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IRIDOID GLUCOSIDES FROM *ASYSTASIA BELLA*

HELLE DEMUTH, SØREN ROSENDAL JENSEN and BENT JUHL NIELSEN

PharmaBiotek Research Center, Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

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Key Word Index—*Asystasia bella*; Acanthaceae; iridoid glucosides; catalpol; 8-epideoxyloganin; gardoside methyl ester; mussaenoside; asystasioside A–E; chlorinated iridoid.

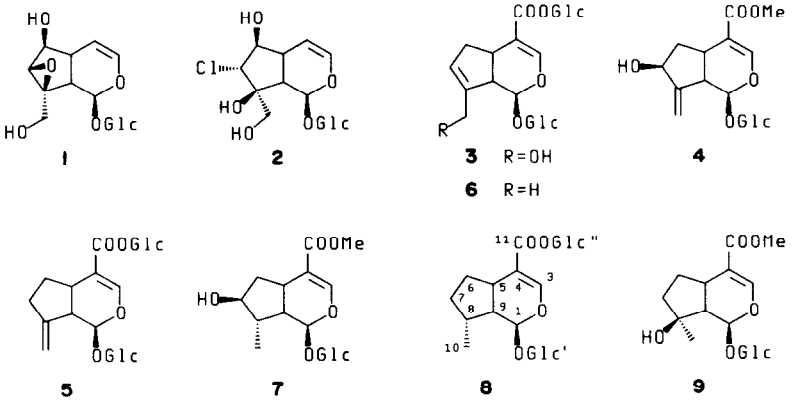
Abstract—Five new iridoid glucosides named asystasioside A–E together with the known compounds catalpol, gardoside methyl ester, 8-epiloganin and mussaenoside were isolated from *Asystasia bella* (= *Mackaya bella*). Asystasiosides A–D are 1-β-glucopyranosyl esters of the four iridoids 8-epideoxyloganic acid, 7-deoxygardoside, 10-deoxygeniposidic acid and geniposidic acid, respectively. Asystasioside E is the chlorohydrin of catalpol with the chlorine atom in the 7α-position. The structural elucidations were mainly performed by NMR spectroscopy, and the structure of asystasioside E was proved by conversion to catalpol. The nine known chloride containing iridoids are all chlorohydrins.

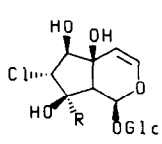
INTRODUCTION

In a recent paper on the chemotaxonomy of the Acanthaceae [1] we reported the presence of four iridoid glucosides in *Asystasia bella* (Harv.) Benth. & Hook. f. (= *Mackaya bella* Harv.). Kooiman [2] had initially isolated catalpol (**1**) from the plant, but we identified three more compounds (**4**, **7** and **9**) and found that some new compounds were also present in smaller amount. Here we present details about the isolation and identification of these minor constituents which we have named asystasioside A–E in the order of presumed biosynthetic complexity.

RESULTS AND DISCUSSION

By reversed phase chromatography of the crude aqueous extract of *Asystasia bella*, asystasioside E (**2**) partly co-chromatographed with catalpol, but repeated fractionation provided the pure compound as a foam (see Experimental). The ^{13}C NMR spectrum (Table 1) showed 15 signals of which six could be assigned to a β-glucopyranosyl moiety and five to an iridoid dihydropyrane ring similar to that in catalpol (**1**). The last four signals were found in the region δ62–81, proving electronegative substituents to be present on the remaining sites, namely C-6, C-7, C-8 and C-10. The ^{1}H NMR spectrum (500 MHz, see Experimental) confirmed this conclusion as the proton signals for H-6, H-7 and CH_2-10 were all seen at low field. Acetylation under mild conditions provided a hexaacetate (**2a**) and the ^{1}H NMR spectrum showed notable downfield shifts for H-6 and CH_2-10 (1.1 and 0.6 ppm, respectively) but only a minor one for H-7 (0.1 ppm), when comparing with the spectrum of **2**. Similarly, in the ^{13}C NMR spectrum of **2a** downfield acetylation shifts were seen for C-6 and C-10 (1.2 and 1.7 ppm, respectively), while an upfield shift was seen for C-7 (3.9 ppm). Such behaviour suggested that **2** might be the chlorohydrin of catalpol (**1**) with the chlorine atom at C-7, and this was in keeping with the elemental analysis. Proof was obtained by conversion of **2** to **1** by treatment with dilute aqueous base (see Experimental).





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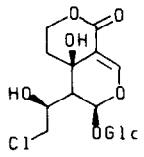

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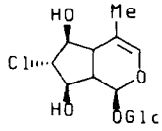

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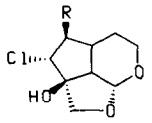

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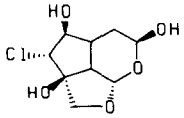

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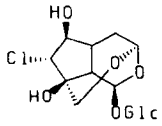

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Table 1 ^{13}C NMR data for asystasiosides A–E and their acetates*

C	2	3	5	6	8	2a	3a	5a	6a	8a
1	92.6	98.3	97.2	97.2	97.1	90.6	96.8	94.8	96.2	94.7
3	139.6	155.6	155.2	155.6	154.7	138.8	153.6	153.5	153.1	152.6
4	106.1	111.6	112.2	110.5	112.7	104.4	110.6	109.5	111.0	111.6
5	35.4	35.1	31.0	35.6	32.5	34.5	34.0	30.6	33.3	32.1
6	81.1	38.9	30.5	38.6	31.5	82.3	38.3	29.9	38.0	30.7
7	71.6	130.2	34.2	127.9	33.1	67.7	131.0	33.4	126.8	32.5
8	79.3	142.1	150.6	139.1	36.1	78.2	137.3	147.5	137.7	35.7
9	47.0	46.6	45.8	49.8	43.4	46.2	46.0	44.5	48.6	42.5
10	62.4	60.7	109.9	15.5	16.3	64.1	61.9	109.6	15.3	15.9
11		168.6	168.4	168.2	168.4		164.7	164.5	164.7	164.8
1′	98.9	99.8	99.5	99.4	99.2	95.3	96.6	95.6	96.0	95.5
2′	73.4	73.7	73.5	73.6	73.5	70.4	70.7	70.5	70.6	70.6
3′	76.4	76.6	76.5	76.5	76.5	71.9	72.0	72.0	72.0	72.0
4′	70.4	70.4	70.4	70.4	70.4	68.0	68.2	68.1	68.1	68.2
5′	76.9	77.1	77.2	77.2	77.1	72.2	72.6	72.4	72.4	72.5
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5
1″		94.7	94.6	94.6	94.5		91.5	91.4	91.3	91.4
2″		72.9	72.8	72.8	72.8		70.1	69.9	69.9	70.0
3″		76.5	76.4	76.4	76.4		72.4	72.2	72.3	72.4
4″		70.1	70.0	70.0	70.0		67.8	67.7	67.7	67.8
5″		77.7	77.6	77.6	77.6		72.7	72.6	72.6	72.7
6″		61.4	61.3	61.3	61.3		61.4	61.3	61.3	61.4

*Glucosides were recorded in D_2O, acetates in $CDCl_3$ at 125 MHz (except for **3** and **3a** at 63 MHz). The spectra were aligned to C-6′ = 61.5 ppm [7].

Asystasioside D (**3**) was isolated as a glass in very small amount (0.003%). The ^{1}H NMR spectrum was similar to that of geniposide, except for the unusually low field shift of H-3, namely δ7.71 which is more than 0.2 ppm downfield from the corresponding shift in geniposide, and for the presence of a doublet (J = 8 Hz) at δ5.60. Additionally, the carbohydrate signals were apparently doubled and no methyl ester signal could be seen. A structure such as **3** seemed a likely possibility, because the anomeric proton of the second β-glucopyranosyl moiety would be expected to resonate at *ca* δ5.5 when acylated at the anomeric position. The ^{13}C NMR spectrum contained 22 signals of which 16 could be assigned to a geniposidic acid moiety by comparison with a spectrum of geniposide [3]. The remaining six signals were consistent with a second β-glucopyranosyl moiety acylated at the anomeric position as seen by the high field shift (δ94.7) of the anomeric carbon. Acetylation provided the crystalline nonaacetate (**3a**), in keeping with the structure **3**.

Asystasioside B and C (**6** and **5**) were obtained in admixture with 8-epiloganin (**7**) in a fraction from reversed phase chromatography. Preparative thin layer chromatography on silica gel separated **7** from the mixture of **5** and **6**, but the two latter showed no separation on this medium, nor on the Merck Lobar column initially used. However, using a Merck Hibar 7μ reversed phase preparative column, baseline separation was obtained and **5** and **6** were isolated in the pure state, although in

very small amount (0.005 and 0.007%, respectively).

Like the other new compounds, asystasioside C (**5**) was obtained as an amorphous glass. The ^{13}C NMR spectrum was similar to that of **3**, except for the six signals arising from C-5 to C-10. Two signals at δ150.6 and 109.9 suggested the presence of an exocyclic double bond. In the ^1H NMR spectrum this was confirmed by the presence of two vinylic signals at δ5.17 and 5.19 assigned to the protons at C-10. This suggested the structure **5**, and comparison with the published [4] spectrum of 7-deoxygardoside showed only minor differences, except for the 'extra' β-glucopyranosyl moiety. Acetylation provided the crystalline octaacetate (**5a**), in keeping with the proposed structure.

The ^{13}C NMR spectrum of asystasioside B (**6**) showed that **5** and **6** were isomeric compounds. In **6** the double bond was in the 7,8-position, as seen from the signals at δ139.1 and 127.9. In fact, when compared with the published [5] spectrum of 10-deoxygeniposide, only minor deviations were seen for this part of the molecule. In the ^1H NMR spectrum of **6** the expected signals for the vinylic proton at C-7 and the allyllic C-10 methyl group were seen at δ5.58 and 1.82, respectively, the same as reported [6] values. As was the case for **5**, compound **6** produced a crystalline octaacetate (**6a**) by acetylation.

The last new compound, asystasioside A (**8**) was again shown to contain a β-glucopyranosyl moiety, in this case acylated at the anomeric centre with 8-epideoxyloganic acid. When comparing the NMR spectra with those of authentic 8-epideoxyloganin [7, 8] and 8-epideoxyloganic acid [9], a good correspondence was seen, except for the expected changes for H-3 in the ^1H NMR spectrum and for C-3, C-4 and C-11 in the ^{13}C NMR spectrum. Also in this case a crystalline octaacetate (**8a**) was obtained.

Asystasioside E (**2**) belongs to a group of chlorine containing iridoids, the number of which has been increasing steadily in recent years and nine compounds are now known. Linarioside (**10**), the chlorohydrin of antirrhinoside was first isolated from *Linaria japonica* (Scrophulariaceae) [10] and later from two closely related genera, namely *Cymbalaria* [11] and *Kickxia* [12]. Recently, the compound avicennioside [13] from *Avicennia officinalis* (Verbenaceae) has been shown to be identical to linarioside (private communication from Prof. H. Rimpler). Eustoside (**11**) from *Eustoma russelianum* (Gentianaceae) [14] is the chlorohydrin of eustomoside and the only chlorinated secoiridoid glucoside known so far. 7-Chlorodeutziol (**12**) is known from *Mentzelia decapetala* (Loasaceae), which is the chlorohydrin of deutzioside [15]. Cistachlorin (**13**) from *Cistanche salsa* (Orobanchaceae) [16] is apparently a transformed chlorohydrin of 6-deoxycatalpol also present in the plant [17]. Rehmaglutins B (**14**) and D (**15**) as well as glutinoside (**16**) have all been isolated [18, 19] from *Rehmannia glutinosa* (Scrophulariaceae) which also contains catalpol (**1**). The compounds **14**–**16** all seem to be (at least formally) derived from asystasioside E (**2**) isolated in the present work. Finally, we have recently published [20] the isolation of thunbergioside (**17**) from *Thunbergia fragrans* (Acanthaceae) which also contains stilbericoside, the corresponding epoxide. Each of the known chlorinated iridoids listed above are chlorohydrins derived from an iridoid epoxide which is consistently present in the same plant. It seems therefore safe to assume that the known members of this class of iridoids are (at least formally) produced in the plant by addition of chloride ion to the corresponding iridoid epoxide.

Asystasioside A–D are examples of an otherwise rare iridoid structure. A number of iridoids carrying more than one sugar moiety are known, but only in a single reported case [21] is the sugar moiety attached to the C-4 carboxyl group, namely in plumenoside, a minor constituent of *Plumeria acutifolia* (Apocynaceae).

In the paper on the chemotaxonomy of Acanthaceae [1] we postulated a biosynthetic pathway for the iridoid glucosides so far known in the family. Following this, 8-epideoxyloganic acid, deoxygeniposidic acid and geniposidic acid were proposed as key intermediates in the biosynthesis of all the iridoids, even though none of these compounds had been isolated from the family. The finding in *Asystasia bella* of the asystasiosides A, B and D support such a biosynthetic scheme, although the compounds actually found are most probably not precursors themselves, but rather end-products removed from the main pathway by esterification with a glucose. We have no ready explanation for the presence of the fourth compound, asystasioside C, with an exocyclic double bond.

EXPERIMENTAL

Microanalyses were performed by LEO Microanalytical Laboratory, Ballerup, Denmark. Mps: corr. The plant material was grown in a greenhouse in The Botanical Garden, The University of Copenhagen. The voucher no. is given in ref. [1]. Except when otherwise specified, prep chromatography was performed on Merck LOBAR reversed phase columns eluted with the H_2O–MeOH mixtures specified in each case. For ^1H NMR the standards used were the HDO-peak (δ4.75 in D_2O) or TMS.

Frozen foliage (430 g) of *Asystasia bella* was homogenized with EtOH and the concd extract partitioned in Et_2O–H_2O. The aq. phase was passed through alumina (300 g) which was washed with H_2O (750 ml). Evaporation followed by trituration with MeOH (40 ml) and passage through act. C provided a white foam (6 g). Chromatography in 2 portions (RP-8, size C; 22 ml/min) eluting with H_2O–MeOH (25:1 to 1:1) gave first a polar fraction which was discarded. The next fraction (A, 885 mg) consisted of pure catalpol (**1**). Fraction B (175 mg, see below) was a mixture of **1** and asystasioside E (**2**) in the proportion *ca* 3:1, according to ^1H NMR. Fraction C (45 mg) contained asystasioside D (**3**). Rechromatography on the same column (5:1) provided the pure compound as a foam (13 mg, 0.003%), characterized only by NMR. ^1H NMR(250 MHz, D_2O): δ7.71 (*d*, J = 0.7 Hz, H-3), 5.84 (*m*, H-7), 5.60 (*d*, J = 7.6 Hz, H-1''), 5.30 (*d*, J = 6.8 Hz; H-1), 4.80 (*d*, J = 8.0 Hz, H-1'), 4.25 and 4.22 (*br* AB-system, J = *ca* 14 Hz, 10-CH_2), 3.22 (*q*-like, J = 7.5 Hz, H-5), *ca* 2.85 (2H, H-9 and H-6), 2.16 (*m*, H-6). ^{13}C NMR data in Table 1. Fraction D consisted of gardoside methyl ester (**4**, 70 mg; 0.02%), identified by comparison with an authentic sample [22]. Fraction E (100 mg, see below) was a mixture of iridoids (**5**–**7**), as was fraction F (190 mg; **7** and **8**), while the last fraction consisted of pure mussaenoside (**9**, 250 mg; 0.06%).

Fraction B rechromatographed twice on a RP-18 column (size C, 25:1) gave catalpol (**1**; total 1.00 g, 0.23%), an intermediate fraction and pure asystasioside E (**2**; total *ca* 40 mg, 0.01%), isolated as a foam $[\alpha]_D^{20}$ −140° (MeOH; *c* 0.3); ^1H NMR 500 MHz, D_2O): δ6.28 (*dd*, J = 6.3 and 1.7 Hz, H-3); 5.53 (*d*, J = 2.2 Hz, H-1); 5.17 (*dd*, J = 6.1 and 3.3 Hz; H-4); 4.10 (*d*, J = 9.0 Hz, H-7); 3.91 and 3.70 (AB-system, J = 12.5 Hz, 10-CH_2);

ca 3.88 (*m*, H-6); 2.67 (*m*, H-5); 2.61 (*br d*, $J = 11$ Hz; H-9). ^{13}C NMR data in Table 1. (Found: C, 43.6; H; 6.1; Cl; 8.4. $C_{15}H_{23}O_{10}Cl$, H_2O requires: C, 43.2; H; 6.1; Cl; 8.5%).

Rechromatography of fraction E by prep. TLC (silica gel; $CHCl_3$–MeOH, 3:1) gave as the faster moving band 8-epiloganin (**7**, 21 mg) identified by comparison with an authentic sample [22]. The slower band consisted of a mixture of **5** and **6** (67 mg) separated by chromatography on a Merck HIBAR RP-18 (7 μm; 25 × 250 mm) column eluting with 4:1. First eluted was asystasioside C (**5**, 22 mg; 0.005%), solely characterized by NMR. ^{1}H NMR(500 MHz, D_2O): δ7.72 (*d*, $J = 0.7$ Hz, H-3), 5.65 (*d*, $J = 8.1$ Hz; H-1''), 5.59 (*d*, $J = 4.6$ Hz; H-1), 5.19 and 5.17 (*m*'s, 10-CH_2), 4.90 (*d*, $J = 8.0$ Hz, H-1'), 3.11 (*q*-like, $J = ca$ 6 Hz; H-5), 2.98 (*m*, H-9), 2.39 (2H, 7-CH_2), 2.07 and 1.82 (each 1H, *m*'s, 6-CH_2). ^{13}C NMR in Table 1. The second compound was asystasioside B (**6**, 30 mg; 0.007%) characterized only by NMR. ^{1}H NMR(500 MHz, D_2O): δ7.71 (*br s*; H-3), 5.64 (*d*, $J = 8.0$ Hz, H-1''), 5.58 (*m*, H-7); 5.51 (*d*, $J = 4.8$ Hz; H-1), 4.84 (*d*, $J = 8.0$ Hz, H-1'), 3.23 (*dt*, $J = 5$ and 8 Hz, H-5), 2.90 (*m*, H-9); 2.77 (*br dd*, $J = 16$ and 7 Hz, H-6), 2.18 (*br d*, $J = 16$ Hz, H-6), 1.82 (*br s*, 10-CH_3). ^{13}C NMR data in Table 1.

Rechromatography of fraction F by prep. TLC as above gave an additional amount of 8-epiloganin (38 mg, total 0.01%) as the faster moving band, followed by asystasioside A (**8**, 108 mg; 0.02%), isolated as a foam, $[\alpha]_D^{20}$ −74° (MeOH; *c* 0.6); ^{1}H NMR (500 MHz, D_2O): δ7.69 (*s*, H-3), 5.64 (*d*, $J = 8.0$ Hz, H-1''), 5.58 (*d*, $J = 3.6$ Hz, H-1), 4.83 (*d*, $J = 8.0$ Hz, H-1'), 2.97 (*br dt*, $J = 5.0$ and 8.5 Hz, H-5), 2.44 (*dt*, $J = 3.7$ and 8.6; H-9), 2.35 (*m*, H-8), 2.07 (*dq*, $J = 13.2$ and 8.0; H-6), 1.82 (*m*, H-7), 1.64 (*ddt*. $J = 13$, 8 and 5 Hz, H-6), 1.33 (*dq*, $J = 12.6$ and 8.0 Hz, H-7), 1.04 (*d*, $J = 7.1$ Hz, 10-CH_3). ^{13}C NMR data in Table 1. (Found: C, 48.2; H, 7.1. $C_{22}H_{34}O_{14}$, $1\frac{1}{2}$ H_2O requires: C, 48.1; H, 6.8%).

Asystasioside A octaacetate (**8a**). Prepared by acetylation with pyridine–Ac_2O (2:1, 2 hr at room temp.). Crystd from EtOH, mp 183–184°; $[\alpha]_D^{20}$ −62° ($CHCl_3$; *c* 0.5); ^{1}H NMR (500 MHz, $CDCl_3$): δ7.45 (*d*, $J = 0.9$ Hz, H-3), 5.74 (*d*, $J = 8.2$ Hz, H-1''), 5.27 (*d*, $J = ca$ 4 Hz; H-1), 5.0–5.3 (6H; H-2', H-2'', H-3', H-3'', H-4' and H-4''), 4.87 (*d*, $J = 8.2$ Hz, H-1'), 4.29, 4.25, 4.20 and 4.10 (*dd*'s, 6'-CH_2 and 6''-CH_2), 3.85 and 3.72 (*ddd*'s, H-5' and H-5''), 2.88 (*q*-like, $J = 7$ Hz, H-5), 2.23 (2H; H-8 and H-9), 1.94–2.08 (8 × AcO), 1.78 (*m*, H-6), 1.44 (*m*, H-7), 1.27 (*m*, H-6), 1.02 (*d*, $J = 6.8$ Hz; 10-CH_3). ^{13}C NMR data in Table 1. (Found: C, 53.2; H, 6.1. $C_{38}H_{50}O_{22}$ requires: C, 53.2; H, 5.9%).

Asystasioside B octaacetate (**6a**). Prepared as above, mp 186–187°; $[\alpha]_D^{20}$ −18° ($CHCl_3$; *c* 0.5); ^{1}H NMR(500 MHz; $CDCl_3$): δ7.44 (*d*, $J = 1.1$ Hz, H-3), 5.74 (*d*, $J = 8.2$ Hz, H-1''), 5.45 (*m*, H-7), 5.09 (*d*, $J = 5.8$ Hz, H-1), 4.85 (*d*, $J = 8.2$ Hz, H-1'), 3.84 and 3.71 (*ddd*'s; H-5' and H-5''), 3.13 (*q*-like, $J = 7$ Hz, H-5), 2.70 (*m*, H-6), 2.64 (*m*, H-9), 1.99–2.09 (8 × AcO), 1.77 (*br s*, 10-CH_3). ^{13}C NMR data in Table 1. (Found: C, 52.8; H, 5.7. $C_{38}H_{48}O_{22}$, $\frac{1}{2}H_2O$ requires: C, 52.7; H, 5.7%).

Asystasioside C octaacetate (**5a**). Mp 200–201°; $[\alpha]_D^{20}$ −40° ($CHCl_3$; *c* 0.4); ^{1}H NMR(500 MHz, $CDCl_3$): δ7.46 (*d*, $J = 1.1$ Hz, H-3), 5.74 (*d*, $J = 8.2$ Hz, H-1''), 5.27 (*d*, $J = ca$. 4.5 Hz, H-1), 5.09 and 5.05 (*m*'s; 10-CH_2), 4.86 (*d*, $J = 8.0$ Hz, H-1'), 3.74 and 3.86 (*ddd*'s, H-5' and H-5''), 2.97 (*q*-like, $J = 6.5$ Hz, H-5), 2.79 (*m*, H-9), 2.32 (2H, 7-CH_2), 1.94-2.10 (8 × AcO), 1.64 (*m*, H-6). ^{13}C NMR data in Table 1. (Found: C, 53.2; H, 5.8. $C_{38}H_{48}O_{22}$ requires: C, 53.3; H, 5.7%).

Asystasioside D nonacetate (**3a**). Mp 177–179°; $[\alpha]_D^{20}$ −7° ($CHCl_3$; *c* 0.4); ^{1}H NMR (500 MHz, $CDCl_3$): δ7.43 (*d*, $J = 1.1$ Hz, H-3), 5.77 (*m*, H-7), 5.69 (*d*, $J = 8.2$ Hz, H-1''), 4.96 (*d*, $J = 7.5$ Hz, H-1), 4.79 (*d*, $J = 8.1$ Hz, H-1'), 4.64 and 4.62 (*br* AB-system, $J = 14.0$ Hz, 10-CH_2), 3.14 (*br q*, $J = 7.8$ Hz, H-5), *ca* 2.75 (*m*, 2H, H-9 and H-6), 1.93–2.02 (9 × AcO). ^{13}C NMR data in Table 1. (Found: C, 51.9; H, 5.6. $C_{40}H_{50}O_{24}$, $\frac{1}{2}H_2O$ requires: C, 52.0; H, 5.6%).

Asystasioside E hexaacetate (**2a**). Amorphous foam, $[\alpha]_D^{20}$ −119° ($CHCl_3$; *c* 0.5); ^{1}H NMR (500 MHz; $CDCl_3$): δ6.16 (*dd*, $J = 6.2$ and 2.0 Hz, H-3), 5.46 (*d*, $J = 2.2$ Hz, H-1), 5.22 (*br dd*, $J = 6$ and 3 Hz, H-4), 4.86 (*d*, $J = 8.1$ Hz, H-1'), 4.81 (*dd*, $J = 7.9$ and 3.8 Hz, H-6), 4.48 and 4.31 (AB-syst., $J = 12.0$ Hz, 10-CH_2), 4.20 (*d*, $J = 7.9$ Hz, H-7), 2.67 and 2.64 (*m*'s, H-5 and H-9), 1.99-2.13(6 × AcO). ^{13}C NMR data in Table 1. (Found: C, 49.5; H, 5.5; Cl, 5.7; $C_{27}H_{35}O_{16}Cl$ requires C, 49.8; H, 5.4; Cl, 5.5%).

Conversion of asystasioside E (**2**) *to catalpol* (**1**). A mixture of **1** and **2** (1:4) was dissolved in D_2O (0.4 ml) in an NMR tube and the spectrum recorded. NaOD (25 μl 25% in D_2O) was then added to the mixture and after 10 min the spectrum was recorded again showing the presence of catalpol (**1**) only.

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