CYCLIC PHENYLBORONATES OF ECDYSTEROIDS AS PRODUCTS OF REGIOSPECIFIC REACTION WITH PHENYLBORONIC ACID

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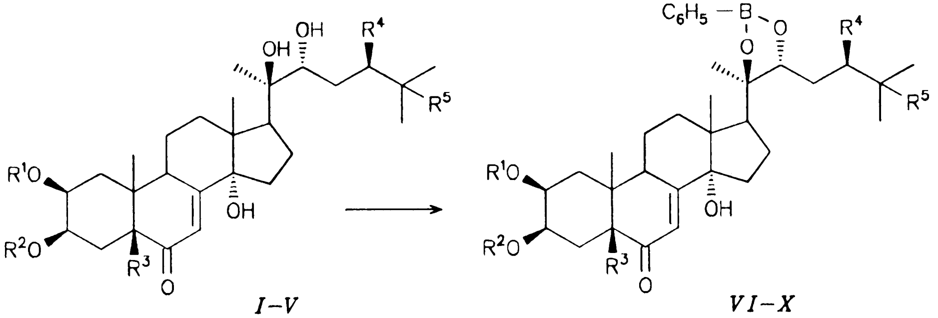
The diol system in the side-chain of ecdysteroids reacts with phenylboronic acid under formation of cyclic esters. Phenylboronates of ecdysteroids *I* – *V* were prepared. The regiospecific reaction course was confirmed by ^{1}H NMR spectra of the products. The reaction conditions were studied for 20-hydroxyecdysone (*I*). The phenylboronate can be prepared in high yields even in aqueous solutions. The liberation of the ecdysteroid *I* from the corresponding phenylboronate *VI* was performed using various reagents – diols or carboxylic acids.

Ecdysteroids represent an important group of natural compounds derived from the insect moulting hormone ecdysone. These compounds play a significant role in the physiology and ontogenesis of many groups of invertebrates[1]. They are also present in relatively high concentrations in various plant species[2]; here, however, their function and importance are not entirely clear. So far, about 150 compounds of this type have been described which differ mainly in the number and position of the hydroxyl groups and in the functionalities attached to them. For the isolation of ecdysteroids a whole number of procedures has been suggested, the latest one being the solid phase extraction method, performed with a support containing chemically bound dihydroxyborane groups[3]. This procedure is based on the general reaction of alkyl or arylboronic acids with diols[4]. In the case of ecdysteroids, phenylboronic acid was used for the protection of the diol grouping of cyasterone[5], for the proof of diol system by chromatographic method[6], and in the regioselective preparation of 20-hydroxyecdysone (*I*) glycosides[7]. Further aromatic boronic acids such as phenanthreneboronic[8] or 3-aminodansylboronic[9] acid were used in order to enhance sensitivity in determination of ecdysteroids or brassinosteroids in samples of biological origin. Most of ecdysteroids contain two diol systems, one in the ring A of the steroid skeleton and the other in the side-chain. Only the diol system in the side-chain forms a cyclic boronate with phenylboronic acid. In order to confirm this fact and the assumption that this reaction is typical for all ecdysteroids containing the diol grouping in position 20,22, this reaction was performed with a series of ecdysteroids *I* – *V*. For isolation of ecdysteroids by extraction on a solid phase containing dihydroxyborane groups it is important to know the conditions under which the boronates can be prepared. This question is particularly important in the case of protic solvents, especially water, because generally boronates are hydrolytic unstable. The necessity of using just these solvents follows from the usual methods of processing compounds of biological origin. Moreover, for the formed boronates it is necessary to find suitable methods for removal of the boronate group, i.e. recovery of the free ecdysteroid. These questions of binding ecdysteroids to the solid phase and their liberation have been studied on 20-hydroxyecdysone (*I*) and its boronate *VI* as models.



	R^1	R^2	R^3	R^4	R^5
I, VI	H	H	H	H	OH
II, VII	H	H	H	H	H
III, VIII	H	H	OH	H	OH
IV, IX	$-C(CH_3)_2-$		H	H	OH
V, X	H	H	H	CH_3	OH

SCHEME 1

Ecdysteroids *I* – *V* were converted into the corresponding boronates *VI* – *X* (see Scheme 1). The ^{1}H NMR data of ecdysteroids *I* – *V* and their cyclic phenylboronates *VI* – *X* are given in Table I. The structural assignment of the signals was done on the basis of chemical shifts, splitting patterns, homocorrelated 2D-COSY experiments and mutual comparison of the spectra. The number and position of free hydroxyl groups were proved using the in situ reaction with trichloroacetyl isocyanate the use of which in the structural analysis of ecdysteroids will be discussed in a separate communication. The structure of the cyclic phenylboronates follows unequivocally from comparison of

TABLE I
Proton NMR parameters of ecdysteroids *I* – *V* and their cyclic phenylboronates *VI* – *X* in hexadeuterioacetone (for other conditions see Experimental)

Proton	Chemical shifts, ppm (coupling constants, Hz)									
	I	*VI*	*II*	*VII*	*III*	*VIII*	*IV*	*IX*	*V*	*X*
H-2	3.86 ddd (12; 4; 3.5)	3.84 um	3.85 um 12; 4.5; 3)	3.84 um	3.85 ddd 12; 4.5; 3.5)	3.84 m	4.23 m	4.24 m	3.82 td (11.5; 4; 4)	3.83 m
H-3	3.93 bq (3; 3; 3)	3.91 bq (3; 3; 3)	3.92 bq (3; 3; 3)	3.91 um	3.87 um	3.84 m	4.24 m	4.26 m	3.90 m	3.91 m
H-5	2.33 dd (11; 6.5)	2.34 dd (12; 5.5)	2.33 dd (10.5; 6.5)	2.34 dd (11; 6)	–	–	[a]	2.21 dd (11.5; 6)	2.33 dd (10; 7)	2.34 dd (12; 5.5)
H-7	5.74 d (2.5)	5.73 d (2.5)	5.73 d (2.5)	5.73 d (2.5)	5.86 d (2.5)	5.88 d (2.5)	5.70 d (2.5)	5.74 d (2.5)	5.71 d (2.5)	5.74 d (2.5)
H-9	3.16 ddd (11.5; 7; 2.5)	3.18 ddd (11; 7.5; 2.5)	3.15 ddd (10; 7; 2.5)	3.18 ddd (11; 7.5; 2.5)	3.24 ddd 11.5; 7; 2.5)	3.27 ddd (11; 7; 2.5)	2.96 ddd (11.5; 7; 2.5)	2.98 ddd (11.5; 7; 2.5)	3.16 ddd (10; 7; 2.5)	3.18 ddd (11; 7; 2.5)
H-12α	2.21 td (13; 13; 5)	[a]	2.18 td (13; 13; 5)	2.26 td (13; 13; 5)	2.21 td (13; 13; 4.5)	2.28 td (13; 13; 4.5)	[a]	2.25 td (13; 13; 5)	2.20 td (13; 13; 5)	2.26 td (13; 13; 4.5)
H-17	2.45 t (9; 9)	2.50 t (9; 9)	2.43 dd (9.5; 8.5)	2.49 t (9; 9)	2.44 dd (9.5; 8.5)	2.50 t (9; 9)	2.45 t (9; 9)	2.50 t (9; 9)	2.43 t (9; 9)	2.50 t (8; 8)
H-22	3.37 dd (10.5; 8)	4.22 dd (8; 4.5)	3.35 dd (10; 1.5)	4.22 dd (9.5; 2.5)	3.36 dd (10.5; 2)	4.22 dd (9; 4)	3.36 bd (10.5; <2)	4.22 dd (9; 4)	3.46 bd (11; <2)	4.37 dd (11.5; 2)
Me-18	0.91 s	1.02 s	0.90 s	1.01 s	0.91 s	1.02 s	0.91 s	1.01 s	0.92 s	1.02 s
Me-19	0.94 s	0.97 s	0.93 s	0.96 s	0.88 s	0.90 s	0.96 s	0.98 s	0.94 s	0.96 s
Me-21	1.20 s	1.40 s	1.18 s	1.39 s	1.20 s	1.41 s	1.19 s	1.40 s	1.19 s	1.38 s
Me-26	1.19 s	1.23 s	0.88 d (6.5)	0.95 d (6.5)	1.18 s	1.23 s	1.18 s	1.23 s	1.15 s	1.19 s
Me-27	1.18 s	1.22 s	0.87 d (6.5)	0.94 d (6.5)	1.17 s	1.21 s	1.17 s	1.21 s	1.11 s	1.16 s
Me-28	–	–	–	–	–	–	–	–	0.92 d (6.5)	1.01 d (6.5)
$(CH_3)_2C$	–	–	–	–	–	–	1.42 s 1.26 s	1.43 s 1.26 s	–	–
C_6H_5 *o*-	–	7.77 m	–	7.78 m	–	7.78 m	–	7.77 m	–	7.78 m
m-	–	7.38 m	–	7.39 m	–	7.39 m	–	7.39 m	–	7.39 m
p-	–	7.49 m	–	7.49 m	–	7.49 m	–	7.49 m	–	7.49 m

[a] The value of parameter could not be determined.

their ^1H NMR spectra with those of the starting ecdysteroids. The presence of the cyclic phenylboronate grouping is confirmed by the aromatic proton signals in the spectra of compounds *VI* – *X* (δ 7.78 m (2 H, *ortho*-); 7.39 m (2 H, *meta*-); 7.49 m (1 H, *para*-)) and its location in position 20,22 follows from the characteristic induced downfield shifts of protons in the vicinity of the substituent (Δδ(H-22) ≈ 0.87 ppm, Δδ(Me-21) ≈ 0.21 ppm, Δδ(Me-18) ≈ 0.11 ppm, Δδ(H-12a) ≈ 0.07 ppm, Δδ(H-17a) ≈ 0.06 ppm and Δδ(Me-26,27) ≈ 0.05 ppm). The signals of protons on the rings A and B (including those of the diol grouping on the ring A) remain practically unchanged (Δδ < 0.02 ppm).

The reaction of 20-hydroxyecdysone with phenylboronic acid was studied in various solvents and the results are summarized in Table II. As seen, the boronates of ecdysteroids can also be prepared in protic solvents. In water-containing solvent mixtures the yields are lower. However, using a tenfold excess of phenyl boronic acid the boronate *VI* can be prepared in a 97% yield even in a 1 : 9 (v/v) methanol–water mixture. This fact makes it possible to utilize the mentioned reaction directly for water-containing biological samples. The reaction is sufficiently fast even in solvents in which one or both reactants are of limited solubility. Methanol appears to be the solvent of choice: it dissolves well both reactants and the reaction is very fast.

In cases of ecdysteroid boronates that are hydrolytic stable, the boronate group can be best removed by the oxidative splitting with hydrogen peroxide in methanol[10]. This method, however, is not suitable for dihydroxyborane groups chemically bound to a solid support. Therefore we performed a model removal of the boronate group in

TABLE II
Yields of reaction of 20-hydroxyecdysone (*I*) with phenylboronic acid (1.2 equivalent) in various solvents (for other conditions see Experimental)

Solvent	Time, min	Yield, %
Methanol	7	100
Dichloromethane[a]	20	100
Acetone[a]	20	100
Benzene[a]	35	100
Dimethylformamide	60	94
Tetrahydrofuran	50	98
Dioxane[a]	60	95
Methanol–water (4 : 1)	60	80
Methanol–water (1 : 9)[b]	15	97
Acetone–water (4 : 1)	60	85
Pyridine	15	95

[a] Solvent in which 20-hydroxyecdysone is sparingly soluble; [b] 10 equivalents of phenylboronic acid.

compound *VI* with nondestructive reagents such as diols or carboxylic acids. The results obtained in methanol are summarized in Table III, the results of the reaction with diols selected on the basis of their efficiency and solubility in tetrahydrofuran and dichloromethane are given in Table IV.

TABLE III
Removal of the phenylboronic group in compound *VI* in methanol (for other conditions see Experimental)

Reagent	Yield (in %) after	
	20 h	48 h
Ethylene glycol	9	11
1,3-propanediol	10	11
2,2-Dimethyl-1,3-propanediol	23	24
2,3-Dimethyl-2,3-butanediol	39	39
cis-1,2-Cyclohexanediol	8	9
Catechol	6	7
Bis(2-hydroxyethyl)amine	44	44
Oxalic acid	10	11
Malonic acid	7	7
Tartaric acid	7	7
Phthalic acid	6	9
Salicylic acid	6	9

TABLE IV
Removal of phenylboronic group of compound *VI* in tetrahydrofuran and dichloromethane (for other conditions see Experimental)

Reagent	Yield[a] (in %) after			Yield[b] (in %) after		
	2 h	3 h	19 h	2 h	3 h	19 h
2,2-Dimethyl-1,3-propanediol	37	43	45	35	35	38
2,3-Dimethyl-2,3-propanediol	20	27	32	18	25	39
Bis(2-hydroxyethyl)amine	27	31	46	–	–	–

[a] In tetrahydrofuran; [b] in dichloromethane.

EXPERIMENTAL

Infrared spectra were recorded on a Bruker IFS-88 instrument in chloroform (wavenumbers in cm^{-1}). Mass spectra were measured on a ZAB-EQ spectrometer (FAB technique). ^{1}H NMR spectra were measured on an FT-NMR spectrometer Varian UNITY 500 (500 MHz for ^{1}H) in hexadeuterioacetone. Chemical shifts were referenced to the residual solvent signal (δ(CHD_2COCD_3) 2.05 ppm).

The yields of the reactions with phenylboronic acid were determined by high-performance liquid chromatography (HPLC), using a 4 × 250 mm column packed with Separon SGX 7 μm, mobile phase dichloromethane–methanol–water 85 : 14 : 1.4, flow rate 1.2 ml/min. The compounds were detected by a UV detector at 254 nm. The starting 20-hydroxyecdysone (*I*) (about 1 mg) was dissolved or suspended in the appropriate solvent so that its concentration was 20 μg/μl. A solution of phenylboronic acid (1.2 equivalent) in the same solvent (concentration 100 μg/μl) was then added.

The yields in the removal of boronate group in boronate *VI* were determined by HPLC under the same conditions as described above. The boronate *VI* (about 1 mg) was dissolved in an appropriate solvent (methanol, tetrahydrofuran or dichloromethane) so that its concentration amounted to 30 μg/μl. The reagent (5 equivalents) in the same solvent (concentration 100 μg/μl) was then added.

20-Hydroxyecdysone 20,22-Phenylboronate (*VI*)

20-Hydroxyecdysone (*I*, 3 mg, 6.25 μmol) was dissolved in methanol (25 μl). Phenylboronic acid (0.84 mg, 6.9 μmol) was added to this solution and the reaction mixture was stirred for 10 min. After evaporation of the solvent the excess solvent was removed by HPLC on an 8 × 250 mm column packed with Separon SGX 7 μm, mobile phase dichloromethane–methanol–water (89 : 10 : 1 v/v/v), flow rate 3 ml/min. The product *VI* was detected at 254 nm. The product-containing fractions on evaporation afforded 3.3 mg (93%) of *VI*. IR spectrum: 3 600, 3 448, (O–H); 1 663 (C=O); 1 626 (C=C), 1 603, 1 498 (C–C arom.); 1 358 (B–O). Mass spectrum (*m/z*) for $C_{33}H_{47}BO_7$ calculated: 566; found: 567 (M + H), 549 (M + H – H_2O), 531 (M + H – 2 H_2O). For ^{1}H NMR spectrum see Table I.

Ponasterone A 20,22-Phenylboronate (*VII*)

The boronate *VII* was prepared from ponasterone A (*II*, 2.2 mg, 4.74 μmol) and phenylboronic acid (0.69 mg, 5.66 μmol) as described for compound *VI*. Yield 2.2 mg (86%) of *VII*. IR spectrum: 3 601, 3 454 (O–H); 1 661 (C=O); 1 629 (C=C); 1 603, 1 499 (C=C arom.); 1 356 (B–O). Mass spectrum (*m/z*) for $C_{33}H_{47}BO_6$ calculated: 550; found: 551 (M + H), 533 (M + H – H_2O). For ^{1}H NMR spectrum see Table I.

Polypodine B 20,22-Phenylboronate (*VIII*)

The boronate *VIII* was prepared from polypodine B (*III*, 2.9 mg, 5.85 μmol) and phenylboronic acid (0.86 mg, 7.05 μmol) as described for compound *VI*; yield 3.1 mg (91%) of compound *VIII*. IR spectrum: 3 600, 3 442 (O–H); 1 675 (C=O); 1 624 (C=C); 1 603, 1 498 (C–C arom.); 1 358 (B–O). Mass spectrum (*m/z*) for $C_{33}H_{47}BO_8$ calculated: 582; found: 583 (M + H), 565 (M + H – H_2O), 547 (M + H – 2 H_2O). For ^{1}H NMR spectrum see Table I.

20-Hydroxyecdysone 2,3-Acetonide 20,22-Phenylboronate (*IX*)

The boronate *IX* was prepared from acetonide *IV* (3.1 mg, 5.96 μmol) and phenylboronic acid (0.87 mg, 7.13 μmol) as described for compound *VI*; yield 3.0 mg (85%) of *IX*. IR spectrum: 3 600 (O–H); 1 666 (C=O); 1 625 (C=C); 1 603, 1 498 (C–C arom.); 1 382 (B–O). Mass spectrum (*m/z*) for $C_{36}H_{51}BO_7$ calculatedlated: 606; found: 607 (M + H), 589 (M + H – H_2O), 571 (M + H – 2 H_2O), 549 (M + H – $(CH_3)_2CO$). For ^1H NMR spectrum see Table I.

Makisterone A 20,22-Phenylboronate (*X*)

Boronate *X* was prepared from makisterone A (*V*, 1.6 mg, 3.24 μmol) and phenylboronic acid (0.47 mg, 3.85 μmol) as described for compound *VI*. Yield 1.5 mg (80%) of compound *X*. IR spectrum: 3 597, 3 437 (O–H); 1 658 (C=O); 1 632 (C=C); 1 603, 1 498 (C–C arom.); 1 357 (B–O). Mass spectrum (*m/z*) for $C_{34}H_{49}BO_7$ calculated: 580; found: 581 (M + H), 563 (M + H – H_2O), 545 (M + H – $2H_2O$). For ^1H NMR spectrum see Table I.

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