Chem. Pharm. Bull. 16(12)2426—2429(1968)

UDC 591.147.08 : 591.34.044.08 : 581.19 : 547.92

Stimulatory Effect of Insect-Metamorphosing Steroids from *Achyranthes* and *Cyathula* on Protein Synthesis in Mouse Liver[1]

Tadahiko Otaka, Mitsuru Uchiyama, *Late* Seiichi Okui, Tsunematsu Takemoto, Hiroshi Hikino,[2a] Shuntaro Ogawa and Nobushige Nishimoto[2b]

Pharmaceutical Institute, Tohoku University School of Medicine[2a] and Research Laboratories, Rohto Pharmaceutical Co., Ltd.[2b]

(Received May 7, 1968)

The insect-metamorphosing steroids (ecdysterone, inokosterone and cyasterone) and the related substance (rubrosterone) isolated from *Achyranthes* and *Cyathula* (Amaranthaceae) were assayed in terms of their ability to stimulate protein synthesis in mouse liver. High stimulation was introduced by all steroids, administered intraperitoneally, in which cyasterone showed the highest activity. Oral administration of ecdysterone produced similar activation. Ecdysterone stimulated protein synthesis not only in male mice but also in female mice.

Recent isolations of steroids possessing insect-metamorphosing activity from plant sources have revealed that such steroids are widely distributed in the plant kingdom. *Inter alia*, we have isolated the metamorphosing substances, ecdysterone and inokosterone, first from *Achyranthes fauriei* Léveillé et Vaniot (Amaranthaceae)[3] and later from a number of *Achyranthes* species.[4] We have also reported the isolation of the unique analogues cyaste-

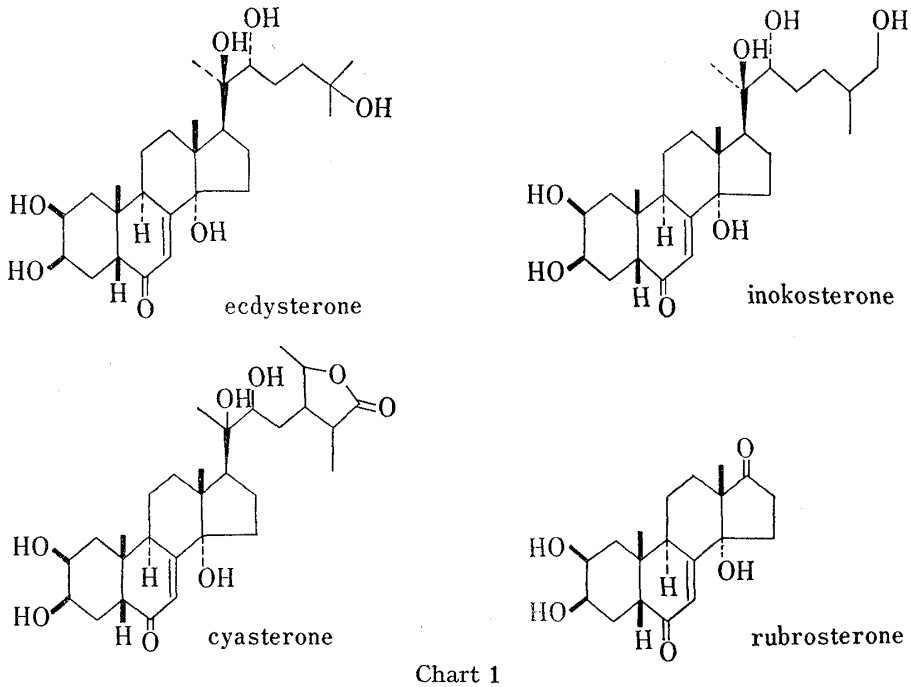

Chart 1

1) This paper forms Part II in the series on Steroids. Part I: H. Hikino, Y. Hikino, K. Nomoto and T. Takemoto, *Tetrahedron*, **24**, 4896 (1698).
2) Location: *a*) *Kita-4-bancho, Sendai*; *b*) *Ikuno, Osaka.*
3) T. Takemoto, S. Ogawa and N. Nishimoto, *Yakugaku Zasshi*, **87**, 325, 1469, 1474 (1967).
4) T. Takemoto, S. Ogawa, N. Nishimoto and S. Taniguchi, *Yakugaku Zasshi*, **87**, 1478 (1967); T. Takemoto, S. Ogawa, N. Nishimoto, K.-Y.Yen, K. Abe, T. Sato, K. Osawa and M. Takahashi, *ibid.*, 1521 (1967); T. Takemoto, S. Ogawa, N. Nishimoto, H. Hirayama and S. Taniguchi, unpublished data.

rone and rubrosterone from *Cyathula capitata* MOQUIN-TANDON (Amaranthaceae)[5] and *Achyranthes rubrofusca* WIGHT,[6] respectively. It has been shown that all of these steroids exhibit substantial insect-metamorphosing activity except rubrosterone which shows little activity in the *Sarcophaga* test.

Since the crude drugs Radices Achyranthis and Cyathulae obtained from *Achyranthes* and *Cyathula* have long been used as a tonic and a diuretic in Oriental medicine, and since the structures of these insect-metamorphosing substances, depicted in Chart 1, are fairly related to corticoids and sex hormones, we have taken an interest in investigating the effect of these steroids on mammalian metabolic systems, especially protein synthesis in liver.

In the present work, the steroids obtained from *Achyranthes* and *Cyathula* were assayed in terms of their ability to stimulate protein synthesis in mouse liver. A part of this work was preliminarily reported.[7]

Experimental

Materials——Uniformly labelled ^{14}C-*Chlorella* hydrolysate (specific activity: 4.2 mCi/mg) was obtained from Daiichi Chemical Co., Japan. ATP, GTP, creatine phosphate and creatine kinase [EC 2.7.3.2.] were obtained from Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Animals and Treatment——Male mice (18—22 g) or female mice (16 g) of dd strain were used with no particular restriction on their food intake throughout the experiments.

Each steroid was generally dissolved in 0.9% saline solution and injected intraperitoneally in a dose of 0.05 mg or 0.005 mg per 100 g of body weight at a.m. 9. For oral application ecdysterone dissolved in water was administered in a dose of 0.5 mg per 100 g of body weight.

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, weighed and homogenized with 1.5 volumes of medium K_1[8] by Potter Elvehjem teflon homogenizer. The homogenate was centrifuged at $20000 \times g$ for 15 minutes. The supernatant fluid (S-20 fluid) was used as enzyme source for the measurement of ^{14}C-amino acid incorporation *in vitro*.

Incubation——The reaction mixture consisted of 50 μmoles of sucrose, 25 μmoles of tris-(hydroxymethyl) aminomethane (pH 7.6), 5 μmoles of $MgSO_4$, 12.5 μmoles of KCl, 2.0 μmoles of ATP, 0.2 μmole of GTP, 10 μmoles of phosphocreatine, 10 μg of creatine kinase [EC 2.7.3.2], 0.1 μCi of ^{14}C-labelled *Chlorella* hydrolysate, and 0.2 ml of S-20 fluid (approx. 10mg of protein) in total 0.5 ml. Incubation was carried out in test tubes under air at 37° for 25 minutes.

Preparation and Counting of Radioactive Protein——After incubation the reaction was stopped by adding 4 ml of 6% perchloric acid. The precipitate was separated by centrifugation, washed three times with 6% perchloric acid and treated with the same reagent at 90° for 15 minutes and finally extracted twice with ether-ethanol-chloroform (2:2:1). The resulting labelled proteins were dissolved in 90% (v/v) formic acid, plated on planchets and dried under an infrared lamp. A 2π gas flow counter with a thin window was used for radioactive counting. Protein content was determined by gravimetric analysis. The incorporation of ^{14}C-amino acids was calibrated after correction for self-absorption effect.

Results

As summarized in Table I, all four steroids stimulated protein synthesis in male mouse liver, when administered intraperitoneally in a dose of 0.05 mg per 100 g body weight.

When the dose of each steroid was lowered to 0.005 mg per 100 g body weight, only cyasterone showed anabolic activity as indicated in Table II.

The elevated dose of the steroid administration over 0.05 mg per 100 g body weight elongated the duration of the enhanced protein synthesis, but did not alter the absolute ratio of increment at the maximum protein synthesis (Fig. 1).

5) T. Takemoto, Y. Hikino, N. Nomoto and H. Hikino, *Tetrahedron letters*, **1967**, 3191.
6) T. Takemoto, Y. Hikino, H. Hikino, S. Ogawa and N. Nishimoto, *Tetrahedron letters*, **1968**, 3053.
7) late S. Okui, T. Otaka, M. Uchiyama, T. Takemoto, H. Hikino, S. Ogawa and N. Nishimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 384 (1968).
8) K. Koike, T. Otaka and S. Okui, *J. Biochem.* (Tokyo), **59**, 201 (1966).

TABLE I. Effect of Insect–Metamorphosing Steroids on Protein Synthesis

	Treatment	Time after injection (hr)	cpm/mg protein	Stimulation (%)
(A)	Control		307±4	100
	Ecdysterone	1	400±62	130
		2	553±31	180
		4	603±12	196
	Inokosterone	1	327±4	107
		2	522±10	170
		4	625±13	206
	Cyasterone	1	350±10	114
		2	540±9	176
		4	632±21	206
(B)	Control		202±2	100
	Rubrosterone	1	201±4	100
		2	249±6	123
		3	316±3	156
	Ecdysterone	3	271±3	134

dose: 0.05 mg/100 g body weight
Results in (A) and (B) are obtained from experiments at different time.
(A) Each results is the mean ± standard error for ten mice used.
(B) Each results is the mean ± standard error for six mice used.

TABLE II. Effect of Insect–Metamorphosing Steroids (0.005 mg/100 g body weight) on Protein Synthesis

Treatment	cpm/mg protein	%
Control	435±10	100
Ecdysterone	412±21	95
Inokosterone	380±25	87
Cyasterone	541±31	124
Rubrosterone	465±32	107

The mice were sacrificed 2 hours after the administration.
Each result is the mean ± standard error for eight mice used.

TABLE III. Effect of Ecdysterone on Protein Synthesis by Oral Adminstration

Treatment	cpm/mg protein
Control	386±5
Ecdysterone 4 hr	584±14

dose: 0.5 mg/100 g body weight
Each result is the mean ±standard error for six mice used.

TABLE IV. Effect of Ecdysterone on Protein Synthesis in Female Mice Liver

Treatment	Time after injection (hr)	cpm/mg protein	Stimulation (%)
Control		123±2	100
Ecdysterone	2	182±5	148
	4	200±7	163

dose: 0.05 mg/100 g body weight Each result is the mean ± standard error for six mice used.

Ecdysterone stimulated protein synthesis not only in the intraperitoneal injection, but in the oral administration (Table III).

Although all the above results were obtained using male mice, the stimulation of protein synthesis by ecdysterone was also effective on female mice (Table IV).

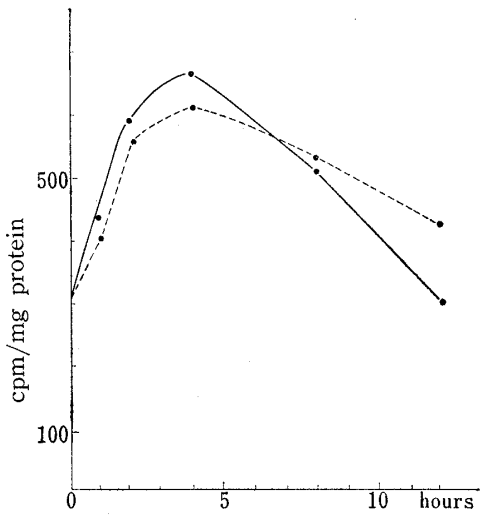

Fig. 1. The Time Course of the Stimulation of Amino Acid Incorporation after the Injection of Ecdysterone

——— ecdysterone 0.05 mg/100 g body weight
····· ecdysterone 0.5 mg/100 g body weight

Discussion

The steroids, used in this work, can be classified into three groups based on the side chain structures (Chart 1): (1) ecdysterone and inokosterone have the cholestane side–chain, (2) cyasterone has the stigmastane side–chain and contains, in particular, a lactone ring, and (3) rubrosterone has the carbonyl instead of a side chain. The above results, however, show that all these steroids stimulate protein synthesis in mouse liver disregarding the differences in their side–chains.

The fact that, when administered intraperitoneally in a dose of 0.005 mg per 100 g body weight, only cyasterone showed enhancement of protein synthesis demonstrates that cyasterone is the most potent anabolic agent in these steroids which may be ascribable to the lactone ring in the side–chain.

It is interesting to note that rubrosterone exhibits little metamorphosing activity in the *Sarcophaga* test, while it shows the anabolic activity. Therefore, it seems that for the metamorphosis of insects a certain side–chain structure may be required but for the stimulation of protein synthesis in mouse liver the nucleus structure may be essential and no side–chain may be neccessary.

The absolute increment was about the same both in a dose of 0.05 and 0.5 mg of ecdysterone per 100 g body weight. This indicates that the degree of stimulation of protein synthesis is maximum in a dose of 0.05 mg.

The observation that ecdysterone was effective even by the oral administration suggests that it is absorbable and not completely degraded in digestive organs.

Sex difference in the response to ecdysterone was not observed.

The mechanism of the stimulation mentioned here is under investigation and has been partly reported.[9]

9) T. Otaka, S. Okui and M. Uchiyama, *Chem. Pharm. Bull.* (Tokyo), in press.