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Stimulatory Effect of Insect-Metamorphosing Steroids from Ferns on Protein Synthesis in Mouse Liver[1]

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The insect-metamorphosing steroids obtained from ferns were assayed in terms of their ability to stimulate protein synthesis in mouse liver. Ponasterone A, pterosterone, ecdysterone, shidasterone and lemmasterone possessing hydroxyls at C-20 and C-22, showed high activity. However, polypodine B (5β-hydroxyecdysterone) was much less active in acceleration. Ponasteroside A (ponasterone A 3-β-D-glucopyranoside) also revealed anabolic potency. On the other hand, ecdysone, having a hydroxyl at C-22 but not at C-20, exhibited little stimulation.

We have recently reported that various steroids possessing insect-metamorphosing hormone activity which have been isolated from *Achyranthes* and *Cyathula* (Amaranthaceae) stimulate protein synthesis in mouse liver.[3] On the other hand, we have found that the crude extracts of ferns show the insect-metamorphosing activity in high frequency, and hitherto isolated from them a number of active substances[4]: *i.e.* ecdysone, ponasterone A, pterosterone, ecdysterone, lemmasterone, shidasterone, and ponasteroside A (Chart 1). Among these steroids ecdysone and ecdysterone are the genuine insect-metamorphosing hormones originally isolated from the silkworm (*Bombyx mori*) and other arthropods.[5] Meanwhile, Jizba, *et al.* have isolated an insect-metamorphosing substance, polypodine B, from a fern.[6]

The present paper describes the effect of these phytoecdysones, obtained from the ferns, on protein synthesis in mouse liver.[7]

1) This paper constitutes Part V in the series on Steroids. Part IV: T. Takemoto, Y. Hikino, H. Hikino, S. Ogawa, and N. Nishimoto, *Tetrahedron*, **25**, 1241 (1969).
2) Location: *Aobayama, Sendai.*
3) T. Otaka, M. Uchiyama, S. Okui, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2426 (1968).
4) T. Takemoto, Y. Hikino, T. Arai, M. Kawahara, C. Konno, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **15**, 1816 (1967); T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *Tetrahedron Letters*, **1968**, 375; T. Takemoto, S. Arihara, Y. Hikino, T. Arai, C. Konno, S. Nabetani, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **16**, 759 (1968); T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *ibid.*, **16**, 762 (1968); T. Takemoto, Y. Hikino, T. Arai, and H. Hikino, *Tetrahedron Letters*, **1968**, 4061; T. Takemoto, S. Arihara, and H. Hikino, *ibid.*, **1968**, 4199; T. Takemoto, Y. Hikino, H. Jin, T. Arai, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **16**, 1636 (1968); T. Takemoto, Y. Hikino, T. Okuyama, S. Arihara, and H. Hikino, *Tetrahedron Letters*, **1968**, 6095.
5) *cf.* T. Takemoto, S. Ogawa, and N. Nishimoto, *Yakugaku Zasshi*, **87**, 1469 (1967). The references cited therein.
6) J. Jizba, V. Herout, and F. Šorm, *Tetrahedron Letters*, **1967**, 5139.
7) Part of the material presented here has been reported in a preliminary form, S. Okui, T. Otaka, M. Uchiyama, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 384 (1968).

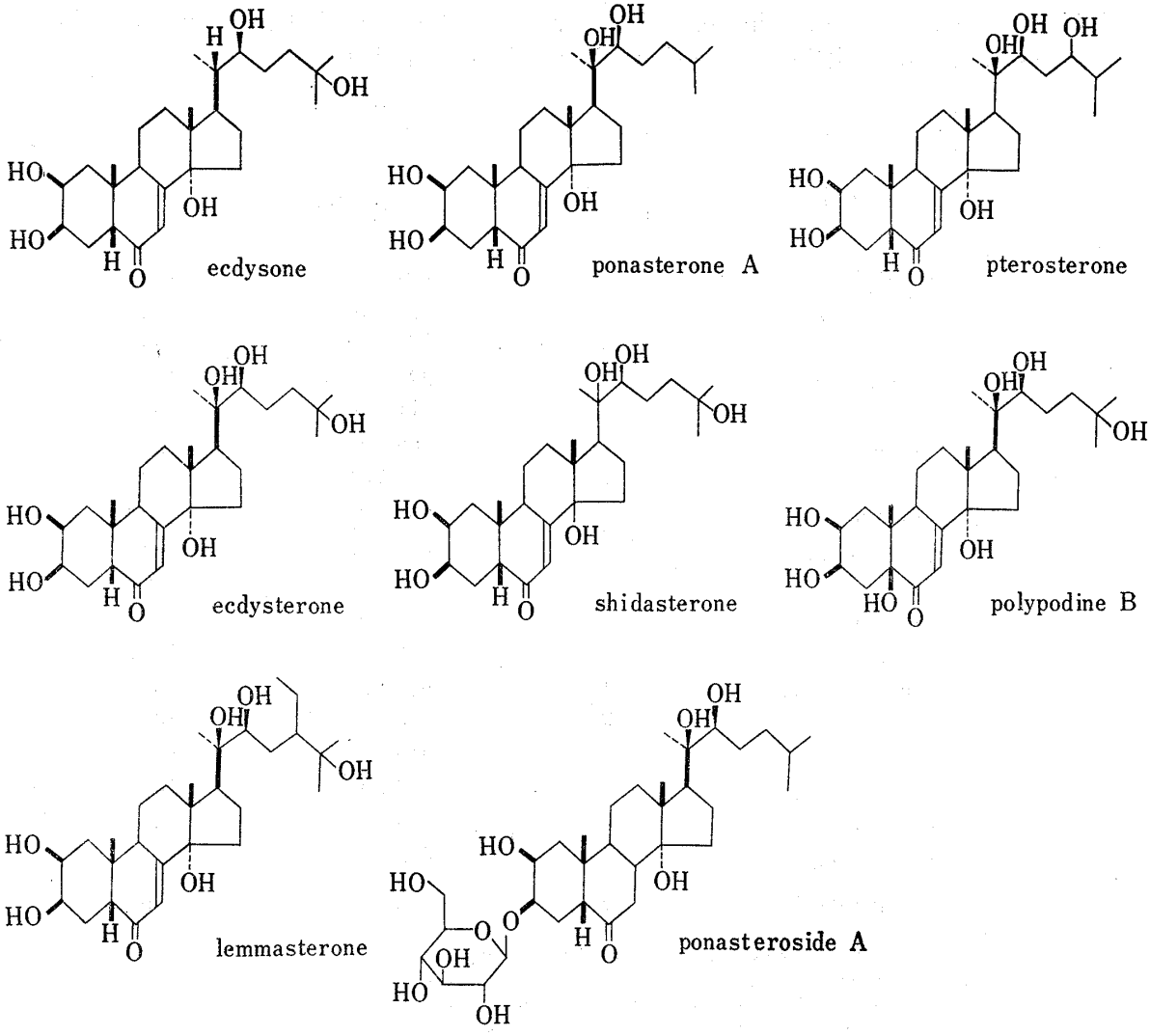

Chart 1

Experimental

Materials——The reagents used are described previously.[3]

Animals and Treatment——Male mice (18—20 g) of *dd* strain were used with no particular restriction on their food intake throughout the experiments. Each steroid was dissolved in 0.9% saline solution and injected to mouse intraperitoneally in a dose of 0.05 mg per 100 g of body weight at a.m. 9.

Preparation of Cell-free Fractions——The mice were decapitated at the indicated time after treatment. Livers were removed rapidly and rinsed in an ice-cold 1.15% KCl solution, weighed and homogenized with 1.5 volumes of medium K_1[8] by a Potter Elvehjem teflon homogenizer. The homogenate was centrifuged at 20000 $\times g$ for 15 min. The supernatant fluid was used as enzyme source for the measurement of ^{14}C-amino acid incorporation *in vitro*.

Incubation——The composition of the reaction mixture was the same as reported previously.[3] Incubation was carried out in a test tube under air at 37° for 25 min.

Preparation and Counting of Radioactive Protein——The procedures were the same as described previously.[3]

Results

As summarized in Table I, all steroids except ecdysone stimulated protein synthesis in mouse liver. Thus, though no marked effect was observed at one hour after the administration,

8) K. Koike, T. Otaka, and S. Okui, *J. Biochem.* (Tokyo), **59**, 201 (1966).

the significant increase of protein anabolic activity was found after two hours. On the other hand, ecdysone showed little stimulation of protein synthesis.

Table I. Effect of Insect-Metamorphosing Steroids on Protein Synthesis

	Treatment	Time after injection (hr)	cpm/mg protein	Stimulation (%)
(A)	Control		307±4	100
	Ponasterone A	1	290±10	95
		2	582±15	190
	Pterosterone	1	328±20	107
		2	571±16	186
	Ecdysterone	1	400±62	130
		2	553±31	180
(B)	Control		257±9	100
	Shidasterone	1	241±16	94
		2	401±6	156
	Lemmasterone	1	273±8	107
		2	468±12	182
	Ponasteroside A	1	271±5	106
		2	356±6	139
	Ecdysone	1	270±4	105
		2	279±5	108
(C)	Control		195±10	100
	Ecdysterone	2	348±7	I78
	Polypodine B	2	233±4	120

The experiments of (A), (B) and (C) were done at different periods.
(A) Each result is the mean ± standard error for ten mice used.
(B) Each result is the mean ± standard error for six mice used.
(C) Each result is the mean ± standard error for four mice used.

Discussion

In a previous paper, the stimulatory effect of a number of insect-metamorphosing steroids, ecdysterone, inokosterone, cyasterone, and rubrosterone, on protein synthesis in mouse liver was reported.[3] In the present investigation, it was found that six additional phytoecdysones also elevate the anabolic activity in mouse liver. From a structural point of view, ponasterone A, pterosterone, ecdysterone and lemmasterone have the common feature, the 2β,3β,14α,-20(*R*),22(*R*)-pentahydroxy-7-en-6-one system in the 5β(H)-steroid skeleton. Therefore, some of the structure must be indispensable to exhibition of stimulatory effect on protein synthesis. Shidasterone which is a stereoisomer of ecdysterone, exhibits the high activity, indicating that alteration of some stereochemistry does not seem to affect much change on anabolic activity. The observation that polypodine B, 5β-hydroxyecdysterone, was much less active in stimulating the protein synthesis, suggests that 5β-hydroxyls in ecdysterols participate less potently than 5β-hydrogens in elevation of anabolic activity. Ponasteroside A, ponasterone A 3-β-D-glucopyranoside, still shows the activity. However, the effect is less than that of its aglycone, ponasterone A. In this case, however, the possibility that ponasteroside A reveals the activity only after enzymatic hydrolysis in mouse, can not be excluded. If it were the case, the apparent anabolic activity must depend on the rate of its hydrolysis in animals. Ecdysone, different from all the steroids mentioned above, showed little anabolic activity. This result may agree with the observation of Lukács and Sekeris that ecdysone

exhibited no effect on RNA polymerase activity on rat liver nuclei.[9] The difference in activities among these steroids may be explained by the following difference in structures: ecdysone has a hydroxyl group at C-22 but not at C-20, while the other steroids which show the anabolic activity have hydroxyl groups at C-20 and C-22. However, for a complete understanding of the structure–activity relationship, further study is required. It is of interest to note that ecdysone has the high activity for metamorphosis of insects, while it gives no effect on protein synthesis in mammalian cells. This fact, as with the case of rubrosterone,[3] also indicates that the structures responsible for the metamorphosing hormone activity in insects are not always the same as those for the anabolic activity in mouse.

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9) I. Lukács and C. E. Sekeris, *Biochem. Biophys. Acta*, **134**, 85 (1967).