

The licorice (*Glycyrrhiza glabra L.*) metabolite derivatives as bioinsecticides against *Anacanthotermes turkestanicus* termites

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Abstract. This article presents the results on the termiticidity of the compounds produced by the reactions of glycyrrhizic acid (GA) with 2-aminothiazole, 3-amino-1,2,4-triazole and 2-aminobenzothiazole. The biological effect of the studied compounds has been found to be dose-dependent. Among the GA derivatives the maximum insecticidal activity has been exhibited by the GA derivative with 2-aminobenzothiazole. Based on the results of the experiments, it has been concluded that the structural-functional approach to the search for biotermiticides is one of the alternative ways to develop environmentally safe means of termite control.

1. Introduction

There are about three thousand species of termites in the world [1], whose harmful activity is related to eating wood containing cellulose. Many termites destroy building structures where wood or cellulose-containing materials are present. Large-scale development of steppe zones in Kashkadarya, Surkhandarya, Bukhara, Andijan, Namangan, Ferghana, Samarkand, Khorezm regions as well as in the Autonomous Karakalpakstan Republic in Uzbekistan has led to the change of their habitats [2]. This has brought to an increase in the damage caused by termites. There are two species of termites of the genus *Anacanthotermes* in Central Asia. They are *Anacanthotermes turkestanicus* and *Anacanthotermes ahngerianus* termites [3-4]. More than 30 thousand structures in private households of rural population, 135 monuments of cultural heritage, as well as structures on the territory of various industrial enterprises have been infested with termites in Uzbekistan.

For a long time, the large-scale use of synthetic preparations belonging to different classes of chemical compounds has been the preferred method of termite control [2, 5]. However, termites that appeared more than 1.5 million years ago quickly developed resistance to them. The detrimental effect of synthetic chemicals on human health and the environment is of a big concern in many countries, including Uzbekistan [6]. On May 23, 2001 according to the decision of the Stockholm Convention, the use, production and sales of number of chemical preparations were banned due to their ability to accumulate in the objects of the environment, the lack of selectivity of their action and their negative impact on humans and useful animals. In order to weaken the chemical burden and find alternative ways to protect buildings from termites, the Government of Uzbekistan adopted the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan from September 4, 2019 No. 725 "On acceleration of work on termite control and reduction of their harm in the Republic".

In order to reduce the chemical load on the environment, the search for alternative ways of protecting buildings from termites with entomopathogenic fungi, bacteria, nematodes, plants, etc. has begun [7]. Considering the close ecological relationship between the termite world and plants, the researchers began to exploit the metabolites from various plant organs as a means of protection against termites [8-11]. Out of the reports on preliminary termiticide

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properties of plant metabolites, it is worth mentioning those on anabasine, alkaloid from *Anabasis aphylla* plant and gossypol, a polyphenol from cotton plant *Gossypium hirsutum* [12].

Since glycyrrhizic acid, a metabolite of licorice - *Glycyrrhiza glabra* L. has been isolated, there have been developed many biologically active preparations with useful properties such as antifungal and antibacterial activity have been developed [13, 14]. This paper presents the results of experimental studies on the termiticidity of the products of chemical transformations of glycyrrhizic acid.

2. Materials and Methods

The termites (*Anacanthotermes turkestanicus*) (Figure 1) were collected from Zarbdor (13.07.2021; 20.09.2021) and Forish (29.10.2021) rural households of Jizzak region of Uzbekistan.



Fig. 1. *Anacanthotermes turkestanicus*



Fig. 2. *Glycyrrhizic glabra* L

The commercial glycyrrhizic acid, [15] the licorice *Glycyrrhizic glabra* L (Figure 2). was purified to a purity of $93 \pm 2\%$ by HPLC.

2.1 The preparation of glycyrrhizic acid derivatives with heterocyclic amines

A mixture of 1 mmol of glycyrrhizic acid and 1 mmol of a heterocyclic amine was dissolved in 25 ml of absolute ethanol and boiled for 10-15 minutes. The solvent was removed in vacuum (1 mmHg, at 30-40°C), and the solid residue was recrystallized in the ethanol-water system. The percentage yield is 93-95%. All of the synthesized salts are homogeneous brittle porous powders.

2.2 Termiticide activity of the synthesized GA

The reaction of termites to the prepared salts has been determined using filter paper (2x2 cm in size) impregnated with the solutions of test substances and distilled water (in the control experiment). Then, the filter paper has been placed as a rolled-up tube in sterilized Petri dishes where 50 working termites have been put beforehand. These Petri dishes with termites have been kept in a dark place during the experiments. Then they have been taken out of the dark to record and observe the condition of termites, the filter paper they ate, and the number of living, paralyzed, and dead termites. The termites have been provided with distilled water all throughout the experiment. The experiment has lasted for 3-7 days. Experimental and control samples have been tested after five replications. The percentage of mortality was calculated according to Abbott's formula, taking into account the percentage of mortality in the control [16, 17].

3. Results and Discussion

The glycyrrhizic acid (GA) salts with 2-aminothiazole (I), 3-amino-1,2,4-triazole (II) and 2-aminobenzothiazole (III, Figure 3) have the following structures:

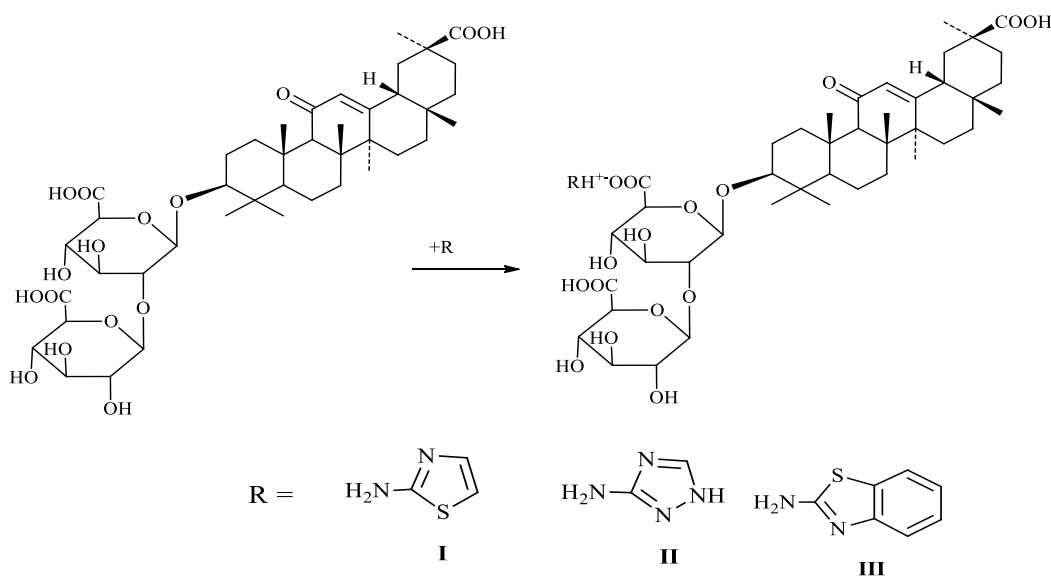


Fig. 3. The structures of glycyrrhizic acid salts

The resulting salts have been characterized by some physicochemical parameters (Table 1).

Table 1. Some physicochemical properties of amino salts of HA

No.	The GA salt with	Yield, %	M.p., °C (decomposed)	Empirical formula	Solubility	Molar mass, gmol ⁻¹
1	I	96	185±1	C ₄₅ H ₆₆ O ₁₆ N ₂ S	in water	923
2	II	97	180±1	C ₄₄ H ₆₆ O ₁₆ N ₄	in water	907
3	III	95	195±1	C ₄₉ H ₆₈ O ₁₆ N ₂ S	in water	973

3.1 Spectra of the of glycyrrhizic acid compound.

The structures of the salts prepared have been studied using IR-spectroscopy and mass-spectrometry. The IR-spectra of the synthesized compounds were recorded in the vibrational frequency range of 400–4000 cm⁻¹ on a Perkin Elmer-10.6.1 spectrometer (USA). Mass-spectrometric studies have been carried out on a Q-TOF UHPLC-MS device, Agilent Technologies series 6520B.

Experiment conditions:

- ionization method - ESI+;
- drying gas flow - 5 l/min;
- drying gas temperature: 300°C;
- voltage on the skimmer cone: 20V;

- voltage on the fragment 125V.
- mass range in MS mode - 100 - 400 m/z;
- in Targeted MS/MS 25 – 400 m/z mode;
- column Zorbax SB C18, 3 μ m, 0.5x150 mm;
- mobile phase: A - 0.1% formic acid solution, B – acetonitrile + 0.1% formic acid;
- eluent rate in isocratic mode -15 μ l/min;
- injection - 1 μ l (autosampler Agilent Technologies Micro WPS).

Thus, in the IR-spectra of the synthesized compounds there have been observed the valent vibrations of -OH and -NH groups at 3500-3100 cm^{-1} (Table 2). Valent vibrations of the carbonyl group of the initial GA have been observed at 1720 cm^{-1} , while in its salts these vibrations have been observed at 1685-1705 cm^{-1} . The shift in these vibrations indicates the presence of electrostatic interactions between the carboxylate group of the GA and the protonated amino group of heterocyclic amine fragments. The IR-spectra strongly suggest the existence of electrostatic (-COO $^{-}$ -+NH $_{3}^{+}$) interactions, and hence, the formation of ionic compounds, which the GA salts with the heterocyclic amines are.

Table 2. The IR-spectroscopic parameters of amino salts of GA

No.	The GA salt with	The observed signals in IR-spectra, cm^{-1}
1	I	$\nu(\text{OH}, \text{NH})=3200\text{-}3450$, $\nu(\text{CH}_3, \text{CH}_2)=2924, 2900$, $\nu(\text{C}=\text{O})=1699$, $\nu(\text{C}=\text{O})=1638$, $\delta(\text{NH}_3^+)=1439$, $\nu(\text{O-H})=1040$, $\nu(\text{C}=\text{C}, \text{C}=\text{N})=1603, 1542, 1523$
2	II	$\nu(\text{OH}, \text{NH})=3100\text{-}3500$, $\nu(\text{CH}_3, \text{CH}_2)=2930, 2890$, $\nu(\text{C}=\text{O})=1688$, $\nu(\text{C}=\text{O})=1660$, $\delta(\text{NH}_3^+)=1420$, $\nu(\text{O-H})=1041$, $\nu(\text{C}=\text{C}, \text{C}=\text{N})=1602, 1508, 1542$
3	III	$\nu(\text{OH}, \text{NH})=3100\text{-}3500$, $\nu(\text{CH}_3, \text{CH}_2)=2925, 2895$, $\nu(\text{C}=\text{O})=1701$, $\nu(\text{C}=\text{O})=1645$, $\delta(\text{NH}_3^+)=1440$, $\nu(\text{O-H})=1041$, $\nu(\text{C-H})=750$ (benzene ring)

3.2 The analysis of Mass-spectrum of GA derivatives

Using the UHPLC-MS method, the molecular weight of the synthesized compounds, the molar masses of the main molecular ions and their daughter ions have been found, based on which the chemical structures of the synthesized compounds have been deduced. An example has been shown in Figure 4.

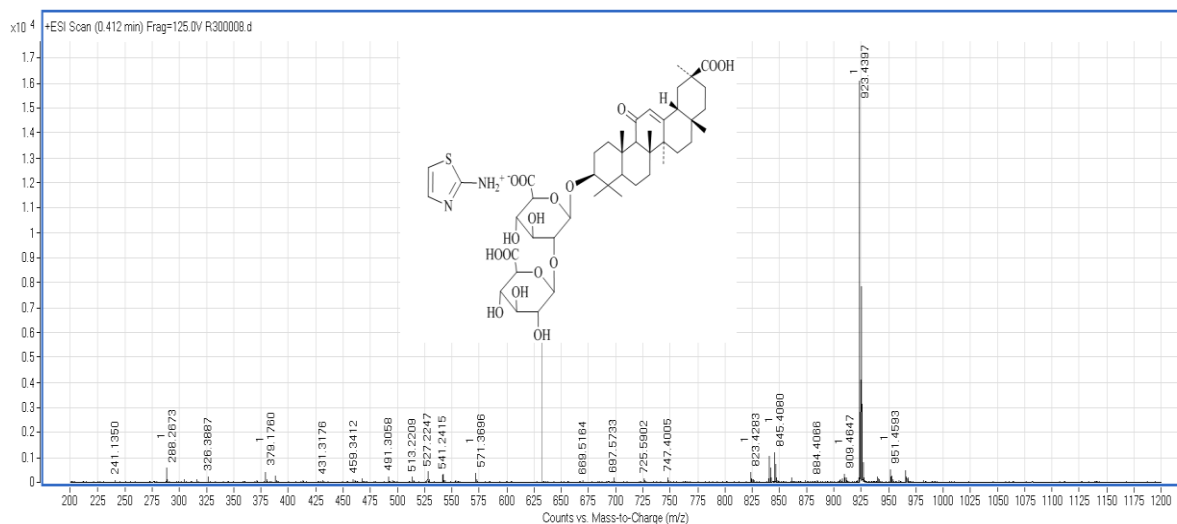


Fig. 4. Mass-spectrum of GA derivative with 2-aminothiazole

Mass-spectrometric data have shown that the GA salt with 2-aminothiazole has the molecular ion [M⁺] with m/z of 923.4397. This molecular ion was subjected to repeated MS/MS analysis. The m/z ratios of 102.12, 453.34, 471.35 and 823.41 correspond to the fragmented ions presented in Figure 5.

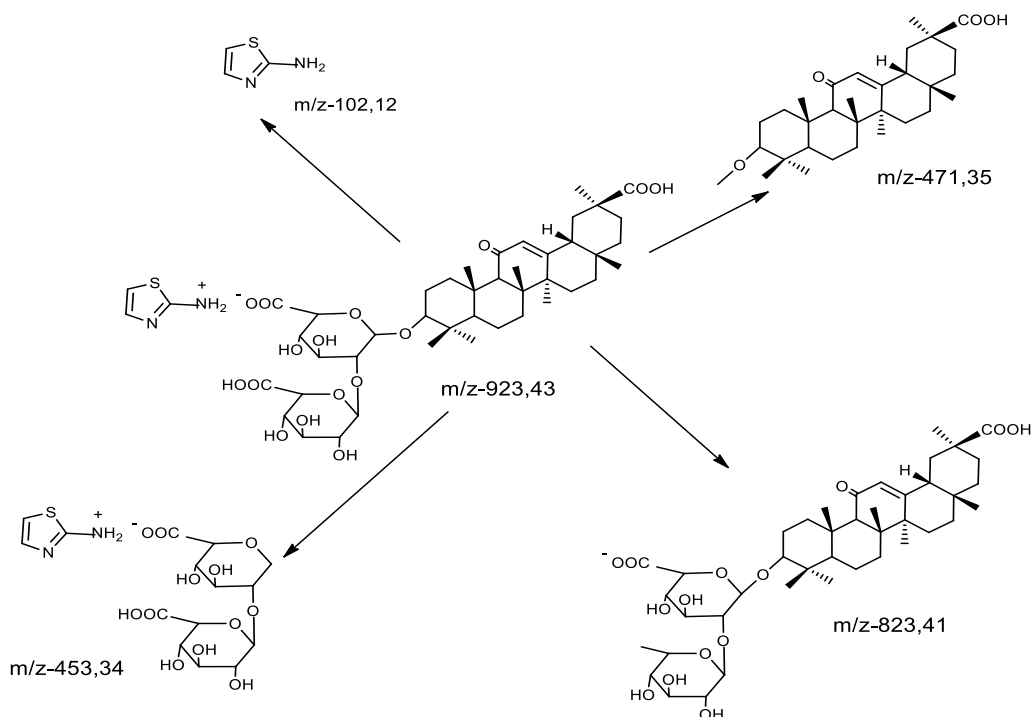


Fig. 5. Fragmentation patterns of the GA salt with 2-aminothiazole

3.3. The reaction of termites to the prepared compounds

The results of the effect of the GA salts with I, II and III on the viability of working termites are presented in Table 3. In order to study the termiticide properties of the salts, 50 termites have been used in each experiment. The effect of the salts obtained has been studied by dissolving them in water and obtaining solutions of two concentrations: 0.5 % and 1.0 %.

Table 3. Termiticide activity of the synthesized GA salts ($M \pm m$, $n=5$, $P<0,5$)

GA salt with	Concentration n, (%)	The number of termites				Mortality of termites (%) from the GA salts on the certain day of recording		
		Before the treatment	Days after the treatment			3	5	7
			3	5	7			
I	0.5	50.0	33.0	27.0	24.0	34.0±0.54	43.8±0.49	50.0±0.44
	1.0	50.0	38.5	24.5	10.5	23.0±0.45	49.0±0.51	78.1±0.53
II	0.5	50.0	16.5	12.0	1.0	65.6±0.58	73.3±0.53	97.8±0.53
	1.0	50.0	17.0	3.0	0	64.6±0.57	93.3±0.59	100.0±0.53
III	0.5	50.0	40.5	34.0	15.0	15.6±0.25	23.3±0.27	66.7±0.43
	1.0	50.0	26.5	15.5	5.3	44.8±0.48	65.6±0.51	88.2±0.50
Control (water)		50.0	48.0	45.0	45.0	0	0	0

The analysis of the effect of 0,5% solution of GA salt with I (containing 2-aminothiazole fragment) on termites suggests that on the third day of the record, termite mortality was 34,0%, on the fifth day it was 43,8%, and on the seventh day it was 50,0%. When the concentration of the GA salt with I doubles, termite mortality on day 7 becomes 78%, i.e., this efficiency is more than 1.5 times higher than at 0.5% concentration of GA salt with I. It should be noted that termite mortality from the GA salt with I depends both on the concentration and on the time of the experiment, which runs from three to seven days. In experiments involving the GA salt with II, the 0.5% solution has been used, and the toxicity against the termites ranged from 65.6% (day 3) to 97.8% (day 7). When its 1.0% solution has been used, 100% mortality (on day 7) of the termites has been noted. Importantly, the GA salt with II, having in its composition a 3-amino-1, 2, 4-triazole group, has a pronounced insecticidal effect compared to the other

salts. In the next series of experiments, there have been studied the toxic effect of the GA salt with III on *Anacanthotermes turkestanicus* termites. The results of the experiments suggest that at a concentration of 0.5%, 15.6% mortality has been observed on the third day, 23.3% on the fifth day and 66.7% mortality of termites was observed on the seventh day. At 1% concentration, the GA salt with III also has exhibited a toxic effect on termites. However, their mortality by the day of recording was 44.8% on day 3, 65.6% on day 5 and 88.2% on day 7, respectively. These termite mortality rates are significantly lower than those caused by the GA salt with II.

4. Conclusion

Thus, based on the experiments conducted, it can be noted that biological effect of all studied compounds has a dose-dependent character. Based on the data given in Fig. 8, it can be concluded that the GA salt with II has the most effective action against termites than those with I and III. Pharmacological studies have shown that thiazole derivatives of GA, according to the classification of substances by toxicity [18], belong to harmless chemical compounds.

To understand the mechanism of action of compounds it is necessary to conduct SAR studies. Such an approach in bioinsecticides' research will lead to a significant shift in development of new insecticidal agents with specified properties.

This research allows conducting a broad search for biotermicides among natural compounds, which can be considered as an alternative way to develop environmentally safe means of controlling termites and other insects.

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