Acta Biochimica Polonica

Vol. 41 No. 4/1994

QUARTERLY

Insect growth regulators. XXV. Chemical approach to the correlation of dynamic structure and biological activity of juvenile hormone analogues*

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Received: 30 January, 1994; Revised: 30 August, 1994

Key words: juvenoids, insects juvenile hormones, structure/biological correlation

On the basis of flexibility of the carbon skeleton of a juvenoid molecule, an analysis of its steric properties required to induce a biological effect in insects *Dysdercus cingulatus* and *Tenebrio molitor*, is presented. Steric analyses of "branched" juvenoids and some derivatives of farnesoic acid differing in position of the double bonds, are also described.

Compounds which show a biological activity similar to that of natural juvenile hormones (JH) are commonly called juvenoids. Position and configuration of double bonds in the carbon skeleton of juvenoids belong to the most important factors which determine their biological activity. Apart from a small variation in electron density inside the molecule, this influence is connected with a reduction of flexibility of a particular part of the carbon chain. Therefore, introduction of the double bonds into some specific positions in the juvenoid structure can make impossible (or at least restrict) formation of such conformations which are required to fit the biological receptor, and as a consequence diminish (or even suppress) the biological activity of juvenoids. However, demonstration of these correlations, in a simple way, is rather difficult and demands the use of rather sophisticated computer programs [1–6]. In this paper we propose a relatively simple approach to the correlation between the double bond position and the possible conformations and biological activity of juvenoids.

MATERIAL AND METHODS

The syntheses and spectral data which confirmed the structures of the juvenoids discussed were presented earlier [7–11]. The purity of the preparations was not lower than 98%, except for the esters **1–4** and **7–10**. These JH-analogues, as a result of two chiral carbon atoms C-3 and C-7 present in their structures, were racemic mixtures of two diastereoisomers, which it is very difficult to separate from each other. Unfortunately, neither GC nor ^{1}H-NMR spectra (300 MHz) gave any data about the composition of these mixtures. Note

*This work was partially supported by the Technical University of Wrocław (grant no. 341138).

[1]The symbols "*R/S*", describing absolute configuration of chiral carbon atoms, the symbols "*E/Z*" and "*re/si*", which characterize stereochemistry of double bonds, and the symbols "*P/M*" which concern helicity of carbon chain, were used according to the IUPAC rules.

that in the natural juvenile hormones, C-3 and C-6 are trigonal carbon atoms.

The biological tests were performed on larvae of *Dysdercus cingulatus* and pupae of *Tenebrio molitor* according to the procedure of Sláma *et al.* [12]. The JH activity was measured and compared for juvenoids in racemic forms. For presentation of the molecular models the computer programme DTMM (Version 1.2, Oxford, 1989) was used.

RESULTS AND DISCUSSION

The results of biological tests, performed in our laboratory, are presented in Tables 1–3. Almost all investigated compounds exhibited moderate hormonal activity in *D. cingulatus* and were inactive in *T. molitor* (at least at the dose below 80 μg/specimen). To systematize the sterical analysis, we distinguished in the juvenoid molecule three structural segments: (a) from C-1 to C-3, (b) from C-3 to C-7, and (c) from C-7 to C-12; the conformational analysis was carried out, and the structure-biological activity correlations were determined separately for each segment. To simplify the analysis of the effects of the double bond position on conformational properties of the juvenoid molecule, first, only those of the possible rotamers were taken into consideration which could be arranged on one plane. Such sterical analysis, carried out for juvenoids **1–10**, is presented in Figs. 1 and 2. In the molecules **1–4** and **7–10**, the structural fragment from C-1 to C-7 is the same, therefore, the differences in flexibility can be observed only in the remaining part of the molecule; thus, free rotations around the single C–C bonds from C-5 to C-11 were taken into account. In considering the three-dimensional picture, one should take into account additional rotations of the marked carbon chain around the single C-6 bond. Comparison of dimensions and shape of the area, into which some part of the analysed C7–C12 fragment of a particular compound could be projected, makes evident the similarities and differences in conformation between individual juvenoids. As it was easy to predict, the most flexible molecules are those of the isomers **1** and **2** or **3**

Table 1

Biological activity in D. cingulatus larvae of juvenoids with different double bond positions in the isoprenoid chain.

In this and subsequent Tables the results are expressed as inhibition doses (ID_{50}), i.e. that amount of the indicated juvenoid (in μg/specimen) which inhibited by 50% metamorphosis of the insect. The topical applications were performed at the very beginning of the sensitive period, i.e. to larvae of *D. cingulatus* at the last instar or pupal instar of *T. molitor*. Ventral side of the insect abdomen was treated topically with 1 μl of acetone-juvenoid solutions. After 6 to 8 days the percentage of retention of the epidermal patterns was evaluated and used for calculation of the ID_{50} Morph. units of activity. A triangle (Δ) indicates inactive or low activity compounds (ID_{50} > 80 μg/specimen).

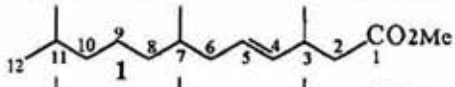
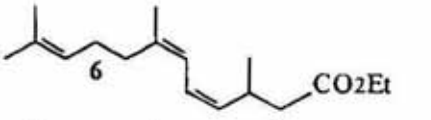
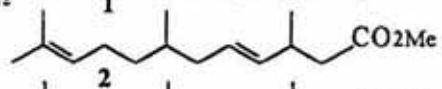
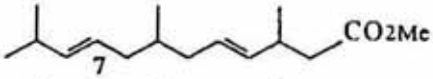
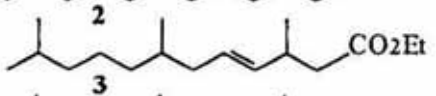
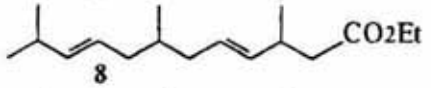
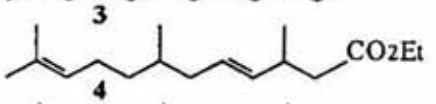
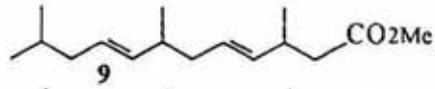
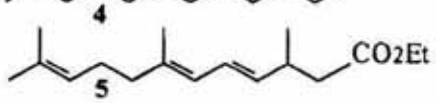
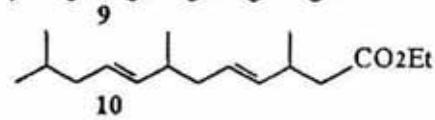
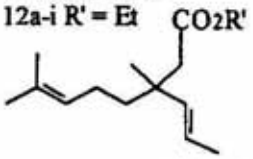
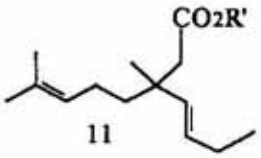
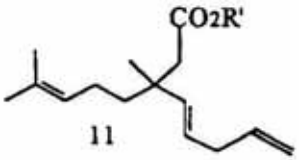
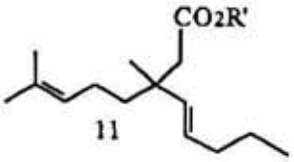
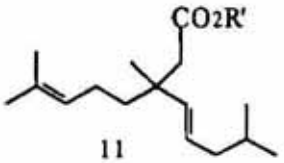
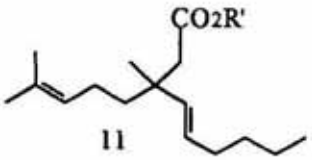
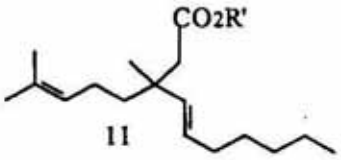
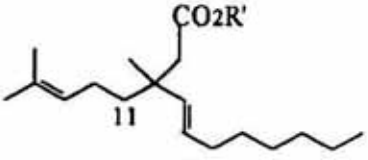
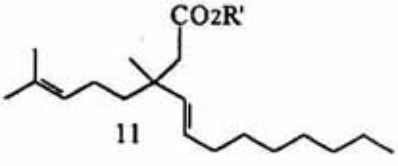
Compound (No.)	ID_{50}, μg/sp. *D. cingulatus*	Compound (No.)	ID_{50}, μg/sp. *D. cingulatus*
 **1**	1.6	 **6**	1.6
 **2**	0.25	 **7**	1.0
 **3**	0.8	 **8**	0.4
 **4**	0.25	 **9**	2.0
 **5**	0.8	 **10**	2.0

Table 2
Biological activity in D. cingulatus larvae of the "branched" juvenoids.
For details see Table 1.

Compound	No.	ID_{50}, µg/sp. *D. cingulatus*
11a-i: R' = Me 12a-i R' = Et 		
	11a	80.0
	12a	Δ
		
	11b	80.0
	12b	Δ
		
	11c	80.0
	12c	Δ
		
	11d	0.8
	12d	8.0
		
	11e	80.0
	12e	80.0
		
	11f	80.0
	12f	80.0
		
	11g	80.0
	12g	80.0
		
	11h	0.8
	12h	16.0
		
	11i	80.0
	12i	Δ

and **4**. In the case of two other isomers **7**, **9** or **8**, **10**, in which the double bond is shifted from the C-10 to C-9 or C-7 position, the flexibility is considerably restricted, and the volumes of spaces where one could insert the possible rotamers of these juvenoids are located in different areas. Since isomers **7** and **8** are more active than isomers **9** and **10**, one can suppose that an important area, where some structural elements should be placed to induce biological activity, seems to be the region 4A (see projection on Fig. 1), where compounds **1–4**, or **7** and **8** are able to place the fragment of their carbon chain. On the other hand, the presence of the structural fragment in the region 2A does not seem to contribute to the biological activity, at least in *D. cingulatus*. This observation confirms almost the same biological activity (in *D. cingulatus*) of the cyclic analogues (**14**, **16**, **18**) of juvenoids **13**, **15** and **17**, where the carbon atoms C-10 and C-14 are bonded by an additional single bond, and a considerably lower

Table 3

Biological activity in D. cingulatus larvae and T. molitor pupae of juvenoids with farnesane skeleton and their cyclic analogues.

For details see Table 1.

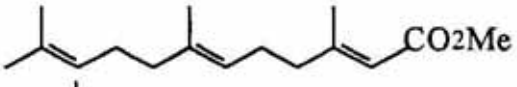
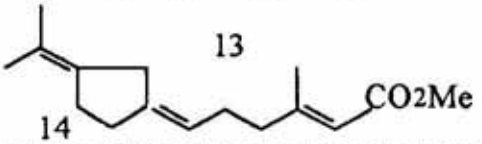
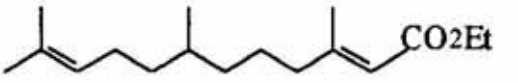
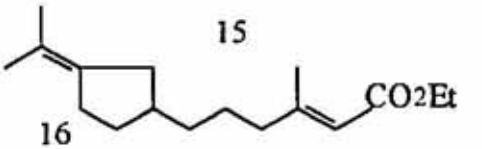
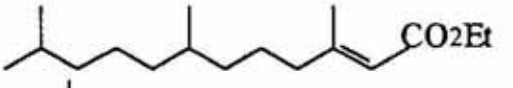
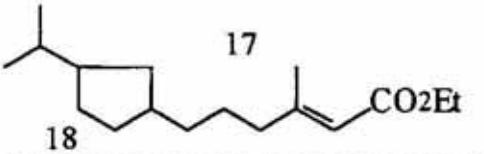
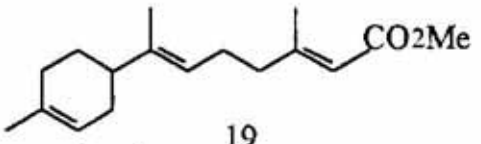
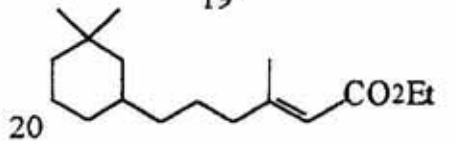
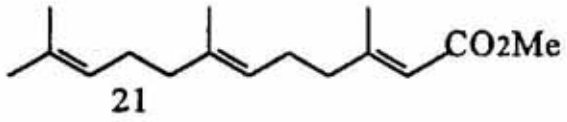
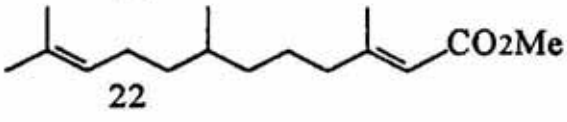
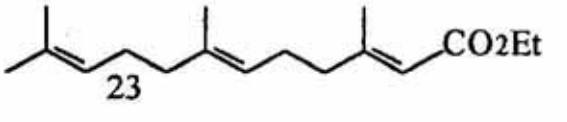
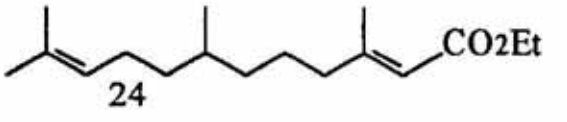
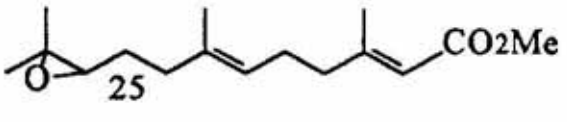
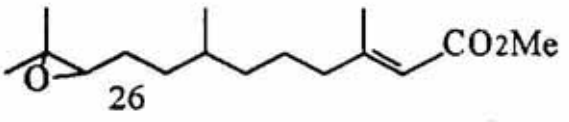
Compound (No.)	ID_{50}, µg/sp. *D. cingulatus*	*T. molitor*
 13	0.8 [13]	80 [13]
 14	1.6 [10]	Δ [10]
 15	0.16 [13]	1.7 [13]
 16	0.16 [10]	Δ [10]
 17	0.8 [13]	0.9 [13]
 18	0.16 [10]	Δ [10]
 19	16.0 [8]	Δ [8]
 20	8 [10]	Δ [10]

Table 4
Influence of double bond in the C-6 position on biological activity of juvenoids.
For details see Table 1.

Compound (No.)	ID_{50}, µg/sp.	
	D. cingulatus [17]	*T. molitor* [17]
 21	50	50
 22	10	30
 23	10	5(i)
 24	4	10(i)
 25	1	10 (i)
 26	0.05	5 (i)

(i) The application of juvenoid was performed by injection.

activity of compounds with other structural arrangements of carbon chain (**19** and **20**), as it is presented in Table 3.

A similar steric analysis applied to the juvenoids with different position and configuration of the double bonds in the middle part of the molecule is presented in Fig. 2. The chemical formulae of the 6*E* (**5**) and the 6*Z* (**6**) isomers[1] suggest that the opposite double bond geometry just in the middle of a molecule should exert a great influence on the general shape of molecules and, as a consequence, on biological activity. However, the activity (at least as concerned *D. cingulatus*) of both these compounds was of the same order (Table 1). The chain flexibility of the two isomers allows to assume their similar shape, including the presence of some structural elements in region 4A (Fig. 2).

Biological activity of the "branched" juvenoids (compounds **11a–i**, and **12a–i**) afforded new interesting information about the correlation between the shape of a molecule and its biological effects (especially the steric arrangements in the vicinity of the ester group). These compounds can be considered derivatives of citronellic esters, with additional carbon chain at position C-3. They do not resemble any other presently known juvenoids, although one could find some structural similarity with juvocimene [14]. The "branched" juvenoids did not act on *T. molitor* (at a dose below 80 µg/specimen) and only a few of them (**11d, 11h, 12d**), with a specific number of carbon atoms in the side chain, were active in *D. cingulatus* (Table 2). Assuming that the mechanism of biological activity of both types of juvenoids is the same, one

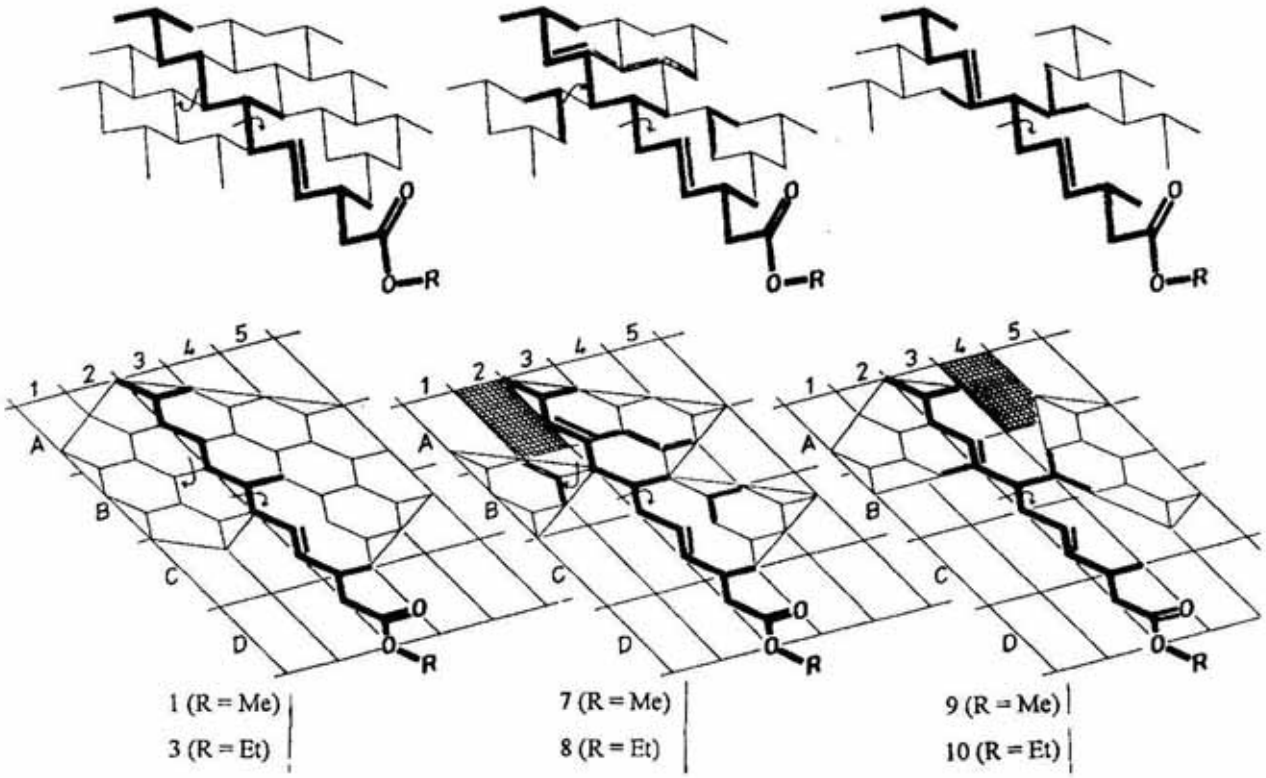


Fig. 1. Flexibility of carbon skeleton of juvenoids with different double bond position in the third isoprenoid segment.

In the upper part of the figure, the sterical view of all possible rotamers arranged on one plane are shown, and in the lower part, their projection onto plane (marking the possible double bond positions). Shapes of the area characteristic for compounds **2** and **4** are the same as for compounds **1** and **3**; only rotation around the C=C double bond at position C11 is restricted. For each juvenoid, only one of the possible diastereoisomers (Fig. 1), or enantiomers (Fig. 2 and 3) was presented.

should look for the shapes which could be possibly adopted by both "chain" and "branched" JH-analogues. Such sterical arrangements of the carbon skeleton of the "branched" and "long-chain" juvenoids are shown in Fig. 3. Thus, the carbon chain in the juvenoids with farnesane skeleton (when interacting with biological JH-receptor or other binding proteins of *D. cingulatus*), seems to be bent at the central carbon atom (C-7). Usually, in the series of juvenoids with farnesane skeleton, when the methyl in the ester group was replaced by the ethyl, the JH-mimic activity was increased. In the "branched" juvenoids the same structural change resulted in an opposite effect. Certainly, the more voluminous ethyl substituent in the ester group prevents the molecule from assuming the shape **11b** presented in Fig. 3. However, it is difficult to explain the relatively high biological activity of compound **11h**. The suggestion that the JH molecule should be able to be bent at the C5–C8 carbon chain fragment could explain the fact that the juvenoids without a double bond at position C-6 (and, as consequence, with higher flexibility at this part of molecule), exhibit higher biological activity (in *D. cingulatus*) than those possessing the double bond at C-6 (Table 4).

Almost all JH-analogues we have presented in this paper include at least one chiral center, mainly in the middle of the molecule, but in most cases we obtained them in racemic forms, only. In the molecules of natural juvenile hormones, depending on their type (JH-1, JH-2 or JH-3), there is one or two chiral centres, which determine the stereochemistry of the oxirane ring, only. Therefore, one could expect that the specific JH-receptor should not be sensitive to the chirality of the other parts of the juvenoid molecule. However, Henrick *et al.* [15] obtained selected juvenoids, in pure enantiomeric forms, with chiral carbon atom situated in the middle

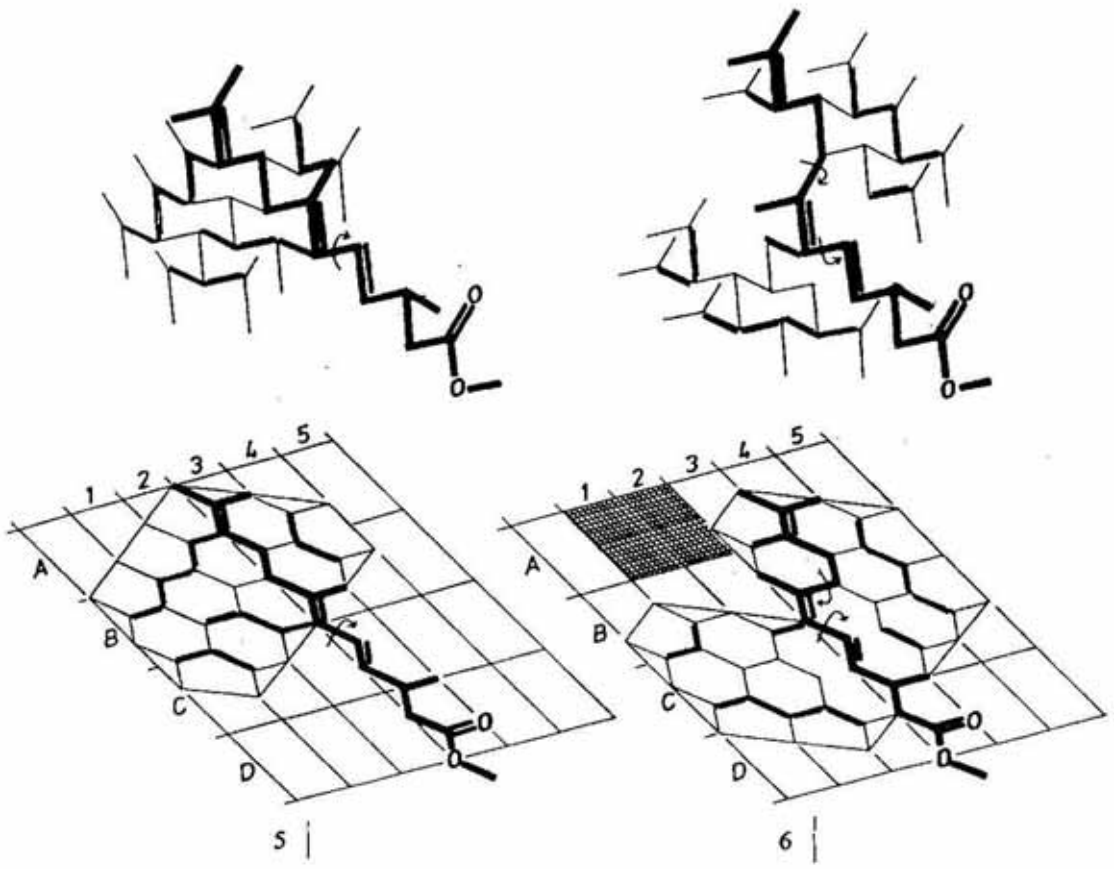


Fig. 2. Flexibility of carbon skeleton of juvenoids with different double bond position in the second isoprenoid segment.
For details see legend to Fig. 1.

of the molecule. He found that the two enantiomers differed in biological activity on *Aedes aegypti, Musca domestica, Tenebrio molitor* and *Galeria mellonella*. In the case of juvenoids with the farnesoic skeleton the 7*S* enantiomers were more active than the 7*R* ones. On the other hand, in the case of juvenoids with the Bowers ethers structure, the more active appeared to be the enantiomers with the opposite sign of chirality. Henrick explained this fact by suggesting that the two types of juvenoids bound to different chiral JH-receptors, or alternatively, both bound to the same biological JH-receptor but in a reverse fashion. In the case of juvenoids with the Bowers ethers structure the epoxide ring in the molecule plays the same role as the ester group in the farnesoic type juvenoid.

In our laboratory we obtained several citronellyl- and menthocitronellyl-*p*-substituted phenyl ethers in forms of pure enantiomers with 3*R* configuration and their racemic forms [16]. The biological tests performed on *D. cingulatus* pointed to a distinctly higher JH activity of pure 3*R* enantiomers in comparison with their racemic forms, thus confirming Henrick's observations made on other insects. The two enantiomeric "branched" juvenoids **11d** and **12d** exhibited also different JH-mimic activity in *D. cingulatus* [18]. The (*S*)-enantiomers were more active than (*R*)-enantiomers. The fact that the specific JH-receptor distinguishes the chirality of the middle part of a juvenoid molecule seems to suggest that although the natural juvenile hormones do not have a chiral centre in this position, the overall shape of this part of carbon chain should be chiral. Such a chiral shape of the JH-III molecule is presented in Fig. 4, where the chirality is introduced by the right or left bending of the isoprenoid chain. In this case, the double bond in the C6 position, depending on the *P* or *M* helicity (cf. Fig. 4), could interact with JH-biological receptor alternatively, by the *re-si* or *si-re* side. Taking into account Henrick's observations and higher

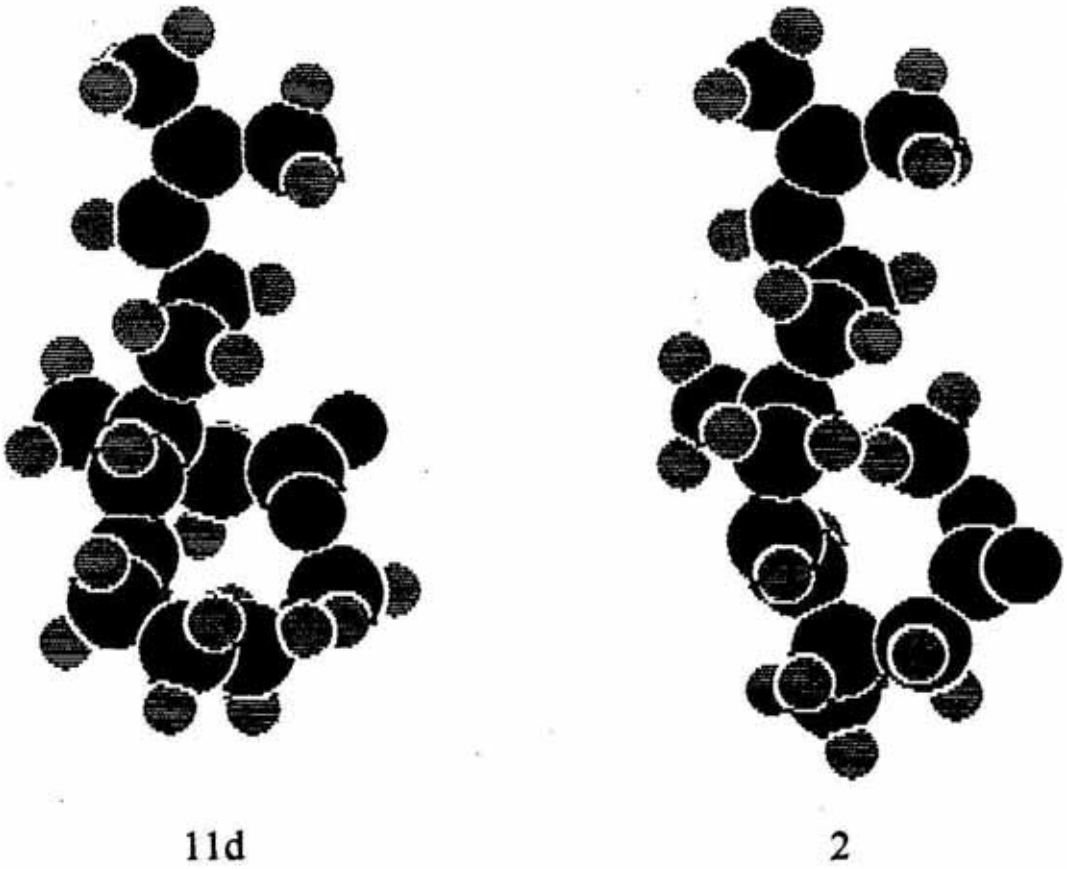
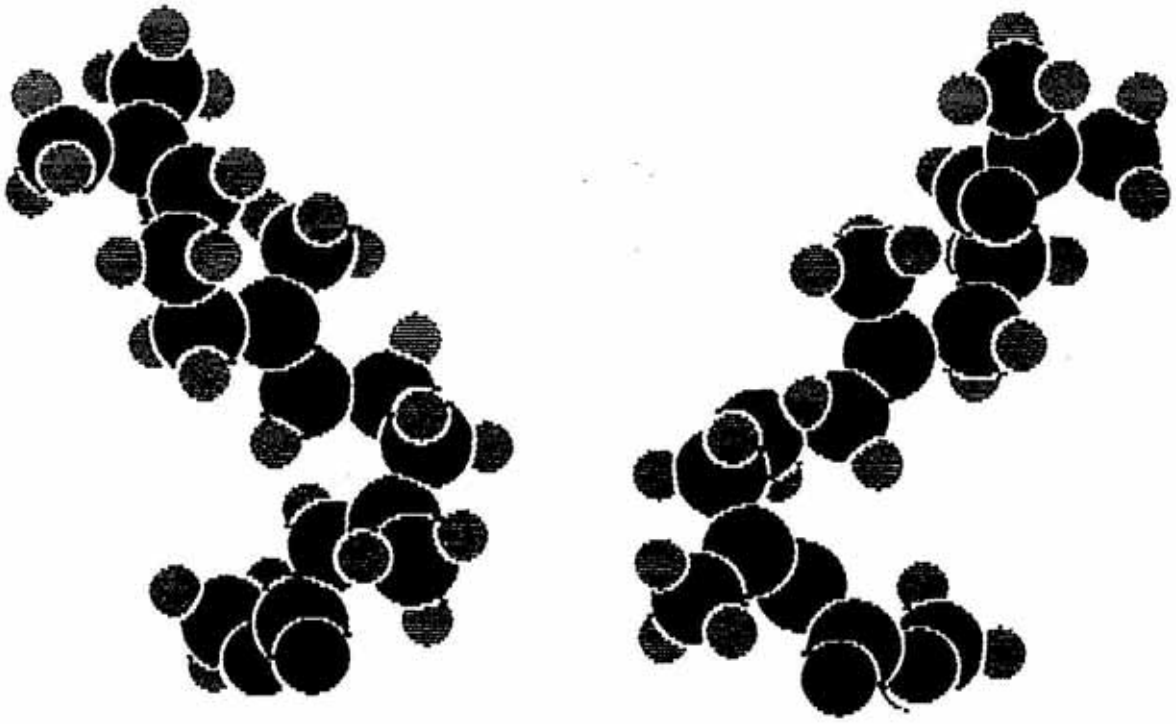


Fig. 3. Similarity of shapes of juvenoids with farnesane skeleton and the "branched" juvenoid **11d**.

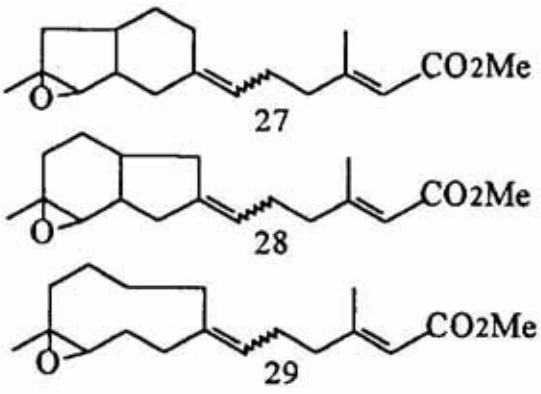
JH-activity of the (*S*)-enantiomer of the "branched" juvenoid **11b**, we suggest that, in the case of *D. cingulatus*, "the biologically active shape" of the JH-molecule would correspond to the model shown on the left side of Fig. 4.

The discussion presented above concerned the juvenoids which exert biological activity in *D. cingulatus*. In the case of *T. molitor*, we can only note that the carbon chain of the juvenoid, active in this insect, should be elongated as much as possible. The cyclization of the carbon chain, as it was evidenced for the juvenoids **14**, **16**, **18**, **19** and **20** (Table 3) reduced (or cancelled) the biological activity. Even in the cases when the basic carbon chain was arranged in one line and contained 12 carbon atoms, but the fragment C7–C10 was sterically stiffened by cyclic systems (see Scheme 1) — as it was proved by Patel *et al.* [19] — no biological activity was observed in this insect. Conjugation of the ester group with double bond is the other structural element which is needed to induce biological activity in *T. molitor*. This type of ester group is more stable, and one can suppose that the JH-esterase, or other esterases present in *T. molitor*, are more active than those present in *D. cingulatus*, and very easily hydrolyse the ester group not stabilised by conjugation with double bond in α-position. "The biologically active shape" of the juvenoid molecule, described in this paper, does not pretend to correspond to the shape of the JH molecule at the time when it binds to the biological receptor. The JH molecule secreted by JH glands, or a juvenoid artificially introduced into the insect organism, has to overcome a long way before reaching the final receptor, and interacts with many other proteins and cell-membranes. The "biologically active shape" presented here, indicates only that some specific stereochemical properties of a molecule (e.g. preservation of free rotation at particular C–C bonds) should be assured to induce (or reinforce) JH-mimic activity in particular insects. We hope that this very simple steric analysis of molecules with relatively high flexibility, in connection with their biological activity, could be also applied to other groups of biologically interesting compounds.



JH-III [(R) - **25**]

Fig. 4. Models of JH-III [(R)-25] molecule.



Scheme 1. The juvenoids with sterically stiffened cyclic system in the C7-C11 fragment.

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