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Antifeedant activity of *neo*-clerodane diterpenoids from *Scutellaria galericulata* against Colorado potato beetle larvae

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ABSTRACT

Nine *neo*-clerodane diterpenoids and a mixture of C-16 epimers - scutegalerins C and D, isolated from *Scutellaria galericulata* (*Labiatae*), were tested for insect antifeedant activity against *Leptinotarsa decemlineata* (Say) larvae in choice feeding assays. Treatment of potato leaf disks with small amounts of the natural products (1000, 100, 10 ppm) demonstrated a significant antifeedant activity. Results were compared with one of the used in feeding assays compounds - 14,15-dihydrojodrellin T, which is known as a very powerful feeding suppressor and deterrent against insect pests. Activity was established by calculating the feeding ratio (FR) between the consumed areas of treated disks (CTD) and control disks (CCD). To obtain comparison results was established a FR_{50} determined as the FR at a CCD of 50% was established. Some of the compounds exhibited activity at dose 10 ppm (0.33 µg/cm^2). The purpose of this study was to define the structural features of the clerodanes and to determine their antifeedant activity.

Key words: *Scutellaria galericulata*, *neo*-clerodane diterpenes, *Leptinotarsa decemlineata* (*Say*), antifeedant activity.

Introduction

Colorado potato beetle (*Leptinotarsa decemlineata* Say) is a very common and one of the most serious insect pest of vegetable-gardens, that cause considerable damage on the both, back yard and commercial potato growers. Both the striped beetle and the black-spotted and their red larvae feed on potato leaves. They can greatly reduce yield and even kill plants. Except potato, *Leptinotarsa decemlineata* infests also tomato, pepper and eggplant.

To rid the crops from the Colorado potato beetle different methods are utilized (both conventional and organic). In spite of the insect has ability to rapidly develop a resistance to most classes of synthetic insecticides they remain the major control methods (Ewing et al., 1981; Hare, 1990). It is necessary chemical insecticides to be used repeatedly and in combination with majority of different manners to suppress population of the pest. Recently, efforts have directed for the search of substances of plant origin as a promising source of new control agents with low mammalian toxicity (Alford et al., 1987). Terpenoids are the most diverse group of plant allelochemicals, some of them showing biological activities against some economically important pests like *Leptinotarsa decemlineata* Say, or *Spodoptera littoralis* (Huang et al., 1995; Mizutani, 1999). Diterpenoids with *neo*-clerodane skeleton (Figure 1a) have attracted interest on account of their biological activities against some economically significant pests, especially like insect-antifeedants (Gaspar et al., 1995; Lopez-Olguin et al., 1999). The plants belonging to the genus *Scutellaria* (Labiatae) are abundant source of this kind of compounds, from which about 150 *neo*-clerodanes have been isolated (Bruno et al., 2002; Shang et al., 2010; Shun-Fa et al., 2011). From *Scutellaria woronowii* has been obtained jodrellin B (Figure 1b) – by far the most potent clerodane antifeedant known to date. Priviously, neoajugapyrin A has been isolated from *Ajuga piramidalis*, but reported as 1β-hidroxyscutecyprin with trivial name ajugapyrin A (Boneva et al., 1998). Scutecolumnin C is found in *Scutellaria columna* and scutalbin A in *Scutellaria* *albida* (De la Torre et al., 1992; Bruno et al., 1996). The rest diterpenes are new structures. The novel compounds which we obtained from this species are scutegalerins A-E.

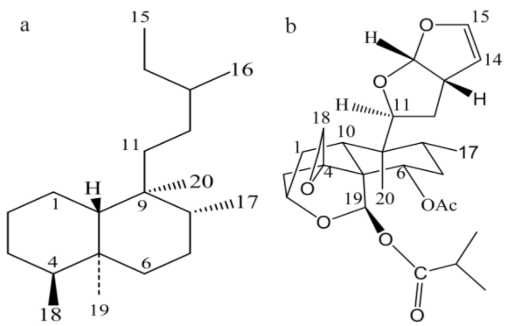


Figure 1. *Diterpenoids with neo-clerodane skeleton.a – base structure of neo-clerodane skeleton; b – jodrellin B.*

We report here on the effects of eleven natural *neo*-clerodanes, isolated in our laboratory from *S. galericulata*, on the feeding behavior of larvae of Colorado potato beetle in order to establish the functional groups determine antifeedant activity.

Materials and Methods

Insects

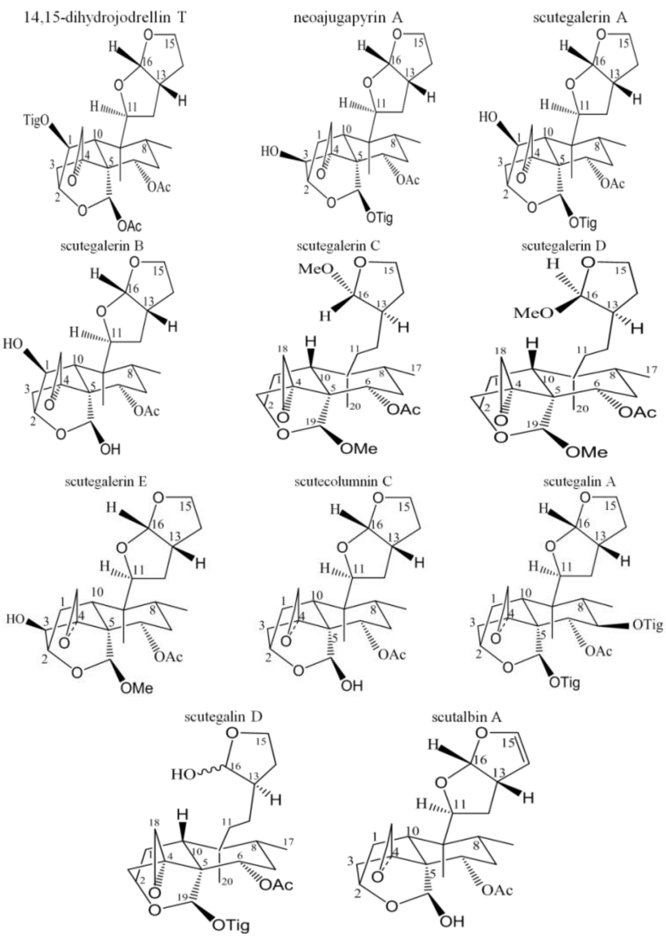
Newly emerged fourth instar larvae used for bioassays were collected from potato fields located around Plovdiv (Bulgaria).

Compounds

Natural *ne*o-clerodane diterpenoids were available from previous works (Figure 2). Samples neoajugapyrin A, scutegalerin A, scutegalerin B and scutecolumnin C were received as described in Bozov et al. (2014a), compounds 14,15-dihydrojodrellin T, scutegalin A, scutegalin D, scutalbin A in Bozov et al. (2014b), whereas substances scutegalerin C, scutegalerin D, scutegalerin E in (Bozov et al., 2014c).

Antifeeding bioassays

Bioassays were carried out in petri dishes (15 x 85 mm), with covered bottom with filter paper, using potato leaf disks with 2 cm^2 area. The upper surface of the disks was treated with 20 µl acetone solution of the test compounds with a microsyringe and after evaporation of the solvents received treated disks (TD). The control disks (CD) were manipulated analogously with pure solvent. In each petri dish alternatively were placed four treated and four control disks. The newly emerged fourth instar larvae were starved for 5 h and placed eight in each dish, where they were allowed to feed. At regular intervals (20 min) during 4-6 h were simultaneously measured consumed areas of treated disks (CTD) and those of control disks (CCD), to calculate the relevant feeding ratio FR = CTD/CCD. To receive comparative results we used FR_{50}, the ratio when 50% of the CD areas in every dishes has been consumed (CCD_{50}). Bioassays were performed under the same temperature, humidity conditions and constant darkness.



Firure 2. *Neo-clerodane diterpenoids tasted against Colorado potato beetle larvae.*

Experiments were terminated, when 75% of the potato disks area has been consumed in each plate.

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Results and Discussion

All tested eleven *neo*-clerodane diterpenoids displayed a 2α,19-hemiacetal or acetal functionality in the decalin ring, C-4 - C-18 spiroepoxide and an acetate group at C-6 position. At the C_{11}-C_{16} substructure there is a very common, for clerodanes isolated from *Scutellaria* species, hexahydrofuro [2,3-b] furan moiety with the exception of compound scutalbin A with tetrahydrofurfuran ring and scutegalerin C, scutegalerin D, scutegalin D in which C-15 and C-16 are involved in the construction of a single ring, hemiacetal (scutegalin D) or acetal (scutegalerin C and scutegalerin D).

Table 1. *Feeding ratios of the tested natural neo-clerodane diterpenes.*

Compound	Dose (ppm)	№	$FR_{50} \pm SE$	$FR_{75} \pm SE$
14,15-dihydrojodrellin T	1000	3	0.03 ± 0.00	0.03 ± 0.01
	100	5	0.12 ± 0.04	0.18 ± 0.06
	10	5	0.37 ± 0.10	0.37 ± 0.10
neoajugapyrin A	1000	3	0.05 ± 0.01	0.07 ± 0.04
	100	5	0.14 ± 0.09	0.21 ± 0.11
	10	5	0.44 ± 0.11	0.49 ± 0.14
scutegalerin A	1000	3	0.04 ± 0.02	0.05 ± 0.01
	100	5	0.14 ± 0.07	0.24 ± 0.09
	10	5	0.42 ± 0.13	0.52 ± 0.16
scutegalerin B	1000	3	0.11 ± 0.04	0.17 ± 0.07
	100	5	0.26 ± 0.08	0.33 ± 0.13
	10	5	0.63 ± 0.17	0.74 ± 0.17
scutegalerin C + scutegalerin D	1000	5	0.31 ± 0.07	0.40 ± 0.09
	100	3	0.59 ± 0.10	0.67 ± 0.17
	10	3	0.88 ± 0.19	0.99 ± 0.22
scutegalerin E	1000	3	0.14 ± 0.04	0.17 ± 0.05
	100	3	0.32 ± 0.12	0.38 ± 0.10
	10	5	0.76 ± 0.16	0.89 ± 0.18
scutecolumnin C	1000	3	0.14 ± 0.03	0.20 ± 0.03
	100	3	0.25 ± 0.10	0.29 ± 0.12
	10	5	0.39 ± 0.14	0.43 ± 0.11
scutegalin A	1000	3	0.09 ± 0.02	0.10 ± 0.05
	100	3	0.17 ± 0.10	0.22 ± 0.16
	10	5	0.47 ± 0.12	0.65 ± 0.18
scutegalin D	1000	5	0.28 ± 0.06	0.32 ± 0.07
	100	3	0.46 ± 0.15	0.51 ± 0.19
	10	3	0.80 ± 0.17	0.88 ± 0.18
scutalbin A	1000	3	0.07 ± 0.02	0.09 ± 0.05
	100	3	0.16 ± 0.10	0.18 ± 0.13
	10	5	0.45 ± 0.12	053 ± 0.16

Legend: № - number of replications; SE - standard error; FR - feeding ratios; FR = CTD/CCD (where CTD and CCD represent consumed areas of treated disks and control disks, respectively).

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Individual compounds difer each other by the variability of the substitute at C-19 – hydroxyl (OH), acetyloxy (OAc), methoxyl (OMe), E-2-methy-2-butenoyloxy (OTig). Some of the substances have additional substitute at C-1, C-3, C-7 or C-16. Neoajugapyrin A and scutegalerin A are position isomers, whereas scutegalerin C and scutegalerin D are epimers at C-16.

As shown in Table 1., most of the tested natural diterpenoids exhibited very good to excellent deterring of *Leptinotarsa decemlineata* larvae to feed (FR_{50} < 0.1) at 1000 ppm dose (~33 μg/cm^2), with observable exception of compounds scutegalerin C, scutegalerin D and scutegalin D. The low activity of these three clerodanes might be assigned to the absence of the furofuran substructure in the C-11−C-16 fragment of the basic clerodane skeleton.

From the positional isomers scutegalerin A and neoajugapyrin A bigger activity showed first one. Scutegalerin A has substitute at C-1 as 14,15-dihydrojodrellin T which demonstrated the highest activity among the tested *neo*-clerodane diterpenoids. In decalin ring as substitutes at C-1, C-3, C-7 and C-19 position vary ester moiety, hydroxyl or methoxy groups. In this order activity decreases and it increases when there is double bond between C-14 and C-15.

Conclusions

According achieved in this work results, on studying of antifeedant activity of natural *neo*-clerodane diterpenoids against Colorado potato beetle larvae, we can conclude that the presence in the clerodane structure of a spiroepoxide substituent at C-4 and two ester groups at C-6 and C-19, together with hexahydro- or tetrahydrofurofuran moiety at C-9, is condition for development of activity. Such dependence was reported in previously investigations on other insect (Blaney et al., 1988; Houghgoldstein & Whalen, 1993).

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