SYNTHESIS OF 2,24-DIEPICASTASTERONE AND 3,24-DIEPICASTASTERONE AS POTENTIAL BRASSINOSTEROID METABOLITES OF THE COCKROACH *Periplaneta americana*

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Investigations of the metabolic conversion of the phytohormone 24-epicastasterone (**1**) in the cockroach *Periplaneta americana* (L.) required the synthesis of 2,24-diepicastasterone (**4**), 3,24-diepicastasterone (**7b**) and 2-dehydro-3,24-diepicastasterone (**9**) as reference standards. 2,24-Diepicastasterone (**4**) was synthesized from 2α,3α-epoxy derivative **2** as well as from the 2β,3β-epoxy-22,23-diol **3** by acid-catalyzed water addition to the epoxy function leading to the desired 2β,3α-*trans* functionality. 3,24-Diepicastasterone (**7b**) was prepared by $NaBH_4$-reduction of the 3-oxo derivative **6**. Upon deprotection conditions from the ketol acetonides **6** and **8** in both cases 2-dehydro-3,24-diepicastasterone (**9**) was obtained. The structure of 2,24-diepicastasterone (**4**) was confirmed by X-ray analysis.

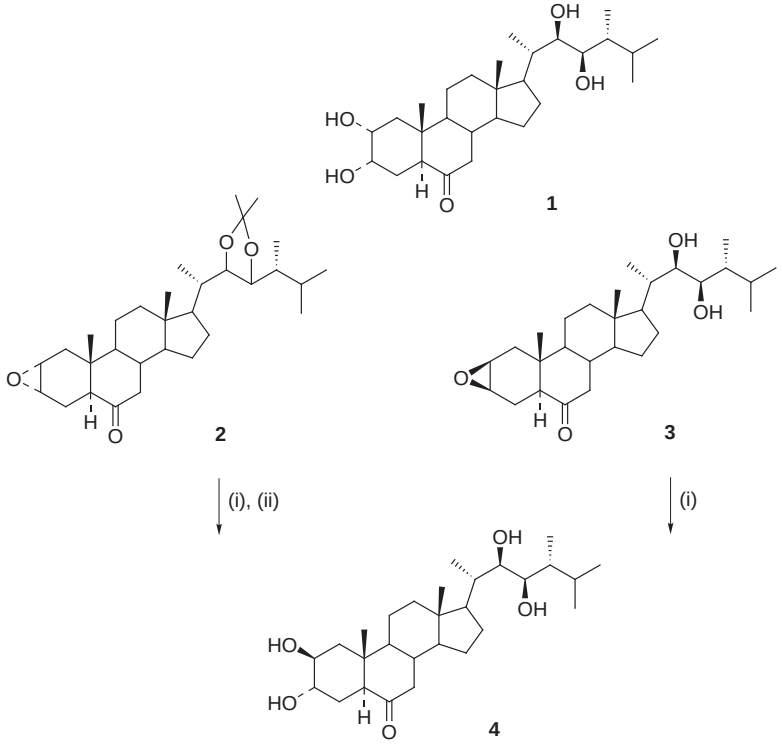
Keywords: Steroids; Phytohormones; Ecdysteroids; Oxidations; Brassinosteroids; 2,24-Diepicastasterone; 3,24-Diepicastasterone; Metabolism; *Periplaneta americana*.

The brassinosteroids represent a new class of steroidal phytohormones of ubiquitous occurrence in the plant kingdom with high growth-promoting and antistress activity[1]. The striking structural similarity of brassinosteroids with moulting hormones of the ecdysone type[2] encouraged us to investigate metabolic transformations in insects. In the course of such studies, we reported recently the organ-specific epimerization of the native phytohormone 24-epicastasterone (**1**) to 2,24-diepicastasterone (**4**) in ovaries of the cockroach *Periplaneta americana* (L.), which represent the first metabolic

transformation of a brassinosteroid observed in an insect[3]. In this paper we present the synthesis of metabolite **4** as well as 3,24-diepicastasterone (**7b**) and 2-dehydro-3,24-diepicastasterone (**9**), required as essential reference standards in these studies.

RESULTS AND DISCUSSION

For the synthesis of 2,24-diepicastasterone (**4**) the ketal derivative of (22*R*,23*R*,24*R*)-2α,3α-epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one (**2**) was used, which is available in seven steps from ergosterol[4] (Scheme 1). Hydrolytic opening of the oxirane ring in **2** with 2.5 M sulfuric acid in tetrahydrofurane–water 9 : 1 at room temperature followed by deprotection of the side chain with 4 M HCl in MeOH at 50 °C gave 2,24-diepicastasterone (**4**) in good yield, prepared also from the corresponding 2α,3α-epoxy



SCHEME 1
Reagents and conditions: (i) 2.5 M H_2SO_4, THF/H_2O; (ii) 4 M HCl, MeOH, 50 °C

22,23-diol by Levinson *et al.*[5]. Acid-catalyzed epoxide opening of 2β,3β-epoxydiol[4] **3**, detected also very recently as native phytohormone 24-episecasterone in *Lychnis viscaria*[6], led likewise to compound **4**. Thus, upon acid-catalyzed ring opening of both epimeric epoxides **2** and **3**, in agreement with the Fürst–Plattner-rule the same compound **4** with *trans*-diaxial 2β,3α-diol function was formed. The structure of **4** was confirmed by X-ray analysis[7] (Fig. 1), showing an intramolecular O(22)–H···O(23) hydrogen bond as well as three intermolecular hydrogen bridges to nearest neighbour molecules within the cell.

For the synthesis of 3,24-diepicastasterone (**7b**) the diisopropylidene derivative of 24-epicastasterone **5** was used as starting compound (Scheme 2). Reaction of **5** with methyl(trifluoromethyl)dioxirane[8] (TFD) in dichloromethane during 20 h at room temperature afforded 3-dehydro-24-epicastasterone-22,23-acetonide (**6**) as main product (52%). As minor components the corresponding 2-dehydro-3,24-diepicastasterone acetonide **8** (8%), reflecting simultaneous isomerisation of **6**, as well as the 22,23-acetonide of 24-epicastasterone[9] (10%) were obtained.

In earlier investigations we have shown the selective C-25 side-chain oxyfunctionalization of the 22,23-monoacetonide of 2,3-diacetyl-24-epicastasterone with TFD (ref.[9]). However, in the case of bisacetonide **5** the ketal function in position 2α,3α is considerably more reactive towards TFD than the stronger shielded ketal in the side chain. The first step of the reaction cascade is the deprotection to the 2α,3α-diol, followed by oxidation of

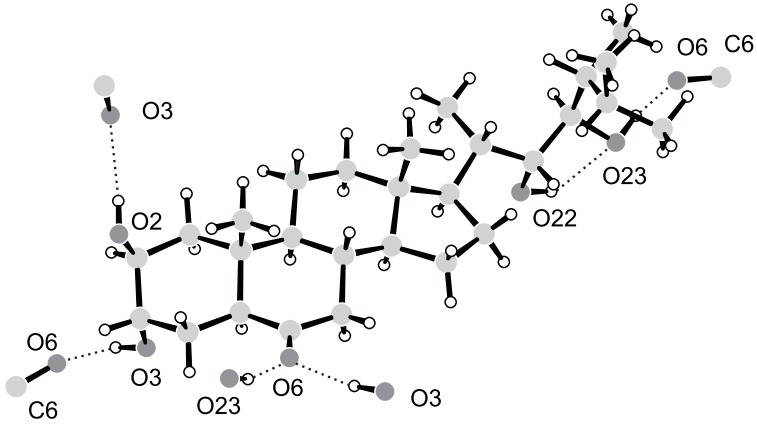
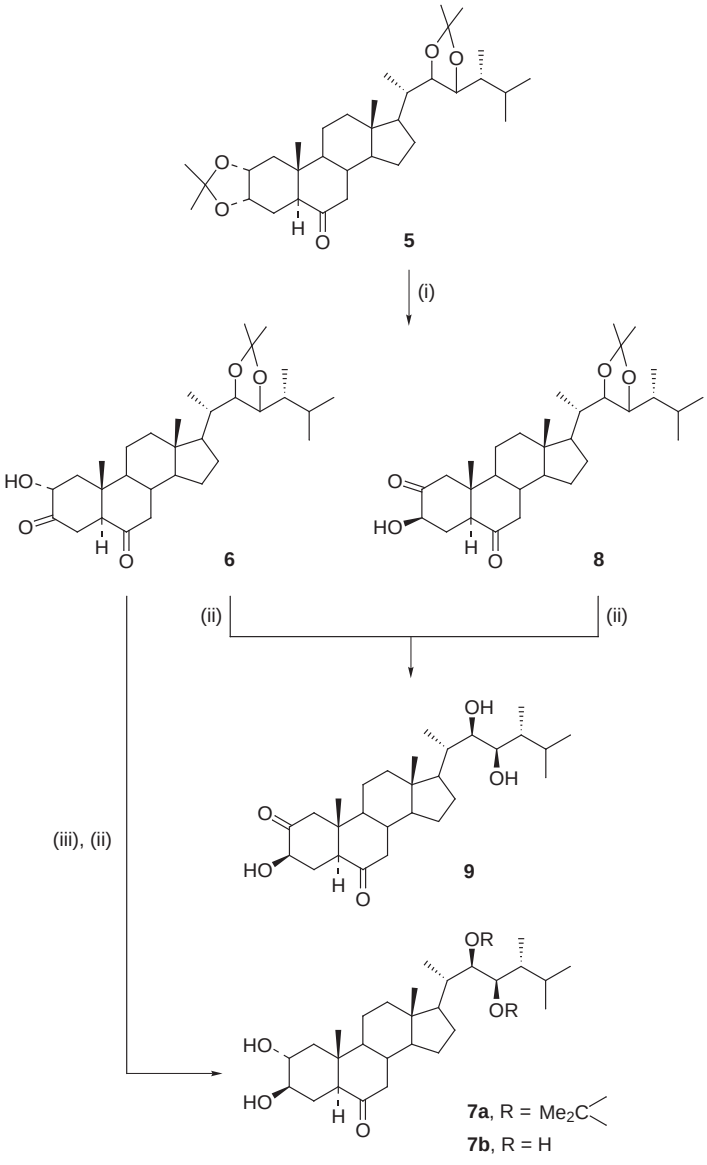


FIG. 1
Molecular structure of **4** with the hydrogen bridges



SCHEME 2
Reagents and conditions: (i) TFD, CH_2Cl_2, rt; (ii) 4 M HCl, MeOH, 50 °C; (iii) $NaBH_4$, EtOH, –25 °C

one of the hydroxy groups to afford mainly the 3-oxo derivative **6** besides 2-ketone **8** and the 22,23-acetonide of 24-epicastasterone. Similar results were described by Bovicelli *et al.*[10] and Curci *et al.*[11], who used dimethyldioxirane for the monooxidation of *sec*-1,2-diols to the corresponding keto alcohols, which exploits the inhibiting effect of the carbonyl group to prevent further oxidation.

Careful reduction of compound **6** with sodium borohydride in ethanol at –25 °C furnished stereoselectively the 3β-hydroxy derivative **7a**. Recovery of the 22,23-diol function by treatment of **7a** with 4 M HCl in MeOH at 50 °C led to the desired 3,24-diepicastasterone **7b**. Compound **7b**, available also from a 2α-bromo-3-oxo derivative[12], was detected as a free and acyl-conjugated metabolite of 24-epicastasterone (**1**) in cell suspension cultures of *Ornithopus sativus*[13]. Very recently 3,24-diepicastasterone was detected in immature seeds of *Phaseolus vulgaris*[14]. Also the 24*S*-epimer of **7b**, 3-epicastasterone, was described to be naturally occurring in *Phaseolus vulgaris* seeds[15].

Deprotection of ketals of **6** and **8** with 4 M HCl in MeOH at 50 °C led in both cases to the same 2-dehydro-3,24-diepicastasterone (**9**), which indicates that simultaneous isomerisation of the 2-hydroxy-3-oxo function has taken place in case of **6**. Similar rearrangements under acetic conditions to the preferred 3β-hydroxy-2-oxo compounds have been reported in the cholestane series[16,17]. The spectral data of the new compounds are in agreement with the given structures (see Experimental). The unequivocal assignments of the ^{1}H and ^{13}C NMR signals were established by the combined use of one- and two-dimensional NMR experiments (COSY, HSQC, HMBC). The configuration at C-2 and/or C-3 was established by NOE difference experiments (Tables I and II).

The phytohormone activity of 2,24- and 3,24-diepicastasterone (**4** and **7b**) as well as 2-dehydro-3,24-diepicastasterone (**9**) was studied using the highly sensitive and specific rice lamina inclination assay[18]. The obtained results showed that the 2-epimer **4** at a concentration of 0.1 ppm has 87%, the 3-epimer **7b** 80% and the 2-dehydro derivative **9** 60% activity related to 24-epicastasterone as standard (100%). Investigations of compounds **4**, **7b** and **9** for an activity as moulting hormone showed no agonist nor antagonist properties[19].

TABLE I
^{1}H NMR data of compounds **4**, **6**, **7a**, **7b**, **8** and **9** (in $CDCl_3$)

Position	δ_H [a,b] (*J*, Hz)					
	4[c]	**6**	**7a**	**7b**[c]	**8**	**9**
1	1.68/1.77	1.46/2.542 dd (12.7/7.0)	1.242 dd (12.9/11.4)/2.05	1.215 dd (12.7/11.5)/2.02	2.364 d (13.3)/2.594 d (13.3)	2.361 d (13.2)/2.593 d (13.2)
2	3.828 m	4.258 ddd (12.1/7.0/3.2)	3.598 ddd (11.4/9.0/4.8)	3.525 ddd (11.5/9.1/4.9)	–	–
3	3.864 m	–	3.389 ddd (11.6/9.0/4.9)	3.314 ddd (11.7/9.1/5.0)	4.158 ddd (12.1/7.4/3.3)	4.161 dd (12.0/7.5)
4	1.63/1.98	2.518 dd (14.0/2.9)/2.705 ddd (14.0/13.6/13.4)	1.95/1.60	1.91/1.54	2.484 ddd (13.9/7.4/3.7)/1.76	2.485 ddd (13.9/7.5/3.2)/1.76
5	2.747 dd (12.4/2.3)	2.650 dd (13.4/2.7)	2.332 dd (12.6/3.0)	2.339 dd (12.6/2.9)	2.803 dd (12.7/3.1)	2.799 dd (12.7/3.2)
7	2.03/2.273 dd (13.2/4.6)	1.998 dd (13.1/12.3)/ 2.388 dd (13.1/4.4)	1.96/2.315 dd (13.2/4.6)	2.00/2.300 dd (13.3/4.6)	2.04/2.403 dd (13.4/4.5)	2.06/2.407 dd (13.4/4.5)
8	1.80	1.848 dddd (12.3/10.7/10.7/4.4)	1.784 m	1.794 m	1.75	1.76
9	1.34	1.35	1.31	1.34	1.55	1.56
11	1.65/1.35	1.69/1.44	1.65/1.34	1.64/1.36	1.53/1.38	1.54/1.38
12	1.26/2.03	1.32/2.06	1.30/2.04	1.28/2.03	1.32/2.06	1.30/2.06
14	1.33	1.32	1.31	1.32	1.32	1.33
15	1.57/1.111 m	1.58/1.11	1.58/1.097 m	1.57/1.110 m	1.59/1.116 m	1.60/1.123 m
16	1.99/1.30	2.04/1.36	2.03/1.34	2.00/1.30	2.03/1.37	2.01/1.32
17	1.57	1.55	1.54	1.57	1.55	1.60

TABLE I
(*Continued*)

Position	δ_H [a,b] (*J*, Hz)					
	4[c]	**6**	**7a**	**7b**[c]	**8**	**9**
18	0.687 s	0.692 s	0.663 s	0.682 s	0.668 s	0.681 s
19	0.955 s	1.045 s	0.804 s	0.794 s	0.713 s	0.712 s
20	1.45	1.50	1.50	1.45	1.52	1.47
21	0.969 d (6.7)	0.986 d (6.3)	0.981 d (6.2)	0.964 d (6.7)	0.983 d (6.3)	0.979 d (6.7)
22	3.666 d (4.4/1.3)	3.935 d (7.0)	3.936 (br) d (7.0)	3.660 dd (4.8/1.6)	3.940 d (6.9)	3.698 dd (4.6/1.5)
23	3.36	3.567 dd (9.4/7.0)	3.563 dd (9.4/7.0)	3.359 dd (6.0/4.8)	3.566 m	3.416 dd (6.0/4.6)
24	1.47	1.56	1.56	1.47	1.57	1.50
25	1.992 m	2.08	2.10	1.90	2.08	1.901 sept. d (6.8/3.8)
$26^{pro\text{-}R}$	0.859 d (6.8)	0.813 d (6.8)[d]	0.811 d (6.8)	0.859 d (6.8)	0.812 d (6.8)[d]	0.872 d (6.8)
$27^{pro\text{-}S}$	0.917 d (6.9)	0.911 d (7.0)[d]	0.909 d (7.0)	0.915 d (6.9)	0.911 d (7.0)[d]	0.922 d (6.9)
28	0.833 d (7.0)	0.707 d (7.0)	0.704 d (7.0)	0.833 d (7.0)	0.707 d (7.0)	0.851 d (7.0)
Me	–	1.342 s	1.343 s	–	1.348 s	–
Me	–	1.387 s	1.389 s	–	1.392 s	–

[a] H-α/H-β. [b] ^{1}H chemical shifts without multiplet specification are chemical shifts of HSQC correlation peaks. [c] In $CDCl_3$ + CD_3OD. [d] Diastereotopic methyl groups 26/27 are not assigned.

TABLE II
^{13}C chemical shifts of compounds **4**, **6**, **7a**, **7b**, **8** and **9** (in $CDCl_3$)

Position	δ_C					
	4[a]	**6**	**7a**	**7b**[a]	**8**	**9**
1	38.7	47.8	44.3	44.1	50.5	50.6
2	69.5	72.0	72.1	71.7	209.9	209.9
3	68.6	211.0	75.8	75.3	74.5	74.5
4	22.6	35.1	27.8	27.5	31.0	31.0
5	51.4	58.6	56.6	56.6	55.2	55.2
6	214.2	208.0	210.1	210.9	208.4	208.4
7	46.3	46.4	46.5	46.4	46.5	46.5
8	37.7	37.6	37.6	37.6	37.7	37.7
9	54.2	53.4	53.8	53.7	53.3	53.2
10	40.8	42.7	42.9	42.8	46.4	46.4
11	20.8	21.8	21.6	21.5	21.4	21.4
12	39.2	39.1	39.1	39.3	38.9	39.1
13	42.6	42.9	42.9	42.8	42.7	42.6
14	56.3	56.2	56.3	56.4	56.2	56.4
15	23.6	23.8	23.8	23.8	23.8	23.8
16	27.4	27.6	27.6	27.6	27.6	27.7
17	52.4	53.4	53.4	52.6	53.3	52.5
18	11.5	11.8	11.8	11.8	11.7	11.7
19	14.6	13.8	14.4	14.2	14.2	14.2
20	40.1	37.9	38.0	40.1	37.9	40.2
21	12.0	12.6	12.6	12.3	12.6	12.4
22	71.9	82.3	82.4	72.4	82.3	72.6
23	75.5	80.3	80.4	76.0	80.4	76.4
24	41.4	43.7	43.8	41.4	43.7	41.4
25	26.6	27.8	27.7	26.9	27.7	27.0
$26^{pro\text{-}R}$	16.8	16.1[b]	16.0	17.2	15.9[b]	17.2
$27^{pro\text{-}S}$	21.7	21.1[b]	21.1	22.0	21.1[b]	21.1
28	10.3	9.9	9.8	10.7	11.7	10.8
Cq		108.0	108.0		108.0	
Me		27.4	27.4		27.3	
Me		27.1	27.2		27.1	

[a] In $CDCl_3$ + CD_3OD. [b] Diastereotopic methyl groups 26/27 are not assigned.

EXPERIMENTAL

General

Melting points were determined on a Boetius hot-stage microscope and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 28 instrument. Optical rotations were measured on a DIP 1000-polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were measured on a Uvikon 941 Kontron instrument. CD spectra were recorded with a Jasco J 710 spectrometer. Mass spectra (EI MS, 70 eV) were obtained with a AMD 402 spectrometer. The GC MS data of trimethylsilyl derivatives were obtained with a MD-800 Fisons instrument. The relative retention times (RR_t) values were calculated with respect to 5α-cholestane. ^{1}H and 2D NMR spectra were recorded on a Varian UNITY 500 spectrometer at 499.8 MHz, whereas ^{13}C and APT spectra were determined on a Varian GEMINI 300 spectrometer at 75.5 MHz. $CDCl_3$ was used as solvent unless otherwise noted. TMS (δ 0) and $CDCl_3$ (δ 77.0) were used as internal reference for ^{1}H and ^{13}C spectra, respectively. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. TLC plates precoated with silica gel 60 PF254 0.2 mm (Merck) and for column chromatography silica gel 60, 0.04–0.063 mm (Merck), were used. The preparative HPLC analysis was carried out on a Knauer instrument, supplied with a YMC-column, ODS, 5 mm, 20 × 150 mm, with MeCN–H_2O as eluent and UV detection at 210 nm. The elemental analyses were carried out on a LECO CHNS-932 instrument (LECO Instrumente GmbH, Kirchheim/München.

For the X-ray crystal structure determination, the data were collected on a STOE-IPDS diffractometer by using MoKα radiation (λ = 0.71073 Å) at room temperature. The structure was solved by direct methods (SHELXS86)[20] and all non H-atoms were refined anisotropically by full-matrix least-squares on F^2; H-atoms were included in calculated positions and refined as riding atoms (SHELXL93)[21]. For the graphical representations the program DIAMOND was used[22].

2,24-Diepicastasterone (**4**)

Method A. From **2**: A solution of epoxide **2** (49 mg, 0.1 mmol) in THF–H_2O (9 : 1 v/v, 15 ml) was treated with 2.5 M H_2SO_4 (0.2 ml) and the mixture was stirred at room temperature for 2 h. After evaporation of the solvent the residue was extracted with $CHCl_3$ to give 46 mg crude product, which was heated at 50 °C with 4 M HCl (1 ml) in MeOH (10 ml) for 3 h. Work-up and flash chromatography by elution with ethyl acetate gave **4** (37 mg, 80%) with m.p. 234–235 °C and $[\alpha]_D^{29}$ –11.90 (*c* 1.04, MeOH). HPLC: R_t 5.92, MeCN–H_2O 65 : 35 v/v. IR (Nujol), ν_{max}: 3 560, 3 526, 3 454 (OH), 1 685 (CO). UV (*c* 1.04, MeOH), λ_{max} (ε): 289 (50). CD: $\Delta\varepsilon_{294}$ –1.35 (MeCN). EI MS, *m/z* (rel.%): 446 (M^+ – 18, 4), 393 (M^+ – 71, 5), 375 (393 – 18, 7), 364 (M^+ – 100, 100), 345 (M^+ – 119, 55). GC MS: RR_t 1.89. EI MS of the methylboronate–TMS–ether, *m/z*: 632 (M^+, 3), 617 (M^+ – 15, 6), 515 (617 – 98, 67), 426 (32); HR MS, *m/z*: 364.2619 (calculated for $C_{22}H_{36}O_4$ 364.2624), 345.2428 (calculated for $C_{22}H_{33}O_3$ 345.2426). For $C_{28}H_{48}O_5$ calculated: 72.37% C, 10.42% H; found: 72.21% C, 10.20% H. For ^{1}H and ^{13}C NMR spectra see Tables I and II.

Method B. From **3**: A solution of 2β,3β-epoxide **3** (23 mg, 0.05 mmol) was treated with 2.5 M H_2SO_4 as described under method *A*. After 5 min at room temperature the reaction was complete; work-up and crystallization from ethyl acetate–hexane gave **4** (21 mg, 86%), whose data are identical with those of **4**, synthesized as described under method A.

X-Ray Crystal Structure Determination of **4**

$C_{28}H_{48}O_5$; orthorhombic; space group $P2_12_12_1$; unit cell dimensions: a = 6.265(2) Å, b = 14.976(3) Å, c = 28.035(8) Å, $\alpha = \beta = \gamma = 90°$, V = 2 630.4(12) Å^3, Z = 4, density (calculated) = 1.173 Mg m^{-3}; absorption coefficient 0.078 mm^{-1}; $F(000)$ = 1 024. θ range: 1.99 to 26.05°; index ranges: $-7 \le h \le 7$, $-18 \le k \le 18$, $-34 \le l \le 34$; reflections collected: 22 246; independent reflections: 5 096 [R(int)= 0.1167]; data/restraints/parameters: 5 096/0/490. S: 0.924; final R indices [$I > 2\sigma(I)$]: R_1 = 0.0430, wR_2 = 0.0705; R indices (all data): R_1 = 0.0849, wR_2 = 0.0801; absolute structure parameter: –0.0 (11); largest difference peak and hole: 0.168 and –0.143 e Å^{-3}. Compound **4** has three intermolecular and one intramolecular hydrogen bridges: O(2)–H(45)···O(3) = 2.1352; O(3)–H(46)···O(6) = 1.9162; O(23)–H(48)···O(6) = 1.9300; O(22)–H(47)···O(23) = 2.1263 (ref.[7]).

3-Dehydro-24-epicastasterone 22,23-Acetonide (**6**) and 2-Dehydro-3,24-diepicastasterone 22,23-Acetonide (**8**)

A solution of 24-epicastasterone diacetonide (**5**; 300 mg, 0.55 mmol) in CH_2Cl_2 (15 ml) was treated with a 0.2 M solution of TFD (ref.[8]) in CH_2Cl_2 (3.6 ml). After 20 h standing at room temperature the peroxide test was negative. Work-up, SiO_2 chromatography and elution with hexane–ethyl acetate 75 : 25 v/v gave the 3-oxo derivative **6** as main product (144 mg, 52%), m.p. 189–192 °C and $[\alpha]_D^{26}$ +4.32 (*c* 1.27, MeOH). IR (Nujol), ν_{max}: 3 500 (OH), 1 721 (CO). UV (*c* 1.27, MeOH), λ_{max} (ε): 290 (105), 256 (175). EI MS, *m/z*: 502 (M^+, 2), 487 (M^+ – 15, 88), 431 (M^+ – 71, 28), 387 (431 – 44, 24), 301 (32), 171 (96), 142 (100); HR MS, *m/z*: 487.3442 (calculated for $C_{30}H_{47}O_5$ 487.3461), 171.1391 (calculated for $C_{10}H_{19}O_2$ 171.1397), 142.1356 (calculated for $C_9H_{18}O$ 142.1355). For $C_{31}H_{50}O_5$ calculated: 74.06% C, 10.02% H; found: 74.12% C, 10.03% H. For ^{1}H and ^{13}C NMR spectra see Tables I and II.

Further elution with hexane–ethyl acetate 6 : 4 v/v furnished the 2-dehydro derivative **8** (22 mg, 8%) with m.p. 121–124 °C and $[\alpha]_D^{25}$ +2.10 (*c* 1.01, MeOH). IR (Nujol), ν_{max}: 3 478 (OH), 1 704 (CO). UV (*c* 1.01, MeOH), λ_{max} (ε): 259 (270). CD: $\Delta\varepsilon_{311}$ –0.65 (MeOH); $\Delta\varepsilon_{303}$ –0.56; $\Delta\varepsilon_{276}$ +0.72; $\Delta\varepsilon_{234}$ +0.22; $\Delta\varepsilon_{202}$ –1.08. EI MS, *m/z*: 503 (M^+ + 1, 23), 487 (M^+ – 15, 78), 431 (M^+ – 71, 29), 387 (431 – 44, 27), 301 (38), 171 (100), 142 (87), 99 (64). HR MS, *m/z*: 487.3438 (calculated for $C_{30}H_{47}O_5$ 487.3452), 431.2813 (calculated for $C_{26}H_{39}O_5$ 431.2828), 301.1819 (calculated for $C_{19}H_{25}O_3$ 301.1835), 171.1380 (calculated for $C_{10}H_{19}O_2$ 171.1375), 142.1349 (calculated for $C_9H_{18}O$ 142.1341), 99.0809 (calculated for $C_6H_{11}O$ 99.0808). ^{1}H and ^{13}C NMR spectra see Tables I and II.

3,24-Diepicastasterone 22,23-Acetonide (**7a**)

A solution of **6** (20 mg, 0.04 mmol) in dry EtOH (10 ml) and $NaBH_4$ (2 mg, 1 equivalent) was stirred under argon at –25 °C for 5 min. TLC monitoring showed two new more polar products, which were separated by preparative HPLC. The fraction eluted with MeCN–H_2O 95 : 5 had R_t 8.95 and was the acetonide **7a** (17 mg, 85%) with m.p. 228–231 °C (ref.[12] gives m.p. 235–236 °C) and $[\alpha]_D^{24}$ –51.4 (*c* 1.02, MeOH). UV (*c* 1.27, MeOH), λ_{max} (ε): 280 (483). CD: $\Delta\varepsilon_{299}$ –0.84 (MeCN). EI MS, *m/z*: 489 (M^+ – 15, 38), 433 (M^+ – 71, 15), 389 (489 – 100, 17), 301 (18), 171 (69), 142 (100), 99 (80). For ^{1}H and ^{13}C NMR spectra see Tables I and II.

The fraction with R_t 9.58 was identical with the known 22,23-acetonide of 24-epicastasterone (ref.[9]).

3,24-Diepicastasterone (**7b**)

The acetonide **7a** (12 mg) was deprotected by stirring of the methanolic solution (6 ml) with 4 M HCl (0.6 ml) at 50 °C for 1 h. Work-up and crystallization ($CHCl_3$) gave the desired 3,24-diepicastasterone (**7b**; 9 mg, 80%) with m.p. 209–212 °C (ref._ gives m.p. 213–215 °C) and $[\alpha]_D^{23}$ –52.16 (*c* 0.533, MeOH). HPLC: R_t 5.47 (MeCN–H_2O 65 : 35 v/v). UV (*c* 0.53, MeOH), λ_{max} (ε): 285 (195). CD: $\Delta\varepsilon_{298}$ –0.86 (MeCN). EI MS, *m/z*: 364 (M^+ – 100, 100), 345 (45), 319 (38). GC MS: RR_t = 2.04. EI MS of the methylboronate–TMS–ether, *m/z*: 512 (M^+ – 120, 8), 358 (9), 287 (10), 155 (100). HR MS, *m/z*: 364.2597 (calculated for $C_{22}H_{36}O_4$ 364.2580), 363.2520 (calculated for $C_{22}H_{35}O_4$ 363.2505), 362.2453 (calculated for $C_{22}H_{34}O_4$ 362.2449), 346.2479 (calculated for $C_{22}H_{34}O_3$ 346.2450), 345.2400 (calculated for $C_{22}H_{33}O_3$ 345.2370), 319.2262 (calculated for $C_{20}H_{31}O_3$ 319.2251). For ^1H and ^{13}C NMR spectra see Tables I and II.

2-Dehydro-3,24-diepicastasterone (**9**)

Method A. From **6**: Acetonide **6** (27 mg, 0.05 mmol) in MeOH (5 ml) was stirred with 4 M HCl (0.5 ml) at 50 °C for 2 h. Work-up and SiO_2 chromatography gave **9** (20 mg, 80%) with m.p. 125–128 °C and $[\alpha]_D^{26}$ + 2.50 (*c* 0.80, MeOH). UV (*c* 0.08, MeOH), λ_{max} (ε): 275 (578). CD: $\Delta\varepsilon_{299}$ –0.04 (MeOH); $\Delta\varepsilon_{234}$ +0.02. EI MS, *m/z*: 462 (M^+, 4), 458 (12), 391 (M^+ – 71, 8), 362 (M^+ – 100, 100), 361 (M^+ – 101, 99), 343 (361 – 18, 86). HR MS, *m/z*: 362.2419 (calculated for $C_{22}H_{34}O_4$ 362.2381), 361.2357 (calculated for $C_{22}H_{33}O_4$ 361.2335), 343.2255 (calculated for $C_{22}H_{31}O_3$ 343.2236), 332.2339 (calculated for $C_{21}H_{32}O_3$ 332.2327), 101.0981 (calculated for $C_6H_{13}O$ 101.0996). For ^1H and ^{13}C NMR spectra see Tables I and II.

Method B. From **8**: Acetonide **8** (25 mg, 0.05 mmol) was deprotected in the same manner as described in method *A*. After SiO_2 chromatography, a product was separated (15 mg, 60%), whose physical data were identical with that of **9**, described under method *A* and was synthesized from **6**.

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