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Ecdysteroids. Selective Protections and Synthesis of Potential Tools for Biochemical Studies*

Denis Guédin-Vuong, Yoichi Nakatani and Guy Ourisson

Centre de Neurochimie CNRS, Université Louis Pasteur, 5 rue Blaise Pascal, 67084, Strasbourg, France

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β-Ecdysone and cyasterone have been transformed into the ecdysterone analogue *4*, which carries a [3-(*p*-hydroxyphenyl)--propoxyl]- group at the end of the side chain. This derivative should be useful for various biochemical investigations (I-labelling, affinity chromatography), since it exhibits a marked ecdysterone--like activity in two independent biological tests. The synthetic scheme makes use of highly selective protections of the hydroxyl groups of phytoecdysteroids.

The mechanism of the activity of the insect moulting hormones, the ecdysteroids, is relevant to the fundamental questions of molecular biology (in particular concerning the regulation of gene expression); it is not well understood at the molecular level[1]. There is, therefore, a need for ecdysteroid derivatives which, while retaining a biological activity close to that of the natural hormones, would have structures enabling one to use them for, say, competitive assay, affinity purification or photoaffinity labelling, etc., of the hormone-binding partners in the target cells. We describe here such an attempt, at least partially successful but not yet put to biological use: the synthesis of an ecdysteroid carrying a C—C linked »arm« potentialy useful for further binding, and yet still biologically active, though not at a high level.

The syntheses of such analogues may start from easily available phyto--ecdysteroids such as ecdysterone (β-ecdysone) *1*, cyasterone *2*, or inokosterone *3*[2–4]. The presence, in these potential starting materials, of a 20,22--dihydroxy grouping suggests that it should be possible to cleave selectively the side-chain and to reconstruct it in a form carrying a suitable terminal substitution. This would of course necessitate differentiation of the numerous hydroxyl groups present in the molecule, using either highly selective reactions, or highly selective protection-deprotection sequences.

We report here four convenient and high yield methods enabling one: — either to protect the 20,22-diol only, — or to protect the 2,3-diol only-, — or to protect both diols-, — or to protect both the 2,3-diol and (after cleavage of the side-chain) the 14-hydroxyl.

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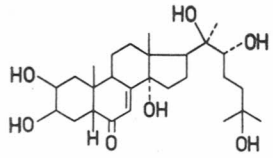
* Dedicated to Professor Mihailo Lj. Mihailović on the occasion of his 60th birthday.



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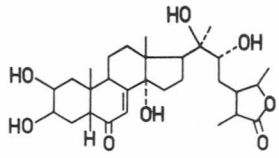

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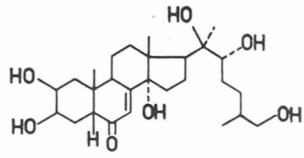

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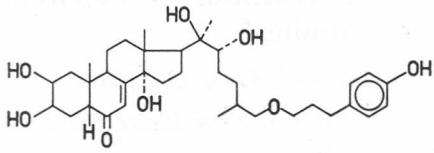

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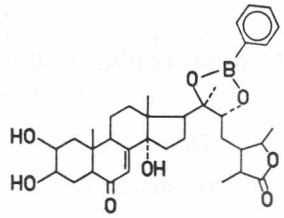

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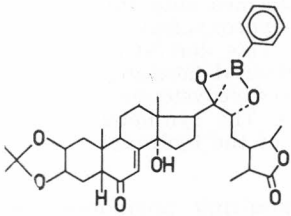

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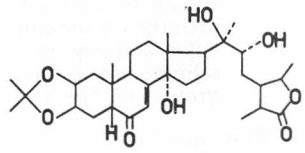

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These procedures enabled us to obtain derivative *4* [R+S at C-25, similar to naturally occurring inokosterone[5]], which could now be labelled ^{125}I, and/or linked to a polymeric support; as we hoped, *4* shows some biological activity, only slightly weaker than that of ecdysone in two independent tests.

Protection of the 20,22-Diol Only

The use of acetonide-forming reagents leads to only marginally preferential protection of the 20,22-diol: for example, treatment of cyasterone *2* in dry acetone for 2 hrs at room temperature with 3 eq of TsOH converts it into the 20,22-monoacetonide in 60% yield, along with 34% of the 2,3;20,22-bis-acetonide.[3] However, we found that cyasterone gives quantitatively the 20,22-phenylboronate *5* when treated with phenylboronic acid (1.1 eq or more) in dry THF of DMF at room temperature for 30 min; the assigned structure is fully supported by the spectroscopic data summarised in the experimental part, and by the smooth and quantitative recovery of cyasterone by treatment with aqueous neutral hydrogen peroxide (see below). Phenylboronates like *5* are stable to acidic acetalisation conditions, highly crystalline, and easily handled.

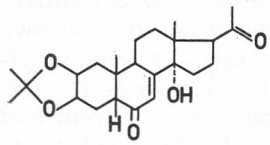
Protection of Both Diols

The 20,22-phenylboronates such as *5* are further protected at the 2, 3 position by suitable acetonide-forming reagents. The reaction [as indicated by the experiments reported above with the unprotected cyasterone[3]] requires

rather strong conditions; treatment for 2 hrs at room temperature, with 1 to 3 eq of TsOH, H_2O in dry acetone, does not bring it to completion, but longer reaction times or higher temperatures lead to the formation of less polar dehydration products. However, the joint use[6] of a catalytic amount (0.01 eq) of fused TsOH and of a trans-acetalisation combination (10 eq dimethoxypropane in dry acetone for a few hours at room temperature) leads quantitatively to the formation of the 2,3-acetonide *6*. The acetonide protecting group is fully stable in the conditions used to remove the phenylboronate moiety, but may be cleaved easily under slightly acidic conditions (see below).

Protection of the 2,3-Diol Only

Our preferred procedure for the selective protection of the 2,3-diol is a three-step process, the first two leading to the fully protected *6*, and the third one being the oxidative hydrolysis of the phenylboronate with neutral aqueous hydrogen peroxide in EtOH or THF at room temperature for a few hours[7]. Again, the reaction is essentially quantitative, giving the 2,3-mono--acetonide 7.



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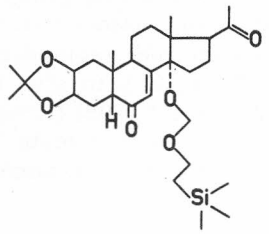

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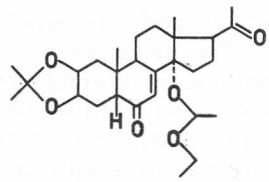

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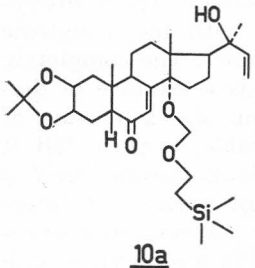

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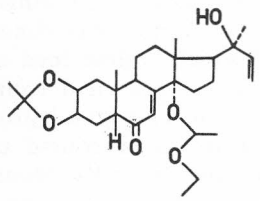

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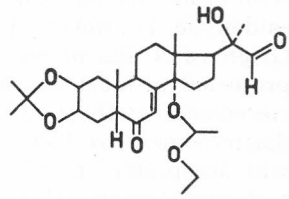

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A mono-protected ecdysteroid such as 7 can be cleaved at C-20; we used preferentially a phase-transfer catalytic process [THF/half-saturated brine, $NaIO_4$, benzyl-dimethyl-hexadecyl-ammonium chloride[8]], giving 89% of the 20-ketone *8*. The four-step sequence leading from *1* or *2* to *8* can be realised without isolation of intermediates, by introducing the phenylboronate and the acetonide groups in DMF, and removing the phenylboronate and cleaving the side-chain in THF; the overall yield in pure, crystallised product *8* is 74% from *1* and 69% from *2*.

Protection of the 14-hydroxy Group

The diketone *8* cannot be used directly to obtain regioselectively products of nucleophilic addition at C-20: in accordance with ref. 9, we found that direct addition of vinyl magnesium bromide led to considerable addition onto the enone group. Faced with this problem, Mori and coworkers, in their synthesis of ecdysterone *1*[10], hydrolysed the acetonide, thus obtaining subsequently a heterogeneous Grignard reaction which was indeed regiospecific in the desired sense, but only at low conversions (40%). Our solution is quite different: we have further protected the intermediate *8* at the 14-hydroxyl, hoping that the presence of a larger group around the α-face of ring B might improve the selectivity of attack at C-20; this has indeed been found to be the case.

Reaction of *8* with SEM-Cl [2-(trimethylsilyl)ethoxymethyl chloride] according to the procedure of Lipshutz et al.[11] gave *9a* (88%). However, the SEM protecting group turned out to be very difficult to remove after reconstruction of the side-chain. We then turned to the alternate 14-protection with ethyl--vinyl-ether giving *9b*, although in a somewhat lower yield (70%). This is, of course, a mixture of diastereomers at the newly introduced asymmetric carbon, and does not crystallise. Both acetal groups of *9b* or of its derivatives can be easily removed by an acidic resin-assisted methanolysis (see below).

The 14-ethers *9* react, as expected, regioselectively at C-20 with vinyl--magnesium bromide (THF, —50 °C, 3h), to give the vinyl-carbinols *10: 10a*, (20S) + *10a* (20R) (81%), *10b* (20R + *10b* (20S) (75%). In each case, a mixture of diastereomers was obtained in an approximate 9 : 1 ratio. By analogy to reactions on similar substrates[10], we have assumed the major isomers to be the desired 20R ones.

Completion of the Synthesis of 4

The completion of the synthesis of *4* followed largely the procedures of Hikino et al. in their synthesis of inokosterone *3*.[5] The vinyl group was cleaved with ozone, the ozonide being decomposed with dimethylsulfide[12]. The hydroxy--aldehyde *11*, obtained in a 65% yield, was condensed with the acetylenic Grignard *A*, the preparation of which is described elsewhere[13]. The completely protected product *12*, obtained in a 52% yield from *10b*, is a mixture of diastereomers at the two temporary acyclic acetal groups, but also a mixture of diastereomers at C-25; this feature is of course undesirable, but we felt it was acceptable in this case, as the naturally occurring inokosterone itself is a diastereomeric mixture at C-25[5]. Of course, we did not purify any intermediate or the end-product by crystallization, but only by low resolution chromatography. The intermediate *12* can be deprotected to *13* in a 57% yield with an acidic resin, and hydrogenated catalytically to *4* (66%), the ^{13}C NMR spectrum of which showed it to be a 1 : 1 mixture of C-25 diastereomers (Cf., for inokosterone, ref. 5). The overall yield of the 10-step sequence leading from *1* to *4* is 7.5%.

Biological Activity

The final product *4* was tested on *Drosophila* chromosomes by a puff-test[14] by Dr. G. Richards (Prof. P. Chambon's laboratory); it shows a dose-dependant activity similar to, but slightly lower than that of ecdysone, and is 10^{-2} and

10^{-3} less active in this test than ecrysterone *1* and ponasterone, respectively. It has also been tested in the imaginal disk system by Dr. J. P. Roussel (Dr. J. Hoffmann's laboratory), and again found to be biologically active at a level comparable with ecdysone, whereas its acetylenic precursor *13* is inactive in this test. These results will be described in detail later; let us only note now that they justify the hopes placed on the synthesis of such ecdysone analogues, despite their marked structural divergence from the naturally occurring hormones.

EXPERIMENTAL

Melting points were measured on a Reichert hot-stage microscope and are uncorrected. $[\alpha]_D$ were measured on a Perkin-Elmer 141 polarimeter, infra-red spectra on a Perkin-Elmer 177 instrument, NMR spectra on a Bruker SY apparatus (200 MHz) in $CDCl_3$ or in d_5-pyridine, with TMS as internal standard. Mass spectra were measured on a double-focusing Thompson-CSF THN-208 B instrument or an LKB 9000 S apparatus by direct introduction. HPLC was carried out on a Du Pont instrument incorporating a 870 pump module with a Model 8800 gradient controller. Analytical TLC was performed on Merck, Darmstadt, F 254 silica gel plates, 0.25 mm thick. Microanalyses were conducted by Mrs. M. François, Institut de Chimie, Strasbourg.

Cyasterone-20,22-phenylboronate 5 *from cyasterone* 2

Cyasterone *2* (5.2 g, 10 mmoles) was placed in a 500 ml, argon-flushed vessel, and dissolved in 25 mL dry (distilled over LAH) THF. Phenylboronic acid $PhB(OH)_2$ (1.35 g, 11 mmole was added, and the vessel was gently shaken to achieve dissolution. After 20 min at room temperature, a TLC check (elution with CH_2Cl_2/MeOH 85/15) showed the reaction to be complete. The solvent was removed under reduced pressure at 30 °C; the solid residue was collected, and finely pulverized in a mortar; it was deposited in a minute amount of chloroform on top of the chromatographic column (SiO_2: 100 g, in AcOEt, diameter 6 cm, height 7 cm). Elution with a gradient of MeOH in AcOEt (0—5%) eluted first the excess phenylboronic acid, and then the desired phenylboronate *5* (5.49 g, 9 mmoles). The analytical sample was obtained crystalline from cyclohexane-AcOEt; m. p. 164—167 °C; $[\alpha]_D$ ($CHCl_3$, 20 °C) = + 115° (*c* = 0.8 mg/mL).

Anal. $C_{35}H_{47}O_8B$ (606.18) calc'd.: C 69.31; H 7.81%
found: C 69.3 ; H 7.95%

^{1}H-NMR, 200 MHz (d_5Py): δ ppm: 1.12 (6H, s, Me-18 and -19), 1.37 (3H, d, J = = 7Hz, Me-27 or -29), 1.45 (3H, s, Me-21), 1.49 (3H, d, J = 6 Hz, Me-29 or -27), 3.06 (1H, dd, J_1 = 12 Hz, J_2 = 4 Hz, H-5), 3.64 (2H, m, H-9 and -22), 4.20 (1H, m broad, H-2), 4.26 (1H, m sharp, H-3), 4.30 (1H, m, H-28), 6.28 (1H, d, J = 2 Hz, H-7), 6.60 (1H, s, OH-14), 7.50 and 8.09 (5H, m, aromatic).

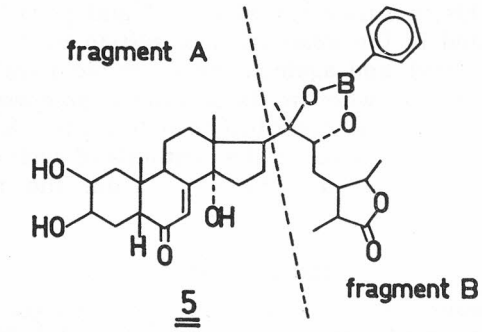
^{13}C-NMR, 50 MHz (d_5Py): δ ppm: 15.6 (C-29), 17.3 (C-18), 20.0 (C-27), 21.1 (C-11), 22.2 (C-16), 22.8 (C-21), 24.5 (C-19), 31.7 (C-12), 31.7 (C-15), 32.5 (C-23), 34.5 (C-9) 38.1 (C-1), 38.8 (C-10), 42.3 (C-24), 47.9 (C-13), 49.4 (C-25), 51.5 (C-5), 52.4 (C-17), 68.2 (C-2), 68.2 (C-3), 80.1 (C-28), 82.7 (C-22), 84.3 (C-14), 87.1 (C-20), 122.1 (C-7), 128.5 (ar.), 131.8 (ar.), 165.2 (C-8), 178.8 (C-26), 203.4 (C-6); C-4 may be at 33.8.

IR ($CHCl_3$): ν (cm^{-1}): 3420, 2940, 1760, 1655, 1600, 1500, 1440, 1355, 1100, 1050, 700, 650.

MS (EI, 70 eV, 250 °C): *m/e* = 606 (8%, M^+), 588 (74, M^+—H_2O), 413 (34), 335 (37), 327 (89), 301 (30, A^+—H_2O, see below), 287 (100, B^+), 249 (94), 173 (94), 161 (31), 105 (86).

Cyasterone-20,22-phenylboronate-2,3-acetonide 6 *from* 5

The phenylboronate *5* (5.44 g, 9 mmole) was placed in a 250 mL, argon-flushed vessl, then dissolved in a minimal amount of anhydrous DMF (ca 20 mL), which



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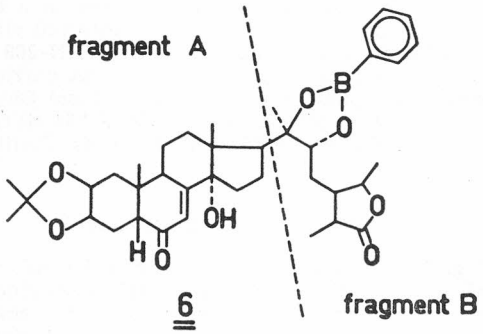

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was diluted with dry acetone (60 mL), 2,2-dimethoxypropane (10 mL, 10 eq), and fused TsOH (30 mg, 0.02 eq.). The vessel was gently shaken to achieve dissolution, and left at room temperature; after 3 hrs, a TLC check (elution with CH_2Cl_2/MeOH 95/5) showed the reaction to be complete. The acetone and dimethoxypropane were removed under reduced pressure at 30 °C. The remaining DMF solution was diluted with 150 mL AcOEt, transferred to a separatory funnel, washed with saturated brine six times to extract the DMF, dried over Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave 5.28 g (91% if pure) of a product shown by TLC to contain approximately 5% of 7, the substance expected from the next step, as the only significant contaminant. The crude product was used directly for the next step.

Estimated yield: 85%. An analytical sample was crystallized in MeOH; m. p. 248—250 °C; $[\alpha]_D$ ($CHCl_3$, 20 °C) : +48.5° (c = 3 mg/mL).

Anal. $C_{38}H_{57}O_8B$ (646.21) calc'd.: C 70.50; H 7.95%
found: C 69.4; H 7.9 %

^{1}H-NMR, 200 MHz (d_5Py): δ ppm: 1.06 (3H, s, Me-19), 1.11 (3H, s, Me-18), 1.35 (3H, s, acetonide), 1.36 (3H, d, J = 7 Hz, Me 27 or 29), 1.48 (3H, s, Me-21), 1.49 (3H, d, J = 6 Hz, Me-29 or 27), 1.58 (3H, s, acetonide), 3.21 (1H, m, H-5), 3.60 (1H, m, H-9), 4.15 (2H, m, H-2 and -3), 4.30 (2H, m, H-22 and -28), 6.23 (1H, d, J = 2 Hz, H-7), 6.63 (1H, s, OH-14), 7.50 and 8.09 (5H, m, aromatic H's).

^{13}C-NMR, 50 MHz (d_5Py): δ ppm: 15.6 (C-29), 17.2 (C-18), 20.0, (C-27), 21.2 (C-11), 22.2 (C-16), 22.8 (C-21), 23.9 (C-19), 26.8 (acetonide), 27.1 (C-4), 28.9 (acetonide), 31.5 (C-12), 31.7 (C-15), 33.8 (C-23), 35.3 (C-9), 38.2 (C-1), 38.3 (C-10), 42.4 (C-24), 48.2 (C-13), 49.4 (C-25), 51.6 (C-5), 52.5 (C-17), 72.3 (C-2), 72.6 (C-3), 80.1 (C-28), 82.7 (C-22), 84.3 (C-14), 87.1 (C-20), 108.3 (acetonide), 121.7 (C-7), 128.5 (aromatic C's), 131.8 (id.), 164.7 (C-8), 178.8 (C-26), 202.1 (C-6).

IR ($CHCl_3$): analogous to that of 5, except for a noticeable decrease in the intensity of the OH band at 3420 cm^{-1}.

MS (EI, 70 eV, 270 °C): m/e = 646 (1%, M^+), 628 (8, $M^+—H_2O$); 613 (5, $M^+—H_2O—CH_3$), 570 (15), 500 (5), 341 (15, $A^+—H_2O$, see formula *6*), 309 (50), 287 (100, B^+, see formula 5), 267 (30).

Cyasterone-2,3-acetonide 7 *from* 6

The crude product resulting from the previous step (5 g, ca. 7.7 mmoles) was dissolved in aqueous THF (THF/H_2O 9/1, v/v, 50 mL) and treated with 1.2 eq H_2O_2 (as a 30% solution in water). After 3 hrs at room temperature, a TLC check (eluent: CH_2Cl_2/MeOH 9/1 v/v) showed the reaction to be complete. Water (50 mL) was added, and the THF was removed under reduced pressure at 30 °C. The remaining system was extracted repeatedly in a separatory funnel with AcOEt; the combined organic phases were washed twice with aqueous 1N NH_4Cl, with 1N $Na_2S_2O_3$, with 0.1 M HCl, with 1N NH_4Cl, and finally with saturated brine. After drying over Na_2SO_4 and removal of the solvent, the crude reaction mixture was chromatographed over silica gel (200 g, CH_2Cl_2/MeOH 96/4 v/v) on a wide column (diam. 6 cm, height 13 cm). Elution with a gradient of MeOH in CH_2Cl_2 (4—6%) eluted first phenol and then the desired product 7 (3.85 g, 6.9 mmole, 89%). An analytical sample was crystallized from AcOEt; m. p. 254—256 °C;

Anal $C_{32}H_{48}O_8$ (560.38) calc'd.: C 68.54; H 8.63%
found: C 69.3; H 8.7 %

^{1}H-NMR, 200 MHz (d_5Py): δ ppm: 1.03 (3H, s, Me-19), 1.26 (3H, s, Me-18), 1.35 (3H, s, acetonide), 1.36 (3H, d, J = 7 Hz, Me-27 or -29), 1.49 (3H, d, J = 6 Hz, Me-29 or -27), 1.58 (3H, s, acetonide), 1.62 (3H, s, Me-21), 3.25 (1H, m, H-5), 3.65 (1H, m, H-9), 4.10 (4H, m, H-2, 3, 22 and 28), 5.34 (1H, s, OH-20 or -22), 6.13 (1H, s, OH-22 or -20), 6.26 (1H, d, J = 2 Hz, H-7), 6.54 (1H, s, OH-14).

IR (KBr): ν (cm^{-1}) 3400, 2920, 1740, 1640, 1370, 1050.

MS (CI, NH_3, 40 °C): m/e = 578 (100%, $M^+NH_4^+$), 562 (40, $M^+NH_4^+—H_2O$), 544 (13, $M^+NH_4^+—2H_2O$).

Poststerone Acetonide 8 *from* 7

Cyasterone 2,3-acetonide 7 (1g, 1.8 mmole) was dissolved in THF (20 mL); a vigorous stirring was started and benzyldimethylhexadecylammonium chloride (80 mg, 0.1 eq.) was introduced. A solution of $NaIO_4$ (430 mg, 1.2 eq) in 10 mL of water and 10 mL of saturated brine was added slowly with stirring, while controlling the temperature. The resulting two-phase system was stirred at room temperature for 5 hrs, and a TLC check (elution with CH_2Cl_2/MeOH) showed the reaction to be complete. The reaction mixture was taken up into AcOEt (200 mL), washed 3 times with saturated brine, once with $Na_2S_2O_3$ 1N, once with HCl (1M) and twice with saturated brine; it was then dried over Na_2SO_4. After removal of the solvent under reduced pressure at 30 °C, the crude product was deposited on top of a chromatography column (SiO_2 in $CHCl_3$, diam. 2 cm, height 12 cm). Elution with a gradient of MeOH in $CHCl_3$ (0 to 5%) eluted the desired product (650 mg, 1.6 mmole, 89%). An analytical sample was crystallized from AcOEt/cyclohexane; m. p. 196—198 °C; $[\alpha]_D$ ($CHCl_3$, 20 °C) = +56° (c = 26 mg/mL).

Anal. $C_{24}H_{34}O_5$ (402.27) calc'd.: C 71.61; H 8.51%
found: C 72.7; H 9.6 %

^{1}H-NMR, 200 MHz (d_5Py): δ ppm: 0.68 (3H, s, Me-18), 0.96 (3H, s, Me-19), 1.35 (3H, s, acetonide), 1.57 (3H, s, acetonide), 2.17 (3H, s, Me-21), 2.58 (3H, m, H-17+?), 3.18 (1H, m, H-5), 3.60 (1H, t, J = 8 Hz, H-9), 4.14 (2H, m, H-2 and -3), 6.15 (1H, d, J = 2 Hz, H-7), 6.74 (1H, s, 0H-14).

^{13}C-NMR, 50 MHz (d_5Py): δ ppm: 17.2 (C-18), 21.2 (C-11), 22.0 (C-16), 23.9 (C-19), 26.8 (acetonide), 27.0 (C-4), 28.9 (acetonide), 30.6 (C-12), 30.9 (C-21), 31.8 (C-15), 35.2 (C-9), 38.0 (C-10), 38.3 (C-1), 48.5 (C-13), 51.6 (C-5), 59.5 (C-17), 71.1 (C-2), 72.5 (C-3), 84.0 (C-14), 108.2 (acetonide), 121.7 (C-7), 164.0 (C-8), 202.1 (C-6), 209.2 (C-20).

IR ($CHCl_3$: ν (cm^{-1}) 3450, 2950, 1700, 1660, 1060.

MS (EI, 70 eV, 40 °C): m/e = 402 (66%, M^+), 387 (100, $M^+—CH_3$, 345 (17), 326 (80), 290 (33), 284 (33).

Poststerone 2,3-Acetonide 14-SEM-ether 9a *from* 8

Poststerone acetonide 8 (201 mg, 0.5 mmole) was placed in a 50 mL, argon-flushed flask, and dissolved in dry CH_2Cl_2 (0.5 mL). Anhydrous diisopropylamine (650 mg, 086 mL, 5 mmoles) and SEM-chloride (500 mg, 0.54 mL, 3 mmoles) were introduced. The mixture was stirred under argon, carefully warmed to 35 °C, and kept at that temperature for 8 hrs, after which a TLC check (CH_2Cl_2/MeOH 97/3) showed it to be complete. The reaction mixture was cooled, diluted with AcOEt (30 mL) in a separatory funnel, and washed repeatedly with 0.1 N HCl, and then with saturated brine. After drying over Na_2SO_4 and removing the solvent under reduced pressure at 30 °C, the crude product was deposited on top of a chromatographic column (SiO_2/hexane). Elution with a gradient of AcOEt in hexane (0 to 100%) eluted the desired product *9a* at ca. 50% AcOEt (234 mg, 0.44 mmole, 88%). An analytical sample was crystallized from hexane; m. p. 114—118 °C; $[\alpha]_D$ ($CHCl_3$, 20 °C) = +60° (*c* = 5.8 mg/mL).

Anal. $C_{30}H_{48}O_6Si$ (532.46) calc'd.: C 67.61; H 9.09%
found: C 68.6; H 8.8 %

^{1}H-NMR, 200 MHz (d_5Py): δ ppm: 0.07 (9H, s, Me_3Si), 0.65 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.04 (2H, t, J = 8 Hz, CH_2—Si), 1.41 (3H, s, acetonide), 1.61 (3H, s, acetonide), 2.16 (3H, s, Me-21), 2.30 (3H, m, H-17+?), 2.88 (1H, m, H-5), 3.42 (1H, t, J = 8 Hz, H-9), 3.83 (2H, t, J = 8 Hz, CH_2—C—Si), 4.40 (2H, m, H-2+H-3), 4.72 (2H, s, acetal CH_2), 6.05 (1H, d, J = 2 Hz, H-7).

^{1}H-NMR, 200 MHz ($CDCl_3$): δ ppm: 0.03 (9H, s, Me_3Si), 0.63 (3H, s, Me-18), 0.89 (2H, t, J = 8 Hz, CH_2—Si), 0.99 (3H, s, Me-19), 1.33 (3H, s, acetonide), 1.50 (3H, s, acetonide), 2.15 (3H, s, Me-21), 2.30 (3H, m, H-17+?), 2.68 (1H, m, H-5), 3.27 (1H, t, J = 8 Hz, H-9), 3.61 (2H, t, J = 8 Hz, CH_2—C—Si), 4.21 (2H, m, H-2 and H-3), 4.56 (2H, s, acetal CH_2), 5.83 (1H, d J = 2 Hz, H-7).

^{13}C-NMR, 50 MHz ($d_5$py): δ ppm: —1.3 (Me_3Si), 17.5 (C-18), 18.5 (C-Si), 20.9 (C-11), 21.9 (C-16), 24.4 (C-19), 26.6 (C-4), 26.7 (acetonide), 27.6 (C-15), 29.0 (acetonide), 30.0 (C-12), 31.3 (C-21), 34.6 (C-9), 38.1 (C-1), 39.0 (C-10), 49.0 (C-13), 51.6 (C-5), 59.4 (C-17), 66.3 (*C*—C—Si), 72.4 (C-2), 72.6 (C-3), 90.1 (acetal), 91.0 (C-14), 108.4 (acetal), 126.0 (C-7), 159.3 (C-8), 202.7 (C-6), 208.7 (C-20).

IR ($CHCl_3$): ν (cm^{-1}) 2920, 1700, 1660, 1440, 1370, 1240, 1050, 1000, 860, 830.

MS(EI, 70 eV, 60 °C): *m/e* = 532 (3%, M^+), 517 (19, M^+—CH_3), 446 (43), 402 (17, M^+-protection in 14), 384 (100, M^+-protection in 14-H_2O).

Poststerone 2,3-Acetonide 14-(1-ethoxyethyl)ether 9b *from* 8

Poststerone acetonide *8* (0.20 g, 0.5 mmole) was dissolved in ethyl-vinyl-ether (5 mL); freshly fused p. toluenesulfonic acid (9 mg) was introduced, and the reaction was allowed to proceed at room temperature; after 40 min, a TLC check (elution with cyclohexane/ether 3/1) showed it to be complete. Excess solid potassium carbonate was introduced, and after a few minutes the rection mixture was diluted with ether (50 mL), washed with 10% aqueous ammonium chloride and with saturated brine. After drying over sodium sulfate and removal of the solvent under reduced pressure at 30 °C, the crude product was chromatographed (Silicagel in cyclohexane/ether 3/1); elution with the same solvent gave the desired product *9b* (167 mg, 70%).

^{1}H-NMR 200 MHz ($CDCl_3$) : δ ppm: 0.60 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.33 (3H, s, acetonide), 1.50 (3H, s, acetonide), 2.15 (3H, s, Me-21), 2.89 (1H, m, H-5), 3.25 (1H, m, H-9), 3.42—3.61 (2H, m, CH_2O), 4.12—4.22 (2H, m, H-2 and H-3), 4.51—4.77 (1H, m, acetal CH), 5.71 (1H, d, J = 1.5 Hz, H-7).

MS (CI, NH_3, 60 °C): m/e = 492 (50%, $M.NH_4^+$), 475 (13, MH^+), 404 (85), 385 (100, MH^+ — protection en 14 — H_2O).

Conversion of 9a *and* 9b *into* 10a *and* 10b *Respectively*

One of the protected intermediates *9a* or *9b* (0.8 mmole) was placed in an argon-flushed flask and dissolved in anhydrous THF (3 mL). The solution was

cooled to —50 °C and 0.8 mmole of vinylmagnesium bromide in THF was introduced under argon with stirring. After 90 min at —50 °C, the same amount of vinylmagnesium bromide was added and the mixture was again left at —50 °C for 90 min. It was then quenched by adding a minimal quantity of aqueous saturated sodium-potassium tartrate necessary to precipitate the magnesium species and agglutinate the precipitate. The resultant clear supernatant solution was filtered, and the residue was washed repeatedly with ethyl acetate. The combined organic phases were evaporated in vacuo below 30 °C, and the crude product was chromatographed on a silicagel column, prepared in hexane (for *10a*) or in cyclohexane/ether 3/1 (for *10b*). Elution with a gradient of ethyl acetate in hexane (4% to 30%) in the first case, and an isocratic flow cyclohexane/ether 3/1 in the second case gave 81% of *10a* or 75% of *10b*. HPLC analysis of *10a* showed it to be a 9/1 mixture of epimers.

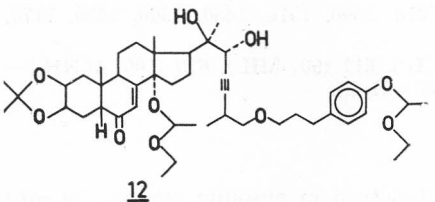
10a:

^{1}H-NMR 200 MHz ($CDCl_3$): δ ppm: 0.03 (9H, s, Me_3Si), 0.80 (3H, s, Me-18), 0.90 (2H, t, J = 7.4 Hz, CH_2—Si), 0.99 (3H, s, Me-19), 1.33 (3H, s, acetonide), 1.36 (3H, s, Me-21), 1.50 (3H, s, acetonide), 2.65 (1H, m, H-5), 3.60 (2H, t, J = 7.4 Hz, CH_2—C—Si), 4.21 (2H, m, H-2 and H-3), 4.53 (2H, s, acetal CH_2), 5.00 (1H, dd, J_1 = 10.7 Hz, J_2 = 1.4 Hz, H-23 cis/H-22), 5.16 (1H, dd, J_1 = 16.3 Hz, J_2 = 1.4 Hz, H-23 trans/H-22), 5.80 (1H, d, J = 2 Hz, H-7), 6.01 (1H, dd, J_1 = 16.3 Hz, J_2 = 10.7 Hz, H-22).

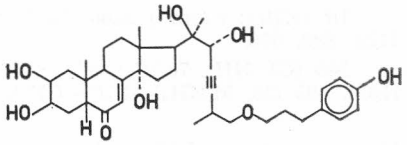
^{13}C-NMR 50 MHz ($d_5$py): δ ppm: —1.3 (Me_3Si), 18.5 (C-18), 18.8 (C—Si), 21.2 (C-11), 22.6 (C-16), 23.9 (C-19), 26.8 (acetonide + C-4), 27.0 (C-15), 28.9 (acetonide), 29.7 (C-21), 31.3 (C-12), 35.4 (C-9), 38.0 (C-10), 38.1 (C-1), 49.4 (C-13), 51.6 (C-5), 54.7 (C-17), 66.1 (C—C—Si), 72.3 (C-2), 72.6 (C-3), 74.8 (C-20), 90.0 (acetal), 91.6 (C-14), 108.3 (acetal), 110.6 (C-23), 125.0 (C-7), 148.0 (C-22), 160.6 (C-8), 201.4 (C-6).

10b:

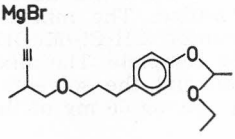
^{1}H-NMR 200 MHz ($CDCl_3$): δ ppm: 0.80 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.33 (3H, s, acetonide), 1.35 (3H, s, Me-21), 1.49 (3H, s, acetonide), 2.85 (1H, m, H-5), 3.44—3.56 (3H, m, H-9 and CH_2O), 4.55—4.72 (1H, m, acetal CH), 4.99 (1H, dd, J_1=10.7 Hz, J_2 = 1.4 Hz, H-23 cis/H-22), 5.16 (1H, dd, J_1 = 17.3 Hz, J_2 = 1.4 Hz, H-23 trans/H-22), 5.79 (1H, d, J = 1.5 Hz, H-7), 6.00 (1H, dd, J_1 = 17.3 Hz, J_2 = 10.7 Hz, H-22).



12



13



A

Conversion of 10b *into* 12

The vinyl alcohol *10b* (150 mg, 0.3 mmole) was dissolved in methylene chloride (10 mL); the solution was cooled to —80 °C and ozonized oxygen was bubbled slowly through the stirred solution (2 bubbles per 3 mn). After 40 min, TLC monitoring showed the reaction to be nearly complete; argon was bubbled through to flush away excess ozone, and a solution of dimethylsulfide (1.5 mL) in methylene chloride (3 mL) was introduced. The mixture was stirred at —80 °C for another 45 min, then at 0 °C for 45 min, and finally at room temperature for 1 h. After addition of ethyl acetate (250 mL), it was washed several times with saturated aqueous sodium-

-potassium tartrate, dried over sodium sulfate, and evaporated in vacuo at —30 °C. The residue was dissolved in dry THF (3 mL) and introduced dropwise with a syringe, over 30 min, into a solution of the acetylenic Grignard A, the preparation of which will be described elsewhere[13] (3eq in 5 mL THF, 0 °C, argon atmosphere, stirring). After another 10 min, the reaction was stopped and treated as described for the previous step. The residue was chromatographed on a silicagel column prepared in methylene chloride, eluted with a step-gradient of ether in methylene chloride (0, 1.5, 5, 10, 15, 20, 25%); elution of the product began at 15%, and gave 124 mg of *12* (yield for the sequence ozonolysis + Grignard: 52%).

MS (CI, NH_3): *m/e* = 812 (35%, $M.NH_4^+$), 795 (20, MH^+), 740 (70, HNH_4^+ — ethylvinylether), 723 (80, MH^+ — ethylvinylether), 705 (50, 723 — ethylvinylether — H_2O), 668 (70, $M.NH_4^+$ — 2 ethylvinylether), 651 (11, MH^+ — 2 ethylvinylether), 633 (100, MH^+ — 2 ethylvinylether — H_2O).

Deprotection of 12 *to* 13

The fully protected derivative *12* (0.5 mmole, 398 mg) was dissolved in methylene chloride (5 mL); methanol (5 mL) was added, and followed by 5 g of acidic resin (Dowex 50 W X4). The flask was gently rotated at an angle, at room temperature, for 3 days. The reaction mixture was filtered, the resin was rinsed with ethanol, and the solvents were removed under reduced pressure. The residue was chromatographed on silicagel ($MeOH/CH_2Cl_2$ 0 to 6%, then 6%), to give 174 mg (57%) of the product *13*. This could not be obtained crystalline.

^{1}H-NMR 200 MHz ($d_5$py): δ ppm: 1.09 (3H, s, Me-19), 1.25 (3H, s, Me-18), 1.29 (3H, d, J = 6Hz, Me-27), 1.88 (3H, s, Me-21), 2.68 (2H, t, J = 6Hz, CH_2—30), 3.05 (1H, dd, J_1 = 8Hz, J_2 = 4Hz, H-5), 3.34 to 3.60 (4H, m, CH_2—O), 4.18 (1H, m, H-2), 4.28 (1H, m, H-3), 6.27 (1H, d, J = 2Hz, H-7), 6.33 (1H, s, OH-14).

^{13}C-NMR 50 MHz ($d_5$py): δ ppm: 17.8 (C-18), 18.2 (C-25), 21.2 (C-11), 21.4 (C-16), 22.0 (C-21), 24.6 (C-19), 27.4 (C-27), 31.9 (C-30), 32.0 (C-29), 32.0 (C-15), 32.6 (C-4), 34.6 (C-9), 38.1 (C-10), 48.1 (C-13), 50.3 (C-5), 51.5 (C-17), 68.2 (C-2), 68.2 (C-3), 68.8 (C-22), 70.5 (C-26), 75.2 (C-28), 77.7 (C-20), 84.3 (C-14), 116.3 (aromatic C's), 121.7 (C-7), 130.0, 135.1, 157.1 (aromatic C's), 166.1 (C-8), 203.4 (C-6).

IR (KBr): ν (cm^{-1}) 3400, 2920, 2230, 1650, 1610, 1590, 1510, 1450, 1360, 1230, 1170, 1120, 880, 830.

MS (CI, NH_3, 70 °C): *m/e* = 628 (30%, $M.NH_4^+$), 611 (90; MH^+), 610 (100, $M.NH_4^+$—H_2O), 595 (25, $M.NH_4^+$—H_2O—CH_3), 593 (59, MH^+—H_2O).

Hydrogenation of 13 *to* 4

The acetylenic substance *13* (30 mg) was dissolved in absolute ethanol (30 mL) containing 150 µL of dry piperidine; 90 mg of 5% Pd/C was added and a hydrogen atmosphere (slightly more than 1 bar) was settled. The mixture was stirred at room temperature for 5 hrs. A TLC check (elution CH_2Cl_2/MeOH 91/9, three successive developments) showed the reaction to be complete. The mixture was filtered, the catalyst rinsed several times with ethanol, and the solvents evaporated. The same chromatography as for the preceding step, giving 60 mg of the desired product 4 (66%).

^{1}H-NMR 200 MHz ($d_5$py): δ ppm: 0.95 (3H, d, J = 7Hz, Me-27), 1.08 (3H, s, Me-19), 1.24 (3H, s, Me-18), 1.61 (3H, s, Me-21), 191 (2H, m, CH_2-29), 2.70 (2H, t, J = 7.5Hz, CH_2-30), 3.05 (1H, m, H-5), 3.23 (2H, m, CH_2-26), 3.29 (2H, t, J = 7Hz, CH_2-28), 3.61 (1H, m, H-9), 3.88 (1H, m, H-22), 4.16 (1H, m, H-2), 4.24 (1H, m, H-3), 6.28 (1H, s, H-7), 6.40 (1H, OH-14), 7.20 (4H, s, aromatic H's), 11.34 (1H, s, ArOH).

^{13}C-NMR 50 MHz ($d_5$py): δ ppm: 17.4 (C-27), 18.0 (C-18), 21.3 (C-11), 21.6 (C-16), 21.7 (C-21), 24.6 (C-19), 30.0 (C-23), 30.2 (C-24), 32.0 (C-30), 32.2 (C-29), 32.3 (C-15), 32.5 (C-12), 32.5 (C-4), 33.9 and 34.2 (C-25), 34.6 (C-9), 38.1 (C-10), 38.8 (C-1), 48.3 (C-13), 50.2 (C-5). 51.5 (C-17), 68.2 (C-2), 68.2 (C-3), 70.4 (C-28), 76.5 (C-20), 76.9 (C-26), 77.1 and 77.3 (C-22), 84.3 (C-14), 116.3 (aromatic C's), 121.8 (C-7), 130.1, 133.0, 157.2 (aromatic C's), 166.0 (C-8), 203.4 (C-6).

IR (KBr): ν (cm^{-1}) 3400, 2910, 1650, 1510, 1440, 1380, 1230, 1110, 1050, 870.

MS (CI, NH_3): *m/e* = 632 (10%, $M.NH_4^+$), 615 (100, MH^+), 597 (66, $MH^+—H_2O$), 579 (40, $MH^+—2H_2O$).

MS (EI, 70 eV, 80 °C): *m/e* 614 (1%, M^+), 612 (1), 596 (54, $M^+—H_2O$), 578 (47, $M^+—2H_2O$), 560 (43, $M^+—3H_2O$), 548 (3), 542 (3, $M^+—4H_2O$), 463 (3, $M^+—O\text{-}(CH_2)_3—Ar$), 445 (5, $M^+—O—(CH_2)_3—Ar—H_2O$), 427 (7, $M^+—O—(CH_2)_3—Ar—2H_20$), 411 (5), 363 (100, cleavage between C-20 and C-22), 345 (86, cleavage between C-20 and C-22—H_2O), 331 (85), 302 (60), 251 (93).

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IZVOD

Ekdisteroidi

D. Guédin-Vuong, Y. Nakatani i G. Ourisson

β-Ekdison i ciasteron transformisani su u ekdisteronov analog *4*, koji na kraju bočnog niza sadrži [3-(*p*-hydroksifenil)-propoksi]-grupu. Ovaj derivat mogao bi se korisno upotrebiti za različita biološka proučavanja (jodno markiranje, afinitetnu hromatografiju, jer u dva nezavisna biološka testa pokazuje izraženu ekdisteronsku aktivnost. U sintetičkoj sekvenci upotrebljeni su postupci visoko-selektivne zaštite hidroksilnih grupa kod fitoekdisterona.