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Structure elucidation of cyasterone stereoisomers isolated from *Cyathula officinalis*†

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Chemical investigation of ecdysteroidal constituents of the roots and stems of *Cyathula officinalis* led to the isolation of two cyasterone stereoisomers, **2** and **3**, together with the known cyasterone **1**. The structures of compounds **2** and **3** were determined to be 28-epi-cyasterone and 25-epi-28-epi-cyasterone, respectively, by means of spectroscopic analysis. X-Ray structures of **1** and **2** confirmed the 24*S*,25*S*,28*R* configuration for **1** and 24*S*,25*S*,28*S* for **2**.

Introduction

Among a number of ecdysteroids isolated from plants, approximately one fifth has two-carbon substituents at their C-24 positions.[1] Varied structures of C_{29}-phytoecdysteroids are mainly due to modifications at the side chain, while common ecdysteroidal structures, *i.e.* a *cis* A/B ring (5β-H) juncture, a 7-en-6-one chromophore and hydroxyl substituents at C-2, C-14, C-20, C-22, and C-25 positions, can be seen for most of the phytoecdysteroids. Plant C_{29}-sterols seem to be precursors of C_{29}-phytoecdysteroids. Several C_{29}-phytoecdysteroids such as cyasterone,[2,3] capitasterone,[4] and makisterone C [5] possess potent insect molting hormone activity. They, however, were proposed to be non-toxic feeding deterrents to certain insects and to affect growth and development upon ingestion from artificial diets by certain insects.[6] These phytoecdysteroids do not appear to have any appreciable plant hormonal function within plants, as tested in several plant bioassays.[7]

Cyasterone **1**, first isolated from *Cyathula capitata* (Amaranthaceae) by Hikino's group, is one of the most popular phytoecdysteroids. The stereochemistry at the chiral centers in the γ-lactone moiety of **1** was determined to be 24*S*,25*S*,28*R* by NMR studies and the application of the Hudson–Klyne lactone rule to the lactone derivative **7** (Fig. 1).[8] Cyasterone was identified in *C. officinalis*[9] as well as many *Ajuga* species (Labiatae) such as *A. reptans*.[10,11] It is reported that **1** possesses antitumor-promoting activity.[12]

Isocyasterone **4** was also isolated from *C. capitata* and its configuration was assigned as 24*S*,25*R*,28*R* on the basis of NMR studies and the application of the Hudson–Klyne lactone rule.[13,14] Isocyasterone is a less common phytoecdysteroid, and the hairy roots of *A. reptans* var. *atropurpurea* is the only other reported source of **4**.[15] It is rather strange that isocyasterone is characterized only from the *Ajuga* hairy roots, although extensive studies have been done for a number of *Ajuga* species. A related 5-hydroxylated compound, 28-epi-sengosterone **6**, was described from *A. reptans* var. *atropurpurea*, for which the 24*S*,25*S*,28*S* configuration was assigned.[16] 'Isocyasterone from the *Ajuga* hairy roots' and 28-epi-sengosterone displayed essentially identical ^{13}C-NMR data for the side-chain signals, thus suggesting the same lactone structure. These conflicting findings need to be clarified.

We have recently demonstrated that cyasterone and 'isocyasterone' (*vide infra*) are biosynthesized from clerosterol [(24*S*)-stigmasta-5,25-dien-3β-ol] **8** in the *Ajuga* hairy roots.[17] It should be noted that the orientation (24β) of the C-24 ethyl group of clerosterol, a major sterol found in *Ajuga* sp.,[18] is the same as that of cyasterone **1**. In contrast, the orientation (24α) of the 24-ethyl group of the major sterols (sitosterol and

† Electronic supplementary information (ESI) available: ^{1}H-NMR data (500 MHz, CD_3OD) for compounds **1**, **2** and **3**, and ^{1}H-NMR data (500 MHz, $CDCl_3$) for compounds **1a**–**4a**. See http://www.rsc.org/suppdata/ob/b4/b416868b/

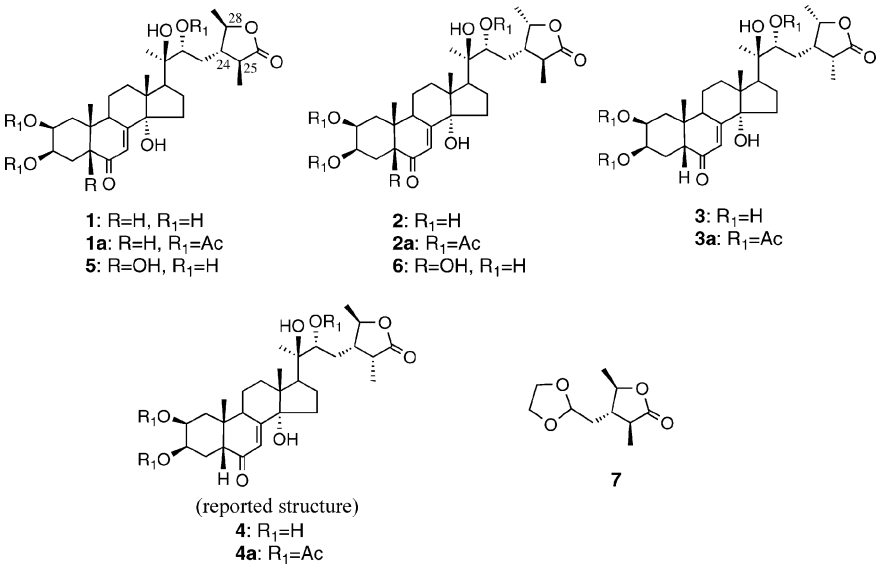

Fig. 1 Chemical structures of compounds **1**–**7**.

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stigmasterol) found in *Cyathula* species is opposite to that of **1** and **4**.[17,19] This implies that the C-24 stereochemistry has to be inverted during the biosynthesis of **1** and **4** from a 24α-ethyl sterol unless a trace amount of a precursor sterol with the correct 24β stereochemistry is utilized. In order to shed light on these questions, we have been interested in reinvestigating phytoecdysteroids of the *Cyathula* species. Although *C. capitata* was not available to us, dried chips of the roots and stems of the closely related *C. officinalis* are commercially available as a Chinese herb preparation. Chemical investigation of the MeOH extract led to the isolation of two γ-lactone-containing C_{29}-ecdysteroids **2** and **3**, together with cyasterone **1** (Fig. 1). In this paper we describe the structure analysis of **2** and **3**, including an NMR data comparison of **1**–**4**.

Results and discussion

The butanol-soluble fraction obtained from the methanolic extract of the dried chips of roots and stems of *Cyathula officinalis* was subjected to repeated column chromatography on silica gel, Sephadex LH-20 and reverse-phase HPLC to afford compounds **1** (0.048% based on the chips), **2** (0.0037%) and **3** (0.00037%).

Compound **1** was isolated as needles, mp 162.0–164.0 °C (lit.[2] 164–166 °C). It was directly identified with cyasterone obtained from the *Ajuga* hairy roots. The ^{13}C-NMR data (in d_5-pyridine[20]) of **1**, listed in Table 1, are in good agreement with those reported.[10] In the ^{1}H-NMR spectrum (in d_5-pyridine[20]) (Table 2), compound **1** showed vicinal coupling constants J_{24-25}: 10.9 Hz, and J_{24-28}: 9.3 Hz. The *J* values recorded in CD_3OD (ESI†) were the same as in d_5-pyridine (seen later in Fig. 4). The *J* values for the 2,3,22-tri-acetate derivative **1a** (in $CDCl_3$) were slightly smaller than those of **1** (ESI†).[8] NOE correlations between H-24 and H-27, and H-24 and H-29 observed for **1** further supported the 24*S**,25*S**,28*R** relative configuration. To confirm the configurations at these chiral centers, X-ray analysis of **1** was carried out. The X-ray structure of **1** is illustrated in Fig. 2 which allowed us to determine the relative configuration of the molecule. On the basis of the known chirality of the steroid nuclei, the 24*S*,25*S*,28*R* absolute configuration was confirmed.

Table 1 ^{13}C-NMR data[a] (125 MHz, py-d_5) for compounds **1**–**3**

	1[b]	**2**	**3**
C-1	37.9	37.9	37.9
C-2	68.1	68.1	68.1
C-3	68.0	68.0	68.0
C-4	32.4	32.4	32.4
C-5	51.3	51.4	51.3
C-6	203.4	203.6	203.4
C-7	121.8	121.7	121.6
C-8	165.7	166.0	165.8
C-9	34.4	34.4	34.4
C-10	38.7	38.6	38.6
C-11	20.9	21.1	21.0
C-12	32.0	32.0	31.9
C-13	48.1	48.2	48.0
C-14	84.1	84.1	84.0
C-15	31.9	31.7	31.7
C-16	21.3	21.2	21.6
C-17	50.0	50.2	49.9
C-18	17.8	17.9	17.7
C-19	24.4	24.4	24.4
C-20	76.7	76.8	76.6
C-21	21.1	21.3	21.3
C-22	74.0	76.4	74.9
C-23	34.4	30.8	26.6
C-24	48.7	45.9	39.8
C-25	42.4	38.7	39.5
C-26	179.1	179.2	180.0
C-27	15.8	16.3	17.4
C-28	79.8	78.8	78.6
C-29	19.3	14.4	12.8

[a] Assignments were based on HMQC spectra. [b] Identical to the data reported in ref. 21 to within 0.2 ppm error.

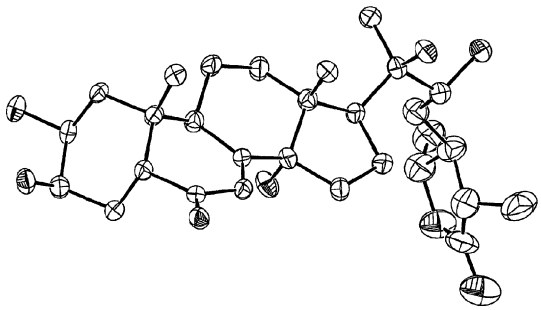


Fig. 2 X-Ray structure of cyasterone **1**.

Sengosterone **5**,[21] isolated from *A. reptans*, is a C-5-hydroxylated derivative of **1**. The ^{13}C-NMR data for the lactone moieties and the J_{24-25} and J_{24-28} values reported for **5**[16] were in good agreement with those of **1**. Compound **5** has recently been characterized in the *Ajuga* hairy roots.[22]

Compound **2** was isolated as needles, mp 248–250 °C. Its molecular formula was determined to be $C_{29}H_{44}O_8$ on the basis of HRFABMS of **2**. The ^{1}H- and ^{13}C-NMR data resembled those of compound **1** except for the signals of the side chain. The H_3-27 and H_3-29 resonated at δ 1.18 and 1.29, respectively. The resonance of H-28 was diagnostically downfield-shifted to δ 4.98 compared to that of **1**. The J_{24-25} value (11.5 Hz) of **2** was close to that (10.9 Hz) of **1**, whereas the J_{24-28} value (6.8 Hz) was much smaller than that (9.3 Hz) of **1**. Acetylation of **2** yielded the 2,3,22-tri-acetate **2a**. The chemical shift (in $CDCl_3$) of H-25 (δ 2.37) of **2a** was very close to that (δ 2.33) of **1a**, whereas H-28 (δ 4.64) of **2a** resonated downfield by *ca.* 0.5 ppm compared to that of **1a** (δ 4.13) (ESI†). These ^{1}H-NMR data suggested that compound **2** should be a C-28 epimer of **1**. This was further supported by NOE correlations from H_3-29 to H-25 and from H_3-27 to H-24. These findings indicated that compound **2** possesses the 24*S**,25*S**,28*S** relative configuration. X-Ray analysis of **2** confirmed the 24*S*,25*S*,28*S* relative configuration, as can be seen in the X-ray structure of **2** (Fig. 3). In reference to the known chirality of the steroid nuclei, the 24*S*,25*S*,28*S* absolute configuration was established. Thus, compound **2** was determined to be a C-28 epimer of cyasterone and named 28-epi-cyasterone.

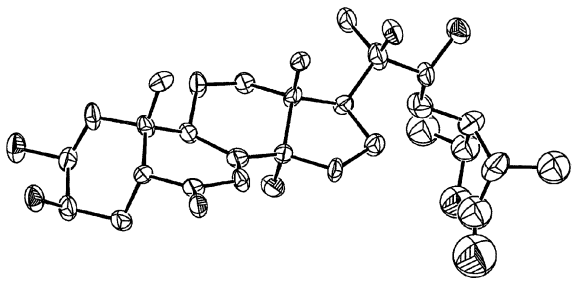


Fig. 3 X-Ray structure of 28-epi-cyasterone **2**.

With a sample of 28-epi-cyasterone with the defined structure in hand, 'isocyasterone from the *Ajuga* hairy roots' was compared with **2**. 'The isocyasterone sample' was identical with **2** in all aspects (^{1}H- and ^{13}C-NMR, TLC, HPLC). Thus, identification of isocyasterone as reported by Matsumoto and Tanaka[15] was found to be erroneous and 'isocyasterone from the *Ajuga* hairy roots' should be revised to 28-epi-cyasterone **2**. The revision accounts for the identical ^{13}C data for the side chains of 28-epi-sengosterone **6** and 'isocyasterone from the

Table 2 ^{1}H-NMR data[a] (500 MHz, py-d_5) for compounds **1**–**4**

	1[b]	**2**	**3**	**4**[c]
1-Hax	1.94	1.92	1.94	
1-Heq	2.15 (dd, 13.5, 4.2)	2.13 (dd, 13.3, 4.6)	2.15 (dd, 13.1, 4.7)	
2-Hax	4.16 (brd, 10.1)	4.16 (dt, 11.0, 3.6)	4.19 (brd, 11.5)	4.06 (m)
3-Heq	4.22 (brs, $W_{1/2} = 9.4$)	4.22 (brs, $W_{1/2} = 9.4$)	4.23 (brs, $W_{1/2} = 7.4$)	4.10 (m)
4-Hax	1.81	1.81	1.81	
4-Heq	2.04	2.03	2.03	
5-H	3.01 (dd, 13.7, 3.4)	3.00 (dd, 13.2, 3.7)	3.02 (dd, 13.2, 3.7)	2.85 (dd, 12, 4)
7-H	6.28 (d, 2.1)	6.25 (d, 2.3)	6.27 (d, 2.1)	6.16 (d, 2)
9-H	3.61 (brt, 8.8)	3.59 (brt, 8.6)	3.59 (brt, 9.9)	3.45 (m)
11-Hax	1.78	1.75	1.74	
11-Heq	1.93	1.90	1.89	
12-Hax	2.65 (td, 12.8, 4.8)	2.60 (td, 13.0, 4.7)	2.57 (td, 13.0, 4.2)	
12-Heq	2.07	2.03	1.98	
15-Ha	1.80	1.92	1.89	
15-Hb	2.22	2.22	2.18	
16-Ha	2.48 (m)	2.47	2.49 (m)	
16-Hb	2.07	2.03	2.05	
17-H	2.87 (t, 9.2)	2.86 (t, 9.2)	2.89	
22-H	3.95 (brd, 9.8)	3.89 (dd, 11.0, 2.1)	3.82 (brd, 11.5)	3.72 (m)
23-Ha	1.73	1.64	1.69	
23-Hb	1.94	1.80	1.85	
24-H	2.23	2.47	3.14	
25-H	2.39 (dq, 10.9, 7.1)	2.34 (dq, 11.5, 7.0)	2.89 (m)	
28-H	4.03 (dq, 9.3, 6.1)	4.98 (dq, 6.8, 6.6)	4.65 (qui, 6.7)	4.76 (qui, 7.0)
18-H_3	1.25 (s)	1.21 (s)	1.22 (s)	1.16 (s)
19-H_3	1.08 (s)	1.05 (s)	1.08 (s)	1.04 (s)
21-H_3	1.58 (s)	1.58 (s)	1.59 (s)	1.52 (s)
27-H_3	1.37 (d, 7.1)	1.18 (d, 7.0)	1.28 (d, 7.5)	1.24 (d, 6.0)
29-H_3	1.33 (d, 6.1)	1.29 (d, 6.6)	1.29 (d, 6.6)	1.19 (d, 7.0)

[a] Assignments were based on ^{1}H–^{1}H COSY and HMQC spectra. [b] Partial data were reported in ref. 21. [c] Adopted from ref. 14.

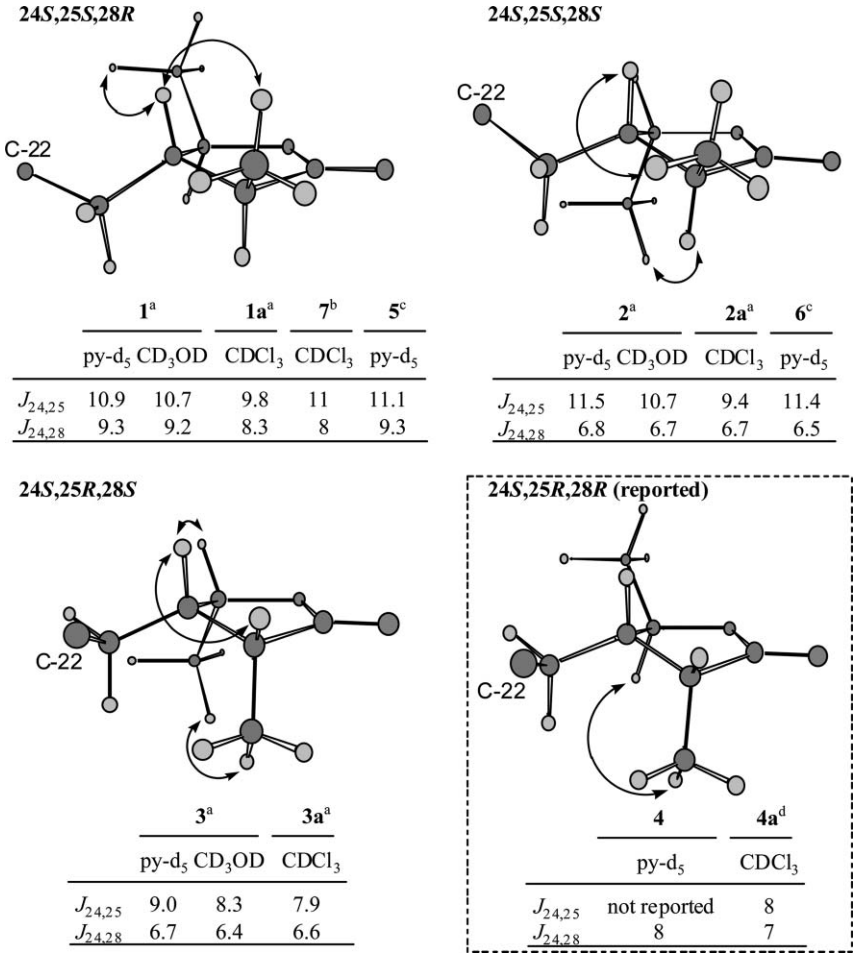
Ajuga hairy roots'. It would be safe to mention that *Ajuga* plants biosynthesize 25*S*-type lactones, but not the 25*R*-type. It is recommended that epi-cyasterone,[13] previously named a C-5 epimer (5α-H) of cyasterone, should be designated as 5-epi-cyasterone to avoid confusion.

Compound **3** was isolated as a white amorphous solid. The molecular formula of **3**, obtained by HRFABMS, was identical to that of **2**. The NMR data of **3** were again close to those of **1** except for the signals due to the side chain, suggesting that **3** is a diastereomer of **1** and **2**. In the ^{1}H-NMR spectrum of **3**, two doublet methyl signals of H_3-27 (δ 1.28) and H_3-29 (δ 1.29) resonances almost overlapped. The J_{24-25} value (9.0 Hz, determined by decoupling experiment irradiating H_3-27) was smaller than those of **1** and **2**, while the J_{24-28} (6.7 Hz) value is very close to that of **2**. NOE correlations were observed between H-24 (δ 3.14) and H-25 (δ 2.89), and H-24 and H-28 (δ 4.65) for **3**, while a ROESY correlation (in $CDCl_3$) between H_3-27 (δ 1.28) and H_3-29 (δ 1.44) was detected in 2,3,22-tri-acetate **3a** which was obtained upon acetylation of **3**. The chemical shift of H-28 (δ 4.65) of **3a** was very close to that (δ 4.64) of **2a**, whereas the chemical shift of H-25 (δ 2.71) of **3a** was significantly different from those of **1a** (δ 2.33) and **2a** (δ 2.37) (ESI†). These observations indicated that H-24 is *syn* to both H-25 and H-28, assigning a 24*S**,25*R**,28*S** configuration. We were not able to obtain evidence for the absolute configuration due to the scarcity of the material, but the 24*S*,25*R*,28*S* configuration of **3** was assumed from a biosynthetic analogy of **1** and **2**. Compound **3** was named 25-epi-28-epi-cyasterone. The ^{13}C resonances of **3** showed the following diagnostic difference. The C-23 signal of **3** was upfield-shifted by *ca.* 8 ppm compared to that of compound **1**. This upfield-shift can be reasonably explained by the steric proximity of C-23 to C-27 and C-29 groups.

Isocyasterone **4** was previously assigned as the 24*S*,25*R*,28*R*-isomer. The *syn*-orientation of H_3-27 and H-28 was based on an NOE observation between the two groups for 2,3,22-tri-acetate **4a**.[14] However, somewhat strangely, the chemical shifts of H-25 (δ 2.70) and H-28 (δ 4.64) reported for **4a** are essentially identical to those (δ 2.71 and δ 4.65) of **3a** (ESI†). Furthermore, the reported J_{24-25} and J_{24-28} values of **4a**, listed in Fig. 4, are very close to those of compound **3a**. It is difficult for us to conclude that the close similarity in the NMR data of **3a** and **4a** is due to the identical nature of **3a** and **4a** or is merely coincidental. If isocyasterone is indeed the 24*S*,25*R*,28*R*-isomer **4** as reported, compound **3** is a new phytoecdysteroid. Otherwise, the structure of the isocyasterone needs to be revised. Our attempt to isolate **4** from *C. officinalis* was not successful. Re-isolation of isocyasterone and measurements of accurate NMR spectra are highly recommended.

In the present study, we have established the structures of compounds **2** and **3**, isolated from *C. officinalis*, and have confirmed the structure of cyasterone **1**. 'Isocyasterone from the *Ajuga* hairy roots' was identified with 28-epi-cyasterone **2**, while 'isocyasterone from *C capitata*' requires rigorous structure elucidation. We recommend use of 28-epi-cyasterone for the 24*S*,25*S*,28*S*-isomer and 25-epi-28-epi-cyasterone for the 24*S*,25*R*,28*S*-isomer hereafter. The accurate NMR data presented here will be helpful for further identification of related ecdysteroids.

Fig. 4 illustrates the most stable conformations for the lactone moieties of the four isomers which were deduced from conformational analysis [Conformer 2.0 (Princeton Simulations) together with MM2 energy minimization by Chem3D program]. All isomers consistently bear envelope conformations with the C-24 carbon atom up which allows the C-23 substituent to occupy a pseudo-equatorial orientation. The X-ray structure (Fig. 2) for the lactone moiety of **1** is similar to that shown for **1** in Fig. 4. However, the lactone conformation in the X-ray structure (Fig. 3) and that shown in Fig. 4 for **2** are significantly different. The dihedral angles of C-23–C-24–C-25–C-27 of **2** are −148° (the most stable conformation) and −80° (X-ray) and C-23–C-24–C-28–C-29 are +36° (the most stable conformation) and −36° (X-ray). In crystals of **2** the



	1[a]		1a[a]	7[b]	5[c]
	py-d_5	CD_3OD	$CDCl_3$	$CDCl_3$	py-d_5
$J_{24,25}$	10.9	10.7	9.8	11	11.1
$J_{24,28}$	9.3	9.2	8.3	8	9.3

	2[a]		2a[a]	6[c]
	py-d_5	CD_3OD	$CDCl_3$	py-d_5
$J_{24,25}$	11.5	10.7	9.4	11.4
$J_{24,28}$	6.8	6.7	6.7	6.5

	3[a]		3a[a]
	py-d_5	CD_3OD	$CDCl_3$
$J_{24,25}$	9.0	8.3	7.9
$J_{24,28}$	6.7	6.4	6.6

	4	4a[d]
	py-d_5	$CDCl_3$
$J_{24,25}$	not reported	8
$J_{24,28}$	8	7

Fig. 4 The most stable conformations of the lactone moieties of compounds **1**–**4** and related compounds **5**–**7** and their acetates **1a**–**4a**. The conformations were deduced for model lactones with a C-24 ethyl substituent. Arrows indicate pertinent NOE correlations. Coupling constants are given in Hz. [a]Present study. [b]Adopted from ref. 8. [c]Adopted from ref. 16. [d]NOE correlation for **4a** is adopted from refs. 13 and 14.

lactone moiety bears an envelope conformation in which the C-24 carbon atom is down. This allows the C-23 substituent to occupy a pseudo-axial position. The conformation essentially equal to the X-ray structure (a model lactone with a C-24 ethyl substituent), deduced from conformational analysis, was less stable by *ca.* 1.6 kcal than the most stable conformation, thus suggesting that there is some reason for adopting the X-ray conformation.

Fig. 4 also summarizes the vicinal coupling constants, $J_{24—25}$ and $J_{24—28}$. It seems difficult to assign a *syn*- or *anti*-relationship in these γ-lactone rings only from the coupling constants, unless the *J* value is more than 10 Hz, *e.g.* $J_{24—25}$ of **1** and **2**. In order to differentiate and identify cyasterone stereoisomers, the chemical shifts of H-25 and H-28 appear to be much more diagnostic, particularly when the spectra are measured in $CDCl_3$ for acetate derivatives or in CD_3OD (ESI†) for non-acetylated materials (H-25 and H-28: δ 2.49 and 4.19 for **1**, δ 2.44 and 4.82 for **2**, δ 2.89 and 4.73 for **3**).

Since the structure of 'isocyasterone from the *Ajuga* hairy roots' has been revised, 'isocyasterone' in our previous papers[17,23] must be read as 28-epi-cyasterone. This requires modification of the biosynthetic route leading to 28-epi-cyasterone (Fig. 5) in *Ajuga* sp. Biosynthesis of cyasterone and 28-epi-cyasterone (not isocyasterone) in the hairy roots of *Ajuga reptans* var. *atropurpurea* would involve non-stereospecific hydroxylation at C-28 and stereospecific hydrogenation (from the 25-*Re* face)

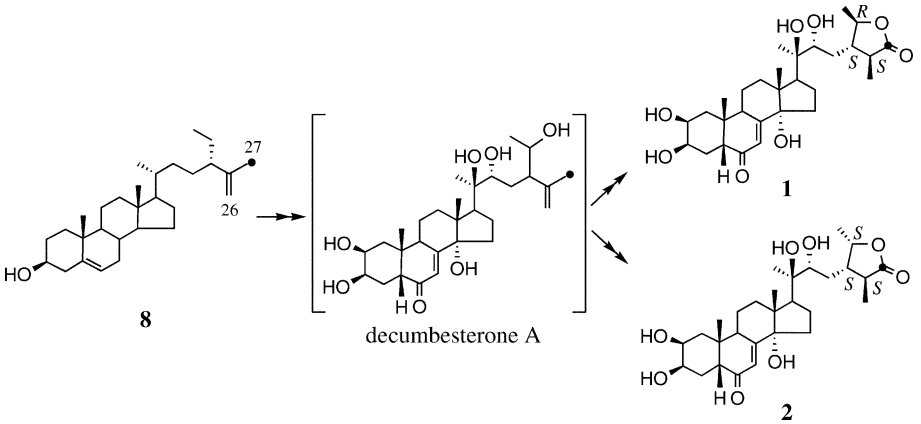


Fig. 5 Postulated biosynthesis of **1** and **2**. Dots represent carbon atoms which are metabolically correlated with respect to C-26 and C-27.

of the $\Delta^{25(26)}$-double bond. A 28-hydroxylated intermediate, decumbesterone A, was previously isolated from *Ajuga decumbens*.[12] We also isolated a small amount of amarasterone A[24] from *C. officinalis*. This compound can be regarded as a biosynthetic intermediate leading to cyasterones in the *Cyathula* species. Study on the C-24 stereochemistry[25] of amarasterone A is in progress in our laboratory to elucidate the puzzling C-24 stereochemical aspects in biosynthesis.

Experimental

Melting points were determined on a Yazawa BY-1 hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker 500 (500 MHz) spectrometer in C_5D_5N, CD_3OD or $CDCl_3$ solution. Chemical shifts are referenced to an internal TMS signal (0.00 ppm) for $CDCl_3$ solution, a residual solvent signal (7.19 ppm) for C_5D_5N and a residual solvent signal (3.30 ppm) for CD_3OD. ^{13}C-NMR spectra were recorded on the same spectrometer (125 MHz) and chemical shifts are referenced to C_5D_5N (149.20 ppm). All δ values are given in ppm and J values in Hz. Mass spectra were taken on a JEOL JMS-700 spectrometer. Column chromatography was performed using Merck Kieselgel 60 (230–400 mesh) or Sephadex LH-20. HPLC was performed on a Shimadzu LC-6A instrument with an SPD-6A UV detector using an ODS column (Shimadzu STR PREP-ODS column, 6 × 15 mm i.d.).

Isolation of ecdysteroid fraction

The chips of roots and stems of *C. Officinalis* (600 g) in benzene (1.8 l) were heated at reflux for 2 h and the solvent was discarded. The defatted chips in MeOH (1.8 l) were refluxed for 1 h and the MeOH was filtered and stored. The chips were extracted with MeOH once more in the same manner. The combined MeOH extract was concentrated *in vacuo*. The residue (117 g) was mixed with water (1 l) and extracted with *n*-BuOH (1 l). The water layer was extracted with the same solvent once again. The combined BuOH extract was concentrated to dryness. The residue (31 g) was chromatographed on silica gel using a $CHCl_3$–MeOH gradient system to give the ecdysteroid-containing fraction (eluted with $CHCl_3$–MeOH 10 : 1, 524 mg) as a brown solid. This was further chromatographed on Sephadex LH-20 using MeOH to give the ecdysteroid fraction (412 mg) as a pale yellow solid. This fraction was separated by HPLC (solvent, MeOH–H_2O 10 : 8; flow rate, 1.0 ml min^{-1}; detector, 243 nm; typical retention times, 13.8, 15.2 and 17.2 min in the order) to give **1** (144 mg), **3** (1.1 mg) and **2** (11.2 mg). R_f values on TLC (developed three times with $CHCl_3$–MeOH 10 : 1) were 0.38 for **1**, 0.38 for **2** and 0.35 for **3**.

Cyasterone (1)

White needles, mp 162.0–164.0 °C (from MeOH) (lit.[2] 164–166 °C). UV λ_{max}^{MeOH} nm (log ε): 241.4 (4.11). Cooling a warmed solution of **1** in a capped vial to room temperature slowly grew colorless crystals for X-ray analysis. Tri-acetate derivative **1a** was prepared in a usual method (Ac_2O/pyridine). m/z [FAB] Found: 647.3425 $[M + H]^+$; $[C_{35}H_{51}O_{11}]$ requires: 647.3431.

28-Epi-cyasterone (2)

White needles, mp 248.0–250.0 °C (from MeOH). m/z [FAB] Found: 521.3152 $[M + H]^+$; $[C_{29}H_{45}O_8]$ requires: 521.3114. UV λ_{max}^{MeOH} nm (log ε): 242.0 (4.11). Colorless crystals for X-ray analysis were prepared in the same manner as described for **1**. Tri-acetate derivative **2a** was prepared in the usual method. White needles, mp 224–226 °C (from MeOH). m/z [FAB] Found: 647.3417 $[M + H]^+$; $[C_{35}H_{51}O_{11}]$ requires: 647.3431.

25-Epi-28-epi-cyasterone (3)

White amorphous solid. m/z [FAB] Found: 521.3109 $[M + H]^+$; $[C_{29}H_{45}O_8]$ requires: 521.3114. UV λ_{max}^{MeOH} nm (log ε): 241.2 (4.11). Tri-acetate derivative **3a** was prepared in the usual method. White amorphous solid. m/z [FAB] Found: 647.3461 $[M + H]^+$; $[C_{35}H_{51}O_{11}]$ requires: 647.3431.

Crystallographic data for cyasterone 1

$C_{29}H_{44}O_8 \cdot 3MeOH$, monoclinic, $P2_1$, $Z = 2$, $a = 13.6542(11)$, $b = 7.7755(4)$, $c = 15.7636(12)$ Å, $\beta = 100.215(3)°$, $V = 1647.1(2)$ Å^3, $T = 123$ K, $D_x = 1.244$ g cm^{-3}, λ(CuKα) = 1.54184 Å, $\mu = 0.760$ mm^{-1}. A total of 18 438 reflections was collected by an oscillation photograph method using a Rigaku R-AXIS RAPID Imaging Plate camera and Lp and semi-empirical absorption corrections were applied. The structure was solved by direct methods using SIR-97. The positional and anisotropic thermal parameters were refined by full-matrix least squares SHELXL-97 using 5428 unique reflections ($R_{int} = 0.0459$). The positions of hydrogen atoms were calculated geometrically and refined using the riding model. Final $R = 0.0647$ for 5250 unique reflections with $|I_o| > 2\sigma(I_o)$. Ecdysteroid molecules are linked by hydrogen bonding directly or *via* methanol molecules.

CCDC reference number 255171. See http://www.rsc.org/suppdata/ob/b4/b416868b/ for crystallographic data in .cif or other electronic format.

Crystallographic data for 28-epi-cyasterone 2

$C_{29}H_{44}O_8 \cdot 3H_2O$, monoclinic, $P2_1$, $Z = 2$, $a = 13.3374(17)$, $b = 7.6406(10)$, $c = 15.5158(19)$ Å, $\beta = 98.311(4)°$, $V = 1564.5(3)$ Å^3, $T = 293(2)$ K, $Dx = 1.254$ g cm^{-3}, λ(CuKα) = 1.54184 Å, $\mu = 0.673$ mm^{-1}. A total of 14 751 reflections was collected and the structure was solved as described for **1**. The positional and anisotropic thermal parameters were refined in the same manner as **1** using 3076 unique reflections ($R_{int} = 0.0930$). Final $R = 0.1593$ for 1620 unique reflections with $|I_o| > 2\sigma(I_o)$. Because of the very small crystal size and poor crystallinity, the quality of the reflection data is poor. Also, the thermal motions of the atoms are relatively large. The structure of the lactone ring was composed from the result of direct methods and constrained as a rigid group at the final refinement stage. They showed large rotational motion around the C23–C24 bond and it prevented convergence without constraints. One of three crystalline waters shows disorder and it is modeled by two half occupancy oxygen atoms. Hydrogen atoms of all crystalline waters could not be located, so they are not included in this structure. Ecdysteroid molecules are linked by hydrogen bonding directly or *via* water molecules.

CCDC reference number 255170. See http://www.rsc.org/suppdata/ob/b4/b416868b/ for crystallographic data in .cif or other electronic format.

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