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Synthesis of ponasterone A derivatives with various steroid skeleton moieties and evaluation of their binding to the ecdysone receptor of Kc cells

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ABSTRACT

A series of ponasterone A (PNA) derivatives with various steroid moieties were synthesized to measure their binding activity to the ecdysone receptors of *Drosophila* Kc cells. The activity of compounds was evaluated by determining the concentration required to give the 50% inhibition (IC_{50} in M) of the incorporation of [^{3}H]PNA to *Drosophila* Kc cells. Compounds with no functional groups such as OH and C=O group in the steroid skeleton moiety were inactive. By the introduction of functional groups such as the OH and the C=O group in the steroidal structure, these compounds became active. Some compounds containing the A/B-*trans* ring fusion, which is different from that (A/B-*cis*) of ecdysteroids were also active. The oxidation of CH_2 at 6-position to C=O, enhanced the activity 19 times, but the activity was erased by the reduction of oxo to OH group at 6-position. The activity was enhanced about 250 times by the conversion of A/B ring configuration from *trans* [(20R,22R)-2β,3β,20,22-tetrahydroxy-5α-cholestan-6-one: $pIC_{50} = 4.84$] to *cis* [(20R,22R)-2β,3β,20,22-tetrahydroxy-5β-cholestan-6-one: $pIC_{50} = 7.23$]. The latter *cis*-type compound which is the most potent among compounds synthesized in this study was equipotent to the natural molting hormone, 20-hydroxyecdysone, even though it is 1/50 of PNA.

1. Introduction

Insect molting is regulated by the molting hormone, 20-hydroxyecdysone (20-OH Ecd, **1** in Fig. 1). The biosynthesis of 20-OH Ecd has been studied intensively by Gilbert and co-workers [1,2]. It is well-known that insects have to intake cholesterol and other related steroidal compounds from their diet because they cannot construct the steroid skeleton. *In Drosophila*, ecdysone (Ecd; Fig. 1) is synthesized from cholesterol in the prothoracic gland and converted to 20-OH Ecd in other peripheral tissues, such as fat body and midgut, although 3-dehydroecdysone is secreted in *Bombyx mori* [3]. Recently, halloween genes coding the P450 hydroxylases that catalyze the final four steps of 20-OH Ecd biosynthesis have been identified in *Drosophila* [4–8].

Even though cholesterol has no receptor binding activity [9], Ecd and 2-deoxyecdysone have weak receptor binding and hormonal activities [10,11]. We also demonstrated that Ecd has a weak binding activity to *in vitro* translated ecdysone receptor proteins [12,13] and molting hormonal activity in tissue [14]. In addition, there are many steroidal compounds that have molting hormonal activity in plants [11] and a few animals, which are available in the web site EcdyBase (URL: http://ecdybase.org/). Among them, ponasterone A (PNA, **2** in Fig. 1), which is isolated from *Podocarpus Nakaii*, is well-known as the most potent ecdysteroid [15,16]. To date, many

Abbreviations: Ecd, ecdysone; 20-OH-E, 20-hydroxyecdysone; PNA, ponasterone A; CS, castasterone.

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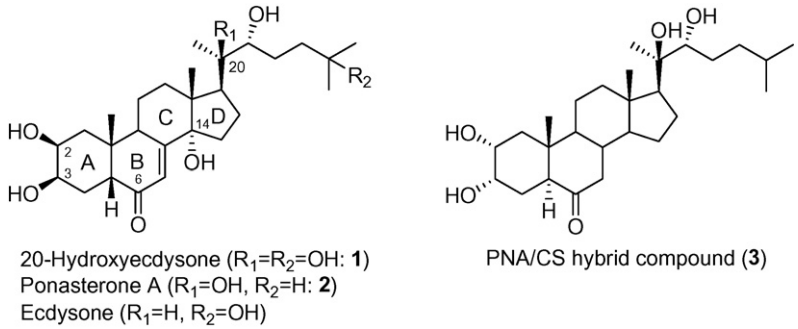

Fig. 1 – Structures of ecdysteroids and PNA/CS hybrid compound.

ecdysteroids have been identified and their hormonal activity has been evaluated in the cell-based assay [17,18]. The structure-activity relationship (SAR) of steroidal compounds is quantitatively analyzed using the three-dimensional quantitative SAR (3D QSAR) method [11,19].

In addition to ecdysteroids, non-steroidal compounds such as diacylhydrazines [20,21], acylaminoketones, [22,23] *N*-benzoyltetrahydroquinolines, [24] 3,5-di-*t*-butyl-4-hydroxybenzamides [25] and oxadiazolines [26] are reported to be ecdsyone agonists. We reported that the side chain moiety of ecdysteroids that is mimicked by the *t*-butylaminobenzoyl moiety of dibenzoylhydrazines is important to express the molting hormonal activity [27-29]. Moreover, crystal structure analyses of the ecdysone receptor proteins with ligand molecules demonstrated that steroidal and non-steroidal ligand molecules are only partially overlapping the ligand binding cavities of ecdysone receptor (EcR) of the tobacco budworm *Heliothis virescens* [30,31]. Thus, a steroid compound PNA and a nonsteroidal ecdysone agonist, a dibenzoylhydrazine-type compound (BYI06830) [30], are overlapped as shown in Fig. 2, which is constructed by fitting the conserved 4 amino acid residues in the ligand binding domain (LBD) of both the PNA- and the BYI06830-bound receptors (http://www.ncbi.nlm.nih.gov/) using the modeling software SYBYL 6.9 (Tripos, USA).

According to Voigt et al., few brassinosteroid/ecdysteroid hybrid compounds have weak ecdysone activity, while some other compounds are antagonists [32]. Previously we also synthesized the brassinosteroid/ecdysteroid hybrid compounds and found that the PNA/castasterone (PNA/CS: Fig. 3) hybrid compound **3** carrying the steroid moiety of a plant steroid hormone, CS, and the side chain of PNA, has a binding affinity to the EcRs and a hormonal activity against lepidopteran tissue [14,33]. Even though the steroid mother skeleton of ecdysteroids is replaceable with that of the brassinosteroids to show the ecdysone activity, the potency of the PNA/CS was 1/40 and 1/250 that of PNA against Diptera Kc and Lepidoptera Sf-9 cells, respectively. [33] This result indicates that the modification of the steroid mother skeleton may change the proper positioning of the side chain moiety of ecdysteroids in ligand binding pocket.

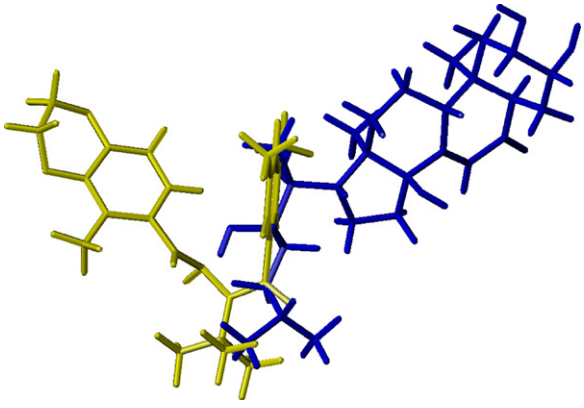

Fig. 2 – Superposition between PNA (black) and BYI06830 (gray).

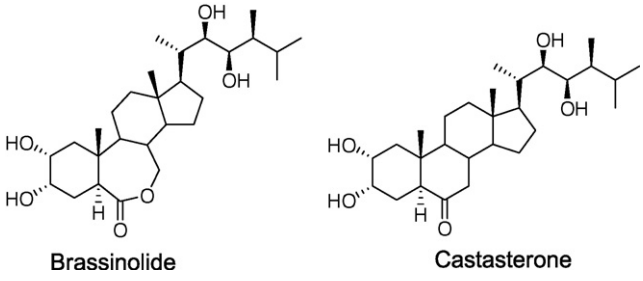

Fig. 3 – Structures of plant steroid hormones.

In this study, we synthesized a number of PNA analogs by modifying the mother skeleton moiety of PNA, and studied the SAR which would be fruitful to design new chemistry. The design of new compounds based on the structure of ecdysteroids will reach to the development of other ecdysone agonists possessing a broad insecticidal activity.

2. Experimental

2.1. *Synthesis*

Chemicals were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin, USA), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), and Nacalai Tesque Inc. (Kyoto, Japan). Dess–Martin periodinane was also prepared according to the conventional method [34,35]. Oven-dried glassware and positive Ar pres-