Chemistry of Natural Compounds, Vol. 36, No. 6, 2000

ZOOECDYSTEROIDS: DISTRIBUTION AND ROLE IN ARTHROPOD LIFE CYCLES

U. A. Baltaev and G. S. Shangaraeva

UDC 547.926:591.147

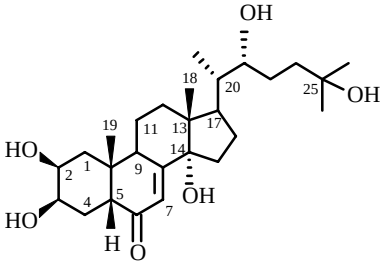
The distribution of ecdysteroids, their metabolism and role in the life cycle of arthropods and the possibility of using exogenic ecdysteroids to regulate pest populations and to increase the productivity and vitality of useful insects are discussed in the review.

Key words: ecdysteroids, zooecdysteroids, molting, insects, arachnids, crustaceans.

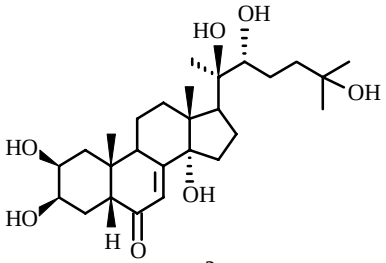
STRUCTURE AND METABOLISM OF ARTHROPOD ECDYSTEROIDS

Molting and metamorphosis of insects is controlled by a hormone that was isolated first from the prothoracic gland and then in a pure form and is called "α-ecdysone" (from the Greek *ecdysis*, molting) [1-3]. A whole class of substances that regulate insect molting was already known by the end of the 1970s. These substances were called "ecdysteroids" [3]. Then is was discovered that several related polyhydroxysteroids or zooecdysteroids in addition to α-ecdysone regulate molting and metamorphosis in arthropods [3, 4].

The most common zooecdysteroids are α-ecdysone (**1**) and ecdysterone (**2**) (Table 1).



1



2

For example, adult males of the cockroach *Blaptica dubua* contain large quantities of ecdysone and ecdysterone [5]. *Bombyx mori* and *Calliphora erythrocephala* contain mainly ecdysone whereas *Calliphora stygia* has mainly ecdysterone [6, 7]. It has been noted that the ecdysone—ecdysterone ratio depends on the insect species and development stage. During postembryonic development, the first four instars of *Bombyx mori*, the small increase of molting hormones is related to the food of the caterpillars. The ecdysteroid content decreases in mature larvae and sharply increases in young wandering-stage caterpillars [8]. Ecdysterone accounted for 2/3 of the total ecdysteroid inventory; ecdysone, the remainder.

The ecdysteroid content may also depend on the sexual dimorphism. Mature female caterpillars of the field cricket *Gryllus bimaculatus* have the maximal titer of ecdysteroids on the fourth day, two days before imaginal molting [9]; imago males of this same species, 8, 12, and 14 h after imaginal molting [10]. The ecdysteroid level in freshly molted imago females of the house cricket *Acheta domesticus* is low, with the exception of the gut and carcass [11]. This is apparently due to the presence of ecdysteroid traces of the preecdysial peak. Females of the desert locust *Schestocerca gregaria* in the two last instars contain 74-84% ecdysterone of the total ecdysteroid inventory; males, 63-74% [12].

Institute of Petroleum Chemistry and Catalysis, Academy of Sciences of the Republic of Bashkortostan, and Ufa Scientific Center, Russian Academy of Sciences, 450075, Ufa, pr. Oktyabrya, 141, Tel/Fax: (3472) 31 27 50. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 433-446, November-December, 2000. Original article submitted August 28, 1999.

0009-3130/00/3606-0543$25.00

TABLE 1. Composition of Ecdysteroids and Their Conjugates from Various Arthropod Species

Species	Organ, tissue, development stage	Ecdysteroids	Reference
1	2	3	4
		Crustaceans	
Callinectes sapidus	Eggs	Ponasterone A	56
	In all stages	Inokosterone (callinecdysone A)	58
		Callinecdysone B	58
		Ecdysterone	58
Carcinis maenas	Y-organ**	25-Dehydroxyecdysone	83
	In all stages	25-Dehydroxy-20-hydroxyecdysonic acid	59
Gecarcinus lateralis	Hemolymph	Ponasterone A	56
Jasus lalandei	In all stages	Ecdysterone	55
		2-Dehydroxyecdysterone	57
		Arachnids	
Boohpilys microplus	Imago**	Ecdyson-22-acylesters (palmitate, palmitoleate, stearate, oleate, linoleate)	63
Ornithodoros moubata	Nymph**	20-Hydroxyecdyson-22-acylesters (palmitate, linoleate, oleate, stearate)	62
Pycnogonum litorale	Adults**	22-Dehydroxyecdysterone	28
		22-Dehydroxy-20,26-dihydroxyecdysone	28
		[25R]- and [25S]-20,26-dihydroxyecdyson-22-acetate	28
		Ecdyson-22-glycolate	28
		20-Hydroxyecdyson-22-glycolate	28
		20-Hydroxyecdyson-22-acetate	28
		Insects	
Acromyrmex octospinosis	Pupae	24-Epimakisterone A	40
Antheraea pernyi	In all stages	Ecdysterone	99
Apis mellifera	Pupae	Makisterone A	49
		Ecdysterone	50
Blaptica dubia	Imago	Ecdysone	5
		Ecdysterone	5
Bombux mori	Ovaries	2,22-Didehydroxyecdysterone	84
		Bombikosterol	38
		2-Dehydroxyecdisone	85
	Larvae**	Poststerone	37
	Pupae	Ecdysone	6, 86
		Ecdysterone	87
	Testicles	Ecdysone	8
	imago	Ecdysterone	8
	Hemolymph	Ecdysone	8
	imago	Ecdysterone	8
	Eggs	22-Dehydroxyecdysterone	88
		3-Epi-22-dehydroxyecdysterone	88
	Diapausing	3-Epi-22-dehydroxyecdysterone	89
	eggs	3-Epi-22-dehydroxyecdysteron-2-phosphate	89
Calliphora erythrocephala	Imago	Ecdysone	39, 117
		Ecdysterone	117
		3-Dehydroecdysone	39
		20-Dehydroxymakisterone A	39

TABLE 1. (continued)

Species	Organ, tissue, development stage	Ecdysteroids	Reference
1	2	3	4
Calliphora vicina	Cell culture**	3-Dehydroecdysone	114
		3-Dehydroecdysterone	115
	In all stages	Inokosterone	116
Calliphora stygia	Imago	Ecdysterone	117
Diadasia rinconis cockerell	Imago	Ecdysterone	41
		Makisterone A	41
		24-Epimakisterone A	41
Leucophae maderae	Fatty body	22-Dehydroxy-26-hydroxyecdysone	118
	imago**	22,25-Didehydroxy-26-hydroxyecdysone	118
Locusta migratoria	Ovaries	2,14,22,25-Tetradehydroxyecdysone	70
		2,22,25-Tridehydroxyecdysone	70
		2,22-Didehydroxyecdysone	70
	Eggs	3-Dehydro-2-dehydroxyecdysone	101
		2-Dehydroxyecdyson-22-phosphate	101
		Ecdyson-3-phosphate	102
		2-Hydroxyecdyson-22-phosphate	103
		2-Dehydroxy-20-hydroxyecdyson-22-phosphate	103
		3-Epi-2-dehydroxyecdyson-3-phosphate	104
		20-Hydroxyecdyson-3-acetate-22-phosphate	103
		2,3-Diacetylecdyson-22-phosphate	102
		Ecdyson-22-adenosinemonophosphate	105
		2-Dehydroxyecdyson-22-adenosinemonophosphate	104
		Ecdyson-22-isopentyl-adenosinemonophosphate	106
	Larvae**	20-Hydroxyecdyson-3-phosphate	107
		20-Hydroxyecdyson-3-acetate-22-phosphate	103
	Imago**	3-Acetyl-20-hydroxyecdyson-2-phosphate	108
		20-Hydroxyecdyson-3-acetate	108
Megonra viciae	Parthenogenetic	Ecdysterone	120
	females	Ecdysone	120
		Makisterone A	120
Manduca sexta	Eggs	26-Hydroxyecdysone	90
		3-Epi-26-hydroxyecdysone	91
		26-Hydroxyecdyson-26-phosphate	92
		26-Hydroxyecdyson-2-phosphate	93
		26-Hydroxyecdyson-22-glycoside	94
	Caterpillars	22-Dehydroxy-26-hydroxyecdysone	95
		22-Dehydroxyinokosterone	95
		Ecdysterone	13
		20,26-Dihydroxyecdysone	13
		26-Hydroxyecdyson	13
	Pupae	26-Hydroxyecdysterone	96
		3-Epiecdysone	97
		3-Epiecdysterone	98
		3-Epi-26-hydroxyecdysterone	97
	Imago	22-Dehydroxyecdysone	95
Oncopeltus fasciatus	Eggs	Makisterone A	42
Pieris brassicae	Imaginal disks**	25-Dehydroxy-20-hydroxyecdysonic acid	77
	Caterpillars**	3-Epi-20-hydroxyecdyson-3-phosphate	100
	Pupae**	Ecdysteronic acid	77

TABLE 1. (continued)

Species	Organ, tissue, development stage	Ecdysteroids	Reference
1	2	3	4
Sarcophaga perigrina	Imago	3-Epiecdysterone	119
Schistocerca gregaria	Eggs	3-Epi-2-deoxyecdysone	109
		Ecdysonic acid	110
		Ecdysteronic acid	110
		Ecdyson-3-acetate	111
		Ecdyson-2-phosphate	112
		3-Epi-2-dehydroxyecdyson-2-phosphate	112
		Ecdyson-22-phosphate	113
		2-Dehydroxyecdyson-22-phosphate	113
		3-Dehydroxy-20-hydroxyecdyson-22-phosphate	76
		20-Hydroxyecdyson-22-phosphate	76
		3-Acetylecdyson-2-phosphate	112
		3-Acetyl-20-ecdyson-2-phosphate	112
		3-(2)-Acetylecdyson-22-phosphate	112
Spodoptera littoralis	Pupae	Ecdysonic acid	110
		Ecdysteronic acid	110

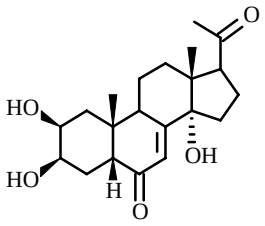
**Ecdysteroids obtained after *in vivo* or *in vitro* experiments, *i.e.*, not endogenic.

Other factors besides the development stage and morphogenesis can affect the change of ecdysteroid content. For example, caterpillars of the tobacco hornworm *Manduca sexta* that are infected with the parasite *Contesia congregata* have the maximal amount of ecdysteroids owing to the production of the parasite ecdysteroids [13, 14]. An analogous phenomenon is observed for *Lymantria dispar* infected with the LdNPV nuclear polyhedrose virus [15]. Caterpillars of the cutworm *Lacenobia oleracea* infected with larvae of *Eulophus pennicornis* do not produce ecdysteroids. Their peak is not observed, in contrast with uninfected caterpillars [16]. Treatment of adult females of *Teleogrullus mitratus* with azadirachtin causes a sharp decrease in the level of ecdysteroids and inhibits the growth of terminal oocytes [17]. The action of other chemicals on the production of ecdysteroids is not always unambiguous. For example, the nonsteroidal ecdysteroid agonist RH-5849 intensified the biosynthesis of ecdysteroids in ovaries of female *Gryllus bimaculatus* but inhibited it in the abdomen shells. Ketoconazole and azadirachtin A inhibited biosynthesis of ecdysteroids in both ovaries and abdomen shells [18].

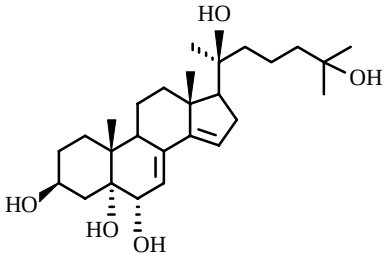
Ecdysteroids are obviously widely distributed. They may function in all arthropod development stages. At least three development stages of the life cycle are known to be controlled by ecdysteroids: embryonic, postembryonic (including pupa in insects with complete conversion), and reproduction [19]. Ecdysteroids in insect larve control molting. The names of these compounds are related to namely this function: molting hormones (MH). Ecdysteroids are observed in both males and females of adult insects. The ecdysteroid titer in the adults is significantly lower than in the pre-imaginal period [20, 21]. Ecdysteroids in crustaceans, in addition to hormonal functions, also act as sex pheromones, which are necessary for normal mating [22]. Publications on the ecdysteroid content of other invertebrates report the presence of them in both juveniles and adults. Such ecological groups as helminths have low ecdysteroid concentrations. Their presence was established only by using very sensitive and specific methods [23].

Ecdysteroids have been isolated from representatives of many species of insects and crustaceans [24, 25] in addition to *Arachnida* {spiders [26], ticks [27], and pycnogonids (*Pantopoda*) [28, 29]}. They have also been detected in other invertebrates: echinoderms (*Echinodermata*) [30], mollusks (*Mollusca, Gastropoda* class) [31, 32], annelidworms (*Annelida*), flatworms (*Platyhelminthes* cystodes and trematodes), roundworms (*Nemathelminthes*) [33-35], and coelenterates (*Coelenterata*) [36].

Zooecdysteroids include mainly 27C- and 28C-ecdysteroids. However, the 21C-ecdysteroid-poststerone (**3**) was isolated from the two insect species *Bombyx mori* and *Calliphora stygia* [7, 37]. The 27C-ecdysteroids include the notable compound bombikosterol (**4**), which was isolated from ovaries of *Bombyx mori* [38].

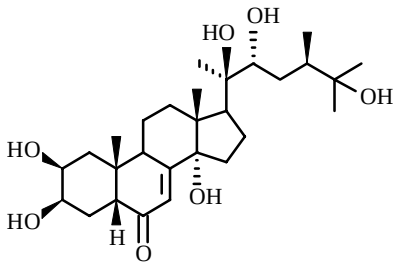


3



4

Makisterone A (24-methylecdysterone) (**5**) is the only insect MH that contains a methyl on C24 of the side chain. Thus, it is a 28C-ecdysteroid. The presence of 20-dehydroxymakisterone [39, 121] and 24-epimakisterone A in this class of arthropods has been reported [40, 41].



5

Makisterone A was observed in representatives of the following orders of arthropods: bugs (*Hemiptera*), hymenopterans (*Hymenoptera*), and dipterans (*Diptera*). Makisterone A was first isolated from large milkweed bug (*Oncopeltus fasciatus*) during embryonic development (egg stage) [42]. It was later established that makisterone A is biologically more active than ecdysterone in adults of this species. It accelerates embryonic development of *Oncopeltus fasciatus* [43]. Then, this zooecdysteroid was detected in other bugs species such as *Nezara viridyla*, *Podisus maculiventris* [44], *Oncopeltus fasciatus* [45], and *Megalotomus quinquespinosus* [46]. It was noted that all species of bugs that are phytophagous contain **5** whereas predators and blood-sucking bugs contain ecdysterone [47].

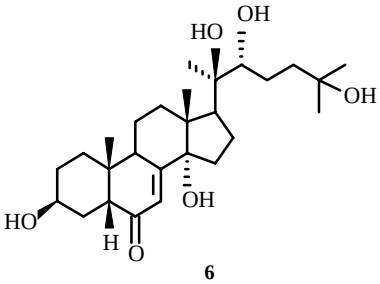
A series of experiments [48] demonstrated that honey bees *Apis mellifera* contain a small quantity of cholesterol, a biosynthetic precursor of all 27C-ecdysteroids. Therefore, it was especially interesting to establish the chemical nature of the MH of this domestically useful insect. Analysis of the developing pupa of *Apis mellifera* revealed the presence of makisterone A [49]. Studies using HPLC and radioimmunological analysis (RIA) of queen-bee ovaries detected makisterone A and ecdysterone [50]. Injection of radioactively labeled steroids into honey-bee pupae established that the 28C-steroid campesterol is converted into a compound that behaves like makisterone A during chromatographic analysis [51]. This proves that certain insect species can use plant 28C-ecdysteroids as precursors of their own MHs. This phenomenon was first noted in an original article [42] in which data from a study of embryogenesis of the large milkweed bug (*Oncopeltus fasciatus*) were reported.

The ratios of ecdysteroid composition in bugs [52] have interesting analogies with hymenopterans. It was found that phytophagous species of the insects, which can produce a C24-alkylated MH (28C,29C-ecdysteroids) that is similar to **5**, contain a large quantity of 28C- and 29C-ecdysteroids and a small amount of cholesterol [53] whereas omnivorous species, which produce ecdysterone, contain a large quantity of cholesterol, a precursor of this hormone.

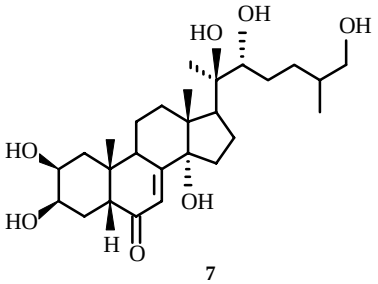
The only representative of dipterans that contains a 28C-ecdysteroid is the fruit fly *Drosophila melanogaster* [38]. However, it was not determined if the makisterone A isolated from the larvae acts as a MH. It is possible that the 24-methylecdysteroid is synthesized in the cephalic glands during preparation of the corresponding steroid substrate [54].

The isolation and identification of ecdysterone in the crab *Jasus lalandei* in 1966 [55] initiated research on ecdysteroids in crustaceans. This was the first compound characteristic of crustaceans. Therefore, it was called crustecdysone.

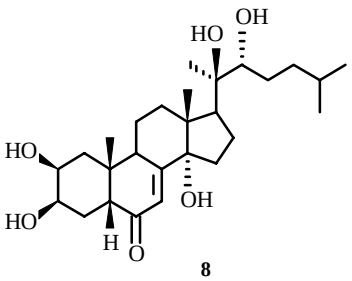
Very specific methods for isolating and analyzing extracts were applied to isolation of α-ecdysone and ecdysterone [56]. Other polar ecdysteroids (more polar than ecdysterone or less polar than ecdysone) cannot be determined if these methods are used. Then, 2-dehydroxy-20-hydroxyecdysone (**6**) [57], inokosterone (callinecdysone A) (**7**), and callinecdysone B (**5**) [56, 58] were isolated.



6



7



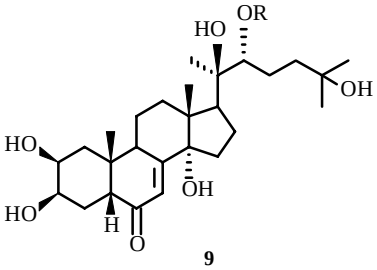
8

Ponasterone A (**8**) was isolated from the crab *Callinectes sapidus* (egg stage) and *Crecarcinus lateralus* (hemolymph) [59].

The concentration of **8** in the hemolymph changes depending on the molting stage [56, 59]; in the ovaries, on the maturation of the ovula [60]. It is hypothesized that this hormone may be more active than ecdysterone in insects [61] and may play an important role in crabs. The absence of this compound in arachnids and primitive insects, which do not molt as adults, and, conversely, the presence in crustaceans and higher insects, which molt at the imago stage, defines the role of ponasterone A as an initiator of imaginal molting.

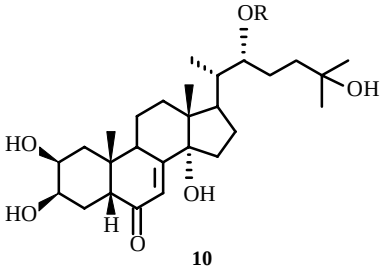
The species *Pycnogonum litorale* is the most studied of the arachnids (*Chelicerata: Pantonoda*). Ecdysteroids were detected in the eggs, juveniles, and adults of this species [28, 29]. However, very little is known about the functions of these compounds. It was noted that the number of juvenile moltings of *Pycnogonum litorale* varies from six to eight and that their durections are different. All these factors make it difficult to determine the molting stage of this species. The detection of ecdysteroids at various development stages of *Pycnogonum* suggests that these hormones are involved in the regulation of molting and embryogenesis.

An ecdysterone metabolite from tick nymphs *Ornithodoros moubata* (Ixodidae), 20-hydroxyecdyson-22-palmitate, has been isolated [62]. Then, other ecdysterone (**9**) conjugates were found: 20-hydroxyecdyson-22-linoleate, 20-hydroxyecdyson-22-oleate, and 20-hydroxyecdyson-22-palmitate [62].



9

R=$COC_{15}H_{31}$
=$COC_{17}H_{31}$
=$COC_{17}H_{33}$
=$COC_{17}H_{35}$

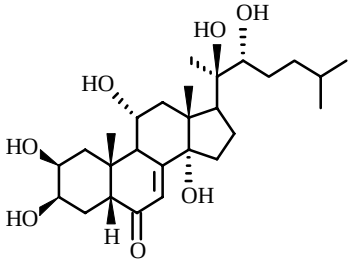


10

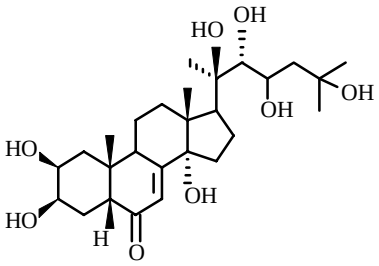
R=$COC_{15}H_{29}$
=$COC_{15}H_{31}$
=$COC_{17}H_{31}$
=$COC_{17}H_{33}$
=$COC_{17}H_{35}$

Ecdysone conjugates that contain fatty-acid residues on C22 (**10**) were detected in representatives of Argasidae, *Boophohilis microplus* [63].

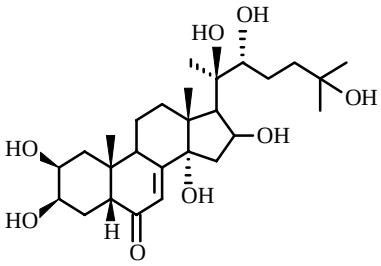
Research on coelenterates (*Coelenterata*), mollusks (*Mollusca*), and worms (*Platyhelminthes, Nematoda, Annelida*) isolated ecdysteroids that are not observed in arthropods: ajugasterone C (**11**) and gerardiasterone (**12**) from *Gerardia savaglia* (Coelenterata) [36, 64] and malakosterone (**13**) from *Cepaea nemoralis* (Mollusca) [65].



11



12



13

The principal ecdysteroids of worms are ecdysone and ecdysterone. Besides these, inokosterone, ponasterone A, 5-epi-ecdysterone, and 20,26-hydroxyecdysone have also been observed. Worm ecdysteroids are very interesting for medicine and agriculture from the viewpoint of battling parasitic worms [66].

Thus, α-ecdysone and ecdysterone are the most widely distributed of the zooecdysteroids. Microorganisms, plants, vertebrates, and certain invertebrates have the ability to synthesize steroids from simple precursors whereas other species of invertebrates require the obtaining of steroids from food [67] that are further converted to ecdysteroids. It was found [68-70] that the conversion of steroids, in particular cholesterol, into ecdysteroids occurs stepwise with addition of hydroxyls.

Each step in the biosynthesis of ecdysteroids is catalyzed by a corresponding enzyme. At present, ecdyson-20-monooxygenase is the most studied.

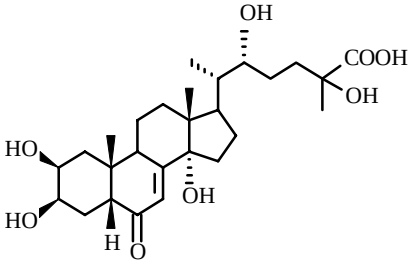
This enzyme was highly active in mitochondrial and microsomal fractions of homogenates of the mid-intestine of the tobacco hornworm caterpillar *Manduca sexta*. It was thought [71] that microsomal ecdyson-20-monooxygenase is the principal, if not the only, enzyme that effects 20-hydroxylation of ecdysone in the mid-intestine of this species of caterpillar.

A peak for mitochondrial activity of ecdyson-20-monooxygenase is observed during the 72nd hour of the sixth instar in the fatty body of *Spodoptera littoralis* larvae [72]. Specific immunoreactive polypeptides that inhibit the activity of mitochondrial ecdyson-20-monooxygenase are detected in mitochondrial extracts of the fatty body. It was hypothesized that ecdyson-20-monooxygenase plays an important role in the regulation of enzyme expression and, therefore, the ecdysterone titer.

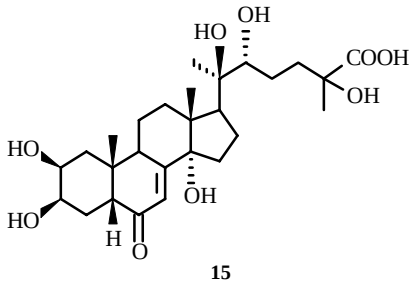
One of the principal routes of α-ecdysone metabolism is hydroxylation at the C20 atom to form ecdysterone, which is biologically more active [4]. Other metabolic routes of α-ecdysone lead to 26-hydroxyderivatives with possible further oxidation of 3-dehydroecdysteroids to ecdysonic and ecdysteronic acids. Enzymatic reduction of the last compounds gives the 3-hydroxyderivatives. Ecdysteroid conjugates are mainly esters of various acids (acetic, phosphoric, or fatty) that are formed at C2, C3, and C22 [44]. The polar compounds formed from the three hydroxyls are reserve or transport forms of the hormones [73].

Ecdysteroids are inactivated, in particular for scaly-winged insects, by transformation of 3-dehydroderivatives into the corresponding 3-epi-ecdysteroid [74, 75]. Further inactivation results from phosphorylation to give ecdysteroid-2-phosphate and a small amount of the corresponding 22-phosphate [74].

The final products of ecdysteroid metabolism in an organism, in particular, *C. erythrocephala* and *L. migratoria* [76, 77] and *S. littoralis* [78], are highly polar ecdysteroids with a side chain, ecdysonic (**14**) and ecdysteronic acids (**15**).



14



15

Mature larvae of *S. littoralis* exhibit a metabolic sequence of ecdysteroids through an aldehyde [78]: ecdysteroid → 26-hydroxyecdysteroid → ecdysteroid-26-aldehyde → ecdysteronic acid (**15**).

Ecdysonic acid is also a final product of the metabolic cycle of ecdysteroids in crustaceans [79].

In addition to these routes, a hypothetical scheme for formation of inokosterone via dehydroxylated ecdysone was proposed [19]: ecdysone → 20-hydroxyecdysone → 25,26-dehydroponasterone → ponasterone A (**8**) → inokosterone (**7**).

As noted above, not only acids but also fatty-acid esters can be metabolites. Experiments using ^{3}H-ecdysone and analysis of its metabolites in *D. melanogaster* demonstrated that the radioactivity is retained in the gut and hemolymph and only insignificantly in the ovaries [80]. Practically all metabolites in the gut were 22-acyl esters of fatty acids. Ecdysone was extensively converted to 3-dehydroecdysone and ecdyson-22-acyl esters in the gut complex, Malpighian tubules. Ecdysterone and its conjugates were observed in the ovaries. Ecdysteroids can be converted to ecdysteroid-22-acyl esters using ecdysteroid-22-O-transferase [81]. This differs from normal metabolism, in which the metabolites are more soluble in water. The conversion of absorbed ecdysteroids was viewed as a possible detoxication pathway in *Heliothis virescens*, the imago of which are resistant to high doses of exogenic ecdysteroids [82].

ROLE OF ECDYSTEROIDS IN ARTHROPOD LIFE CYCLE

Hormonal growth regulation and postembryonic development of insects with complete conversion (Holometabola) (Fig. 1) includes the interaction of three endocrine organs: neurosecretory brain cells, adjoining bodies, and the prothoracic gland. It was proposed [122] that these hormones appear during phylogenesis in the following sequence: ecdysiotropic hormone (ETH), MHs, and juvenile hormones (JHs).

For insects with incomplete conversion (Hemimetabola), removal of the adjoining body during the second, third, or fourth nymph stages produces a nymph that molts excesively and turns into a minature adult. The increase in dimensions and attainment of sexual maturity in such insects might be caused by a decrease in the hemolymph level of JH (Fig. 2).

The prothoracic glands in winged insects atrophy upon reaching the imago stage. Continued molting becomes impossible. This is not observed among wingless insects. The adjoining body in all adults is again activated and produces JH. This is necessary to females for formation of yolks in the eggs; for males, for normal function of prostate glands and formation of the spermatophore, the capsule in which spermatozoids are fed into the vagina of the female [123].

As mentioned above, ETH acts on the prothoracic glands, in which the biosynthesis of ecdysteroids occurs. The prothoracic glands in the tobacco hornworm *M. sexta* respond to ETH through a regulatory mechanism that includes protein phosphorylation and synthesis and increased secretion of ecdysteroids [124]. Changes in the ultrastructure level that are related to secretory activity are noted [124, 125]. Three phases of the cycle were identified based on the identified ultrastructure changes in cells of the prothoracic gland of *Philosamia cynthia vicins* [126]: inactive phase after molting (1), active phase between moltings (2), release of ecdysone before molting (3).

A more detailed study of the mechanism of action of ETH on the prothoracic glands suggested that ETH stimulates ecdysteroidogenesis in the presence of Ca^{2+} [127-129]. Inverse effects are observed in addition to the direct dependence between ETH activity and ecdysteroid production. Ecdysteroid secretion was extremely weak in nm-g mutants of *B. mori*, whose development stops with caterpillars of the first and second instar, for high ETH activity in the brain [130]. However, the dependence of ecdysteroid secretion on ETH activity is not always unambiguous. Thus, ecdysone production in prothoracic glands of mature larvae of the cockroach *Periplaneta americana* is regulated at least in the two stage by an ETH-like hormone [131]. It was found that another different ETH polypeptide hormone, bursicon, participates in imaginal molting for many insect species [132]. The presence in silkworms (*Antherae* and *Hyalophora*) of a hormone called neurotropic molting hormone, which synchronizes molting with the environmental diurnal period, has been demonstrated [133].

Numerous studies have demonstrated that ecdysteroids affect not only molting and metamorphosis but also exchange processes [134-137]. The effect of ecdysteroids on nucleic-acid biosynthesis is specific for every insect species and each development stage. Whereas DNA synthesis in larvae and pupae of various *Lepidoptera* species is directly correlated to the ecdysteroid content, it is suppressed by ecdysterone in, for example, *Drosophila melanogaster* [173]. The increase in ecdysone and ecdysterone production in hornworm *M. sexta* was observed to be related to the expression of ecdysonic genes. Ecdysterone itself can induce the synthesis of ecdysonic genes [134].

Ecdysteroids also influence protein exchange in insects at various development stages. Ecdysterone decreases protein synthesis in honey-bee larvae *A. mellifera* [135] *in vitro* regardless of age and may increase the activity of the corresponding genes during pupation whereas ecdysterone in *D. melanogaster* has no effect on protein synthesis [136]. Ecdysteroids are required for normal spermatogenesis in *Oryctes rhinoceros* [137].

Developing gonads of worker and queen honey-bee larvae are similar in size and contain 100 and more rudimentary ovarioles [138]. The number of rudimentary ovarioles in workers decreases during the fifth instar to 4-7. It was thought that ecdysteroids regulate the expression of specific ovarian proteins and can initiate a physiological apoptopic cycle in worker ovaries [139].

It was found earlier that ecdysteroids can form not only in the prothoracic glands but also in ovaries [140]. Ecdysteroids that are synthesized in ovaries of female silkworms *B. mori* reach the eggs. Nine free ecdysteroids and their conjugates were found. Depending on whether the eggs pass through diapause or not, the ratio of free ecdysteroids and conjugates changed [141]. The content of free ecdysteroids did not change in diapausing eggs. However, the conjugate content increased. On the other hand, the amount of free ecdysteroids, including ecdysterone, increased in eggs that did not reach diapause. The original amount of conjugated forms was present. Diapause was hindered after injection of exogenous ecdysterone into the eggs before diapause.

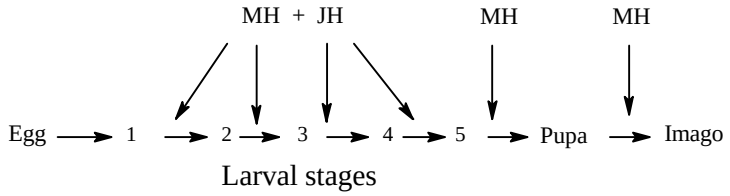


Fig. 1. Hormonal regulation of insect development with complete conversion.

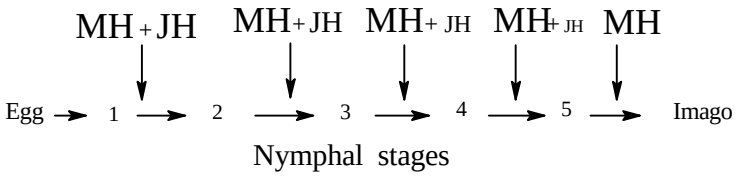


Fig. 2. Hormonal regulation of insect development with incomplete conversion.

Therefore, ecdysteroids are necessary for the normal course of embryogenesis. Injection of ecdysterone or ecdysone facilitated normal metamorphosis of hybrid females of several hornworm species that usually remain in diapuase until death [142]. Exogenous ecdysteroids stopped diapause, metamorphosis, and imago emergence.

Research on embryogenesis of the earwig *Labidura riparia* demonstrated that ecdysteroid titers are directly correlated with secondary cuticle synthesis and its apolysis [143]. Ecdysteroids are necessary for a normal neurogenesis rate and induction of postembryonic glycogenesis in *Drosophila* [144].

The effect of ecdysone on carbohydrate metabolism that relates to mobilization of the internal reserves of an organism during metamorphosis was studied using pupae of the silkworm *B. mori* [145]. A new function of ecdysteroids (ecdysone and ecdysterone) in the hemolymph of *B. mori* caterpillars was found [146]. Ecdysteroids are involved in the removal of sorbitol-6-phosphate (from hemolymph), which is a reserve form of the sugar that is used as an energy source during metamorphosis.

The action of ecdysteroids on various life-cycle processes of insects has been studied in the last decade. Issues of the dependence of protein biosynthesis and ecdysteroid levels [147, 148], the action of exogenic ecdysteroids on the enzymatic system [149-152], the correlation of ecdysteroids and female attractiveness [153], oocyte growth [154], and sexual development [155, 156] have been examined.

The study of a direct, more subtle influence of ecdysteroids on RNA and protein synthesis can be placed in a separate category [157-168].

Ecdysteroids control mainly molting in other classes of arthropods.

Results of several investigations suggested that insect and crustacean molting is controlled by hormones that are similar in structure. Such hormones, in particular ecdysterone, were isolated from *J. lalandei* [169]. It was found that these compounds are produced by Y-organs of crustaceans. Further research [170, 171] elucidated the mechanisms controlling secretion of ecdysteroids and the nature of their biological activity.

The structure and composition of spider ecdysteroids are poorly studied. Steroids were studied only in the two species *Pisaura mirabilis* [26] and *Opilio ravennae* [172]. The ecdysteroids from these species were characterized by TLC and RIA. However, data on the biosynthesis and metabolism were not obtained although it was demonstrated already in 1968 that exogenous ecdysteroids cause molting of these arachnids [173]. The ecdysteroid content during molting of *Pisaura mirabilis* varies the same as in other arthropods. It was shown that the period between moltings can be contracted by introducing ecdysterone. The duration of the intermolting period depends on the ecdysteroid dose. Injections before and after molting are ineffective. Winter diapause can also be disrupted by injections of hormones that control molting [174].

The chemical nature and functions of ecdysteroids in ticks, which belong to the arthropod class, were reorted during the mid-1980s. Several reviews [27, 175, 176] suggest that either molting is accelerated or molting is induced in nymphs without affecting its duration, depending on the species. Supermolting was also observed in *Argasidae* but not in *Ixodidae* ticks. Differences toward assimilation of ecdysteroids were very distinct in different tick species. The effective dose of an ecdysteroid depends on the type and method of administration. Administration of ecdysteroids limits larval diapause. Larger amounts are lethal. The lethal dose depends on the tick species and hormone type. Despite the fact that pheromone and ecdysteroid

TABLE 2. Biological Functions of Ecdysteroids from Invertebrates

Animal group	Ecdysteroid functions	
	assumed	proven
Single-celled	-	Effect on sex cycles
Coelenterates	Protective functions	
Flatworms:		
Trematodes	Growth regulator	
	Sexual maturation	
	Vitellogenesis	
	Cercarium molting	
Cestodes	Growth regulator	
	Vegetative multiplication	
Roundworms	Embryonic development	
	Embryogenesis	
	Vitellogenesis	
	Molting regulation and molting cycles	
	Growth regulator	
Ringworms	Embryogenesis	
	Molting and cuticule formation	
Mollusks	Accumulation of calcium salts during shell formation	
Arthropods:		
Arachnids	Embryogenesis	Regeneration
	Molting	
	Fertility and vitellogenesis	
	Tissue degeneration	
Crustaceans	Embryogenesis	Molting
Centipedes		Molting
Insects		Embryogenesis
		Vitellogenesis
		Molting

concentrations were not correlated, structures changed and pheromone synthesis increased in pheromone glands. Use of exogenous ecdysteroids effects on pheromones formation in the insects such as arachids. However, whereas pheromone synthesis in arachnids increased, injection of ecdysterone to virgin females of *H. virescens* suppressed pheromone production [177]. Such reduction in pheromone production is characteristic of mated females. The nature of the native ecdysteroid that usually suppresses pheromone biosynthesis in mated females has not yet been established.

An important point is the method of administering the hormones. Ticks are more sensitive to injection than to adiminstration *per os* because an ecdysteroid detoxication method is active in the tick organism (gut). Furthermore, the feeding method substantially changes the activity of ecdysteroids in animals.

The effect of ecdysteroids on molting in centipedes (*Myroipodia, Arthropoda*) was first observed in 1964 [170]. Molting and reproduction processes in adult centipedes are highly synchronized. Reproduction (the period of ovary enlargement) decreases before molting. Accelerating imaginal molting in the centipede *Lithobius forficatus* through the action of ecdysterone retards the appearance of spermatid and spermatozoa [178]. Ecdysteroids regulate chitin synthesis, which is a molting component, in Lithobius as in other arthropods [179].

Table 2 presents accrately determined and only postulated functions of ecdysteroids in invertebrates. The activity of ecdysteroids becomes obvious if all manifestations are compared. The principal function of ecdysteroids is to control molting. However, in most instances it is unclear if the effect comes from only humoral regulation or in combination with peptide hormones [180]. A distinguishing feature of ecdysteroids is the control of embryogenesis and vitellogenesis of almost all animal groups.

PRACTICAL USE OF ECDYSTEROIDS

Ecdysteroids are arthropod hormones and facilitate their normal metamorphosis and regulate many important life-cycle processes. It was noted above that different ecdysteroids are produced by living organisms. The highly specific activity of hormones at exceedingly low concentrations provides a basis for developing methods for practical use of MHs to regulate arthropod pest populations, on one hand, and to increase the productivity of domestically useful species, on the other.

It was assumed that the use of various exogenous ecdysteroids would lead to the appearance of various toxic effects in insects. Studies [181, 182] have shown that the degree of ecdysteroid toxicity depends on the species and stage of development, i.e., native hormones are not always highly toxic. Therefore, the use of ecdysteroid analogs, both synthetic and natural (phytoecdysteroids), becomes interesting.

Many phytoecdysteroids and synthetic analogs of zooecdysteroids can specifically suppress cell growth during insect development (i.e., act as antagonists of ecdysteroids that prevent their biosynthesis or functioning) and also exhibit toxic effects on adults [183]. Ecdysone is less of a growth inhibitor than ecdysterone. The combined activity of ecdysterone and JH suppresses cell growth more than the individual compounds [136]. Topical application of ecdysterone and makisterone A on fifth instar nymphs of *Dysdercus cingulatus* reduced the number of hemocytes regardless of the dose. Ecdysterone was more toxic than makisterone A [184].

Injection of various phytoecdysteroids in larvae of the housefly *M. domestica* increases sharply the number of pupal larvae compared with a control [185] (Table 3).

Zooecdysteroid analogs have specific activity depending on the insect species. Spraying a 0.0005% solution of ecdysterone resulted in the death of 40% of leaf aphid larvae whereas treatment with a 0.1% solution of this same compound produced practically no lethal effect on larvae of the house fly, Colorado potato beetle, and lesser apple worm [186]. The ability to use natural hormones and their analogs as chemosterilants is of great practical interest in addition to the appearance of general toxicity [187].

The activity of nonsteroidal ecdysteroid agonists such as RH-5849 (1,2-dibenzoyl-1-*t*-butylhydrazine) [188-192] and RH-5992 (tebufenozide) [193-195] has been well studied. Experimental results indicate that their activity on insect embryogenesis is similar to that of ecdysteroids. Treatment of certain species with the corresponding doses kills caterpillars during molting [192], causes premature molting [193, 194], inhibits diapause, produces imago with deformed wings [190], and makes insects hyperactive with subsequent paralysis and death [189]. An investigation of the activity of several hydrazines [196] revealed that their biological activity can be modulated by substituents on aromatic rings.

On the other hand, the development of biological control methods that are based on disruption of normal ecdysteroid metabolism within the organism is interesting. A synthetic scheme for selective inhibitors of various enzymatic systems was devised based on research on insect biosynthetic mechanisms, in particular, of *L. migratoria* [197]. Classes of inhibitors that affect hyhdroxylation of ecdysone in the C22 and C25 positions were identified. Other classes affect dehydration of ring B in the C7 and C8 positions. It was established that an imidazole derivative (KK-42) is a potential inhibitor of ecdysone synthesis in *B. mori* [198]. Topical application of KK-42 during a certain period of embryogenesis reduces ecdysone synthesis. This leads to incomplete hatching and the inability to fertilize eggs.

Practical use of ecdysteroids should not be viewed only from the viewpoint of reducing the population and destroying pests. The opposite side is possibly more important. This is the use of ecdysteroids to increase the survivability and productivity of useful species, in particular, honey bees and silkworms.

The vitality of bee colonies and their normal development in all stages depend on many factors (natural, climatic, and ecological conditions; observation of beekeeping technology, the degree of infection with various diseases). However, biologically active substances, including MHs, influence substantially the vitality of bees [199]. As noted above, ecdysteroids are produced by the prothoracic gland during postembryonic development. This gland atrophies and steroid hormones are not produced in imago. Bees should receive steroidal compounds from external sources such as nectar and pollen [199, 200] in order to maintain normal vitality. Phytoecdysteroids *in toto*, chemically pure or in combination with other biologically active compounds, can also be used [186, 199-205].

The preparations "Biospon" [200, 201] and VESP [199, 202] and chemically pure ecdysterone [186, 203-205] have been proposed for use in beekeeping. These preparations had a definite effect on the healing of bees, improvement of wintering, spring—summer growth, and development of bee colonies. They increased significantly the mass of infertile queens and the production of mother's milk in educational families.

TABLE 3. Action of Molting Hormones from Various Natural Sources on *Musca domestica* Larvae

Compound, dose	Pupa formation, %
Control	12
Ecdysterone, 5 ng (*Podocarpus andinus*)	80
Control	5
Ecdysterone, 20 ng (*Taxus baccata*)	100
Control	26
Ponasterone, 5 ng (*Podocarpus macrophyllis*)	76

Exogenous ecdysterone, which is used at various stages of silkworm *B. mori* embryogenesis, can increase the size of deposited eggs [206] and the cocoon mass [207] and can synchronize its winding [87]. Biological tests of MHs and their analogs demonstrated that they can be successfully used not only to combat pests but also to increase the productivity and disease resistance of useful insects such as silkworms and honey bees.

REFERENCES

1. C. M. Williams, *Biol. Bull.*, **120**, 103 (1952).
2. A. A. Akhrem, N. S. Levina, and Yu. A. Titov, *Ecdysones - Steroidal Insect Hormones* [in Russian], Nauka i Tekhnika, Minsk (1973).
3. T. W. Goodwin, D. H. S. Horn, P. Karlson, J. Koolman, K. Nakanishi, W. E. Robbins, J. B. Siddall, and T. Takemoto, *Nature (London)*, **272**, No. 5649, 122 (1978).
4. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids. Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
5. S. Reimer and F. Romer, *Mitt. Dtsch. Ges. Allg. Angew. Entomol.*, **10**, 691 (1995).
6. A. Butenandt and P. Karlson, *Z. Naturforsch. 9b*, **6**, 389 (1954).
7. M. N. Galbraith, D. H. S. Horn, E. J. Middleton, J. A. Thompson, J. B. Siddall, and W. Hafferl, *J. Chem. Soc., Chem. Commun.*, No. 19, 1134 (1969).
8. H. Fugo, M. Yamauchi, and S. G. Dedos, *Proc. Jpn. Acad., Ser. B*, **72**, 34 (1996).
9. B. Gerstenlauer and K. H. Hoffmann, *Eur. J. Entomol.*, **92**, 81 (1995).
10. F. Romer and H.-U. Bressel, *Z. Naturforsch. C: Biosci.*, **49**, 871 (1994).
11. P. Whiting, S. Sparks, and L. Dinan, *Arch. Insect Biochem. Physiol.*, **35**, 279 (1997).
12. A. I. Tawfik, A. Matʹhova, F. Sehnal, and S. H. Ismail, *Arch. Insect Biochem. Physiol.*, **31**, 427 (1996).
13. D. B. Gelman, D. A. Reed, and N. E. Beckage, *Amer. Zool.*, **35**, 7 (1995).
14. N. E. Beckage, D. Zitnan, and D. B. Gelman, *Amer. Zool.*, **32**, 130 (1992).
15. T. J. Kelly, E. J. Park, C. A. Maler, and J. P. Burand, *Eur. J. Entomol.*, **92**, 51 (1995).
16. R. J. Weaver, G. S. Marris, S. Olieff, J. H. Mosson, and J. P. Edwards, *Arch. Insect Biochem. Physiol.*, **35**, 169 (1997).
17. G. K. Karnavar, C. Strambi, A. Strambi, and M. A. Sureshkumar, *Entomon*, **19**, 149 (1994).
18. J. Lorenz, H. Lenz, and K. H. Hoffmann, *Z. Naturforsch. C: Biosci.*, **50**, 286 (1995).
19. J. Koolman, *Insect Biochem.*, **12**, 225 (1982).
20. H. H. Hagedron, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 8: Endocrinology II*, G. A. Kerkut and L. I. Gilbert, eds., Pergamon Press, Oxford, UK (1985), p. 181.
21. H. H. Rees and R. E. Isaac, in: *Biosynthesis, Metabolism, and Mode of Action of Invertebrate Hormones*, J. Hoffmann and M. Porchet, eds., Springer-Verlag, Fed. Rep. Ger. (1984), p. 181.
22. G. Levinson, *Usp. Khim.*, **43**, 181 (1975).
23. H. H. Rees, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 28.

24. D. H. S. Horn and R. Bergamasco, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 7: Endocrinology I*, G. F. Kerkut and L. I. Gilbert, eds., Pergamon Press, Oxford (1985), p. 185.
25. K.-D. Spindler, R. Keller, and J. D. O'Connor, in: *Developments in Endocrinology, Vol. 7: Progress in Ecdysone Research*, J. A. Hoffmann, ed., Elsevier, Amsterdam, Neth. (1980), p. 247.
26. J. C. Bonaric and M. De Reggi, *Experientia*, **33**, 1664 (1977).
27. P. A. Diehl, J. L. Connat, and E. Dotson, in: *Morphology, Physciology and Behavior Biology of Ticks*, J. R. Sauer and J. A. Hair, eds., Ellis Horwood, Chichester, West Sussex England; Halsted Press, New York (1986), p. 165.
28. D. Buckmann, G. Starnecker, K. H. Tomaschko, E. Wilhelm, R. Lafont, and J. P. Girault, *J. Comp. Physiol. B*, **156**, 759 (1986).
29. W. Behrens and D. Buckmann, *Gen. Comp. Endocrinol.*, **51**, 8 (1983).
30. P. Karlson *Hoppe-Seyler's Z. Physiol. Chem.*, **364**, 1067 (1983).
31. F. Romer, *Naturwissenschaften*, **66**, 471 (1979).
32. D. L. Whitehead and K. Sellheyer, *Experientia*, **38**, 1249 (1982).
33. H. H. Rees and H. H. Mendis, in: *Biosynthesis, Metabolism, Mode of Action of Invertebrate Hormones*, J. A. Hoffmann and M. Porchet, eds., Springer-Verlag, Fed. Rep. Ger. (1984), p. 338.
34. J. Koolman, J. Walter, and H. Zahner, in: *Biosynthesis, Metabolism, Mode of Action of Invertebrate Hormones*, J. A. Hoffmann and M. Porchet, eds., Springer-Verlag, Fed. Rep. Ger. (1984), p. 323.
35. H. H. Rees and J. G. Mercer, in: *Advances in Invertebrate Reproduction*, M. Porchet, J.-C. Andries, and A. Dhainaut, eds., Elsevier, Amsterdam (1986), p. 173.
36. A. Guerriero and F. Pietra, *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, **80**, 277 (1985).
37. H. Hikino, J. Ohizumi, and T. Takemoto, *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 309 (1975).
38. Y. Fujimoto, S. Miyasaka, N. Ikekawa, E. Ohnishi, T. Mazuno, and K. Watanabe, *J. Chem. Soc., Chem. Commun.*, 10 (1985).
39. E. Budd, G. Kauzer, and J. Koolman, *Arch. Insect Biochem. Physiol.*, **23**, 181 (1993).
40. P. Maurer, J.-P. Girault, M. Larcheveque, and R. Lafont, *Arch. Insect Biochem. Physiol.*, **23**, 29 (1993).
41. M. F. Feldlaufer, S. L. Buchmann, W. R. Lusby, G. F. Weirich, and J. V. Svoboda, *Arch. Insect Biochem. Physiol.*, **23**, 91 (1993).
42. J. N. Kaplanis, S. R. Dutky, W. E. Robbins, M. J. Thompson, E. L. Lindquist, and D. H. S. Horn, *Science*, **190**, 681 (1975).
43. J. R. Aldrich, J. A. Svoboda, and M. J. Thompson, *J. Exp. Zool.*, **218**, 133 (1981).
44. J. R. Aldrich, T. S. Kelly, and C. W. Woods, *J. Insect Physiol.*, **28**, 857 (1982).
45. J. M. Gibson, M. S. I. Majunder, A. H. W. Mendis, and H. H. Rees, *Arch. Insect Biochem. Physiol.*, **1**, 105 (1983).
46. M. F. Feldlaufer, S. A. Svoboda, J. R. Aldrich, and W. R. Lusby, *Arch. Insect Biochem. Physiol.*, **3**, 423 (1986).
47. T. J. Kelly, J. R. Aldrich, C. W. Woods, and A. B. Borkovec, *Experientia*, **40**, 996 (1984).
48. J. A. Svoboda, M. J. Thompson, E. W. Herbert, Jr., and H. Shimanuki, *J. Insect Physiol.*, **26**, 291 (1980).
49. M. F. Feldlaufer, E. W. Herbert, Jr., S. A. Svoboda, M. J. Thompson, and W. R. Lusby, *Insect Biochem.*, **15**, 597 (1985).
50. M. F. Feldlaufer, S. A. Svoboda, and E. W. Herbert, Jr., *Experientia*, **42**, 200 (1986).
51. M. F. Feldlaufer, E. W. Herbert, Jr., S. A. Svoboda, and M. J. Thompson, *Arch. Insect Biochem. Physiol.*, **3**, 415 (1986).
52. S. A. Svoboda, W. R. Lusby, and J. R. Aldrich, *Arch. Insect Biochem. Physiol.*, **1**, 139 (1984).
53. S. A. Svoboda and W. R. Lusby, *Arch. Insect Biochem. Physiol.*, **3**, 13 (1986).
54. C. P. F. Redfern, *Experientia*, **42**, 307 (1986).
55. F. Hampshire and D. H. S. Horn, *J. Chem. Soc., Chem. Commun.*, 37 (1966).
56. J. F. McCarthy, *Steroids*, **34**, 799 (1979).
57. M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *J. Chem. Soc., Chem. Commun.*, 83 (1968).
58. A. Faux, D. H. S. Horn, E. J. Middleton, H. M. Pales, and M. E. Lowe, *J. Chem. Soc., Chem. Commun.*, No. 4, 175 (1969).
59. F. Lashaise and R. Lafont, *Steroids*, **43**, 243 (1984).
60. F. Lashaise, M. Goudeau, C. Hetru, and J. A. Hoffmann, *Hoppe-Seyler's Z. Physiol. Chem.*, **362**, 521 (1981).

61. R. Bergamasco and D. H. S. Horn, in: *Developments in Endocrinology, Vol. 7: Progress in Ecdysone Research*, J. A. Hoffmann, ed., Elsevier, Amsterdam, Neth. (1980), p. 299.
62. P. A. Diehl, J.-L. Connat, S. P. Girault, and R. Lafont, *Int. J. Invertebr. Reprod. Dev.*, **8**, 1 (1985).
63. T. Crosby, R. P. Evershed, D. Lewis, K. P. Wigglesworth, and H. H. Rees, *Biochem. J.*, **240**, 1381 (1986).
64. A. Guerro, P. Traldi, and P. Pietra, *J. Chem. Soc., Chem. Commun.*, No. 1, 40 (1986).
65. M. Carsia, J.-P. Girault, and R. Lafont, *J. Invertebr. Reprod. Dev.*, **9**, 43 (1986).
66. S. Franke and G. Kauser, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 296.
67. D. Gilmour, *The Metabolism of Insects*, San Francisco, W. H. Freeman (1965).
68. F. Guo, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 444.
69. P. Karlson, *Perspect. Biol. Med.*, **6**, 203 (1963).
70. C. Hetru, M. Lagueux, B. Luu, and J. A. Hoffmann, *Life Sci.*, **22**, 2141 (1978).
71. G. F. Weirich, V. P. Williams, and M. F. Feldlaufer, *Arch. Insect Biochem. Physiol.*, **31**, 305 (1996).
72. J.-H. Chen, T. Hara, M. Fisher, and H. H. Rees, *Biochem. J.*, **299**, 711 (1994).
73. J. Pis, J.-P. Girault, V. Grau, and R. Lafont, *Eur. J. Entomol.*, **92**, 41 (1994).
74. T. J. Webb, R. Powels, and H. H. Rees, *Biochem. J.*, **312**, 561 (1995).
75. J. F. Weirich, M. F. Feldlaufer, and J. A. Svoboda, *Arch. Insect Biochem. Physiol.*, **23**, 199 (1993).
76. R. E. Isaac, M. E. Roze, H. H. Rees, and T. W. Godwin, *Biochem. J.*, **213**, 533 (1983).
77. R. Lafont, C. Blais, R. H. Beydon, J. Modde, U. Enderle, and J. Koolman, *Arch. Insect Biochem. Physiol.*, **1**, 41 (1983).
78. J.-H. Chen, M. Kabbouh, M. J. Fisher, and H. H. Rees, *Biochem. J.*, **301**, 89 (1994).
79. F. Lashaise, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 313.
80. V. Gran and R. Lafont, *J. Insect Physiol.*, **40**, 87 (1994).
81. M. Zhang and I. Kubo, *J. Chem. Ecol.*, **18**, 1139 (1992).
82. I. Kubo, M. Zhang, G. de Boer, and K. Uchima, *Entomol. Exp. Appl.*, **70**, 263 (1994).
83. F. Lashaise, M. F. Meister, C. Hetru, and R. Lafont, *Mol. Cell. Endocrinol.*, **45**, No. 2-3, 253 (1986).
84. N. Ikekawa, T. Ikeda, T. Mizuno, E. Ohnishi, and S. Sakurai, *J. Chem. Soc., Chem. Commun.*, 448 (1980).
85. E. Ohnishi, T. Mizuno, F. Chatani, N. Ikekawa, and S. Sakurai, *Science*, **197**, 66 (1977).
86. R. Huber and W. Hoppe, *Chem. Ber.*, **98**, 2403 (1965).
87. O. Ninazi, C. Maruyama, H. Mizusawa, Y. Kozakai, M. Wakabayashi, and M. Maruyama, *Bull. Nat. Inst. Seri. Entomol. Sci.*, **9**, 7 (1993).
88. H. Sonobe, M. Kamba, G. Mamiya, and G. Fujimoto, *Zool. Sci.*, **10**, 144 (1993).
89. G. Mamiya, H. Sonode, K. Yoshida, W. Hara, and G. Fujimoto, *Experientia*, **51**, 363 (1995).
90. J. M. Kaplanis, M. J. Thompson, S. R. Dutky, and W. E. Robbins, *Steroids*, **34**, 333 (1979).
91. J. N. Kaplanis, M. J. Thompson, S. R. Dutky, and W. E. Robbins, *Steroids*, **36**, 321 (1980).
92. M. J. Thompson, G. F. Weirich, H. H. Rees, J. A. Svoboda, M. F. Feldlaufer, and K. R. Wilzer, *Arch. Insect Biochem. Physiol.*, **2**, 227 (1985).
93. M. J. Thompson, J. A. Svoboda, H. H. Rees, and K. R. Wilzer, *Arch. Insect Biochem. Physiol.*, **4**, 183 (1987).
94. M. J. Thompson, M. F. Feldlaufer, R. Lozano, H. H. Rees, W. R. Lusby, J. A. Svoboda, and K. R. Wilzer, *Arch. Insect Biochem. Physiol.*, **4**, 1 (1987).
95. J. N. Kaplanis, M. J. Thompson, and S. R. Dutky, W. E. Robbins, and E. L. Lindquist, *Steroids*, **20**, 105 (1972).
96. M. J. Thompson, J. N. Kaplanis, W. E. Robbins, and R. T. Yamamoto, *J. Chem. Soc., Chem. Commun.*, No. 13, 650 (1967).
97. J. N. Kaplanis, M. J. Thompson, S. R. Dutky, and W. E. Robbins, *Steroids*, **34**, 333 (1979).
98. M. J. Thompson, J. N. Kaplanis, W. E. Robbins, S. R. Dutky and H. M. Nigg, *Steroids*, **24**, 359 (1974).
99. D. H. S. Horn, E. J. Middleton, J. A. Wunderlich, and F. Hampshire, *J. Chem. Soc., Chem. Commun.*, 339 (1966).
100. P. Beydon and R. Lafont, *Arch. Insect Biochem. Physiol.*, **5**, 139 (1987).
101. G. Tsoupras, C. Hetru, B. Luu, E. Constantin, M. Lagueux, and J. Hoffmann, *Tetrahedron*, **39**, 1789 (1983).
102. M. Lagueux, J. Hoffmann, F. Gotlzene, C. Kappler, G. Tsoupras, C. Hetru, and B. Luu, in: *Biosynthesis, Metabolism*

and Mode of Action of Invertebrate Hormones, J. Hoffmann and M. Porchet, eds., Springer-Verlag, Fed. Rep. Ger. (1984), p. 160.
103. G. Tsoupras, B. Luu, and J. Hoffmann, *Steroids*, **40**, 551 (1982).
104. G. Tsoupras, C. Hetru, B. Luu, M. Lagueux, E. Constantin, and J. Hoffmann, *Tetrahedron Lett.*, **23**, 2045 (1982).
105. C. Hetru, D. Fraisse, J. C. Tabet, and B. Luu, *C. R. Acad. Sci., Ser. 2*, **299**, 429 (1984).
106. G. Tsoupras, B. Luu, and J. A. Hoffmann, *Science*, **220**, 507 (1983).
107. G. Tsoupras, B. Luu, C. Hetru, J.-F. Muller, and J. Hoffmann, *C. R. Seances Acad. Sci., Ser. 3*, **296**, 77 (1983).
108. J.-F. Modde, R. Lafont, and J. Hoffmann, *Int. J. Invertebr. Reprod. Dev.*, **7**, 161 (1984).
109. R. E. Isaac, H. H. Rees, and T. W. Goodwin, *J. Chem. Soc., Chem. Commun.*, 418 (1981).
110. R. E. Isaac, N.-P. Milner, and H. H. Rees, *Biochem. J.*, **213**, 261 (1983).
111. R. E. Isaac, H. H. Rees, and T. W. Goodwin, *J. Chem. Soc., Chem. Commun.*, 594 (1981).
112. R. E. Isaac and H. H. Rees, *Biochem. J.*, **221**, 459 (1984).
113. R. E. Isaac, M. E. Roze, H. H. Rees, and T. W. Goodwin, *J. Chem. Soc., Chem. Commun.*, 249 (1982).
114. P. Karlson, H. Bugany, H. Dopp, and G.-A. Hoyer, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1610 (1972).
115. J. Koolman and K. D. Spindler, *Hoppe-Seyler's Z. Physiol. Chem.*, **358**, 1339 (1977).
116. J. Koolman, L. Reum, and P. Karlson, *Hoppe-Seyler's Z. Physiol. Chem.*, **360**, 1351 (1974).
117. M. N. Galbraith, D. H. S. Horn, J. A. Thompson, G. J. Neufeld, and R. J. Hackney, *J. Insect Physiol.*, **15**, 1225 (1969).
118. M. J. Thompson, W. E. Robbins, and S. R. Dutky, *Lipids*, **13**, 783 (1978).
119. A. F. Moribayashi and T. Ohtaki, *J. Insect Physiol.*, **24**, 279 (1978).
120. P. Kulcsar, L. Polgar, B. Darvas, and P. Zavorszky, *Acta Phytopathol. Entomol. Hung.*, **29**, 161 (1994).
121. C. P. F. Redfern, *Proc. Nat. Acad. Sci. USA*, **81**, 5643 (1984).
122. V. Ya. Novak, *Zh. Obshch. Biol.*, **31**, 14 (1970).
123. N. Green, W. Stout, and R. Taylor, *Biology* [Russian translation], Mir, Moscow (1990), Vol. 3, p. 375.
124. W. A. Smith, *Arch. Insect Biochem. Physiol.*, **30**, 271 (1995).
125. E. Balamini and V. S. K. Nair, *Proc. Indian Nat. Sci. Acad. Part B*, **60**, 483 (1994).
126. C. Lee, *Acta Entomol. Sin.*, **35**, 133 (1992).
127. W. K. Hanton, D. R. Watson, and W. E. Bollencher, *J. Morphol.*, **216**, 95 (1993).
128. V. C. Henrich, *Arch. Insect Biochem. Physiol.*, **30**, 239 (1995).
129. S. Wunsch, S. Schneider, A. Schwab, and H. Oberleithener, *Cell Tissue Res.*, **274**, 145 (1993).
130. M. Nagata, K. Tsuchida, C. Orikasa, and A. N. Suzuki, *J. Seri. Sci. Jpn.*, **61**, 400 (1992).
131. K. Richter, *Ladek Zdroj.*, Wroclaw Univ. Inst. Chemistry (1994), p. 11.
132. G. S. Fraenkel and C. Hsiao, *J. Insect Physiol.*, **11**, 513 (1965).
133. J. W. Truman and L. M. Riddiford, *Adv. Insect Physiol.*, **10**, 297 (1974).
134. H. Fujiwara and L. M. Riddiford, *Zool. Sci.*, **6**, 124 (1993).
135. G. Bosquet, J. Fourche, and C. Gullet, *J. Insect Physiol.*, **35**, 1005 (1989).
136. V. A. Gvozdev, V. T. Kakpakov, L. M. Mykhovatova, L. G. Polukarova, and V. Z. Tarantul, *Ontogenez*, **5**, 33 (1971).
137. N. Jacob, *Zool. Sci.*, **9**, 451 (1992).
138. K. Hartfelder, C. Hepperle, K. Kostlin, and C. J. C. Schmidt, *Univ. Paris Nord*, Paris (1994), p. 219.
139. K. Hartfelder, K. Kostlin, and C. Hepperle, *Apidologie*, **24**, 503 (1993).
140. K. N. Hoffmann and B. Gerstenlauer, *Arch. Insect Biochem. Physiol.*, **35**, 149 (1997).
141. H. Sonobe, T. Masumoto, G. Mamiya, T. Kanda, and T. Tamura, *Zool. Sci.*, **9**, 1270 (1992).
142. E. A. Loeliger and F. Kaizer, *Nota Lepideptorolog.*, **19**, 113 (1996).
143. N. Bertella, F. Sayah, and M. Breuzet, *C. R. Acad. Sci., Ser. III*, **318**, 579 (1995).
144. T. A. Awad and J. W. Truman, *Am. Zool.*, **32**, 81 (1992).
145. S. Kimura, *J. Insect Physiol.*, **19**, 115 (1973).
146. O. Gasunori, M. Jwami, and S. Sakurai, *Zool. Sci.*, **12**, 19 (1995).
147. K. Richter and E. Baumann, *Arch. Insect Biochem. Physiol.*, **35**, 111 (1997).
148. T. Burmester, U. Matzner, and K. Scheller, *Eur. J. Entomol.*, **92**, 217 (1995).
149. I. B. Kometiani, N. M. Kutuzova, and Yu. B. Filippovich, Kursk Pedagog. Inst., Kursk (1993), p. 55.
150. N. M. Kutuzova, I. B. Kometiani, N. V. Selina, A. D. Areshev, S. S. Mikhailovskii, and Yu. B. Filippovich, Moscow

Pedagog. Inst., Dep. VINITI, No. 3127-V96.
151. R. Banmeister, M. Ludwig, and B. M. Spindler, *Naturwissenschaften*, **79**, 185 (1992).
152. R. Farkas and J. Knopp, *Arch. Insect Biochem. Physiol.*, **35**, 71 (1997).
153. M. Trablon, M. Campan, N. Hartmann, J.-C. Baehr, P. Porcheron, and J.-L. Clement, *Arch. Insect Biochem. Physiol.*, **25**, 363 (1994).
154. N. I. Kozhanova and F. Segal, *Tsitologiya*, **39**, 789 (1997).
155. P. R. Geetha, *Indian J. Exp. Biol.*, **33**, 81 (1995).
156. F. Sayah, A. Karlinsky, and M. Breuzet, *Tissue Cell*, **27**, 575 (1995).
157. A. S. Yudaev, *Vestn. Akad. Med. Nauk SSSR*, **7**, 33 (1976).
158. P. Karlson and C. Sekeris, *Acta Endocrinol.*, **53**, 505 (1966).
159. F. Jacob and J. Monad, *J. Mol. Biol.*, **3**, 318 (1961).
160. P. Karlson, *J. Cell Comp. Physiol.*, **66**, 69 (1965).
161. H. Kroeger and M. Lezzi, *Annu. Rev. Entomol.*, **11**, 1 (1966).
162. M. Jindra, *Eur. J. Entomol.*, **91**, 163 (1994).
163. E. H. Bachrecke, *Arch. Insect Biochem. Physiol.*, **33**, 231 (1996).
164. C. Wang, A. Dulta-Gupta, and A. K. Kumaran, *Eur. J. Entomol.*, **92**, 215 (1995).
165. K. W. Deitsch, N. Dittmer, M. Z. Kapitskaya, J.-Sh. Chen, W.-L. Cho, and A. S. Raikhel, *Eur. J. Entomol.*, **92**, 237 (1995).
166. M. Jindra, F. Sehnal, and L. M. Riddiford, *Eur. J. Entomol.*, **92**, 235 (1995).
167. K. Kimura, D. A. Currie, and J. W. Truman, *Zool. Sci.*, **9**, 1271 (1992).
168. R. E. Langelan, S. R. Paili, K. Hiruma, D. Champlin, and L. M. Riddiford, *Am. Zool. Sci.*, **32**, 79 (1992).
169. D. H. S. Horn, S. Fabbri, F. Hampshire, and D. E. Lowe, *Biochem. J.*, **109**, 399 (1978).
170. K. D. Spindler, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 290.
171. E. S. Chang, *Annu. Rev. Entomol. 38*, Palo Alto, Calif. (1993), p. 161.
172. F. Romer and W. Gnatzy, *Cell. Tissue Res.*, **216**, 449 (1981).
173. A. Krushnakumaran and H. A. Schneiderman, *Biol. Bull.*, **139**, 520 (1970).
174. J. C. Bonaric, in: *Ecophysiology of Spiders*, W. Nentwig, ed., Springer-Verlag, Berlin, Fed. Rep. Ger. (1987), p. 111.
175. W. H. Dees, D. E. Soenshine, and E. Breidling, *J. Med. Entomol.*, **22**, 22 (1985).
176. R. A. Harris and W. R. Kaufman, *Experientia*, **41**, 740 (1985).
177. S. B. Ramaswamy and N. E. Cohen, *Naturwissenschaften*, **79**, 29 (1992).
178. F. Leubert and H. Scheffel, *Acta Entomol.*, **81**, 22 (1984).
179. K. D. Spindler, in: *The Larval Serum Proteins of Insects: Function, Biosynthesis, Genetics*, K. Scheller, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1983), p. 135.
180. G. Kauzer, in: *Ecdysone: From Chemistry to Mode of Action*, J. Koolman, ed., Georg Thieme Verlag, Stuttgart, Fed. Rep. Ger. (1989), p. 327.
181. L. R. Cleveland, A. W. Burke, and P. Karlson, *J. Protozool.*, **7**, 229 (1960).
182. P. Karlson and D. Buckmann, *Naturwissenschaften*, **43**, 44 (1956).
183. V. T. Kakpakov, A. A. Shamshurin, V. I. Spektor, and G. E. Muntyan, *Izv. Akad. Nauk Mold. SSR, Ser. Biol. Khim. Nauk*, **3**, 67 (1974).
184. A. Ahmad, *Entomol. Croat.*, **1**, 41 (1995).
185. N. M. Sikura, S. N. Pochtar′, Yu. D. Kholodova, and V. P, Vendt, *Ukr. Biokhim. Zh.*, **50**, 105 (1978).
186. Yu. D. Kholodova, *Ukr. Biokhim. Zh.*, **51**, 560 (1979).
187. W. E. Robbins, J. N. Kaplanis, M. J. Thompson, T. J. Shortino, and S. C. Joyner, *Steroids*, **16**, 105 (1970).
188. O. Koul and R. S. Kapril, *Experientia*, **50**, 461 (1994).
189. F. Ortego and W. S. Bowers, *Experientia*, **52**, 42 (1996).
190. M. Sielezniew and B. Cymborowski, *Arch. Insect Biochem. Physiol.*, **35**, 191 (1997).
191. M. A. Pszczolkowski and B. Kuszczak, *Ladek Zdroj*, Wroclaw Univ. Chem. Inst. (1994), p. 30.
192. C. Sakunthala and V. S. K. Nair, *Indian J. Exp. Biol.*, **33**, 690 (1995).
193. G. Smagghe, E. Vinuela, F. Budia, and D. Degheele, *Arch. Insect Biochem. Physiol.*, **35**, 179 (1997).

194. G. Smagghe, E. Vinuela, F. Budia, and D. Degheele, *Arch. Insect Biochem. Physiol.*, **33**, 121 (1996).
195. J. J. Brown, *Arch. Insect Biochem. Physiol.*, **26**, 235 (1994).
196. A. K. Mohammed-Ali, T.-H. Chan, A. W. Thomas, G. N. Strung, and B. Jewett, *Can J. Chem.*, **73**, 550 (1995).
197. J. P. Roussel, *Entomol. Exp. Appl.*, **71**, 193 (1994).
198. K. Kadone-Okuda, W. Amornsak, and O. Yamashita, *Arch. Insect Biochem. Physiol.*, **25**, 121 (1994).
199. V. T. Kakpakov, *Pchelovodstvo*, **5-6**, 8 (1993).
200. E. V. Poluektova, V. G. Mitrofanov, V. M. Karpov, Z. D. Gorbunova, and I. M. Voronkov, *Pchelovodstvo*, **2**, 22 (1989).
201. M. G. Giniyatullin, S. M. Bakhtiyarova, and T. A. Proskurina, *Pchelovodstvo*, **5**, 27 (1996).
202. V. I. Maslennikova, *Pchelovodstvo*, **6**, 20 (1995).
203. G. S. Shangaraeva, U. A. Baltaev, and V. N. Odinokov, RF Pat. No. 2127043, 10 Mar. 1999.
204. G. S. Shangaraeva, U. A. Baltaev, and V. N. Odinokov, RF Pat. No. 2125793, 10 Feb. 1999.
205. G. S. Shangaraeva, U. A. Baltaev, and V. N. Odinokov, *Pchelovodstvo*, **6**, 18 (1998).
206. Y. Kawaguchi, Y. Banno, K. Koga, and H. Fijii, *J. Seri. Sci. Jpn.*, **61**, 145 (1992).
207. H. Takizawa and M. Sudo, *J. Seri. Sci. Jpn.*, **61**, 341 (1992).