*ECDYSONE AND ECDYSONE ANALOGUES: THEIR ASSAY ON THE FLESHFLY SARCOPHAGA PEREGRINA**

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Among the recent rapid developments in insect endocrinology none has been more surprising than the discovery that certain plants contain impressive amounts of substances resembling or even identical with ecdysone, the sterol growth hormone of insects. For example, Nakanishi[1] and his co-workers at Tohoku University report that no less than 0.06 per cent of the dry weight of the leaves of the evergreen tree, *Podocarpus nakaii* (family Taxaceae), consists of a mixture of ponasterones A, B, C, D—all of which are nearly identical to authentic α- and β-ecdysones (Fig. 1). The biological assays which Kobayashi *et al.*[2] have carried out on flies and silkworms have demonstrated, moreover, that the hormonal activities of the ponasterones equal, or even exceed, that of α- or β-ecdysone.

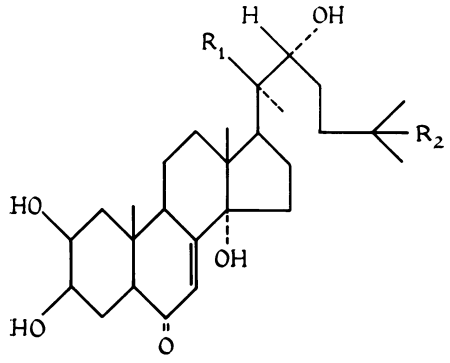
In parallel developments at Tohoku University, Takemoto[3] and his collaborators have found that 0.05 per cent of the dry weight of the roots of the ubiquitous Japanese weed, *Achyranthes fauriei* (family Amaranthaceae), consists of a mixture of β-ecdysone (ecdysterone; crustecdysone) and the closely related inokosterone (see Fig. 1). Meanwhile, the Czechoslovakian workers Jizba *et al.*[4] report the astonishing finding that β-ecdysone constitutes in excess of 1 per cent of the dry weight of the rhizomes of the well-known fern, *Polypodium vulgare*. Even more recently, Takemoto[5] and his co-workers have characterized yet another ecdysone-like compound, cyasterone, which they have extracted from the root of the Chinese weed *Cyathula capitata* (Amaranthaceae).

These surprising developments, coupled with the *de novo* synthesis of α-ecdysone by teams of German and Swiss[6] and American[7] chemists, have paved the way for a fresh appraisal of many aspects of insect endocrinology which have hitherto remained almost inaccessible.

As our first step in this direction we have compared the endocrine activities of seven of these materials in terms of their ability to provoke puparium formation of isolated abdomens of fleshfly larvae.

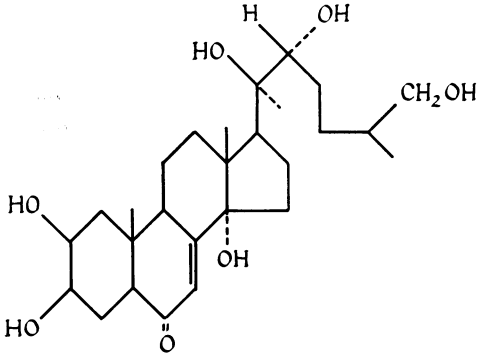
Materials and Methods.—The biological assay: Assays were carried out on larvae of *Sarcophaga peregrina* as previously described.[8] Homogeneous groups of mature larvae were stored in water-containing jars for four days and then transferred to dry filter paper. After six hours under dry conditions at 25°C each larva was ligated with a cotton thread. Twenty-four hours later, about 50 per cent of larvae had formed puparia at their anterior ends. These individuals were collected and aged an additional 16 hours. Individuals showing any trace of puparium formation behind the ligature were discarded. The others were used immediately in the biological assay. The latter was carried out as described elsewhere.[8, 9]

The assays were evaluated 24 hours after injection. As in the *Calliphora* assay,[9] each abdomen was scored as having undergone complete, marked, slight, or no puparium formation; these responses were equated to 100, 75, 50, and 0 per cent, respectively, for the purpose of calculating the average "per cent puparium formation."

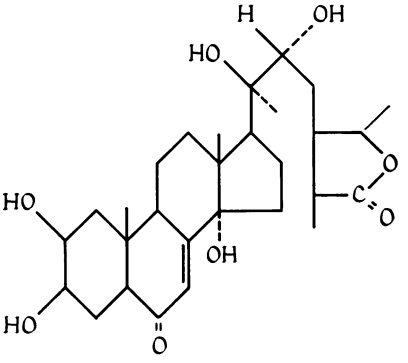


Substance	R_1	R_2
α - Ecdysone	H	OH
β - Ecdysone	OH	OH
Ponasterone A	OH	H

(The configurations at C_{20} and C_{22} have been firmly established only for α - ecdysone)



Inokosterone



Cyasterone

FIG. 1.—The structural formulae of five ecdysones are here depicted. (Data derived from references 1–3, 5, and 11.)

Results.—As summarized in Table 1, all seven of the ecdysones were highly active in the assay. This implies that the injected materials, or products immediately formed from them, were able to supplement the endogenous ecdysone that had been secreted prior to ligation. As shown in Table 1, substantial scatter was encountered among homogeneous groups of test abdomens subjected to the same treatment. Moreover, at nearly all dose levels, a number of abdomens initiated but failed to complete puparium formation.

In the right-hand column of Table 1 the "per cent puparium formation" has been calculated as described under *Methods*. In Table 2 the biological activities of the seven materials have been compared in terms of the estimated critical dose required to provoke a puparium index of 50 per cent. The most potent of the seven materials (ponasterone A) proves to be about 6 times as active as the weakest (inokosterone). And, interestingly enough, all materials except inokosterone were significantly more active than α-ecdysone.

Discussion.—In Table 3 we have compared the results of the present study with assays previously reported for five of these materials. The agreement is remarkable, especially when the different weights of the larval abdomens are taken into

TABLE 1

ASSAYS* OF SEVEN ECDYSONES ON ISOLATED ABDOMENS OF *Sarcophaga peregrina*

Substance	Amount (μg)	Number	Puparium Formation				Weighted average (%)
			Complete	Marked	Slight	None	
H_2O	—	40	0	0	0	0	0
α-Ecdysone	0.1	40	37	2	0	1	96
	0.05	70	34	9	15	12	69
	0.02	80	5	7	14	54	22
	0.01	80	8	5	9	58	20
	0.005	70	5	3	6	56	15
	0.0025	40	1	0	1	38	4
	0.0013	40	0	0	2	38	2
β-Ecdysone	0.1	10	10	0	0	0	100
	0.05	30	18	5	3	4	78
	0.025	30	10	9	6	5	66
	0.013	30	4	5	10	11	42
Cyasterone	0.05	20	16	3	1	0	94
	0.025	20	7	6	6	1	73
	0.013	20	0	5	7	8	36
	0.006	20	0	0	1	19	2
Ponasterone A	0.05	20	15	5	0	0	94
	0.025	20	13	6	1	0	90
	0.013	20	4	6	6	4	58
	0.006	20	1	0	3	16	12
Ponasterone B	0.05	20	12	3	3	2	79
	0.025	20	7	2	5	6	55
	0.013	20	0	4	7	9	32
	0.006	20	0	5	2	13	24
Ponasterone C	0.05	20	17	3	0	0	96
	0.025	20	5	4	4	7	50
	0.013	20	1	1	4	14	19
	0.006	20	0	1	0	19	4
Inokosterone	0.1	20	11	5	2	2	79
	0.05	20	2	6	3	9	40
	0.025	20	0	3	4	13	21
	0.013	20	1	1	0	18	9

* The volume of each injection was 0.5 μliter.

consideration. We suspect that Kobayashi *et al.*[2] may have underestimated the activity of ponasterone C.

Of the entire series of seven compounds, only α- and β-ecdysones have been extracted from insects; the other five, as well as β-ecdysone itself, are synthesized and for some unexplained reason accumulated by certain parts of certain plants. Since α-ecdysone is thought to be the principal growth hormone of insects, it is surprising to find that its activity ranks sixth among the seven compounds tested in the *Sarcophaga* assay; β-ecdysone ranks third.

It is worth recalling that α- and β-ecdysones have been isolated from entire insects and not from prothoracic glands.[10] Until this difficult experiment can be performed, there remains the possibility that one or both of these materials may be formed secondarily from some other ecdysone secreted by the prothoracic glands.

All seven materials were completely inactive when topically applied to test abdomens, the solvents being acetone, methanol, ethanol, cyclopropane, or dimethyl sulfoxide. The same negative result has been observed in unpublished experiments performed on silkworm pupae. Therefore, the presence of high concentrations of these agents in certain plants constitutes something of an enigma.

TABLE 2

RELATIVE ACTIVITIES OF ECDYSONE MATERIALS IN THE *Sarcophaga* ASSAY

Substance	Estimated critical dose (μg)	Activity relative to α-ecdysone
α-Ecdysone	0.035	1
β-Ecdysone	0.018	1.9
Cyasterone	0.016	2.2
Inokosterone	0.064	0.5
Ponasterone A	0.011	3.2
Ponasterone B	0.023	1.5
Ponasterone C	0.025	1.4

TABLE 3

ENDOCRINE ACTIVITIES OF ECDYSONES ASSAYED ON THREE DIFFERENT SPECIES OF FLIES

Substance	*Sarcophaga* (Wt = 55 mg)	*Musca* (Wt = 16 mg)	*Calliphora* (Wt = 36 mg)
Ponasterone A	0.011 μg	0.015 μg†	—
Cyasterone	0.016	—	—
β-Ecdysone	0.018	0.005*	0.017*
Ponasterone B	0.023	0.038†	—
Ponasterone C	0.025	0.75†	—
α-Ecdysone	0.035	0.005*	0.018*
Inokosterone	0.064	—	—

50% of isolated larval abdomens would undergo puparium formation when injected with the recorded doses, which were calculated by interpolation.
* Data of Kaplanis *et al.*[10]
† Data of Kobayashi *et al.*[2]

Summary.—Synthetic α-ecdysone and six ecdysones of plant origin were assayed and compared in terms of their ability to provoke puparium formation of isolated larval abdomens of the fleshfly, *Sarcophaga peregrina*. All materials showed high activity and in the following order: ponasterone A > cyasterone > β-ecdysone (ecdysterone, crustecdysone) > ponasterone B > ponasterone C > α-ecdysone > inokosterone. All the materials are inactive when topically applied to the unbroken skin; therefore, we are unable to account for the synthesis of these complicated and potent sterols by certain plants.

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