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Synthesis and growth regulatory activity of guaianolide estafiatine and its derivatives

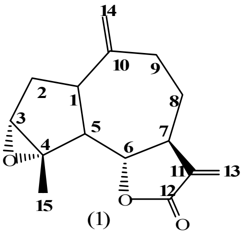
In this work, methods for directed synthesis of practically significant derivatives of guaianolide estafiatine were considered. It is shown that α-epoxy cycle, which is present in the molecule of estafiatine determines the stereo — and regioselectivity of nucleophilic addition and obtaining new biologically active derivatives. Discussed in this paper sesquiterpene lactone estafiatine and its derivatives were studied in terms of obtaining new biologically active compounds and the influence of structural features of the molecules of this series on their biological activity. During of performed bio-experiments, growth regulatory activity of series of estafiatine derivatives was found.

Key words: sesquiterpene γ-lactones, guaianolides, estafiatine, directed synthesis, acetylation, biological activity, epoxy cycle, growth regulatory activity.

Terpenoid compounds, in particular, widespread in nature, sesquiterpene γ-lactones are active principle of many medicinal plants of the Compositae family (Asteraceae). In this regard, the synthesis of new derivatives of natural sesquiterpene γ-lactones with further consideration of the biological activity of the obtained compounds represents a scientific and practical interest [1–3].

In this work, the results of chemical transformations of estafiatine (1) are presented. Estafiatine is available sesquiterpene γ-lactone of guaian-type. Estafiatine was isolated by us from widespread in Central Kazakhstan Noble Yarrow. Considering that there are many literary reports about chemical transformations of guaianolides by exomethylene group of γ-lactone cycle, by double bond at C3=C4, C4=C15, by oxy-function, molecule of estafiatine (1) is a convenient model for targeted modifications by α-epoxy cycle at C3–C4. One of the areas of modifications by epoxy cycle of estafiatine molecule (1) is obtaining on its base fragment with α, β-unsaturated keto-group, as such functional group, being a good alkylating center, can positively affect the biological activity of the obtained compound.

So, the isomerization of α-epoxy cycle of the molecule (1) with subsequent oxidation of the resulting secondary hydroxyl group was carried out by us. At the first stage interaction of estafiatine (1) with aluminum isopropylate in toluene led to derivative (2) in the form of a colorless crystalline compound of composition $C_{15}H_{18}O_3$, mp 142–144 °C, 90 % yield. Performed ^{1}H NMR-spectroscopic research (Table 1) allowed us to establish for compound (2) structure of 3α-hydroxy-guaia-4(15),10(14),11(13)-triene-6,12-olide, which turned out to be identical guaianolide isozaluzanin C, isolated from Saussurea Lappa [4]. At the next stage, the obtained derivative (2) was oxidized with chromium trioxide in pyridine. Thus, a crystalline compound (3) of composition $C_{15}H_{16}O_3$ was obtained, mp 134–135 °C, 95 % yield. The presence in the molecule of the conjugated α, β-unsaturated keto-group (table 1) is also confirmed by UV-spectrum (217 nm, E 1420), which clearly indicates the formation of 3-keto-guaia-4(15),10(14),11(13)-triene-6,12-olide. It turned out to be identical 3-keto-4-methylene-cis-guaianolide, isolated from Brachylaena transvaalensis Hutch.ex Phill. Et Schweick [5].



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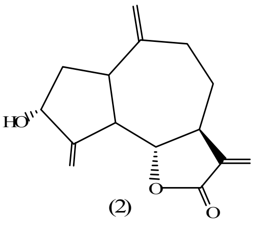

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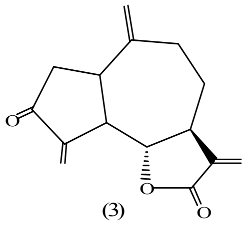


(3)

Acetylation of isozaluzanin C (2) with acetic anhydride in pyridine led to acetyl-derivative (4) in the form of a colorless oil. Composition $C_{17}H_{20}O_4$, $[\alpha]_D^{26}$ +8,8° (0,0045, chloroform), 96 % yield. On the basis of spectral data (Table 1) was found that the compound (4) had the structure of 3α-acetoxy-guaia-4(15), 10(14), 11(13)-triene-6,12-olide.

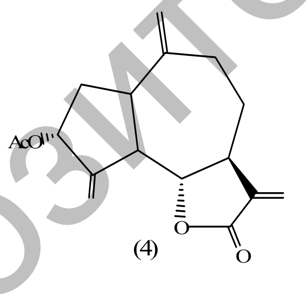
Table 1

^{1}H NMR-spectrum data of estafiatine (1) and compounds (2)-(4), (6)-(8)

Number of compounds	Protons										
	Me-4	H-3	H-4	H-6	H-13a	H-13b	H-14a	H-14b	H-15a	H-15b	Other protons
(1)	1,53 s	3,28 brs	–	4,01 quint (10,5;9)	5,42 d (3,5)	6,12 d (3,5)	4,78 brs	4,78 brs	–	–	–
(2)	–	4,68 t (8)	–	3,98 t (9)	5,48 d (3,5)	6,21 d (3,5)	4,78 brs	4,92 brs	5,35 brs	5,48 brs	–
(3)	–	–	–	3,99 t (8,5)	5,56 d (3)	6,28 d (3)	4,57 s	4,91 s	5,85 d (2,5)	6,23 d (2,5)	–
(4)	–	5,62 brt (6)	–	3,87 t (9)	5,47 d (3)	6,18 d (3)	4,76 brs	4,90 brs	5,36 brs	5,51 brs	–OAc: 2,02 s
(6)	1,34 s	4,26 quint (12,5;7)	–	4,17 quint (11,5;9)	5,58 d (3,5)	6,26 d (3,5)	5,01 brs	5,08 brs	–	-	–
(7)	1,51 s	3,94 brd (3)	–	4,23 quint (10,5;9)	5,48 d (3)	6,20 d (3)	4,94 d (1)	4,94 d (1)	–	-	–
(8)	1,74 s	5,33 d (4)	–	4,39 quint (10,5;9)	5,51 d (3)	6,22 d (3)	5,08 brs	5,08 brs	–	-	–OAc: 2,06 s

Notes. s (singlet); d (doublet); t (triplet); quint (quintet); brs (broad singlet); brt (broad triplet); brd (broad doublet). ^{1}H NMR spectra of all obtained compounds were registered on a spectrometer Bruker Avance-400 (operating frequency 400,13 MHz), solvent $CDCl_3$, internal reference TMS.

In terms of another approach for the preparation of 3-keto-derivative of estafiatine (1), reaction with boron trifluoride in chloroform was carried out by us. In this case, colorless crystalline compound (5) was obtained. Composition $C_{15}H_{18}O_3$, mp 140–142 °C (from ethyl acetate), $[\alpha]_D^{19} + 126°$, (0,005; chloroform). Molecule of the obtained compound (5) contains a carbonyl group (IR-spectrum: 1745 cm^{-1}), which is also confirmed by ^{1}H NMR spectrum (Table 1). Comparison of physico-chemical and spectral data of the molecule (5) with the literature data [5–7], we were able to identify it as guaianolide estafiatine with the structure of 3-keto-1,5,7α(H),4,6β(H)-guaia-10(14),11(13)-dien-6,12-olide (5):



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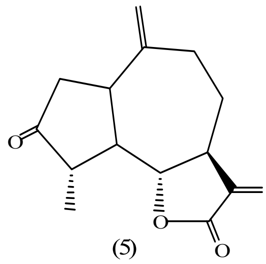
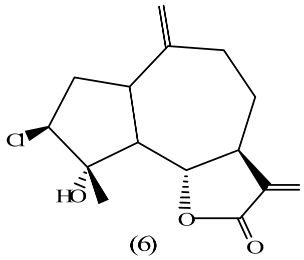


(5)

In order to obtain analogy of chlorine-containing biologically active guaianolides, the interaction estafiatine (1) with gaseous hydrogen chloride in methanol was investigated by us. In this case a mixture of two compounds was formed. Rf value 0,55 and 0,45, TLC system: diethyl ether. After chromatography on silica gel two derivatives (6) and (7) were isolated with 80 % and 15 % yields respectively. On the basis of spectral data (table 1), we can conclude that in the course of this reaction two isomeric chlorohydrins were formed.

In order to confirm the location of the hydroxyl group in these molecules, acetylation reactions were carried out. The interaction of the main chlorohydrin (6) with acetic anhydride in pyridine at room temperature does not lead to the acetate derivative. This shows that the hydroxyl group in the molecule (6) is tertiary. Acetylation of the second derivative (7) in the same conditions led to acetate with 60 % yield. The presence of acetyl group is confirmed by the data of IR-spectrum (1756 and 1250 cm^{-1}) and ^{1}H NMR spectrum (Table 1). Therefore, structure of 3α-acetoxy-4β-chloro-guaia-10(14),11(13)-dien-6,12-olide can be proposed for acetoxy-derivative (8). From this it follows that the initial chlorohydrin (7) has structure of 3α-hydroxy-4β-chloro-guaia-10(14), 11(13)-dien-6, 12-olide, as a consequence, the main isomer (6) has the structure of 3β-chloro-4α-hydroxy-guaia-10(14), 11(13)-dien-6,12-olide: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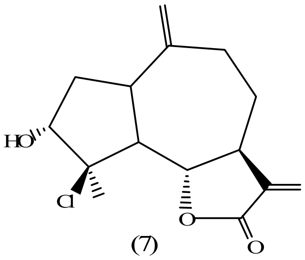
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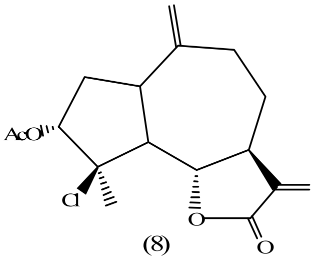
(8)

Thus, the reaction of oxirane ring-opening of estafiatine molecule (1) is stereoselective. It can be explained by the small conformational mobility of this molecule.

Also, growth regulatory activity of estafiatine (1) and its derivatives (2), (5) and (6) was studied by us. Growth regulatory activity was determined by actions on the seed germination of mustard (Sinaps arvensis L.) and common wild oat (Avena fatua). Compounds (1), (5), (6) and (2) were investigated in comparison with drugs triallate and oktalon, which used in practice. Water was control sample. Germination of mustard seeds was determined after 24 hours, wild oats — 72 hours. Determining of least significant difference was carried out with standard biometric methods using computers. The results of the tests are presented in Tables 2 and 3.

Table 2

Influence of estafiatine (1) and its derivatives on germination of mustard seeds

№	Variants of experiments, names of compounds	Concentration, g/cm^2	The number of germinated mustard seeds	% of inhibition
1	Control (water)	–	47,5±3,3	0
2	Standard (oktalon)	$6{,}29\times10^{-6}$	33±14,75	31
3	Estafiatine (1)	$6{,}29\times10^{-6}$	42±5,75	12
4	Keto-estafiatine (5)	$6{,}29\times10^{-6}$	41±6,5	14
5	Chlorohydrin — estafiatine (6)	$6{,}34\times10^{-6}$	35±12,5	26
6	3-Hydroxy-4-methylene-estafiatine (2)	$6{,}45\times10^{-6}$	34±13,5	28,4
Notes. LSD (Least Significant Difference) — 5,017; SX (accuracy of experience) — 4,762.				

Table 3

Influence of estafiatine (1) and its derivatives on germination of wild oat seeds

№	Variants of experiments, names of compounds	Concentration, g/cm^2	The number of germinated wild oat seeds	% of inhibition
1	Control (water)	–	49±5,4	0
2	Standard (oktalon)	$6{,}3\times10^{-6}$	33±16,25	33
3	Estafiatine (1)	$6{,}28\times10^{-6}$	44±5,25	11
4	Keto-estafiatine (5)	$6{,}35\times10^{-6}$	37±12	24,5
5	Chlorohydrin — estafiatine (6)	$6{,}29\times10^{-6}$	34±15,25	31,2
6	3-Hydroxy-4-methylene-estafiatine (2)	$5{,}83\times10^{-6}$	43±6	12
Notes. LSD (Least Significant Difference) — 2,825; SX (accuracy of experience) — 2,506.				

The study of growth regulatory activity of estafiatine (1) and its three derivatives on the germination of wild oats and mustard seeds at a concentration $5{,}83\times10^{-6}$ – $6{,}45\times10^{-6}$ g/cm^2 showed significant inhibitory activity. Moreover, the introduction of a chlorine atom, a hydroxyl group or a double bond in the molecule of estafiatine significantly increases its activity. Thus, inhibition of germination of mustard seeds by 3-chloro-4-hydroxy derivative of estafiatine (6) at a concentration $6{,}34\times10^{-6}$ g/cm^2, and by 3-hydroxy-4-methylene-estafiatine (2) at a concentration $6{,}45\times10^{-6}$ g/cm^2 was 2 times higher than the activity of original estafiatine (1). And introduction of a keto-group in the structure of estafiatine did not affect activity. The effect on the germination of wild oat seeds by 3-chloro-4-hydroxy derivative (6) at a concentration of $6{,}29\times10^{-6}$ g/cm^2 was 3 times higher than the action of estafiatine (1).

Thus, the results of bioscreening of estafiatine and its derivatives show that the introduction of alkylating centers in the molecule of sesquiterpene lactone, such as α,β-unsaturated keto-group, and functional groups such as hydroxyl, halogen atoms contribute to a targeted change of their growth regulatory activity. Synthesized biologically active compounds can be the basis for creation of new practically valuable drugs.

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Эстафиатин және оның туындыларының синтезі мен өсуді реттегіш белсенділігі

Мақалада гваянолид эстафиатиннің практикалық маңызды туындыларының синтезі қарастырылған. Эстафиатин молекуласындағы α-эпоксидтік циклдің болуы нуклеофильді қосылу реакциясының стерео- және региоселективтігін анықтайтынын көрсетті. Авторлар зерттеу барысында алынған сесквитерпенді лактон эстафиатин және оның модификацияланған туындылары жаңа биологиялық белсенді заттарды алу және алынған заттар құрылыстарының оның биологиялық белсенділігіне әсерін анықтады. Жүргізілген биологиялық зерттеулердің нәтижесінде эстафиатин туындыларының өсуді реттегіш белсенділігі байқалды.

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Синтез и рострегулирующая активность эстафиатина и его производных

В статье рассмотрены подходы к направленному синтезу практически значимых производных гваянолида эстафиатина. Показано, что наличие в молекуле эстафиатина α-эпоксидного цикла определяет стерео- и региоселективность нуклеофильного присоединения и получения новых биологически активных производных. Обсуждаемый сесквитерпеновый лактон — эстафиатин и его производные были изучены в плане получения новых биологически активных веществ и влияния структурных особенностей молекул данного ряда на их биологическую активность. В ходе проведенных биоиспытаний обнаружена рострегулирующая активность ряда производных эстафиатина.

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