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Regioselective Synthesis of 20-Hydroxyecdysone Glycosides

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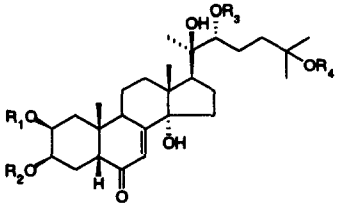
Abstract: Four β-D-glucopyranosides of 20-hydroxyecdysone (**1**) were prepared. The regioselective course of glycosylation was achieved by the combination of hydroxyl and 1,2-diol protective groups, *i.e.* acetates and phenyl boronates, in the aglycone moiety.

INTRODUCTION

The growing number of ecdysteroid conjugates isolated from both animals[1] and plants[2] suggests an active role in the ecdysteroid metabolism, transport or deactivation. The nonpolar esters and more polar ecdysteroid glycosides are the most common conjugates of natural origin. Their possible ecological significance in plant-insect chemical interaction is considered[3]. Since these compounds are mostly inactive in the common ecdysone assays[4], new biological tests must be developed. Some 20-hydroxyecdysone glycosides have been isolated from animal[5] and plant[6] sources, 20-hydroxyecdysone 25-β-D-glucopyranoside (**5**) has been isolated[7] from the roots of *Pfaffia iresinoides* , however the structural variations and the amount available are rather limited. For new bioassays, as well as for analytical correlation, a suitable variety of conjugates must be prepared by chemical synthesis. This paper deals with the preparation of a series of bioanalogical glycosides **2-5**.



1 $R_1 = R_2 = R_3 = R_4 = H$
2 $R_2 = R_3 = R_4 = H$ R_1 = β-D-Glc
3 $R_1 = R_3 = R_4 = H$ R_2 = β-D-Glc
4 $R_1 = R_2 = R_4 = H$ R_3 = β-D-Glc
5 $R_1 = R_2 = R_3 = H$ R_4 = β-D-Glc

Fig.1. Structures of 20-hydroxyecdysone and its glucosides

Regioselective manipulation of hydroxyl groups of polyols is frequently required particularly in the chemistry of natural products. However, only a few examples of protection and deprotection sequences for 20-hydroxyecdysone (**1**) have been reported. They include acetonide[8,9], acetate[9] and boronate[10,11] formation.

Other reagents, *e.g.* N-trimethylsilylimidazole[12] and 1-anthroyl nitrile[13], have been employed for analytical purposes. None of the reported protection procedures allows the whole set of regioisomers to be prepared.

RESULTS AND DISCUSSION

The structure of 20-hydroxyecdysone (**1**) suggests that the problem of regioselective manipulation of hydroxyl groups may be solved in this case by methods which distinguish between isolated hydroxyls and 1,2-diols. We therefore searched for a suitable diol-protective group. The phenylboronate group appeared most suitable, because it can be introduced regioselectively in high yield. The acetate group was chosen for protection of isolated hydroxyls in the aglycone because it corresponded with our intention to control stereochemistry on the anomeric carbon of glucose by acetyl group participation[14]. Suitable protected aglycones **6**, **8**, **12** and **17** were prepared as follows. 20-Hydroxyecdysone (**1**) was acetylated to give known[9] triacetate **6** as the major product along with small amount of 20-hydroxyecdysone 2,3,22,25-tetraacetate. The side chain diol of 20-hydroxyecdysone (**1**) was protected by reaction with phenylboronic acid in methanol to give the phenylboronate **8**. It is worthwhile to note that phenylboronate is formed exclusively on the side-chain diol[11]. The most reactive hydroxyl group (*i.e.* at C-2) of boronate **8** can easily be protected as an acetate under mild acetylation conditions, yielding compound **12** (77 %). Diacetate **13** (16 %) was formed as a by-product. In order to prepare protected compound **13** as the major product, acetylation using DMAP was used giving diacetate **13** in 78 % yield and triacetate **14** as a by-product. Prolonged reaction times furnished triacetate **14** in excellent yield (85 %). Deprotection of the phenylboronate group in compound **13** was accomplished by a methanolic solution of hydrogen peroxide giving aglycone **17**. Having suitably protected aglycones in hand, we turned our attention to glycosylation. Tetra-O-acetylglucopyranosyl bromide and Ag-silicate catalyst were used for the introduction of the glucose unit. All glycosylation reactions were performed in dry dichloromethane under an inert atmosphere. Glycosides were isolated from the reaction mixtures by normal-phase HPLC. Yields of the glycosylation reaction varied from 40 % to 70 %, and the stereochemistry of the resulting glycosides was β (determined by ^{1}H-NMR). Triacetate **6** thus furnished protected glycoside **7** in 69 % yield. Diacetate **17** afforded acetylated glycosides **18** (9 %) and **19** (32 %). A mixture of glycosides **9** (42 %), **10** (21 %) and **11** (7 %) resulted from the reaction of boronate **8**, yielding after separation of individual compounds and removing of the boronate protection group glycosides **20, 21** and **22**. In order to increase the yield of 3-glycoside we also utilized 2-acetate **12**. However, the glycosylation reaction gave the mixture of 3- and 25-glycosides **15** and **16** in a 1 : 1 ratio. Finally, the protective acetate groups were removed from compounds **7**, **18, 19, 20** and **21** by potassium cyanide catalysed transesterification[15]. This method is mild enough to avoid epimerization of the steroid skeleton. Generally an equilibrium of A/B *cis* and *trans* fused steroid rings is reached when ecdysteroids are subjected to basic conditions[16,17].

All compounds **1** - **22** were fully characterised by ^{1}H-NMR (see Table 2) and compounds **1** - **5, 17- 22** also by ^{13}C-NMR spectra (see Table 1). The data for glucoside **5** are in a good accordance with data previously reported[7]. The position of free and/or acetylated glucose was determined from observed glucosylation shifts in ^{1}H and ^{13}C-NMR spectra of corresponding compounds (see Table 3). β-Configuration of glucose followed from J(H-1′,H-2′) ca 8 Hz observed in all glycosides studied (Table 2). The characteristic downfield proton shifts indicating the location of phenylboronate grouping are discussed in our previous paper[11].

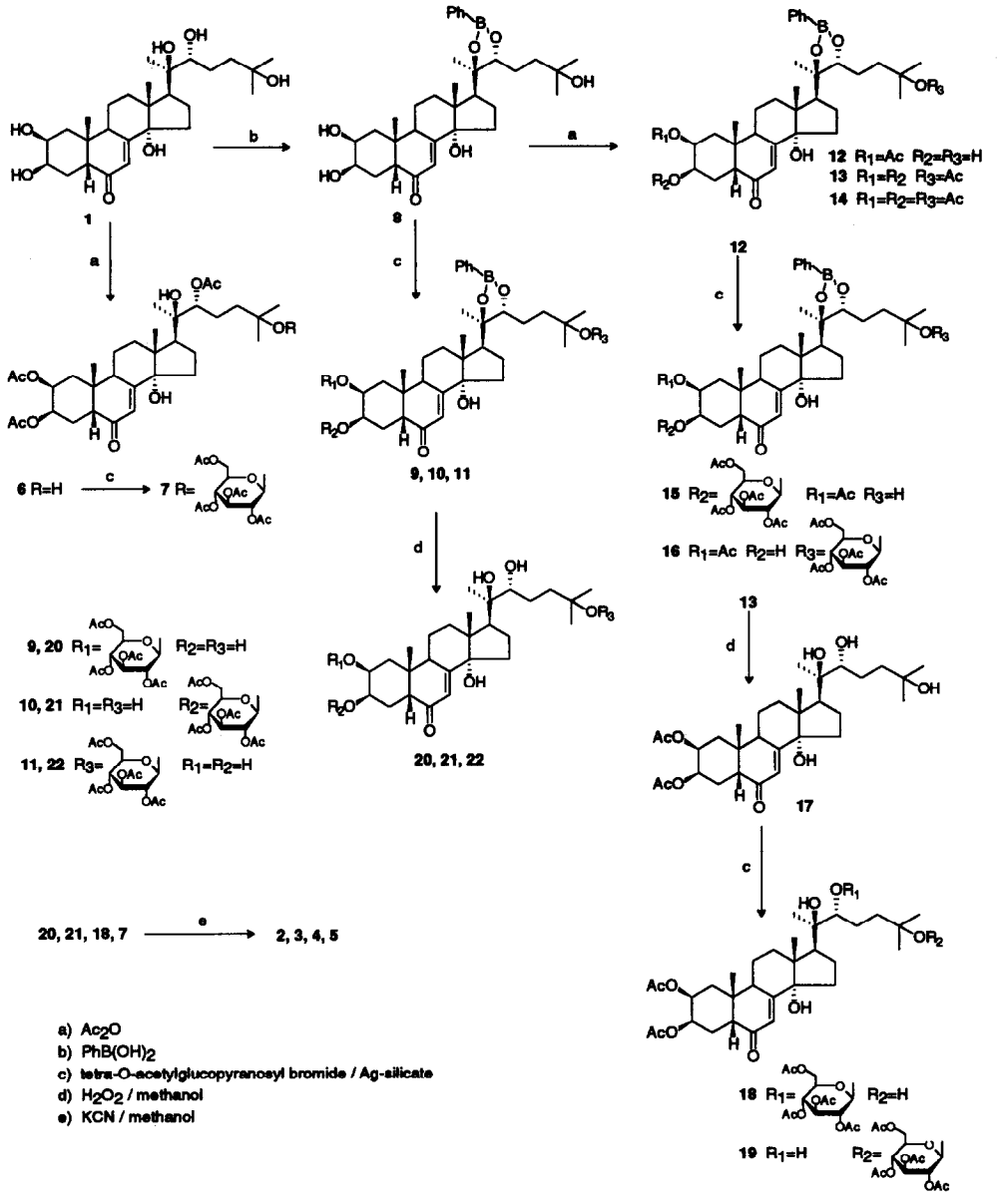


Fig. 2. Synthesis of 20-hydroxyecdysone glucosides

EXPERIMENTAL

General

Starting 20-hydroxyecdysone (1) was isolated from the roots of *Leuzea carthamoides* (Willd.) DC.[18] and was fully characterised by ^{1}H-NMR, MS and IR. Tetra-O-acetylglucopyranosyl bromide was prepared from

D-glucose according to known procedures[19], and was characterised by ^{1}H-NMR. Ag-silicate was prepared from silver nitrate and sodium metasilicate[20]; Ag content was approx. 3.4 mmol/g of support. Hydrogen peroxide (30 % aqueous solution) was from Lachema. Other reagents were from either Lachema or Aldrich and were used without any further purification. Solvents were from Lachema and were purified and dried according to standard procedures. Dichloromethane was freshly distilled from phosphorus pentoxide and glycosylation reactions were performed under an inert atmosphere of dry nitrogen in oven dried glassware. Normal-phase HPLC using a column (8 mm I.D., 250 mm length) packed with Separon SGX 7μm was employed for the isolation of reaction products. Ternary mixtures of dichloromethane-methanol-water (DMW) of various elutropic strengths were used as the mobile phase (concentrations are given as volume/volume). Glucosides **2-5** were purified by a reversed phase HPLC using a column (8 mm I.D., 250 mm length) packed with 7μm Separon SGX C-18 and methanol-water mixtures (for concentrations and retention times see below) as the mobile phase. The flow rate was 4 ml/min in all cases. The compounds were detected by a UV detector at 254 nm. Infrared spectra were recorded on a Bruker IPS-88 in $CHCl_3$ unless stated otherwise. NMR spectra were recorded either on a Varian UNITY-200 (at 200 MHz for ^{1}H and 50.3 MHz for ^{13}C) or Varian UNITY-500 (at 500 MHz for ^{1}H and 125.7 MHz for ^{13}C) in acetone-d_6 (^{1}H) and methanol-d_4 (^{13}C). Chemical shifts were referenced to the residual solvent signal at 2.05 ppm (^{1}H) and 49.00 ppm (^{13}C). NMR spectra of very poor-soluble compound **3** were run in pyridine-d_5. Mass spectra were recorded on a ZAB-EQ spectrometer with fast atom bombardment (FAB) ionisation using a glycerol - thioglycerol mixture as a matrix. The melting points were determined on a Boëtius apparatus and are uncorrected.

20-Hydroxyecdysone 2-β-D-glucopyranoside (2).
(20R,22R)-2β-(β-D-Glucopyranosyloxy)-3β,14α,20,22,25-pentahydroxy-5β-cholest-7-en-6-one
Acetylated glycoside **20** (5.3 mg; 6.5 μmol) was dissolved in methanol (150 μl) and solid potassium cyanide (0.7 mg) was added. The reaction mixture was stirred overnight at room temperature. The resulting solution was concentrated to 30 μl and purified by RP-HPLC (40% MeOH in water, R.T.=13.5 min). Glycoside **2** (3.0 mg, 72 %) was obtained as an amorphous solid (m.p. 180-183°C) after evaporation of solvents. IR spectrum (KBr pellet): 3420 (ν_{O-H}); 1652 ($\nu_{C=O}$); 1050, 1031 (ν_{C-O}) cm^{-1}. Mass spectrum: 665 [M+Na], 647 [M+Na-H_2O], 643 [M+H], 625 [M+H-H_2O], $C_{33}H_{54}O_{12}$ (M+H) requires 643.3694, found 643.3656. For ^{13}C and ^{1}H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 3-β-D-glucopyranoside (3).
(20R,22R)-3β-(β-D-Glucopyranosyloxy)-2β,14α,20,22,25-pentahydroxy-5β-cholest-7-en-6-one
Acetylated glycoside **21** (4.0 mg, 4.9 μmol) was treated with potassium cyanide (0.6 mg) in methanol (150 μl) in the same manner as in the synthesis of glycoside **2**, yielding after RP-HPLC separation (40% MeOH in water, R.T.=13.5 min) 2.2 mg (69 %) of glycoside **3**. Crystallisation from MeOH afforded glycoside **3** as white crystals, m.p. 297-300°C (decomp.). IR spectrum (KBr pellet): 3419 (ν_{O-H}); 1660 ($\nu_{C=O}$); 1051 (ν_{C-O}) cm^{-1}. Mass spectrum: 665 [M+Na], 647 [M+Na-H_2O], 643 [M+H], 625 [M+H-H_2O], $C_{33}H_{54}O_{12}$ (M+H) requires 643.3694, found 643.3658 For ^{13}C and ^{1}H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 22-β-D-glucopyranoside (4).
(20R,22R)-22-(β-D-Glucopyranosyloxy)-2β,3β,14α,20,25-pentahydroxy-5β-cholest-7-en-6-one
Acetylated glycoside **18** (5.0 mg, 5.6 μmol) was treated with potassium cyanide (0.6 mg) in methanol (150 μl) in the same manner as in the synthesis of glycoside **2**, yielding after RP-HPLC separation (40% MeOH in water R.T.=10.2 min) 2.8 mg (78 %) of glycoside **4** as amorphous solid (m.p. 260-265°C). IR spectrum (KBr pellet): 3419 (ν_{O-H}); 1639 ($\nu_{C=O}$); 1050, 1030 (ν_{C-O}) cm^{-1}. Mass spectrum: 665 [M+Na], 647 [M+Na-H_2O], 643

[M+H], 625 [M+H-H_2O], $C_{33}H_{54}O_{12}$ (M+H) requires 643.3694, found 643.3657. For ^{13}C and ^1H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 25-β-D-glucopyranoside (5).
(20R,22R)-25-(β-D-Glucopyranosyloxy)-2β,3β,14α,20,22-pentahydroxy-5β-cholest-7-en-6-one
Acetylated glycoside 7 (7.0 mg, 7.5 μmol) was treated with potassium cyanide (0.8 mg) in methanol (150 μl) in the same manner as in the synthesis of glycoside **2**, yielding after RP-HPLC separation (40% MeOH in water R.T.=11.5 min) 4.1 mg (85 %) of glycoside **5** as amorphous solid (m.p. 158-163°C). IR spectrum (KBr pellet): 3420 (ν_{O-H}); 1652 ($\nu_{C=O}$); 1052 (ν_{C-O}) cm^{-1}. Mass spectrum: 665 [M+Na], 647 [M+Na-H_2O], 643 [M+H], 625 [M+H-H_2O], $C_{33}H_{54}O_{12}$ (M+H) requires 643.3694, found 643.3656. For ^{13}C and ^1H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 2,3,22-triacetate (6).
(20R,22R)-2β,3β,22-Triacetyloxy-14α,20,25-trihydroxy-5β-cholest-7-en-6-one.
20-Hydroxyecdysone (**1**; 48.0 mg, 100 μmol) was dissolved in pyridine (550 μl). DMAP (1 mg) and acetic anhydride (120 μl) were added. The reaction mixture was stirred for 3 hours at room temperature; progress of the reaction was monitored by HPLC. The reaction was stopped by addition of ethyl alcohol and the residue was treated and evaporated with ethyl alcohol (5 x 1 ml). Triacetate **6** was separated using column chromatography (silica-gel, mobile phase 4% MeOH in CH_2Cl_2) . Crystallisation from methanol afforded 41.2 mg (68 %) of the compound **6**, m.p. 145 - 147 °C. IR spectrum: 3601 (ν_{O-H}); 1739 ($\nu_{C=O}$ ester); 1652 ($\nu_{C=O}$ ketone); 1602 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{33}H_{50}O_{10}$: 629 [M+Na], 607 [M+H], 589 [M+H-H_2O], 571 [M+H-2H_2O]. For ^1H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2,3,22-triacetate 25-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (7).
(20R,22R)-25-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,3β,22-triacetyloxy-14α,20-dihydroxy-5β-cholest-7-en-6-one.
The reaction was carried out in an oven dried glassware under nitrogen atmosphere. A solution of triacetate **6** (10.2 mg, 16.8 μmol) in dichloromethane (30 μl) was added to a suspension of Ag-silicate (110 mg) in dichloromethane (700 μl). The mixture was stirred for 1 hour at room temperature; then a solution of tetra-O-acetylglucopyranosyl bromide (30.0 mg, 73 μmol) in dichloromethane (100 μl) was added. The reaction mixture was stirred for 48 hours at room temperature. The solid catalyst was filtered off and the filtrate was passed through a short column of silica-gel. NP-HPLC (DMW 960/40/1, R.T.=3.9 min) afforded 10.9mg (69 %) of compound **7** as an amorphous solid. IR spectrum: 3589, 3513 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{47}H_{68}O_{19}$: 959 [M+Na], 937 [M+H], 919 [M+H-H_2O], 901 [M+H-2H_2O], 589 [M+H-sugar]. For ^1H-NMR spectrum see Table 2.

20-Hydroxyecdysone 20,22-phenylboronate (8).
(20R,22R)-2β,3β,14α,25-Tetrahydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.
Phenylboronic acid (8.4 mg, 69.0 μmol) was added to a solution of 20-hydroxyecdysone (**1**; 30.0 mg, 62.5 μmol) in methanol (300 μl). The reaction mixture was stirred for 20 min. at room temperature. The solvent was evaporated and the dry residue was purified by NP-HPLC (DMW 925/75/1.5, R.T.=19.4 min). Pure boronate **8** (33.0 mg, 93%) was obtained as an amorphous solid after evaporation of solvents. IR spectrum: 3600, 3448 (ν_{O-H}); 1636 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1498 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{33}H_{47}O_7B$: 567 [M+H], 549 [M+H-H_2O], 531 [M+H-2H_2O]. For ^1H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 20,22-phenylboronate (9).
(20R,22R)-2β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-3β,14α,25-trihydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.
The reaction was performed in the same manner as for compound **7**. Boronate **8** (30.0 mg, 53 μmol) was stirred in dichloromethane (1 ml) with Ag-silicate (68.6 mg) for 1 hour. Tetra-O-acetylglucopyranosyl bromide

(15.2 mg, 74 μmol) in dichloromethane (50 μl) was then added. After work-up, a mixture of glycosides **9**, **10** and **11** was obtained. NP-HPLC separation (DMW 925/75/1.5, R.T.=3.6, then DMW 960/40/1 R.T.=12.6 min) afforded glycoside **9** (19.9 mg, 42 %) as an amorphous solid. IR spectrum: 3601 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1498 (ν_{C-C} arom); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{47}H_{65}O_{16}B$: 897 [M+H], 879 [M+H-H_2O], 549 [M+H-sugar]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 20,22-phenylboronate (10).
(20R,22R)-3β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,14α,25-trihydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.
Glycoside **10** was obtained along with glycosides **9** and **11** after glycosylation of **8**. NP-HPLC separation (DMW 925/75/1.5, R.T.=3.6 min, then DMW 960/40/1, R.T.=16.6 min) of the mixture gave pure glycoside **10** (10.0 mg, 21 %) as an amorphous solid. IR spectrum: 3600 (ν_{O-H}); 1740 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1498 (ν_{C-C} arom); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{47}H_{65}O_{16}B$: 897 [M+H], 879 [M+H-H_2O], 549 [M+H-sugar]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 25-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 20,22-phenylboronate (11).
(20R,22R)-25-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,3β,14α-trihydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.
Glycoside **11** was obtained along with glycosides **9** and **10** after glycosylation of **8**. NP-HPLC separation (DMW 925/75/1.5, R.T.=6.1 min) gave pure glycoside **11** (3.4 mg, 7 %) an amorphous solid. IR spectrum: 3600 (ν_{O-H}); 1740 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1498 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{47}H_{65}O_{16}B$: 897 [M+H], 879 [M+H-H_2O], 549 [M+H-sugar]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2-acetate 20,22-phenylboronate (12).
(20R,22R)-2β-Acetyloxy-3β,14α,25-trihydroxy-20,22-[(phenylborylene)bis(oxy)]-5β-cholest-7-en-6-one.
Acetic anhydride (100 μl) was added to a solution of boronate **8** (19.5 mg, 34.4 μmol) in pyridine (500 μl) and the reaction mixture was stirred at room temperature. After 1 hour the reaction was stopped with ethanol (1 ml). The reaction mixture contained starting boronate **8** (7 %) and diacetate **13** (16 %) along with monoacetate **12** (77 %) according to the NP-HPLC analysis. Pure acetate **12** was obtained by NP-HPLC separation (DMW 925/75/1.5, R.T.=6.9 min) of the crude mixture. Crystallisation from methanol gave **12** (14.5 mg, 70 %, m.p. 265-270 °C). IR spectrum: 3600, 3468 (ν_{O-H}) 1738 ($\nu_{C=O}$ ester); 1664 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1498 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{35}H_{49}O_8B$: 631 [M+Na], 609 [M+H], 591 [M+H-H_2O], 573 [M+H-2H_2O]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2,3-diacetate 20,22-phenylboronate (13).
(20R,22R)-2β,3β-Diacetyloxy-14α,25-dihydroxy-20,22-[(phenylborylene)bis(oxy)]-5β-cholest-7-en-6-one
Acetic anhydride (95 μl) was added to a solution of boronate **8** (18.3 mg, 32.3 μmol) and DMAP (0.5 mg) in pyridine (300 μl). The reaction mixture was stirred for 3 hours at room temperature. Excess acetic anhydride was destroyed by ethyl alcohol, and the mixture was treated and evaporated with ethyl alcohol (4 x 1 ml) to remove pyridine. The crude mixture was subjected to NP-HPLC (DMW 960/40/1, R.T.=4.8 min), giving diacetate **13** (16.4 mg, 78 %) as an amorphous solid. IR spectrum: 3600 (ν_{O-H}); 1738 ($\nu_{C=O}$ ester); 1665 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1605, 1499 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{37}H_{51}O_9B$: 651 [M+H], 591 [M+H-AcOH], 573 [M+H-H_2O-AcOH]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2,3,25-triacetate 20,22-phenylboronate (14).
(20R,22R)-2β,3β,25-Triacetyloxy-14α-hydroxy-20,22-[(phenylborylene) bis(oxy)]-5β-cholest-7-en-6-one.
Acetic anhydride (120 μl) was added to a solution of boronate **8** (28.3 mg, 50 μmol) and DMAP (0.5 mg) in

pyridine (100 µl). The reaction mixture was stirred for 6 hours at 40 °C. Excess acetic anhydride was destroyed by ethyl alcohol, and the mixture was treated and evaporated with ethyl alcohol (4 x 1 ml) to remove pyridine. The crude mixture was subjected to NP-HPLC (960/40/1, R.T.=2.8 min), giving triacetate **14** (29.4 mg, 85 %) as an amorphous solid. IR spectrum: 3595 (ν_{O-H}); 1738 ($\nu_{C=O}$ ester); 1664 ($\nu_{C=O}$ ketone); 1603, 1496 (ν_{C-C} arom.); 1360 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{39}H_{53}O_{10}B$: 693 [M+H], 675 [M+H-H_2O], 633 [M+H-AcOH], 615 [M+H-H_2O-AcOH]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2-acetate 3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 20,22-phenyl-boronate (15)

(20R,22R)-2β-Acetyloxy-3β-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-14α,25-dihydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.

The reaction was performed in the same manner as for compound **7**. Aglycone **12** (35.0 mg, 57.7 µmol) was stirred in dichloromethane (1 ml) with Ag-silicate (80.6 mg) for 1 hour . Tetra-O-acetylglucopyranosyl bromide (27.3 mg, 66.4 µmol) in dichloromethane (80 µl) was then added. After work-up, the mixture of glycosides **15** and **16** was obtained in 1:1 ratio. NP-HPLC separation (DMW 960/30/1, R.T.=17.3 min), afforded glycoside **15** (14.6 mg, 27 %) as an amorphous solid. IR spectrum: 3597, 3523 (ν_{O-H}); 1752 ($\nu_{C=O}$ ester); 1660 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1500 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm $^{-1}$. Mass spectrum, $C_{49}H_{67}O_{17}B$: 939 [M+H], 921 [M+H-H_2O], 591 [M+H-sugar]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2-acetate 25-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 20,22-phenyl-boronate (16).

(20R,22R)-25-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β-acetyloxy-3β,14α-dihydroxy-20,22-[(phenylborylene) bis (oxy)]-5β-cholest-7-en-6-one.

Glycoside **16** was obtained along with glycoside **15** after glycosylation of **12**. After NP-HPLC separation (DMW 970/30/1, R.T.=18.1 min), pure glycoside **16** (10.7 mg, 20 %) was obtained as an amorphous solid. IR spectrum: 3597 (ν_{O-H}); 1754, 1142 ($\nu_{C=O}$ ester); 1664 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$); 1603, 1500 (ν_{C-C} arom.); 1358 (ν_{B-O}) cm^{-1}. Mass spectrum, $C_{49}H_{67}O_{17}B$: 939 [M+H], 921 [M+H-H_2O], 591 [M+H-sugar]. For ^{1}H-NMR spectrum see Table 2.

20-Hydroxyecdysone 2,3-diacetate (17).

(20R,22R)-2β,3β-Diacetyloxy-14α,20,22,25-tetrahydroxy-5β-cholest-7-en-6-one.

Hydrogen peroxide (10 µl) was added to a solution of boronate **13** (12.0 mg, 18.5 µmol) in methanol (100 µl). The reaction mixture was stirred for 10 min at room temperature. After evaporation of the solvent, the crude mixture was subjected to NP-HPLC (DMW 925/75/1.5, R.T.=8.2 min) giving acetate **17** (8.7 mg, 89 %) as an amorphous solid. IR spectrum: 3598 (ν_{O-H}); 1737 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1625 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{31}H_{48}O_9$: 565 [M+H], 547 [M+H-H_2O], 529 [M+H-2H_2O], 511 [M+H-3H_2O]. For ^{13}C and ^{1}H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 2,3-diacetate 22-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (18).

(20R,22R)-22-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,3β-diacetyloxy-14α,20,25-trihydroxy-5β-cholest-7-en-6-one.

The reaction was performed in the same manner as for compound **7**. Aglycone **17** (65.6 mg, 116.2 µmol) was stirred in dichloromethane (2 ml) with Ag-silicate (150 mg) for 1 hour. Tetra-O-acetylglucopyranosyl bromide (54.2 mg, 123 µmol) in dichloromethane (150 µl) was then added. After work-up, NP-HPLC separation (DMW 970/30/1, R.T.=17.4 min) afforded glycoside **18** (5.4 mg, 9 %) and **19** as amorphous solids. IR spectrum: 3529 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{45}H_{66}O_{18}$: 895 [M+H], 877 [M+H-H_2O], 859 [M+H-H_2O], 529 [M+H-H_2O-sugar], 511 [M+H-2H_2O-sugar]. For ^{13}C and ^{1}H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 2,3-diacetate 25-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (19). (20R,22R)-25-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,3β-diacetyloxy-14α,20,22-trihydroxy-5β-cholest-7-en-6-one.

Glycoside **19** was obtained along with glycoside **18** after glycosylation of **17**. NP-HPLC purification (DMW 970/30/1, R.T.=8.1 min) gave **19** (17.5 mg, 32 %). IR spectrum: 3529 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{45}H_{66}O_{18}$: 895 [M+H], 877 [M+H-H_2O], 859 [M+H-H_2O], 529 [M+H-H_2O-sugar], 511 [M+H-2H_2O-sugar]. For ^{13}C and ^1H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 2-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (20). (20R,22R)-2β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-3β,14α,20,22,25-pentahydroxy-5β-cholest-7-en-6-one.

The boronate protecting group was removed in the same manner as for compound **17**. Boronate **9** (16.0 mg, 17.8 μmol) in methanol (150 μl) was treated with hydrogen peroxide (10 μl), yielding after NP-HPLC separation (DMW 925/75/1.5, R.T.=11.1 min) 13.2 mg (91 %) of compound **20**. IR spectrum: 3485 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{41}H_{62}O_{16}$: 811 [M+H], 793 [M+H-H_2O], 775 [M+H-2H_2O], 463 [M+H-sugar], 445 [M+H-sugar-H_2O]. For ^{13}C and ^1H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (21). (20R,22R)-3β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,14α,20,22,25-pentahydroxy-5β-cholest-7-en-6-one.

The boronate protecting group was removed in the same manner as for compound **17**. Boronate **10** (6.4 mg, 7.2 μmol) in methanol (150 μl) was treated with hydrogen peroxide (8 μl), yielding after NP-HPLC separation (DMW 925/75/1.5, R.T.=11.1 min) 4.2 mg (75 %) of compound **21**. IR spectrum: 3485 (ν_{O-H}); 1740 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{41}H_{62}O_{16}$: 833 [M+Na], 811 [M+H], 793 [M+H-H_2O], 775 [M+H-2H_2O], 463 [M+H-sugar], 445 [M+H-sugar-H_2O]. For ^{13}C and ^1H-NMR spectrum see Table 1 and 2.

20-Hydroxyecdysone 25-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (22). (20R,22R)-25-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2β,3β,14α,20,22-pentahydroxy-5β-cholest-7-en-6-one.

The boronate protecting group was removed in the same manner as for compound **17**. Boronate **11** (4.9 mg, 5.4 μmol) in methanol (100 μl) was treated with hydrogen peroxide (8 μl), yielding after NP-HPLC separation (DMW 925/75/1.5, R.T.=16.0 min) 4.0 mg (91 %) of compound **22**. IR spectrum: 3485 (ν_{O-H}); 1741 ($\nu_{C=O}$ ester); 1662 ($\nu_{C=O}$ ketone); 1626 ($\nu_{C=C}$) cm^{-1}. Mass spectrum, $C_{41}H_{62}O_{16}$: 811 [M+H], 793 [M+H-H_2O], 775 [M+H-2H_2O], 463 [M+H-sugar] 445 [M+H-sugar-H_2O]. For ^{13}C and ^1H-NMR spectrum see Table 1and 2.

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Table 1 Carbon-13 Chemical Shifts of Ecdysteroid Derivatives in Methanol-d_4

Carbon	1	2	3 [a]	4	5	17	18	19	20	21	22
C-1	37.36	36.11	38.99	37.38	37.36	35.01	34.96	34.96	35.91	38.43	37.38
C-2	68.70	76.40	68.34	68.71	68.70	70.15	70.12	70.16	77.33	67.73	68.70
C-3	68.52	65.98	77.39	68.52	68.51	68.75	68.70	68.67	66.20	77.45	68.53
C-4	32.86	32.10	30.80	32.86	32.86	30.19	30.14	30.17	33.66	30.07	32.90
C-5	51.79	51.84	51.41	51.80	51.78	52.48	52.47	52.46	51.69	52.03	51.81
C-6	206.45	206.16	203.56	206.45	206.44	204.63	204.70	204.72	206.08	205.24	206.50
C-7	122.13	122.11	121.69	122.12	122.17	122.06	122.07	121.98	122.15	122.12	122.10
C-8	167.97	168.14	166.75	167.95	167.92	168.20	168.15	168.37	168.11	167.90	168.09
C-9	35.09	34.99	34.35	35.08	35.10	35.12	35.07	35.12	35.00	35.05	35.16
C-10	39.26	39.51	38.73	39.28	39.27	39.38	39.38	39.32	39.45	39.12	39.26
C-11	21.50	21.50	21.15	21.53	21.46	21.52	21.57	21.56	21.50	21.57	21.56
C-12	32.51	32.47	32.03	32.64	32.50	32.45	32.54	32.45	32.50	32.48	32.55
C-13	b	48.57	48.15	b	b	b	b	48.58	b	b	b
C-14	85.23	85.23	84.30	85.26	85.37	85.19	85.16	85.17	85.17	85.24	85.23
C-15	31.78	31.74	31.80	31.84	31.76	31.83	31.87	31.82	31.75	31.80	31.79
C-16	21.50	21.38	21.57	21.40	21.46	21.52	21.36	21.56	21.50	21.49	21.56
C-17	50.53	50.52	50.20	51.14	50.43	50.56	51.09	50.49	50.55	50.52	50.54
C-18	18.05	18.05	17.99	18.14	18.06	18.04	18.32	18.08	18.01	18.04	18.08
C-19	24.40	24.23	24.12	24.40	24.40	24.18	24.15	24.22	24.30	24.41	24.42
C-20	77.90	77.92	77.03	77.46	77.96	77.91	77.26	77.94	77.90	77.90	77.97
C-21	21.05	21.05	21.80	22.39	21.06	21.08	22.20	20.91	21.06	21.03	21.08
C-22	78.42	78.42	77.73	89.70	78.41	78.44	91.16	78.17	78.44	78.43	78.17
C-23	27.34	27.33	27.56	27.60	26.69	27.37	27.60	27.00	27.37	27.34	26.96
C-24	42.40	42.38	42.73	40.93	40.10	42.40	41.47	40.95	42.40	42.40	41.05
C-25	71.29	71.29	69.79	71.38	78.67	71.30	71.04	79.46	71.30	71.30	79.48
C-26	29.70	29.71	30.12	29.65	27.35	29.70	29.86	26.83	29.72	29.72	26.83
C-27	28.95	28.95	30.04	29.05	27.35	29.01	28.92	26.48	28.96	28.94	26.31
Glc:C-1′	--	102.68	103.98	105.71	98.66	--	102.76	96.30	100.66	100.03	96.32
C-2′	--	75.19	75.03	75.44	75.30	--	73.23	73.16	73.02	72.88	73.16
C-3′	--	77.87	78.74	78.08	78.19	--	72.88	72.47	72.94	72.75	72.51
C-4′	--	71.64	71.73	71.42	71.87	--	69.97	70.13	69.84	69.86	70.17
C-5′	--	77.97	78.54	77.96	779.00	--	74.36	74.34	74.44	74.24	74.41
C-6′	--	62.71	62.70	62.43	63.13	--	63.30	63.44	63.27	63.07	63.43
Ac: CO	--	--	--	--	--	--	172.23	172.32	171.71(2)	172.34	172.33
							172.07	171.99(2)	171.50	171.62	171.69
							172.00	171.67	171.30	171.48	171.34
							171.59	171.34		171.27	171.14
							171.24	171.12			
							171.01				
CH_3	--	--	--	--	--	--	21.10	21.02	20.75(2)	21.03	20.89
							20.90(2)	20.91(2)	20.55(2)	20.60	20.68
							20.69	20.70		20.54(2)	20.57(2)
							20.51(2)	20.60(2)			

[a] Data from d_5-pyridine solution (insoluble in methanol-d_4); [b] overlapped with a strong signal of solvent at ca 49.0 ppm

Table 2 Proton NMR Spectra of Ecdysteroids 1 - 22 in Acetone-d_6

Proton	Chemical shifts / (Coupling constants)										
	1	2	3 [a]	4	5	6	7	8	9	10	11
H-2	3.86 ddd (12.0;4.3;3.4)	4.00 ddd (12.0;4.2;3.0)	4.11 dt (12.3;3.0;3.0)	3.82 ddd (12.0;4.0;3.0)	3.85 m	5.07 ddd (12.4;4.5;3.0)	5.08 ddd (12.5;4.0;3.0)	3.84 m	4.06 ddd (12.0;4.0;3.0)	3.78 m	3.84 m
H-3	3.93 bq (3.3)	4.13 bq (3.0)	4.31 bq (3.0)	3.90 um	3.92 um	5.29 q (3.0)	5.30 um	3.91 bq (3.0)	4.10 m	4.05 um	3.91 um
H-5	2.33 dd (11.0;6.5)	2.33 dd (10.5;6.0)	2.93 dd (13.3;3.5)	b	b	b	2.28 dd (13.0;4.4)	2.34 dd (12.5;5.0)	2.36 dd (9.2;8.0)	b	2.34 dd (12.2;5.0)
H-7	5.74 d (2.6)	5.71 d (2.5)	6.21 d (2.3)	5.71 d (2.4)	5.72 d (2.5)	5.78 d (2.3)	5.77 d (2.2)	5.73 d (2.5)	5.73 d (2.5)	5.75 d (2.4)	5.73 d (2.5)
H-9	3.16 ddd (11.0;7.2;2.6)	3.14 m	3.53 m	3.16 m	3.16 m	3.26 m	3.28 m	3.18 ddd (11.0;7.5;2.5)	3.16 m	3.18 m	3.18 ddd (12.0;7.0;2.4)
H-17	2.45 t (9.2)	2.45 t (9.0)	2.98 t (9.0)	2.33 t (9.0)	2.46 t (9.0)	2.47 t (8.8)	2.46 t (8.6)	2.50 t (8.8)	2.50 t (8.8)	2.49 t (8.8)	2.50 t (9.0)
H-22	3.37 dd (10.7;1.8)	3.36 dd (10.0;1.5)	3.87 dd (9.6;1.0)	3.48 bd (9.6; <2)	3.35 bd (10.0; <2)	4.90 dd (10.2;1.7)	4.87 bd (10.0;<2)	4.22 dd (8.0;4.5)	4.22 dd (9.4;3.7)	4.22 dd (9.0;4.0)	4.20 dd (10.0;2.8)
Me-18	0.914 s	0.910 s	1.170 s	0.900 s	0.910 s	0.918 s	0.92 s	1.015 s	1.004 s	1.009 s	1.010 s
Me-19	0.944 s	0.945 s	0.858 s	0.935 s	0.939 s	1.021 s	1.02 s	0.966 s	0.972 s	0.931 s	0.961 s
Me-21	1.198 s	1.196 s	1.576 s	1.188 s	1.192 s	1.298 s	1.30 s	1.405 s	1.399 s	1.403 s	1.405 s
Me-26	1.185 s	1.179 s	1.374 s	1.160 s	1.245 s	1.161 s	1.21 s	1.233 s	1.222 s	1.228 s	1.305 s
Me-27	1.178 s	1.170 s	1.374 s	1.154 s	1.216 s	1.144 s	1.20 s	1.220 s	1.208 s	1.214 s	1.281 s
H-1'	–	4.53 d (7.8)	4.90 d (7.8)	4.41 d (7.6)	4.50 d (7.7)	--	4.94 d (7.9)	--	4.99 d (8.1)	4.85 d (8.1)	5.00 d (8.0)
H-2'	--	3.18 dd (7.8;9.0)	4.03 dd (7.8;9.0)	3.2-3.4 m	3.14 dd (7.7;9.0)	--	4.87 dd (7.9;9.3)	--	4.88 dd (8.1;9.7)	4.97 dd (8.1;9.8)	4.87 dd (8.0;9.8)
H-3'	--	3.40 t (9.0;9.0)	4.22 t (9.0;9.0)	3.2-3.4 m	3.41 t (9.0;9.0)	--	5.30 dd (9.3;9.8)	--	5.24 dd (9.7;9.5)	5.30 dd (9.8;9.4)	5.28 dd (9.8;9.4)
H-4'	--	3.36 t (9.0;9.0)	4.18 t (9.0;9.0)	3.2-3.4 m	3.24 t (9.0;9.0)	--	4.37 dd (9.8;10.1)	--	5.01 dd (9.5;10.0)	5.04 dd (9.4;10.0)	4.97 dd (9.4;10.0)
H-5'	–	3.32 m (9.0;2.5;5.0)	3.92 ddd (9.0;2.2;5.8)	3.2-3.4 m	3.31 ddd (9.0;2.5;6.7)	--	3.95 ddd (10.1;5.5;2.6)	--	3.97 ddd (10.0;5.6;2.4)	3.98 ddd (10.0;5.5;2.5)	3.96 ddd (10.0;5.7;2.4)
H-6a'	–	3.84 dd (11.5;2.5)	4.53 dd (11.7;2.2)	3.84 dd (12.0;2.4)	3.84 dd (11.7;2.5)	--	4.25 dd (12.1;5.5)	--	4.24 dd (12.2;5.6)	4.28 dd (12.2;5.5)	4.22 dd (12.0;5.7)
H-6b'	--	3.67 dd (11.5;5.0)	4.30 dd (11.7;5.8)	3.68 dd (12.0;4.8)	3.58 dd (11.7;6.7)	--	4.08 dd (12.1;2.6)	--	4.14 dd (12.2;2.4)	4.09 dd (12.2;2.5)	4.07 dd (12.0;2.4)
Ac:	--	--	--	--	--	2.08 s	2.01 s	--	1.991 s	2.156 s	2.021 s
						2.05 s	1.98 s		1.991 s	2.017 s	1.991 s
						1.94 s	1.94 s		1.983 s	1.995 s	1.982 s
							1.93 s		c	1.963 s	1.935 s
							c				
BPh: o-	--	--	--	--	--	--	--	7.77 m	7.77 m	7.78 m	7.78 m
m-	--	--	--		--	--	--	7.38 m	7.38 m	7.39 m	7.39 m
p-	--	--	--		--	--	--	7.49 m	7.48 m	7.49 m	7.48 m

[a] Data from d_5-pyridine (3 is insoluble in methanol-d_4); [b] not determined; [c] other acetate signals are overlapped with solvent peak.

Table 2 - continued

Proton	Chemical Shifts / (Coupling Constants)										
	12	13	14	15	16	17	18	19	20	21	22
H-2	4.96 ddd (12.2;5.0;3.0)	5.08 ddd (12.5;4.4;3.0)	5.09 ddd (12.3;4.3;3.0)	4.88 ddd (12.8;4.0;3.5)	4.96 ddd (12.0;4.0;3.0)	5.07 ddd (12.4;4.3;3.0)	5.06 ddd (12.4;4.5;3.0)	5.08 ddd (12.0;4.5;3.0)	4.04 dm (12.0;4.5;3.0)	3.76 ddd (12.0;4.0;3.0)	3.83 m
H-3	4.10 um	5.30 um	5.30 um	4.23 um	4.10 um	5.29 um	5.29 um	5.30 um	4.10 um	4.04 um	3.91 um
H-5	2.43 dd (13.2;4.2)	2.30 dd (13.3;3.8)	b	b	2.43 dd (13.2;4.0)	b	2.28 dd (13.5;4.0)	2.29 dd (13.0;4.5)	2.36 dd (11.0;6.3)	2.36 m	2.33 dd (11.3;6.0)
H-7	5.76 d (2.5)	5.78 d (2.5)	5.78 d (2.4)	5.78 d (2.4)	5.76 d (2.4)	5.77 d (2.5)	5.75 d (2.5)	5.77 d (2.3)	5.75 d (2.5)	5.73 d (2.6)	5.71 d (2.5)
H-9	3.27 ddd (11.5;7;2.5)	3.29 um	3.29 m	3.24 m	3.27 m	3.29 m	3.26 m	3.27 m	3.14 m	3.15 m	3.17 ddd (11.5;7;2.5)
H-17	2.51 t (8.7)	2.51 t (8.8)	2.51 t (9.9)	2.50 t (8.7)	2.51 t (8.8)	2.46 t (8.6)	2.40 t (9.0)	2.46 t (8.6)	2.45 t (9.0)	2.45 t (9.0)	2.45 t (9.0)
H-22	4.23 dd (9.3;4.0)	4.22 dd (9.4;4.0)	4.23 dd (8.5;4.5)	4.22 dd (9.0;3.4)	4.21 dd (10.5;3.0)	3.36 bd (10.1;<2)	3.52 bd (9.0; <2)	3.35 dd (10.4;1.8)	3.36 dd (10.5;1.5)	3.35 dd (10.4;1.0)	3.35 ddd (10.0;5.0;1.7)
Me-18	1.020 s	1.021 s	1.02 s	1.017 s	1.020 s	0.92 s	0.924 s	0.922 s	0.900 s	0.908 s	0.912 s
Me-19	1.002 s	1.043 s	1.04 s	0.976 s	1.004 s	1.03 s	1.018 s	1.022 s	0.949 s	0.908 s	0.937 s
Me-21	1.408 s	1.404 s	1.42 s	1.400 s	1.411 s	1.20 s	1.186 s	1.195 s	1.195 s	1.167 s	1.194 s
Me-26	1.228 s	1.225 s	1.49 s	1.222 s	1.307 s	1.18 s	1.181 s	1.244 s	1.186 s	1.176 s	1.243 s
Me-27	1.214 s	1.210 s	1.47 s	1.207 s	1.284 s	1.175 s	1.164 s	1.215 s	1.176 s	1.176 s	1.214 s
H-1'	--	--	--	4.75 d (8.1)	5.00 d (8.2)	--	4.93 d (8.2)	4.94 d (8.2)	4.98 d (8.1)	4.84 d (8.0)	4.95 d (8.0)
H-2'	--	--	--	4.95 dd (8.1;9.7)	4.87 dd (8.2;9.7)	--	5.01 dd (8.2;9.6)	4.85 dd (8.2;9.3)	4.88 dd (8.1;9.6)	4.96 dd (8.0;9.7)	4.85 dd (8.0;9.8)
H-3'	--	--	--	5.28 dd (9.7;9.6)	5.28 dd (9.7;10.0)	--	5.31 t (9.6;9.6)	5.29 dd (9.3;9.7)	5.24 t (9.6;9.6)	5.30 dd (9.7;9.5)	5.28 dd (9.8;9.5)
H-4'	--	--	--	5.00 dd (9.6;9.9)	4.97 t (10.0;10.0)	--	5.00 dd (9.6;10.0)	4.96 dd (9.7;10.0)	5.01 dd (9.6;10.0)	5.04 dd (9.5;10.0)	4.95 dd (9.5;10.0)
H-5'	--	--	--	3.89 ddd (9.9;5.8;2.4)	3.97 ddd (10.0;5.6;2.5)	--	4.08 ddd (10.0;7.5;2.5)	3.94 ddd (9.7;5.6;2.5)	3.96 ddd (10.0;5.5;2.5)	3.98 ddd (10.0;5.5;2.5)	3.95 ddd (10.0;5.5;2.5)
H-6a'	--	--	--	4.25 dd (12.4;5.8)	4.22 dd (12.0;5.6)	--	4.22 dd (12.0;7.5)	4.23 dd (12.0;5.6)	4.24 dd (12.3;5.5)	4.28 dd (12.3;5.5)	4.22 dd (12.0;5.5)
H-6b'	--	--	--	4.02 dd (12.4;2.4)	4.07 dd (12.0;2.4)	--	4.11 dd (12.0;2.5)	4.09 dd (12.0;2.5)	4.14 dd (12.3;2.5)	4.08 dd (12.2;2.5)	4.09 dd (12.0;2.5)
Ac:	c	2.086 s	1.94 s	2.196 s	2.022 s	2.06 s	2.081 s	2.082 s	1.99s(2x)	2.154 s	2.03 s
		1.942 s	1.91 s	1.989 s	1.997 s	1.94 s	2.070 s	2.023 s	1.94 s	2.014 s	2.01 s
			c	1.979 s	1.993 s		2.008 s	2.012 s	c	1.994 s	1.99 s
				1.963 s	1.983 s		1.952 s	1.989 s		1.962 s	1.94 s
					1.937 s		1.933 s	1.935s(2x)			
BPh: o-	7.78 m	7.78 m	7.79 m	7.77 m	7.78 m	-	-	-	-	-	-
m-	7.39 m	7.39 m	7.39 m	7.39 m	7.39 m	-	-	-	-	-	-
p-	7.49 m	7.49 m	7.49 m	7.48 m	7.48 m	-	-	-	-	-	-

Table 3 Glucosylation Shifts in Proton and Carbon-13 NMR Spectra of Ecdysteroids

			Proton NMR spectra					Carbon-13 NMR spectra								
Subst.	**Position**	**Comp.**	**H-2**	**H-3**	**Me-19**			**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-10**			
Glc	**2**	(2-1)	0.14	0.20	0.00			-1.25	7.70	-2.54	-0.76	0.05	0.25			
	3	(3-1)[a]	-0.06	0.10	-0.20			0.90	0.01	9.16	-1.73	-0.07	-0.07			
Glc(Ac)	**2**	(9-8)	0.22	0.19	0.01			b	b	b	b	b	b			
		(20-1)	0.18	0.17	0.01			-1.45	8.63	-2.32	0.80	-0.10	0.19			
	3	(10-8)	-0.06	0.14	-0.04			b	b	b	b	b	b			
		(21-1)	-0.10	0.11	-0.04			1.07	-0.97	8.93	-2.79	0.24	-0.14			
		(15-12)	-0.08	0.13	-0.03			b	b	b	b	b	b			
			Me-18	**Me-21**	**H-22**	**Me-26**	**Me-27**	**C-17**	**C-20**	**C-21**	**C-22**	**C-23**	**C-24**	**C-25**	**C-26**	**C-27**
Glc	**22**	(4-1)	-0.01	-0.01	0.11	-0.02	-0.02	0.61	-0.44	1.34	11.28	0.26	-1.47	0.09	-0.05	0.10
	25	(5-1)	0.00	-0.01	-0.02	0.06	0.04	-0.10	0.06	0.01	-0.01	-0.65	-2.30	7.38	-2.35	-1.60
Glc(Ac)	**22**	(18-17)	0.00	-0.01	0.16	0.00	-0.01	0.53	-0.65	1.12	12.72	0.23	-0.93	-0.26	0.16	-0.09
	25	(11-8)	0.00	0.01	-0.02	0.07	0.06	b	b	b	b	b	b	b	b	b
		(7-6)	0.00	0.00	-0.03	0.05	0.06	b	b	b	b	b	b	b	b	b
		(22-1)	0.00	0.01	-0.02	0.06	0.04	0.01	0.07	0.03	-0.25	-0.38	-1.35	8.19	-2.87	-2.64
		(16-12)	0.00	0.00	-0.02	0.08	0.07	b	b	b	b	b	b	b	b	b
		(19-17)	0.00	0.00	-0.01	0.06	0.04	-0.07	0.03	-0.17	-0.27	-0.37	-1.48	8.16	-2.87	-2.53

[a] The shift values from NMR data obtained in pyridine-d_5 solution (data for **1** in pyridine-d_5 taken from ref. 21); [b] carbon-13 spectra of corresponding compounds were not measured.

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