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Synthesis of 24-Epiteasterone, 24-Epityphasterol and their B-Homo-6a-Oxalactones from Ergosterol

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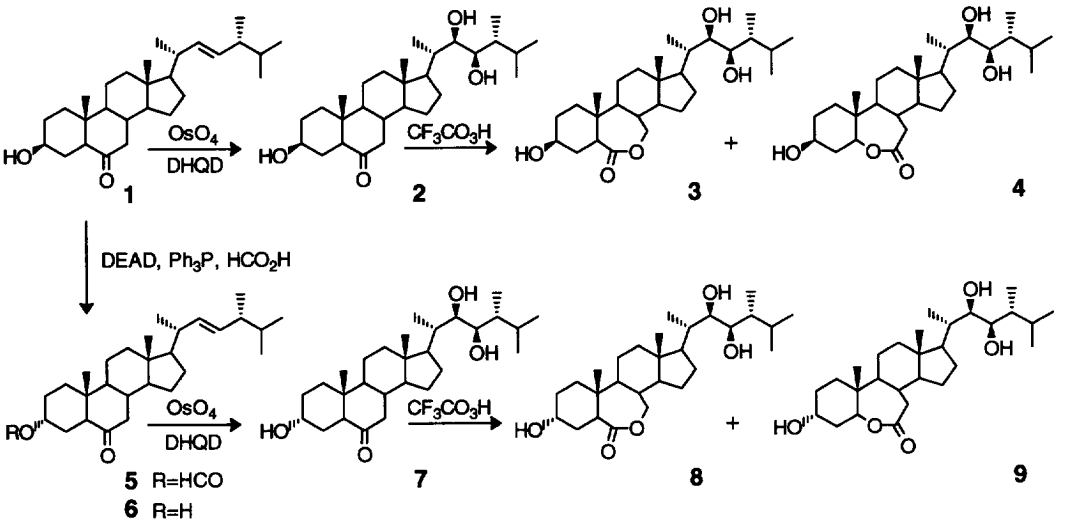
Abstract: Continuing a programme directed toward the synthesis of biologically active analogues and potential biogenetic precursors of brassinosteroids the new members 24-epiteasterone (**2**) and 24-epityphasterol (**7**) as well as their corresponding lactones 2-deoxy-3,24-diepibrassinolide (**3**) and 2-deoxy-24-epibrassinolide (**8**) have been synthesized starting from ergosterol.

The brassinosteroids represent a new class of steroidal phytohormones of an ubiquitous occurrence in the plant kingdom with high promoting and antistress activity.[1-3] Since the discovery of the first member brassinolide in 1979 about 40 other native brassinosteroids have been identified from a broad variety of plants.[4-6] The structural determination of endogenous brassinosteroids, present only in minute amounts in plant material, requires the availability of corresponding reference standards. With regard to their expected occurrence in plants we developed convenient methods for the synthesis of 24-epiteasterone (**2**) and 24-epityphasterol (**7**) as well as their corresponding B-homo-6a-oxalactones 2-deoxy-3,24-diepibrassinolide (**3**) and 2-deoxy-24-epibrassinolide (**8**), respectively, starting from ergosterol.

RESULTS AND DISCUSSION

For the synthesis of 24-epiteasterone (**2**) the known (24R)-3β-hydroxy-24-methyl-5α-cholest-22-en-6-one (**1**), prepared in 5 steps from ergosterol,[7] was used (Scheme 1). Catalytic asymmetric dihydroxylation of the Δ^{22} double bond of **1** (OsO_4, $K_3Fe(CN)_6$, K_2CO_3) with the chiral ligand dihydroquinidine (DHQD) 4-chlorobenzoate[8] in tert. butanol-water afforded 66% 24-epiteasterone **2** with (22R,23R)-vicinal diol function, essential for a high brassinosteroidal bioactivity.[9] Baeyer-Villiger oxidation of **2** with trifluoroperoxyacetic acid followed by preparative HPLC separation led to 2-deoxy-3,24-diepibrassinolide (**3**) and its 5a-oxa-6-oxo isomer **4** in a 1:0.6 ratio.

The corresponding 3α-hydroxylated compounds **7**, **8** and **9** were synthesized from **1** using the Mitsunobu procedure[10] for the inversion of the configuration at C-3. Thus, reaction of **1** with diethyl azodicarboxylate (DEAD), triphenylphosphine (Ph_3P) and formic acid in dry benzene led to the 3α-formyloxy ester **5**, which was hydrolyzed to give the 3α-alcohol **6**. Asymmetric dihydroxylation of the Δ^{22} double bond of **6** and HPLC separation as described for **2** afforded 24-epityphasterol (**7**) as main product, which upon Baeyer-Villiger oxidation led to the desired 2-deoxy-24-epibrassinolide (**8**) and its regioisomer **9** in a 1:0.6 ratio.



Scheme 1

The spectral data of all new compounds are in agreement with the given structures (see Experimental). Especially the ^{1}H NMR spectra confirm the lactone/isolactone structures of **3** and **4** as well as **8** and **9**, respectively. As shown in the (24S)-series[11] both regioisomeric B-homo lactone types are easily to differentiate by their characteristic H-5 and H-7 chemical shifts: Whereas in the 6a-oxa-lactones the H-5 signal appears as a double doublet at 2.86 (**3**) or 3.18 (**8**) and that of H-7 (2 H) at 4.06 (**3**) or 4.10 ppm (**8**), in the 5a-oxa-lactones H-5 resonates at 4.26 (**4**) or 4.62 (**9**) and H-7 at 2.48 (**4**) or 2.49 ppm (**9**). Also the opposite circular dichroism observed in trifluoroethanol allows a clear differentiation between both lactone series (Fig.1; 6a-oxalactones **3** and **8**: $\Delta\varepsilon$ + 0.20 and + 0.27, respectively, at 215 nm; 5a-oxalactones **4** and **9**: $\Delta\varepsilon$ - 0.14 and - 0.12, respectively, at 212 nm).

All four lactone/isolactone compounds **3** and **4** as well as **8** and **9** can be also distinguished as methylboronate-trimethylsilyl derivatives[12] by their GC data and electron impact mass spectra (Table 1). The relative retention times RR_t in the capillary gas chromatography are quite different, indicating the 3α-hydroxy compounds **8** and **9** elute earlier than the corresponding 3β-hydroxy compounds **3** and **4**, respectively. This is in agreement with results obtained for typhasterol and teasterone as well as their 24-epi-isomers **7** and **2**.[13]

The mass spectral behaviour of these compounds is mainly characterized by side chain cleavages and fissions in the AB-ring system (Scheme 2). While ions of type **a, j, f** and **g** are typical for lactone type brassinosteroids with a 24-methyl and 22,23-dihydroxy side chain structure,[14] ion **l** represents a key ion in the mass spectra of trimethylsilylated 3-hydroxy steroids.[15] However, the most important fragments for differentiating the lactone (**3, 8**) and isolactone (**4, 9**) type brassinosteroids are the complementary ions **c** and **m** for lactones as well as **d** and **i** for isolactones, respectively (Scheme 2, Table 1). The mass spectral differences are important for the identification of such compounds in trace amounts. By comparison of such GC/MS data including the here described compounds **2, 3, 7** and **8** very recently the new lactone type brassinosteroid 2-deoxybrassinolide from seeds of *Apium graveolens*, was detected.[13]

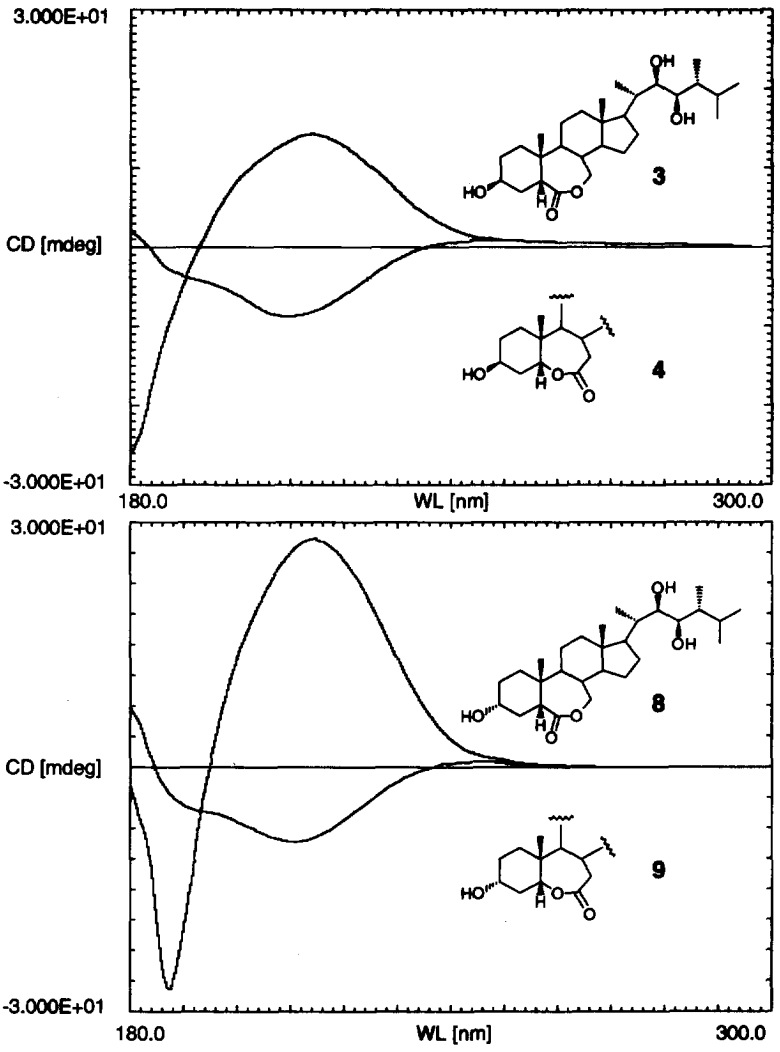
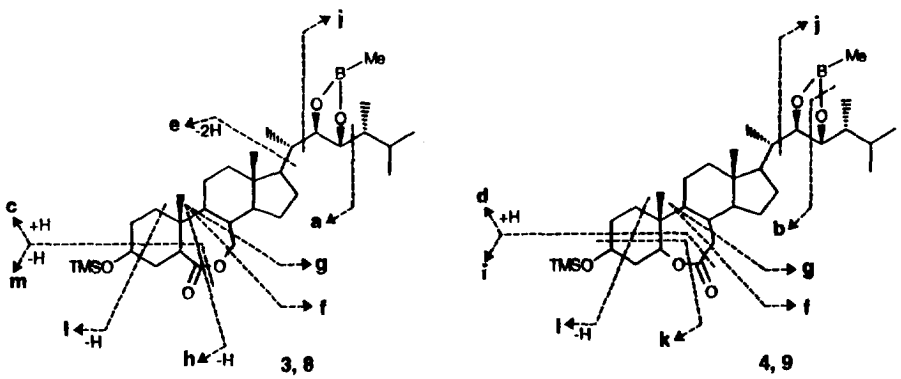


Fig. 1. Circular Dichroism Spectra of the Isomeric Lactones **3** und **4** as well as **8** and **9** (in Trifluoroethanol)

Table 1. GC/MS Data of Compounds 3, 4, 8 and 9 (as Methylboronate-Trimethylsilyl Derivatives)

Compound		3	4	8	9
RR_t [a]		2.59	2.48	2.13	2.10
Ion	m/z	Relative intensity (%)			
M^+	560	1	5	4	5
$[M-Me]^+$	545	100	21	20	-
$[M-H_2O]^+$	542	-	8	-	10
$[M-Me-H_2O]^+$	527	-	15	-	-
(a+H)	490	-	-	13	-
a	489	3	-	-	2
$[M-TMSOH]^+$	470	2	-	5	21
b	460	-	35	-	33
c	404	3	-	3	-
d	388	-	49	-	89
e	375	2	-	2	-
(**d**-Me)	373	-	12	-	15
f	332	1	2	5	3
g	316	2	12	2	7
h	211	5	-	5	-
i	173	-	22	-	17
m	156	67	-	100	-
j	155	26	100	20	74
k	145	-	75	-	65
l	129	12	71	6	100

[a] Relative retention time (RR_t) related to 5α-cholestane (R_t = 5.45 min)



Scheme 2. Mass Spectral Fragmentation of Compounds 3, 4, 8 and 9 (as Methylboronate-Trimethylsilyl Derivatives)

Teasterone and typhasterol were shown to be the biogenetic precursors of castasterone in seedlings of *Nicotiana tabaccum* and *Oryza sativa* and in *Catharanthus roseus* also for brassinolide.[16] It can be expected, that the herewith described (24R)-analogues 24-epiteasterone (**2**) and 24-epityphasterol (**7**) are also intermediates in the biosynthetic pathway leading to the phytohormones 24-epicastasterone and 24-epibrassinolide which were shown to be more wide-spread in higher plants than hitherto assumed.[4] Furthermore, our finding of 2-deoxybrassinolide as native brassinosteroid[13] indicates, that in regard to the substitution pattern at ring A parallel biosynthetic pathways are possible which could led also to oxalactones **3** and **8** as naturally occurring members.

The phytohormone activity of the synthesized (24R)-configurated brassinosteroid analogues was studied using the highly sensitive and specific rice lamina inclination bioassay according to the method of Arima et al.[17] The obtained results showed that 24-epiteasterone (**2**) at a concentration of 0.01 ppm has 94% and 24-epityphasterol (**7**) 87% activity related to 24-epibrassinolide as standard (100%). Also the corresponding 6a-oxalactones **3** and **8** exhibit 103 and 94% activity, respectively. The isomeric 5a-oxa-lactones **4** and **9** were shown to be remarkable less active, namely 74 as well as 66%.

EXPERIMENTAL SECTION

General: Melting points (m.p.)were determined on a Boetius hot stage microscope and are uncorrected. IR spectra were recorded on a Bruker IFS 28 instrument. Optical rotations were measured on a DIP 1000-polarimeter, UV spectra on an 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, Electro spray ionisation (ESI) mass spectra with a Finnigan TSQ 7000 instrument. The GC-MS data of methylboronate-trimethylsilyl derivatives were obtained with a MD-800 Fisons instrument.[13] ^{1}H NMR spectra were recorded on a Varian 500 spectrometer at 499.84 MHz in $CDCl_3$ with TMS as internal standard. For TLC plates precoated with silica gel 60 F_{254} 0.2 mm (Merck) and for column chromatography silica gel 60, 0.04 - 0.063 mm (Merck), were used. The preparative HPLC was carried out on a Knauer instrument, with a LiChrospher 100 RP 18, 10 µm, 10 - 250 mm column, MeOH/H_2O 7:3 v/v as eluent and UV detection at 210 nm.

24-Epiteasterone (2). A mixture of compound **1** (0.139 g, 0.3 mmol), $K_3Fe(CN)_6$ (0.650 g, 2.1 mmol, 6 equiv.), anhydr. K_2CO_3 (0.280 g, 2.1 mmol, 6 equiv.), methanesulfonamide (0.070 g, 0.6 mmol, 2 equiv.), dihydroquinidine 4-chlorobenzoate (0.035 g, 0.06 mmol, 0.2 equiv.) in 10 ml t-BuOH-water 1:1 was stirred at rt for 5 days. Solid sodium sulfite (0.250 g) was added, and the mixture was stirred at rt for 1h. t-BuOH was removed under reduced pressure, and the residue was extracted with ethyl acetate (6 x 15 ml). Combined organic extracts were washed with 0.3 M H_2SO_4 (3 x 15 ml) and brine, dried, and concentrated. The crude product was purified by silica gel chromatography. Elution with hexane-ethyl acetate 3:7 v/v afforded **2** (0.099 g, 66%) with m.p. 233 - 234° C (needles); R_f 0.38 (SiO_2, $CHCl_3$/MeOH 95:5 v/v); $[\alpha]_D^{26}$ -23.8° (c 1.26, MeOH); IR (nujol): ν_{max} 3372 (OH), 1709 cm^{-1} (CO); UV (c 1.26, MeOH): λ_{max} (ε) 290 nm (60); CD (MeOH): $\Delta\varepsilon_{293}$ -1.72; ^{1}H NMR: δ 0.68 (3H, s, 18-H_3), 0.76 (3H, d, J 6.7, 19-H_3), 0.85 (3H, d, J 7.0, 28-H_3), 0.87 (3H, d, J 6.7, 26-H_3), 0.92 (3H, d, J 7.0, 27-H_3), 0.98 (3H, d, J 6.7, 21-H_3), 2.22 (1H, dd, J 12.5/2.7, 5α-H), 2.33 (1H, dd, J 13.3/4.4, 7-H), 3.41 (1H, dd, J 10.7/5.2, 23-H), 3.58 (1H, m, 3α-H), 3.70 (1H, m, 22-H); EI-MS: m/z (relative intensities) 447 (M^+-1, 1), 415 (2), 377 (M^+-71, 6), 359

(377-18, 4), 348 (M^+-100, 100); ESI-MS: m/z 471 ($[M+Na]^+$, 100). Anal. calcd. for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78%. Found: C, 74.76; H, 10.59%.

2-Deoxy-3,24-diepibrassinolide (3) and 5α-oxa-6-oxo-lactone 4. To a solution of 24-epiteasterone (2) (125 mg, 0.28 mmol) in dry chloroform (10 ml) at 0° C a mixture of hydrogen peroxide (0.4 ml, 30%) and trifluoroacetic anhydride (2.5 ml) in chloroform (3 ml) was dropped and the mixture was stirred under argon for 2 h. After addition of chloroform the organic layer was washed with water, aqueous sodium carbonate and brine, dried over sodium sulfate and evaporated to give a crude product. Silica gel chromatography yielded 88 mg (68%) of **3** and **4** in a 1:0.6 ratio (measured from ^{1}H NMR integrals of 5α-protons), separated by preparative HPLC: **3**, m.p. 226 - 227° C; R_f 0.26, $[\alpha]_D^{23}$ +38.1° (c 1.47, MeOH); IR (KBr): ν_{max} 3450 (OH), 1710 cm^{-1} (lactone); CD (trifluoroethanol): $\Delta\varepsilon_{215}$ +0.20; ^{1}H NMR: δ 0.71 (3H, s, 18-H_3), 0.85 (3H, d, J, 7.0, 28-H_3), 0.87 (3H, d, J 6.7, 26-H_3), 0.92 (3H, s, 19-H_3), 0.92 (3H, d, J 6.7, 27-H_3), 0.96 (3H, d, J 6.7, 21-H_3), 2.86 (1H, dd, J 11.8/4.9, 5α-H), 3.40 (1H, m, 23-H), 3.56 (1H, sept., 3α-H), 3.68 (1H, br s, 22-H), 4.01 and 4.06 (1H, m, 7α-H, 7β-H); EI-MS: m/z 465 (M^++1, 2), 393 (M^+-71, 12), 364 (M^+-100, 79), 363 (M^+-101, 100), 345 (52), 334 (68), 327 (43), 287 (38); HR-EI-MS 363.2517 (calcd. for $C_{22}H_{35}O_4$ 363.2535); ESI-MS: m/z 487 ($[M+Na]^+$, 100). Anal. calcd. for $C_{28}H_{48}O_5$: C, 72.37; H, 10.42%. Found: C 72.16; H 10.35%.

Compound **4**: m.p. 225-226° C; R_f 0.24, $[\alpha]_D^{24}$ +26.3° (c 1.03, MeOH), IR (KBr): ν_{max} 3454 (OH), 1700 cm^{-1} (lactone); CD (trifluoroethanol): $\Delta\varepsilon_{212}$ -0.14; ^{1}H NMR: δ 0.70 (s, 3H, 18-H_3), 0.85 (d, 3H, J 7.0, 28-H_3) 0.87 (3H, d, J 6.7, 26-H_3), 0.93 (3H, d, J 6.7, 27-H_3), 0.92 (3H, s, 19-H_3), 0.96 (3H, d, J 6.7, 21-H_3), 2.43 (1H, t, J 11.6, 7α-H), 2.54 (1H, d, J 13.4, 7β-H), 3.41 (1H, d, J 5.5, 23-H), 3.58 (1H, m, 3α-H), 3.69 (1H, 22-H), 4.26 (1H, dd, J 11.3/5.2, 5α-H). ; EI-MS: m/z 465 (M^++1, 1), 393 (M^+-71, 12), 364 (M^+-100, 67), 363 (M^+-101, 44), 346 (50), 345 (66), 334 (40), 327 (100), 287 (74); ESI-MS: m/z 487 ($[M+Na]^+$, 100). Anal. calcd. for $C_{28}H_{48}O_5$: C, 72.37; H, 10.42%. Found: C, 72.30; H, 10.23%.

(22E,24R)-3α-Formyloxy-24-methyl-5α-cholest-22-en-6-one (5). To a solution of ketol **1** (212 mg, 0.5 mmol), HCO_2H (0.035 ml), Ph_3P (212 mg) in dry benzene (10 ml) was droped diethyl azodicarboxylate (DEAD, 170 mg) in benzene (2 ml) with stirring and allowed to stand for 20 h. After evaporation of solvents the crude product was purified by flash chromatography. Elution with hexane-ethyl acetate 9:1 v/v gave **5** (160 mg, 71%), m.p. 167-170° C; $[\alpha]_D^{25}$ -35.7 (c 1.60, MeOH); IR (KBr): ν_{max} 1705 (CO), 1734 (HCO_2-); UV (c 1.60, MeOH): λ_{max} (ε) 287 nm (45); CD ($CHCl_3$): $\Delta\varepsilon_{292}$ -2.30; ^{1}H NMR: δ 0.68 (3H, s, 18-H_3), 0.75 (3H, s, 19-H_3), 0.82 (3H, d, J 6.7, 28-H_3), 0.83 (3H, d, J 7.0, 26-H_3), 0.91 (3H, d, J 7.0, 27-H_3), 1.01 (3H, d, J 7.4, 21-H_3), 2.01 and 2.31 (1H, 7α- and 7β-H), 2.59 (1H, dd, J 12.2/3.4, 5α-H), 5.15 (1H, m, 23-H), 5.20 (1H, m, 22-H), 5.25 (1H, m, 3β-H), 8.03 (1H, s, HCO); EI-MS: m/z 442 (M^+, 95), 427 (M^+-15, 13), 396 (M^+-HCO_2H, 44), 381 (396-15, 13), 358 (32), 353 (43), 344 (M^+-98, 64), 329 (33), 315 (55), 302 (21), 289 (32), 271 (100). Anal. calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47%. Found: C, 78.54; H, 10.26%.

(22E,24R)-3α-Hydroxy-24-methyl-5α-cholest-22-en-6-one (6). 3α-Formyloxy derivative **5** (110 mg, 0.25 mmol) was hydrolyzed with K_2CO_3 (100 mg) in methanol (10 ml) at rt for 5 h under stirring. The reaction mixture was neutralized with 2N HCl, the solvent evaporated and the aqueous layer extracted with

ethyl acetate. The organic extract was washed, dried over Na_2SO_4, filtered, evaporated and crystallized from ethyl acetate-hexane to give compound **6** (81 mg, 79%), m.p. 192-195° C; $[\alpha]_D^{25}$ -28.0° (c 1.68, MeOH); IR (KBr): ν_{max} 3464 (OH), 1710 cm^{-1} (CO); UV (c 1.48, $CHCl_3$): λ_{max} (ε) 286 nm (75); CD ($CHCl_3$): $\Delta\varepsilon_{291}$ -1.60; ^1H NMR: δ 0.68 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 0.82 (3H, d, J 7.0, 28-H_3), 0.83 (3H, d, J 6.7, 26-H_3), 0.91 (3H, d, J 6.7, 27-H_3), 1.01 (3H, d, J 6.7, 21-H_3), 2.02 and 2.30 (1H, dd, J 12.9/4.6, 7α- and 7β-H), 2.72 (1H, m, 5α-H), 4.17 (1H, s, 3β-H), 5.15 (1H, dd, J 15.6/7.9, 23-H), 5.21 (1H, dd, J 15.3/7.3, 22-H); EI-MS: m/z 414 (M^+, 92), 399 (M^+-15, 16), 381 (399-18, 5), 371 (M^+-43, 22), 353 (371-18, 32), 316 (M^+-98, 84), 287 (71), 271 (100). Anal. calcd. for $C_{28}H_{46}O_2$: C, 81.10; H, 11.18%. Found: C, 81.02; H, 10.97%.

24-Epityphasterol (7). Asymmetric dihydroxylation of compound **6** (0.390 g, 0.9 mmol) as described for the synthesis of **2**, afforded **7** (309 mg, 73%), m.p. 228-231° C; R_f 0.34; $[\alpha]_D^{27}$ -13.7° (c 1.46, MeOH); IR (nujol): ν_{max} 3341 (OH), 1702 cm^{-1} (CO); UV (c 1.46, MeOH): λ_{max} (ε) 292 nm (64); CD (MeOH): $\Delta\varepsilon_{292}$ -1.81; ^1H NMR: δ 0.68 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 0.84 (3H, d, J 6.7, 28-H3), 0.86 (3H, d, J 6.7, 26-H_3), 0.92 (3H, d, J 7.0, 27-H_3), 0.97 (3H, d, J 6.7, 21-H_3), 2.03 and 2.30 (1H, 7α- and 7β-H), 2.73 (1H, t, J 7.8, 5α-H), 3.40 (1H, t, 23-H), 3.67 (1H, dd, J 4.9/1.5, 22-H), 4.12 (1H, t, J 3.9, 3β-H); EI-MS: m/z 448 (M^+, 1), 430 (M^+-18, 2), 415 (430-15, 2), 377 (M^+-71, 5), 359 (377-18, 7), 348 (M^+-100, 100). Anal. calcd. for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78%. Found: C, 74.83; H, 10.62%.

2-Deoxy-24-epibrassinolide (8) and 5a-oxa-lactone 9. Baeyer-Villiger oxidation of compound **7** (127 mg, 0.29 mmol) as described for the synthesis of **3** led after silica gel chromatography to a 1:0.6 ratio of **8** and **9** (90 mg, 68%), separated by preparative HPLC: **8**: m.p. 215-218° C; R_f 0.27; $[\alpha]_D^{23}$ +16.3° (c 1.02, MeOH); IR (KBr): ν_{max} 3444 (OH), 1713 cm^{-1} (lactone); CD (CF_3CH_2OH): $\Delta\varepsilon_{215}$ +0.27; ^1H NMR: δ 0.71 (3H, s, 18-H_3), 0.85 (3H, d, J 7.0, 28-H_3), 0.87 (3H, d, J 6.7, 26-H_3), 0.89 (3H, s, 19-H_3), 0.92 (3H, d, J 7.0, 27-H_3), 0.97 (3H, d, J 6.4, 21-H_3), 3.18 (1H, dd, J 11.9/3.7, 5α-H), 3.41 (1H, 23-H), 3.69 (1H, 22-H), 4.10 (2H, m, 7α/7β-H), 4.17 (1H, 3β-H); EI-MS: m/z 465 (M^++1, 3), 393 (M^+-71, 12), 364 (M^+-100, 94), 363 (M^+-101, 100), 346 (28), 345 (40), 334 (72), 327 (28), 287 (39); HR-EI-MS: m/z 393.2667 (calcd. for $C_{23}H_{37}O_5$ 393.2641), 363.2519 (calcd. for $C_{22}H_{35}O_4$ 363.2535); ESI-MS: m/z 487 ($[M+Na]^+$, 100). Anal. calcd. for $C_{28}H_{48}O_5$: C, 72.37; H, 10.42%. Found: C, 72.16; H, 10.34%.

Compound **9**: m.p. 218-221° C; R_f 0.24; $[\alpha]_D^{23}$ +8.4° (c 1.28, MeOH); CD (CF_3CH_2OH): $\Delta\varepsilon_{212}$ -0.12; ^1H NMR: δ 0.70 (3H, s, 18-H_3), 0.84 (3H, d, J 7.0, 28-H_3), 0.87 (3H, d, J 6.7, 26-H_3), 0.89 (3H, s, 19-H_3), 0.92 (3H, d, J 6.4, 27-H_3), 0.96 (3H, d, J 6.1, 21-H_3), 2.49 (2H, m, 7α/7β-H), 3.40 (1H, s, 23-H), 3.69 (1H, s, 22-H), 4.21 (1H, s, 3β-H), 4.62 (1H, m, 5α-H). EI-MS: m/z 465 (M^++1, 1), 393 (M^+-71, 10), 364 (M^+-100, 53), 363 (M^+-101, 30), 346 (35), 345 (38), 334 (21), 327 (100), 287 (40); HR-EI-MS: m/z 393.2638 (calcd. for $C_{23}H_{37}O_5$ 363.2641), 327.2315 (calcd. for $C_{22}H_{31}O_2$ 327.2306); ESI-MS: m/z 487 ($[M+Na]^+$, 100). Anal. calcd. for $C_{28}H_{48}O_5$: C, 72.37; H, 10.42%. Found: C, 72.09; H, 10.26%.

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