resibufagenin hemisuberate
Resibufagenin (bufogenin)
Telocinobufain 3-suberoyl L-glutamine ester
Telocinobufain 3-suberoyl L-arginine ester
Telocinobufagenin

Table 2. Amphibian antimicrobial peptides [1,3,4,5,6,7,10,15,17]

Peptide	Species of origin	Sequence
Caerin 1.1	*Litoria caerulea*	GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2
Caerin 1.9	*Litoria chloris*	GLFGVLGSIAKHVLPHVVPVIAEKL-NH_2
Caerin 4.1	*Litoria caerulea*	GLWQKIKSAAGDLASGIVEGIKS-NH_2
Dahlein 5.6	*Litoria dahlii*	GLLASLGKVFGGYLAEKLKPK
Dermaseptin	*Phyllomedusa sauvagii*	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ
Esculentin-1ARb	*Rana areolata*	GLFPKFNKKKVKTGIFDIIKTVGKEAGMDVLRTGIDVIGCKIKGEC
Esculentin-2P	*Rana pipiens*	GFSSIFRGVAKFASKGLGKDLARLGVNLVACKISKQC
Maculatin 1.1	*Litoria genimaculata*	GLFGVLAKVAAHVVPAIAEHF-NH_2

Peptide	Species of origin	Sequence
Magainin II	***Xenopus laevis***	**GIGKFLHSAKKFGKAFVGEIMNS**
MRP	***Rana tagoi***	**AIGSILGALAKGLPTLISWIKNR-NH_2**
Palustrin-3AR	***Rana areolata***	**GIFPKIIGKGIVNGIKSLAKGVGMKVFKAGLNNIGNTGCNNRDEC**
Ranatuerin-6	***Rana catesbeiana***	**FISAIASMLGKFL-NH_2**
Ranatuerin-2P	***Rana pipiens***	**GLMDTVKNVAKNLAGHMLDKLKCKITGC**
RCCP	***Rana catesbeiana***	**Natural mixture of peptides**
Uperin 3.6	***Uperoleia mjobergii***	**GVIDAAKKWNVLKNLF-NH_2**

Nevertheless, the sequence of the peptides tested and species of origin can be found in Table 2. Caerin 1.1, caerin 1.9, caerin 4.1, dahlein 5.6, maculatin 1.1, and uperin 3.6 have been synthesized, by using L-amino acids and standard 9-fluorenylmethoxycarbonyl chemistry. Pregnenolone is the precursor to the cardenolides, *e.g.* digitoxigenin and the plant-derived bufadienolides, *e.g.* hellebrigenin. The conversion of pregnenolone into digitoxigenin requires the inclusion of an acetate group,whereas in the biogenesis of scilliroside, the α-pyrone is formed by the condensation of a pregnane derivative with one molecule of oxaloacetic acid.

5. Conclusıon

Amphibian chemicals have significant toxic effects on Gammarus pulex.Therefore, toxic effects of alkoloids,bufadienolides and peptides concerning with amphibian can be evaluated in the study of cancer and tumors.However, these substances can tried on other organisms.

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