8. New Pterocarpinoids from *Dolichos marginata* ssp. *erecta*

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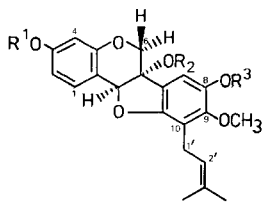
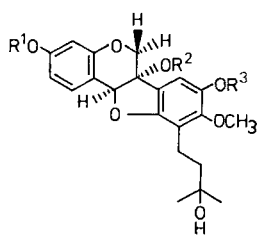
Four new pterocarpinoids, sphenostylin A, B, C, and D (**1–4**), have been isolated from the $CHCl_3$ extract of the root bark of *Dolichos marginata* ssp. *erecta* (Leguminosae) by preparative liquid chromatography. The structures have been established by spectroscopic methods (UV, ^{1}H-NMR, ^{13}C-NMR, EI-MS, DCI-MS, CD) and chemical transformations. The isolated compounds showed weak antifungal activity against *Cladosporium cucumerinum*.

Introduction. – In the course of our systematic screening of African medicinal plants for biological activities, root extracts of *Dolichos marginata* ssp. *erecta* E. MEY. (BAK.) VERDC. (Leguminosae) (syn. *Sphenostylis erecta* E. MEY.) inhibited growth of spores of the fungus *Cladosporium cucumerinum*. The genus *Dolichos* has been studied for the occurrence of lectins, amino acids, and proteins, but the only other constituents of note to be reported are an isoflavone diglycoside [1] and two pterocarpans (= 6a,11a-dihydro-6*H*-benzofuro[3,2-*c*][1]benzopyrans) and dolichins A and B [2] from *D. biflorus* and saponins from *D. falcatus* [3]. The saponins from *D. falcatus* have analgesic [4] and antitumour activities [5]. In the present paper, the isolation of four antifungal pterocarpinoids from the roots of *D. marginata* ssp. *erecta* is described.

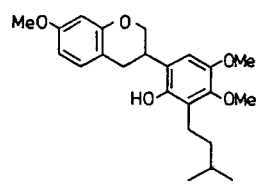
Results. – The $CHCl_3$ extract of *D. marginata* ssp. *erecta* showed by a direct TLC (silica gel, $CHCl_3$/MeOH 10:1) bioassay with *Cladosporium cucumerinum* [6] [7] several inhibition zones. The major antifungal components were isolated and characterised.



1 $R^1 = R^3 = CH_3$, $R^2 = H$
1a $R^1 = R^3 = CH_3$, $R^2 = Ac$
2 $R^1 = R^2 = R^3 = H$
2a $R^1 = R^2 = R^3 = Ac$



3 $R^1 = R^2 = R^3 = H$
3a $R^1 = R^2 = R^3 = Ac$
4 $R^1 = R^2 = H$, $R^3 = CH_3$
4a $R^1 = R^2 = Ac$, $R^3 = CH_3$
5 $R^1 = R^3 = CH_3$, $R^2 = H$



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[1]) Present address: *Analytecon SA*, CH–2108 Couvet.

The crude extract was fractionated by medium-pressure liquid chromatography [8] (see *Exper. Part*). The fractions were further purified by low-pressure liquid chromatography to obtain 4 major active compounds, sphenostylins A, B, C, and D (**1–4**). Their molecular formula and structure could be deduced from their MS, ^{1}H-NMR, and ^{13}C-NMR spectra (**1**: $C_{23}H_{26}O_6$; **2**: $C_{21}H_{22}O_6$; **3**: $C_{21}H_{24}O_7$; **4**: $C_{22}H_{26}O_7$) and from chemical transformations.

The 4 ^{1}H-NMR spectra showed a typical pattern assigned to the heterocycle protons of 6a-hydroxypterocarpans. This included an *AB* pattern ('*q*', 2 H–C(6)) at *ca.* 4 ppm and a low-field *s* (H–C(11a)) at *ca.* 5.2 ppm (see *Table*), as observed for compounds of this class [9]. This was confirmed by easy conversion into their pterocarpene derivatives, a feature characteristic of 6a-hydroxypterocarpans [10]. The reaction was achieved by adding 1 drop of conc. HCl to a solution of pterocarpan in a UV cell. We observed the following bathochromic shifts: 285 to 340 nm for **1**, 284 to 335 nm for **2**, 285 to 335 for **3**, and 286 to 335 nm for **4**. The sphenostylins **1–4** exhibited furthermore an aromatic *ABC* pattern and an aromatic *s*. The presence of 3 MeO groups and an isopentenyl substituent for **1** and 2 MeO groups and an isopentenyl function for **2** is readily apparent (see *Table*). Compounds **3** and **4** have 1 and 2 MeO substituents, respectively. They show, in addition, the presence of a $(CH_3)_2C$ group (1.1 ppm for **3**; 1.2 ppm for **4**), and *m*'s at 1.45 and 2.45 ppm for **3** and 1.6 and 2.65 ppm for **4**, characteristic signals for 3-hydroxyisopentyl groups.

Treatment of **1–4** with CH_2N_2 gave the following results: Compound **1** was unaffected, and **2** yielded a substance identical to **1** (^{1}H-NMR, UV, MS); **3** and **4** yielded the same compound **5** (^{1}H-NMR, UV, MS). The permethylated compounds **1** and **5** exhibited the same aromatic *ABC* pattern (chemical shifts and coupling constants; see *Table*). The values observed are comparable with those of 3-substituted pterocarpans [9]. Thus, substances **1–4** must have the same substitution on ring A. The monosubstitution of ring A was confirmed by hydrogenolysis (10% Pd/C, AcOEt) of **1**. The obtained isoflavan **6** was analyzed by MS. It displayed a typical isoflavan fragmentation pattern [11] with major peaks at *m/z* 250 (46%), *m/z* 249 (52%), and *m/z* 137 (28%).

The positions of the ring-D substituents were determined by acetylation of **1–4** (see *Exper. Part*). The acetates **1a–4a** were unstable (formation of the corresponding pterocarpenes) upon separation on silica gel, but could be purified by rapid *Lobar* chromatography (silica gel) for **2a** and **3a** and by HPLC *(RP-18)* for **4a** and **1a**. In each case, the phenolic functions and the OH group at C(6a) were acetylated. For **3** and **4**, the tertiary OH function in the side chain remained unaffected.

The ^{1}H-NMR chemical shifts of **1a–4a** permitted to deduce the positions of the different substituents. For the aromatic ring A, a MeO group is located at C(3) for **1** and an OH function at C(3) for **2–4** (see *Table;* chemical-shift displacement for H–C(1), H–C(2), and H–C(4) in **2a–4a**). In addition, we observed an important downfield shift of the proton at ring D. In **1a** and **4a**, the shift ($\Delta\delta$ = 0.3 ppm) is only due to the contribution of the AcO group at C(6a) (no phenolic function on ring D for **1a** and **4a**). For compounds **2a** and **3a**, the shift ($\Delta\delta$ = 0.45 ppm) is due to the presence of 2 AcO moieties, one at C(6a) ($\Delta\delta$ = 0.3 ppm) and one at ring D ($\Delta\delta$ = 0.15 ppm). From these values, it can be concluded that the aromatic proton at ring D is next to C(6a) and thus at C(7) for **1–4**.

Finally, from biosynthetic considerations [6] and comparison of chemical shifts with known pterocarpans [9] [12] [13], the isopentenyl and hydroxyisopentyl substituents can be located at C(10). Hence, C(9) of ring D is occupied by the remaining MeO group in all 4 compounds.

The sphenostylins A, B, C, and D **1–4** are new 6a-hydroxypterocarpans. Measurement of their CD spectra [9] showed for each compound a strong negative *Cotton* effect

Table. *^{1}H-NMR Spectra* (200 MHz)

	H–C(1)	H–C(2)	H–C(4)	H–C(7)	H–C(8)
Cristacarpin [9]	7.33 (*d*, *J* = 8.5)	6.56 (*'q'*, *J* = 8.5, 2.3)	6.31 (*d*, *J* = 2.3)	7.18 (*d*, *J* = 8)	6.54 (*d*, *J* = 8)
1 ($CDCl_3$)	7.42 (*d*, *J* = 8)	6.65 (*'q'*, *J* = 8, 2)	6.45 (*d*, *J* = 2)	6.79 (*s*)	–
2 ($CDCl_3$)	7.32 (*d*, *J* = 7)	6.55 (*'q'*, *J* = 7, 2)	6.37 (*d*, *J* = 2)	6.82 (*s*)	–
1a ($CDCl_3$)	7.40 (*d*, *J* = 8)	6.65 (*'q'*, *J* = 8, 2)	6.42 (*d*, *J* = 2)	7.12 (*s*)	–
2a ($CDCl_3$)	7.51 (*d*, *J* = 8)	6.82 (*'q'*, *J* = 8, 2)	6.68 (*d*, *J* = 2)	7.26 (*s*)	–
3 ((D_6)DMSO)	7.20 (*d*, *J* = 8)	6.40 (*'q'*, *J* = 8, 2)	6.20 (*d*, *J* = 2)	6.67 (*s*)	–
4 (CD_3OD)	7.28 (*d*, *J* = 8)	6.48 (*'q'*, *J* = 8, 2)	6.27 (*d*, *J* = 2)	6.88 (*s*)	–
5 ($CDCl_3$)	7.41 (*d*, *J* = 8)	6.65 (*'q'*, *J* = 8, 2)	6.45 (*d*, *J* = 2)	6.80 (*s*)	–
3a ($CDCl_3$)	7.51 (*d*, *J* = 9)	6.81 (*'q'*, *J* = 9, 2)	6.67 (*d*, *J* = 2)	7.25 (*s*)	–
4a ($CDCl_3$)	7.51 (*d*, *J* = 8)	6.82 (*'q'*, *J* = 8, 2)	6.66 (*d*, *J* = 2)	7.10 (*s*)	–

[a]) Chemical shifts are given in ppm and coupling constants *J* in Hz.

(see *Exper. Part*), thus indicating the (6a*S*,11a*S*)-configuration. In a TLC bioassay with the fungus *Cladosporium cucumerinum*, the pure compounds inhibited spore growth at a minimum concentration of 6.25 μg for **1**, 10 μg for **2**, 20 μg for **4**, and 50 μg for **3**.

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Experimental Part

General. Acetylations were carried out by stirring the compounds with Ac_2O in pyridine for 24 h at r.t. Medium-pressure liquid chromatography (MPLC): *Büchi B-681* system. Low-pressure liquid chromatography (LPLC): *Lobar* silica-gel column *(Merck)*. HPLC: *Perkin Elmer Series 3B*, column *RP-18 Knauer* semi-prep. TLC: silica-gel precoated Al sheets *(Merck)*, $CHCl_3$/MeOH 9:1, visualisation with *Godin* reagent [14]. UV: *Perkin Elmer Lambda 3*. CD ($\lambda(\Theta)$ in nm): *Jasco J-500 C*. ^{1}H- and ^{13}C-NMR spectra; *Bruker WP-200* (200 and 50.29 MHz) in $CDCl_3$, CD_3OD, or (D_6)DMSO using TMS as an internal standard. MS: *Nermag R-3010* spectrometer.

Plant Material. D. marginata ssp. *erecta* was collected at Zomba, Malawi. A voucher specimen of the plant material is retained at the Herbarium, Chancellor College, University of Malawi, Zomba.

Extraction and Isolation. The powdered root bark of *D. marginata* (152 g) was extracted at r.t. with petroleum ether (625 mg), followed by $CHCl_3$ (11 g). A part of the crude extract (3.5 g) was separated by MPLC on a 36 mm × 92 cm column (silica gel *Merck 9385*) with $CHCl_3$/MeOH 10:0.8. Five fractions were collected. *Fraction 1* (140 mg) was further purified on a *Lobar* silica-gel column with petroleum ether/AcOEt 5:5 affording **1** (62 mg). *Fraction 4* (580 mg) was purified by MPLC on a 36 mm × 92 cm column (silica gel), with petroleum ether/AcOEt 3:7: **2** (43 mg) and **3** (35 mg). *Fraction 5* was purified on a *Lobar* silica-gel column with petroleum ether/AcOEt 2:8: pure **4** (27 mg).

*Sphenostylin A (= (6aS,11aS)-6a,11a-Dihydro-3,8,9-trimethoxy-10-(3-methyl-2-butenyl)-6*H*-benzofuro-[3,2-c][1]benzopyran-6a-ol;* **1**). Transparent oil. TLC (SiO_2): R_f 0.60. UV ($CHCl_3$): 243, 285, 298. CD ($CHCl_3$): 244 (−35000), 277 (−5700), 286 (0), 290 (+3400), 297 (0), 305 (−1700). ^{1}H-NMR: *Table*. ^{13}C-NMR ($CDCl_3$): 17.96

Sphenostylins A–D (**1–4**) *and of their Derivatives*[a])

H–C(6)	H–C(11a)	2 H–C(1')	1 or 2 H–C(2')	2 CH_3–C(3')	MeO	AcOAr	AcO–C(6a)
.06, 4.16	5.27 (*s*)	3.25 (*d*, *J* = 7)	5.16 (*t*, *J* = 7)	1.65 (*s*)	3.83 (*s*)	–	–
AB, *J* = 12)				1.76 (*s*)			
.05, 4.22	5.24 (*s*)	3.25 (*d*, *J* = 7)	5.17 (*t*, *J* = 7)	1.63 (*s*)	3.77 (*s*, 6 H)	–	–
AB, *J* = 11)				1.73 (*s*)	3.82 (*s*)		
98, 4.18	5.20 (*s*)	3.27 (*d*, *J* = 7)	5.17 (*t*, *J* = 7)	1.65 (*s*)	3.75 (*s*)	–	–
AB, *J* = 12)				1.73 (*s*)			
51, 4.69	5.58 (*s*)	3.25 (*d*, *J* = 7)	5.17 (*t*, *J* = 7)	1.63 (*s*)	3.77 (*s*)	–	2.06 (*s*)
AB, *J* = 12)				1.73 (*s*)	3.78 (*s*)		
					3.83 (*s*)		
35, 4.78	5.59 (*s*)	3.27 (*d*, *J* = 7)	5.14 (*t*, *J* = 7)	1.65 (*s*)	3.75 (*s*)	2.30 (*s*)	2.06 (*s*)
AB, *J* = 12)				1.73 (*s*)		2.29 (*s*)	
80, 4.00	5.05 (*s*)	2.45 (*m*)	1.45 (*m*)	1.10 (*s*)	3.65 (*s*)	–	–
AB, *J* = 11)							
92, 4.10	5.15 (*s*)	2.60 (*m*)	1.60 (*m*)	1.19 (*s*)	3.75 (*s*)	–	–
AB, *J* = 12)				1.20 (*s*)	3.80 (*s*)		
02, 4.22	5.23 (*s*)	2.65 (*m*)	1.67 (*m*)	1.23 (*s*)	3.77 (*s*)	–	–
AB, *J* = 11)					3.80 (*s*)		
					3.83 (*s*)		
35, 4.74	5.60 (*s*)	2.62 (*t*, *J* = 8)	1.68 (*m*)	1.23 (*s*)	3.77 (*s*)	2.28 (*s*)	2.05 (*s*)
AB, *J* = 12)				1.24 (*s*)		2.29 (*s*)	
49, 4.71	5.50 (*s*)	2.65 (*t*, *J* = 7)	1.68 (*m*)	1.22 (*s*)	3.82 (*s*)	2.28 (*s*)	2.05 (*s*)
AB, *J* = 12)					3.83 (*s*)		

(CH_3–C(3')); 23.66 (C(1')); 25.86 (CH_3–C(3')); 55.64 (CH_3O–C(3)); 57.12 (CH_3O–C(8)); 60.97 (CH_3O–C(9)); 70.00 (C(6)); 78.08 (C(6a)); 84.4 (C(11a)); 102.08 (C(7)); 105.83 (C(4)); 110.00 (C(2)); 113.28 (C(11b)); 120.30 (C(6b)); 121.92 (C(1)); 122.59 (C(2')); 131.95 (C(10)); 132.35 (C(3')); 148.70 (C(8)); 150.36 (C(9)); 152.76 (C(10a)); 156.14 (C(4a)); 161.53 (C(3)). EI-MS: 398 (100, $M^{+\cdot}$), 380 (18), 370 (20), 342 (14), 314 (20), 299 (18), 177 (27).

Acetate **1a** was purified by HPLC on *RP-18* with MeOH/H_2O 95%→100% MeOH in 20 min. ^{1}H-NMR: *Table*. DCI-MS (NH_3): 440 ($M^{+\cdot}$).

*6-(3,4-Dihydro-7-methoxy-2*H*-1-benzopyran-3-yl)-3,4-dimethoxy-2-(3-methylbutyl)phenol* (**6**). For 24 h, 1.5 mg of **1** was hydrogenated over 10% Pd/C in AcOEt. After filtration, the mixture was purified on silica gel with petroleum ether/AcOEt 9:1. The main component **6** was analyzed by MS. EI-MS: 386 (100, $M^{+\cdot}$), 249 (52), 250 (46), 137 (28).

Sphenostylin B (= *(6a*S*,11a*S*)-6a,11a-Dihydro-9-methoxy-10-(3-methyl-2-butenyl)-6*H*-benzofuro[3,2-c]-[1]benzopyran-3,6a,8-triol;* **2**). Oil. TLC (SiO_2): R_f 0.45. UV ($CHCl_3$): 245, 284, 298. CD ($CHCl_3$): 246 (−15700), 279 (−3800), 285 (O), 289 (+2400), 294 (O), 303 (−1400). ^{1}H-NMR: *Table*. ^{13}C-NMR ($CDCl_3$): 17.85 (CH_3–C(3')); 23.63 (C(1')); 25.66 (CH_3–C(3')); 61.49 (CH_3O–C(9)); 69.59 (C(6)); 77.73 (C(6a)); 83.98 (C(11a)); 103.80 (C(7)); 107.45 (C(4)); 110.41 (C(2)); 113.21 (C(11b)); 118.99 (C(6b)); 121.94 (C(1)); 123.21 (C(2')); 132.26 (C(10)); 132.50 (C(3')); 144.12 (C(8)); 147.36 (C(9)); 151.88 (C(10a)); 155.95 (C(4a)); 157.26 (C(3)). DCI-MS (NH_3): 370 ($M^{+\cdot}$).

Triacetate **2a** was purified by chromatography on silica gel with petroleum ether/AcOEt 7:3. ^{1}H-NMR: *Table*. DCI-MS (NH_3): 496 ($M^{+\cdot}$).

Sphenostylin C (= *(6a*S*,11a*S*)-6a,11a-Dihydro-10-(3-hydroxy-3-methylbutyl)-9-methoxy-6*H*-benzofuro[3,2-c]-[1]benzopyran-3,6a,8-triol;* **3**). TLC (SiO_2): R_f 0.37. UV (MeOH): 230, 280 (sh), 285, 298. CD (MeOH): 249 (−20500), 286 (−2150), 290 (O), 296 (+2400), 305 (O), 313 (−1700). ^{1}H-NMR ((D_6)DMSO): *Table*. ^{13}C-NMR ((D_6)DMSO): 19.08 (2 CH_3–C(3')); 28.94 (C(2')); 43.41 (C(1')); 60.04 (CH_3O–C(9)); 68.90 (C(6)); 69.40 (C(3')); 75.85 (C(6a)); 83.22 (C(11a)); 102.70 (C(7)); 108.65 (C(4)); 109.86 (C(2)); 111.98 (C(11b)); 119.18 (C(6b)); 123.61 (C(1)); 132.02 (C(10)); 144.24 (C(8)); 147.18 (C(9)); 150.34 (C(10a)); 155.60 (C(11a)); 158.62 (C(3)). DCI-MS (NH_3): 388 ($M^{+\cdot}$).

Triacetate **3a** was purified on silica gel with petroleum ether/AcOEt 5:5. ^{1}H-NMR: *Table*. DCI-MS (NH_3): 514 ($M^{+\cdot}$).

*(6aS,11aS)-6a,11a-Dihydro-10-(3-hydroxy-3-methylbutyl)-3,8,9-trimethoxy-6*H*-benzofuro[3,2-c][1]benzopyran-6a-ol* **5**. Compound **3** or **4** was treated in MeOH with an excess of ethereal diazomethane: **5**. ^{1}H-NMR: *Table*. UV ($CHCl_3$): 242, 280 (sh), 285, 297. DCI-MS (NH_3): 416 ($M^{+\cdot}$).

Sphenostylin D (= *(6aS,11aS)-6a,11a-Dihydro-10-(3-hydroxy-3-methylbutyl)-8,9-dimethoxy-6*H*-benzofuro[3,2-c][1]benzopyran-3,6a-diol;* **4**). TLC (SiO_2): R_f 0.31. UV (MeOH): 230, 280 (sh), 286, 295 (sh). CD (MeOH): 248 (−27300), 283 (−2000), 292 (O), 297 (+1600), 305 (O), 310 (−800). ^{1}H-NMR: *Table*. ^{13}C-NMR (CD_3OD): 20.37 ($2CH_3$–C(3')); 29.04 (C(2')); 44.83 (C(1')); 57.51 (CH_3O–C(8)); 61.53 (CH_3O–C(9)); 70.84 (C(6)); 71.68 (C(3')); 78.21 (C(6a)); 85.57 (C(11a)); 104.23 (C(7)); 107.45 (C(4)); 111.29 (C(2)); 113.58 (C(11b)); 121.55 (C(6b)); 124.42 (C(1)); 133.36 (C(10)); 149.49 (C(8)); 150.78 (C(9)); 153.68 (C(10a)); 157.49 (C(11a)); 160.21 (C(3)). DCI-MS (NH_3): 402 ($M^{+\cdot}$).

Diacetate **4a** was purified by HPLC on *RP-18* with MeOH/H_2O 90%→100% MeOH in 20 min. ^{1}H-NMR: *Table*. DCI-MS (NH_3): 486 ($M^{+\cdot}$).

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