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Research Article

The ecdysteroid agonist/antagonist and brassinosteroid-like activities of synthetic brassinosteroid/ecdysteroid hybrid molecules

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Abstract. A series of synthetic hybrid brassinosteroid/ecdysteroid structures has been assessed for their ecdysteroid agonist/antagonist activities in the *Drosophila melanogaster* B_{II} cell bioassay and for brassinosteroid-like activity in the rice lamina inclination test. Most of the compounds proved inactive for ecdysteroid agonist activity, demonstrating the specificity of the ecdysteroid receptor for compounds closely structurally related to 20-hydroxyecdysone. However, compound **18**, with 14α-hydroxy-7-en-6-one and 22*S*-hydroxy functionalities (as in most active ecdysteroids), possessed distinct agonist activity (median effective concentration = 1.4×10^{-5} M), although this is still almost 2000-fold less active than 20-hydroxyecdysone (**25**). Compounds **13** and **15** possessed weak agonist activity. Compounds **5**, **11** and **14** weakly antagonised the action of 20-hydroxyecdysone (at 5×10^{-8} M) on B_{II} cells. In the brassinosteroid bioassay, most of the tested compounds showed activity. This may reflect the metabolic capability of plant tissue to convert test compounds to more active analogues. However, it is clear that biological activity declines as the structure of the test compound deviates further from that of castasterone (**16**). Three ecdysteroids (**25**, **26** and **27**) are completely inactive in the rice lamina inclination test. These studies demonstrate the high specificities of the insect ecdysteroid receptor and the plant brassinosteroid receptor and indicate that phytoecdysteroids, even in high concentrations, would not interfere with brassinosteroid signalling pathways in plants where the two classes of compounds co-occur. Equally, brassinosteroids would not interfere with ecdysteroid signalling in insects, especially if one takes into account the low concentrations of brassinosteroids in the diet of phytophagous insects.

Key words. Agonist; antagonist; bioassay; brassinosteroid; castasterone; ecdysteroid; 20-hydroxyecdysone; steroid hormone receptor.

Ecdysteroids are the steroid hormones of arthropods and probably of several other invertebrate groups too. In insects they regulate many aspects of development, from moulting and metamorphosis to reproduction and embryogenesis. Approximately 50 ecdysteroid analogues have been isolated from insects, but it is generally accepted that 20-hydroxyecdysone is the major biologically active ecdysteroid in insect systems, where the circulating concentration normally varies between 10^{-11} M and 10^{-6} M [1].

Ecdysteroid analogues are also present in 5–6% of terrestrial plant species [2, 3]. Here the concentrations can, in a few cases, reach very high concentrations [4]: up to 3% of the dry weight (e.g. [5]). A wider variety of analogues has been isolated from plant sources (ca. 250 [6]), but again 20-hydroxyecdysone is the one most com-

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monly encountered. Phytoecdysteroids are believed to contribute to the deterrence of non-adapted invertebrate predators. It has also been suggested that they might have a hormonal role in plants [7], but the evidence for this is unconvincing (reviewed in [8]). Rather, the steroidal hormones of plants appear to be the brassinosteroids, which have been demonstrated to stimulate plant growth, cellular elongation and division, and regulate the expression of specific genes [9–11]. Chemically, brassinosteroids and ecdysteroids possess several features in common. Both families comprise C_{27} to C_{29} polyhydroxylated steroids with an oxygenated B-ring. However, the B-ring in brassinosteroids possesses a carbonyl group at C-6 or may be expanded to form a lactone, whereas the ecdysteroids possess a characteristic 14α-hydroxy-7-en-6-one grouping. Also, hydroxyl groups at C-2, C-3 and C-22 are found in both families, but the orientations of these and the locations of further hydroxyls differ between the two classes. The junction of the A- and B-rings also differs, being A/B-*cis* in the ecdysteroids and A/B-*trans* in the brassinosteroids. Several reports exist on the biological activities of brassinosteroids in insect systems, which have been ascribed to agonism [12, 13] or antagonism [14–16] of ecdysteroid-regulated processes. Such effects are only seen at high concentrations and, owing to the biological complexity of the assay systems used, metabolism of the brassinosteroids by endogenous (ecdysteroid-metabolising?) enzymes remains a distinct possibility (e.g. [17]). When the activity of natural and synthetic brassinosteroid analogues was determined in a simpler in vitro cell-based ecdysteroid-responsive system which does not appear to metabolise ecdysteroids [18, 19], they were found to be essentially inactive [20]. Ecdysteroids possess very weak or no activity in brassinosteroid-responsive plant bioassays [21, 22]. Biochemically, the structural differences between ecdysteroids and brassinosteroids are significant and it is to be expected that ecdysteroid receptors would not recognise brassinosteroids and vice versa. However, the chemical similarities between these two classes of compounds means that similar synthetic approaches can be applied to the generation of analogues. This provides an opportunity to investigate hybrid structures, combining features of ecdysteroids and brassinosteroids, to determine whether they possess ecdysteroid-like, brassinosteroid-like or both activities. Such studies will help to define the minimum structural requirements for ecdysteroid or brassinosteroid activity. Here we report the assessment of a range of such hybrid molecules, modifications of the brassinosteroid castasterone, for ecdysteroid agonist or antagonist activities, using a bioassay which has been characterised with regard to an extensive range of ecdysteroid analogues [23], and for brassinosteroid-like activity, using the standard rice lamina inclination (RLI-) test [24].

Materials and methods

Synthesis and purity of test compounds

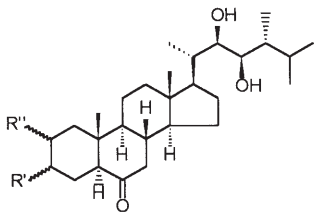
The structures of the test compounds are presented in figure 1 and named in table 1. The synthesis of nine of the analogues was as previously reported: **1** and **2** [25], **7** and **13** [26], **16** [27], **17** [28], **22** [29], **20**, **21** and **23** [30]. The synthesis of the other 14 analogues will be reported separately [B. Voigt et al., in preparation]. The structures of these analogues were confirmed by two-dimensional nuclear magnetic resonance (2D-NMR) and mass spectrometry. Additionally, X-ray crystallographic data were obtained for **4**, **10** and **24**, and these data have been deposited at the Cambridge Crystallographic Data Centre. The test compounds were >95% pure as assessed by NMR and high-performance liquid chromatography (HPLC). Ecdysteroids **25–27** were obtained from Prof. Jan Koolman, University of Marburg, Germany.

Bioassay for ecdysteroid agonist and antagonist activities

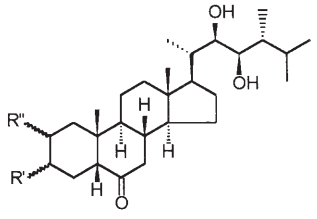
Stock solutions (10^{-2} or 10^{-3} M) of test compounds were prepared gravimetrically in methanol, and 10-fold serial dilutions in methanol were prepared from these. Aliquots (5, 10 and 20 µl) of the dilutions were dispensed in quadruplicate into flat-bottomed 96-well sterile microtitre plates. For the antagonist assay, 20 µl 5×10^{-7} M 20-hydroxyecdysone (in methanol) was also added to the wells. The solvent was allowed to evaporate in a laminar-flow cabinet, and 200 µl of B_{II} cell suspension (5×10^5 cell/ml in Schneider's medium with 10% v/v heat-inactivated foetal calf serum) was added to each well. Plates were covered and incubated in a moist environment for 7 days at 25 °C. The absorbance of the wells was read at 405 nm with a microplate reader (Anthos hIIa) relative to controls in which cells had received either no treatment (no response) or had been treated with 5×10^{-7} M (final concentration) 20-hydroxyecdysone (full response). Responding wells were also examined in situ with an inverted microscope to verify the specificity of the response [19]. Agonists bring about a reduction in cell density together with clumping and enlargement of cells, whereas antagonists prevent the induction of this response by 20-hydroxyecdysone (i.e. cells remain small, numerous and evenly distributed over the bottom of the well).

Bioassay for brassinosteroid activity

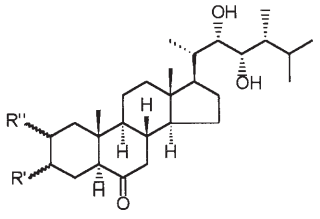
The highly specific and sensitive rice lamina inclination test was used according to Wada et al. [31] and Arima et al. [24]. Etiolated rice seedlings (*Oryza sativa* L. cv. Koshihikari) were grown in the dark for 6 days at 27–30 °C. The seedlings were cut 2 cm below the leaf sheath, and the upper segment was floated on distilled water for 24 h in the dark at 27–30 °C. Then 10 segments were in-



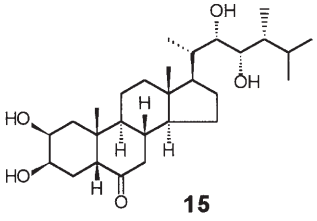
	R'	R''
1	αH/βOH	αH/βH
2	αH/βH	αH/βOH
3	αOH/βH	αH/βH
4	αOH/βH	αH/βOH
5	αH/βOH	αH/βOH
6	αH/βOH	=O
7	αOH/βH	αOH/βH
8	αH/βOH	αOH/βH



	R'	R''
9	αOH/βH	αH/βH
10	αOH/βH	αH/βI
11	αH/βOH	αH/βOH
12	αOH/βH	αH/βOH



	R'	R''
13	αOH/βH	αOH/βH
14	αH/βOH	αH/βOH



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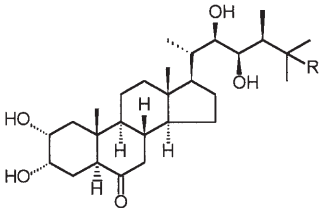
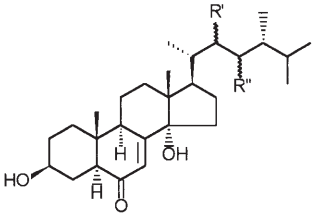
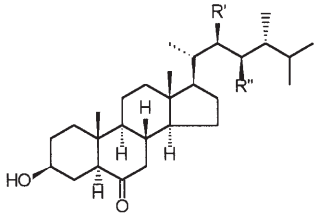


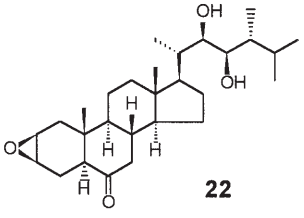
	R
16	H
17	OH



	R'	R''
18	22*S*-OH	23*S*-OH
19	22*R*-OH	23*R*-OH



	R'	R''
20	OH	H
21	H	OH



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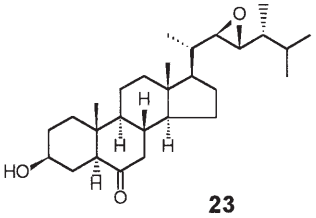

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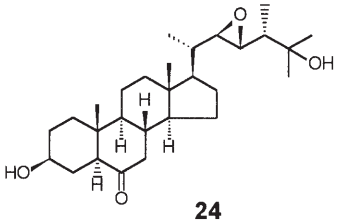

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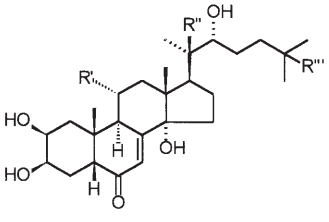

	R'	R''	R'''
25	H	OH	OH
26	H	H	OH
27	OH	OH	H

Figure 1. Structures of the test compounds.

Table 1. Assessment of ecdysteroid agonist and antagonist activities of the test compounds.

Compound[a]		Max. conc.[b]	Agonist response[c]	Antagonist response[c]	Cytotoxicity[c]
1	24-epiteasterone	10^{-3} M	–	–	+ @ $\geq 2.5 \times 10^{-5}$ M
2	3-deoxy-2,24-diepicastasterone	10^{-3} M	–	–	+ @ $\geq 5.0 \times 10^{-5}$ M
3	24-epityphasterol	5×10^{-4} M	–	–	+ @ $\geq 10^{-4}$ M
4	2,24-diepicastasterone	10^{-3} M	–	–	+ @ $\geq 5.0 \times 10^{-5}$ M
5	2,3,24-trisepicastasterone	10^{-4} M	–	w @ $\geq 5 \times 10^{-6}$ M	+ @ $\geq 10^{-5}$ M
6	2-dehydro-3,24-diepicastasterone	(not tested)			
7	24-epicastasterone	10^{-3} M	–	–	+ @ $\geq 10^{-4}$ M
8	3,24-diepicastasterone	10^{-4} M	–	–	w @ 10^{-4} M
9	5,24-diepityphasterol	10^{-3} M	–	–	+ @ $\geq 2.5 \times 10^{-5}$ M
10	2β-iodo-5,24-diepityphasterol	10^{-3} M	–	–	+ @ $\geq 2.5 \times 10^{-5}$ M
11	2,3,5,24-tetraepicastasterone	10^{-4} M	–	w @ $\geq 5 \times 10^{-6}$ M	+ @ $\geq 2.5 \times 10^{-5}$ M
12	2,5,24-trisepicastasterone	10^{-4} M	–	–	+ @ $\geq 5.0 \times 10^{-5}$ M
13	22,23,24-trisepicastasterone	10^{-3} M	+ @ 5×10^{-5} M	–	+ @ $\geq 10^{-4}$ M
14	2,3,22,23,24-pentaepicastasterone	10^{-4} M	–	w @ $\geq 10^{-6}$ M	+ @ $> 5.0 \times 10^{-5}$ M
15	2,3,5,22,23,24-hexaepicastasterone	10^{-4} M	+ @ 10^{-4} M	–	+ @ $\geq 5.0 \times 10^{-5}$ M
16	castasterone	5×10^{-4} M	–	–	– @ 5×10^{-4} M
17	25-hydroxycastasterone	5×10^{-4} M	–	–	– @ 5×10^{-4} M
18	(22*S*,23*S*,24*R*)-3β,14α,22,23-tetrahydroxy-24-methyl-5α-cholest 7-en-6-one	10^{-3} M	+ ($EC_{50} = 1.4 \times 10^{-5}$ M)	–	+ @ 10^{-3} M
19	(22*R*,23*R*,24*R*)-3β,14α,22,23-tetrahydroxy-24-methyl-5α-cholest-7-en-6-one	10^{-3} M	–	–	+ @ 2.5×10^{-4} M
20	24-epicathasterone	10^{-3} M	–	–	+ @ $\geq 5.0 \times 10^{-4}$ M
21	22-deoxy-24-epiteasterone	10^{-3} M	–	–	+ @ $\geq 2.5 \times 10^{-5}$ M
22	24-episecasterone	10^{-3} M	–	–	+ @ $\geq 10^{-4}$ M
23	(22*R*,23*R*,24*R*)-3β-hydroxy-24-methyl-22,23-epoxy-5α-cholestan-6-one	10^{-3} M	–	–	– @ 10^{-3} M
24	(22*R*,23*R*,24*S*)3β,25-dihydroxy-24-methyl-22,23-epoxy-5α-cholestan-6-one	5×10^{-4} M	–	–	– @ 5×10^{-4} M
25	20-hydroxyecdysone[d]	10^{-3} M	+ ($EC_{50} = 7.5 \times 10^{-9}$ M)	–	+ @ 10^{-3} M
26	ecdysone[d]	10^{-3} M	+ ($EC_{50} = 1.1 \times 10^{-6}$ M)	–	+ @ 10^{-3} M
27	muristerone A[d]	10^{-3} M	+ ($EC_{50} = 2.2 \times 10^{-8}$ M)	–	+ @ 10^{-3} M

[a] see Fig. 1. [b] Maximum concentration assessed in the bioassay. [c] –: no response; +: positive response; w: weak response. [d] Data taken from Dinan et al. (1999).

cubated in 20 ml of a solution of the test compound at the appropriate concentration (0.01, 0.05 or 0.1 ppm) for 2 days in the dark. The angle between the lamina and the sheath was measured. Results are expressed as a percentage of the angle obtained with 0.1 ppm 24-*epi*-castasterone (148°). The detection limit for this assay is 10^{-13} M for brassinolide [32]. The values given are the means of eight replicates.

Results and discussion

Ecdysteroid bioassays

The bioassay data for the tested compounds are summarised in table 1, including data for castasterone (**16**: archetypal brassinosteroid) and 20-hydroxyecdysone (**25**: archetypal ecdysteroid). Castasterone and the majority of the synthetic analogues show no specific agonistic or antagonistic activity, although most of these compounds are cytotoxic at high concentrations. This emphasises the highly specific structural requirements necessary for ecdysteroid activity imposed by the ligand binding domain of the ecdysteroid receptor complex. In the discussion which follows, structural modifications are related to the median effective concentration (EC_{50}) values in the B_{II} bioassay for ecdysteroid analogues possessing identical modifications relative to 20-hydroxyecdysone [23]. All the ecdysteroids tested in this bioassay (ca. 100) possess agonist activity; none has yet been found to antagonise 20-hydroxyecdysone.

Summary of the effects of relevant structural modifications on ecdysteroid agonist activity

Side-chain modifications

All the hybrid molecules possess a full sterol side-chain joined to the steroid nucleus by a 17β linkage, as in natural ecdysteroids.

Table 2. Activity of the test compounds in the rice lamina inclination test for brassinosteroid activity.

Compound		Activity % [relative to 24-epicastasterone at 0.1 ppm (= 100%)]		
		0.10 ppm	0.05 ppm	0.01 ppm
1		98	88	61
2		49	60	34
3		59	59	38
4		87	82	36
5		50	23	0
6		60	31	32
7	24-epicastasterone	100	91	65
8		80	61	30
9		63	37	6
10		70	21	0
11		44	16	1
12		not tested		
13		77	74	55
14		47	47	43
15		51	51	42
16		110	105	78
17		93	76	46
		(0.24 ppm)	(0.05 ppm)	(0.005 ppm)
18		42	47	27
19		32	35	26
20		20	16	11
21		58	41	23
22		91	63	38
23		66	47	34
24		40	37	30
25		inactive		
26		inactive		
27		inactive		

Analogues **1**–**24** possess a methyl group at C-24, in either the *R* or *S* configuration. Such a group is not present in 20E (agonist: $EC_{50} = 7.5 \times 10^{-9}$ M), but is present in makisterone A (24*R*-methyl-20-hydroxyecdysone; $EC_{50} = 1.3 \times 10^{-8}$ M) and 24-*epi*-makisterone A (24*S*-methyl-20-hydroxyecdysone; $EC_{50} = 2.2 \times 10^{-7}$ M). Thus, the presence of a 24*R*-methyl group in ecdysteroids does not significantly reduce biological activity at the *D. melanogaster* ecdysteroid receptor, whereas a 24*S*-methyl does. This is in accord with makisterone A being a natural ecdysteroid in *D. melanogaster* [33].

All the hybrid molecules, except **17** and **24**, lack a hydroxyl group at C-25. Absence of this hydroxyl in ecdysteroids is associated with enhanced agonist activity, e.g. ponasterone A (25-deoxy-20-hydroxyecdysone) is highly active ($EC_{50} = 3.1 \times 10^{-10}$ M).

Ecdysteroids are characterised by a 20*R*,22*R*-diol. Active brassinosteroids, on the other hand, are characterised by the presence of a 22*R*,23*R*-diol. Although the notation is 22*R* in both cases, the configuration of the 22-OH differs between the ecdysteroids and brassinosteroids, owing to the presence of a 23-OH altering the priority sequence in the Cahn-Ingold-Prelog rules. A 22*R*-hydroxyl enhances agonist activity in ecdysteroids. For example, taxisterone (22-deoxy-20-hydroxyecdysone) has an EC_{50} of 9.5×10^{-8} M [L. Dinan and L. Khalilov, unpublished]. A 22*S*-hydroxyl reduces activity, as does a 22-carbonyl group (22-dehydro-20-hydroxyecdysone; $EC_{50} = 1.7 \times 10^{-7}$ M). Activity is considerably enhanced by the additional presence of a 20*R*-hydroxyl (EC_{50} for ecdysone = 1.1×10^{-6} M).

A 23*S*-hydroxyl group is present in gerardiasterone (20*R*,23*S*-dihydroxyecdysone), and this reduces biological activity ($EC_{50} = 4.0 \times 10^{-7}$ M) relative to 20-hydroxyecdysone. The 20*R*,22*S*,23*R* analogue (22,23-*diepi*-gerardiasterone) possesses much lower activity ($EC_{50} = 4.0 \times 10^{-5}$ M).

Steroid nucleus

Active ecdysteroids possess a 7-en-6-one in ring B. All the hybrid molecules possess a carbonyl group at C-6, but most lack the α,β unsaturation. Ecdysteroid analogues without the 7,8-double bond possess lower activity (e.g. compare cheilanthone B [2β,3β,14α,22*R*-tetrahydroxycholestan-6-one; $EC_{50} = 1.3 \times 10^{-7}$ M; L. Dinan, J. Harmatha and R. Lafont, unpublished] and 25-deoxyecdysone [$EC_{50} = 1.0 \times 10^{-8}$ M]).

The consequences of the presence or absence of a C-14 hydroxyl on the agonist activity of ecdysteroids has been a moot point, with some evidence indicating that it is very important for high activity [34], whereas other evidence shows that its presence may even be detrimental to activity [35]. This may be attributable in part to comparison of activity data obtained with different assay systems using different insect species. With regard to the B_{II} bioassay, current evidence shows that the 14-hydroxyl enhances, but is not essential for, biological activity. Thus, the EC_{50} for 14-deoxy (14α-H)20E is 3.0×10^{-8} M. More important is the configuration at C-14, rather than the substituent, since 14-deoxy (14β-H)20E has significantly lower activity ($EC_{50} = 8.3 \times 10^{-7}$ M) and 14-*epi*-20-hydroxyecdysone (14β-OH) is almost inactive ($EC_{50} = 1.7 \times 10^{-4}$ M [36]).

The agonist activity of (5α-H)20-hydroxyecdysone ($EC_{50} = 3.3 \times 10^{-6}$ M) is considerably lower than that of 20E. Hydroxyls at C-2 and C-3 enhance agonist activity in ecdysteroids, but are not essential (EC_{50} for 2-deoxy-20-hydroxyecdysone = 6.6×10^{-7} M). The effect of the orientation of these hydroxyls on activity is dependent on whether the A- and B-rings are *cis*- or *trans*-linked; 2β- and 3β-hydroxyls favour activity for A/B-*cis* molecules, whereas 2α- and 3α-hydroxyls favour activity for A/B-*trans* molecules.

The agonist activity of compounds 13, 15 and 18

It is notable that the hybrid molecules possessing agonist activity (**13, 15** and **18**) possess a 22*S*-hydroxyl, although it is surprising that **13** (24*R*-methyl) is more active than **16** (24*S*-methyl), since a 24*R*-methyl (NB: altered prior-

ity sequence, owing to absence of 23-OH) is associated with higher activity in ecdysteroids. It may be that in this case the 24*R*-methyl (**13**) permits better accommodation of the 23*S*-hydroxyl group into the receptor binding pocket. Compound **15** was expected from its structure to possess agonist activity. Evidence for this was obtained at 10^{-4} M, based on the reduced A_{405} value of the wells relative to controls and the presence of clumped cells, but the agonist activity is strongly masked by concurrent cytotoxic activity.

The only hybrid molecule with significant agonist activity (**18**) possesses a 14α-hydroxy-7-en-6-one grouping (as found in active ecdysteroids). **19** is the only other test molecule to possess this grouping, but in this case it is present in association with a 22*R*,23*R*-diol, which can be expected to reduce biological activity.

Compound **18** possesses a 3β-hydroxyl, as present in most biologically active ecdysteroids, but the A-ring is *trans*-linked to the B-ring (5α-H), and this positions O-3 at some distance from its location in active ecdysteroids. However, in **13**, the combination of the 2α,3α-diol with the 5α-H positions O-2 and O-3 close to their normal locations in active ecdysteroids [34].

Antagonist activity of compounds 5, 11 and 14

These compounds possessed at best only weak antagonist activity. This is in contrast to the clear-cut antagonism seen with certain cucurbitacins, withanolides, limonoids, stilbenoids (reviewed in [37]) and phenylalkanoids [20]. Since antagonists may also act at other sites in the ecdysteroid signalling pathway than the ligand binding site of the ecdysteroid receptor, these hybrid molecules may not be receptor antagonists. This may be the same phenomenon as the antagonistic activity of certain brassinosteroid analogues seen in previous studies [14–16]. Unfortunately, the weak activities of **5**, **11** and **14** preclude closer examination of their mode of action.

Brassinosteroid bioassay

Several variants of the RLI test have been developed [24, 31, 38–41]. The three major variations consist of (i) which cultivar of rice is used, (ii) whether cut laminae are floated on an aqueous solution of the test compound or small volumes of an ethanolic solution of the test compound are applied to growing plants, and (iii) whether the laminae are cotreated with IAA, which enhances the response to brassinosteroid by ca. 100-fold. Consequently, it is difficult to compare data from various studies. Structure-activity relationships for natural and synthetic brassinosteroids in the RLI test have been investigated previously [40, 42–44]. Among natural brassinosteroids, biological activity increases according to the order of the biosynthetic pathway, with brassinosteroids in the early C-6 oxidation pathway being more active than the corresponding brassinosteroids in the late C-6 pathway; all the compounds examined here possess a C-6 carbonyl. Even early intermediates in the brassinosteroid pathway show some biological activity, but this may be a consequence of metabolism by the plant material to more active analogues. The most active brassinosteroid analogues reported to date [43, 44] are 25-methoxy-, 24-cyclopropyl- and 24-cyclobutyl derivatives of brassinolide, but the reasons for their high activity are not fully understood.

The results for the test compounds in the RLI test are summarised in table 2. Owing to the nature of the bioassay (especially the possibility of metabolism of test compounds) and the limited range of concentrations of each compound tested, these data can only be regarded as semiquantitative. However, it is clear from the sequential epimerisations from castasterone at C-24, C-2, C-3 and C-5 (**16** $\rightarrow$ **7** $\rightarrow$ **4** $\rightarrow$ **5** $\rightarrow$ **11**) that each of these reduces activity in the RLI-test. Hydroxylation at C-25 (cf. **17** and **16**) reduces biological activity. Removal of O at C-2 (cf. **4** and **2**) or oxidation of a C-2 hydroxyl to an oxo-group (cf. **6** and **8**) also reduce activity. Epimerisation of both C-22 and C-23 hydroxyl groups appears to elevate activity (cf. **5** and **14** and **11** and **15**). The presence of a 2α-OH enhances activity (cf. **3** and **7**). In the absence of a hydroxyl at C-2, conversion of a 3α-OH (**3**) to a 3β-OH (**1**) enhances activity further. Introduction of an iodine atom at C-2 reduces activity (cf. **9** and **10**), probably owing to the bulk of this introduction. Compound **18**, which possesses measurable ecdysteroid agonist activity also possesses moderate activity in the RLI test.

Conclusions

The structural requirements of the ligand binding domain of the ecdysteroid receptor are highly specific. Natural brassinosteroids do not act as ecdysteroid agonists or antagonists in the B_{II} bioassay [20]. Most of the synthetic hybrid molecules show no agonist or antagonist activity. The one analogue to show clear-cut agonist activity (**18**) possesses a 14α-hydroxy-7-en-6-one grouping and a 22*S*-hydroxyl group, but the potency was still almost 2000-fold lower than that of 20-hydroxyecdysone in the same assay. Each structural difference between castasterone and 20-hydroxyecdysone contributes to the difference in biological activity; the difference in potency cannot be attributed predominantly to a specific functionality. Although the synthetic analogues retain some activity in the RLI test, activity reduces as the structural similarity to castasterone decreases. Natural ecdysteroids possess no activity in the RLI test. Thus, as the structural requirements of the brassinosteroid receptor in plants and the ecdysteroid receptor in insects are specific and different, it is to be expected that there will be no cross-reac-

tivity between ecdysteroids and brassinosteroids; the concentrations of brassinosteroids in plants are far too low [10] to affect phytophagous insects, and ecdysteroids will not activate brassinosteroid receptors even when phytoecdysteroids are present at very high concentrations in plants.

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