Arcapitins A – C, First Dammarane-Type Triterpenes from the Convolvulaceae[§]

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Three new dammarane-type triterpenes, named arcapitin A, B and C, were isolated from the roots of *Argyreia capitata* (Convolvulaceae). They turned out to be 11α-acetoxydammar-24-ene-20(*S*)-ol-3-one, dammar-24-ene-11α,20(*S*)-diol-3-one and 11α-acetoxydammar-24-ene-3β,20(*S*)-diol. While the same compounds were also detected in the aerial vegetative parts of this species, their occurrence in a number of other convolvulaceous species could not be confirmed.

Introduction

Nearly 2000 predominantly tropical species belong to the Convolvulaceae. A number of pentacyclic triterpenes have been isolated from members of this family over the years, e. g. friedelan-3-one (friedelin) and friedelan-3β-ol from *Argyreia populifolia* Choisy (Gunatilake and Sultanbawa, 1973), α- and β-amyrine from *Convolvulus lanatus* Vahl (Seif El-Nasr *et al.*, 1984) and lupeol from *Ipomoea purpurea* (L.) Roth (Bhatt *et al.*, 1981). Reports on tetracyclic triterpenoids are less frequent. Apart from the occurrence of ubiquitous sterols like β-sitosterol (Gunatilake and Sultanbawa, 1973) only phytoecdysones have been described so far (Canonica *et al.*, 1975 and references cited). The present study comprises the isolation and structural characterization of three dammarane-type triterpenes from *Argyreia capitata* (Vahl) Choisy, a tropical liana from Southeast Asia.

Results and Discussion

The compounds **1**–**3** (Fig. 1) were detected in the petroleum ether and dichloromethane extracts, giving blue-coloured spots with van Urk's reagent on TLC sheets. They could not only be observed in the roots and in the aerial vegetative parts of

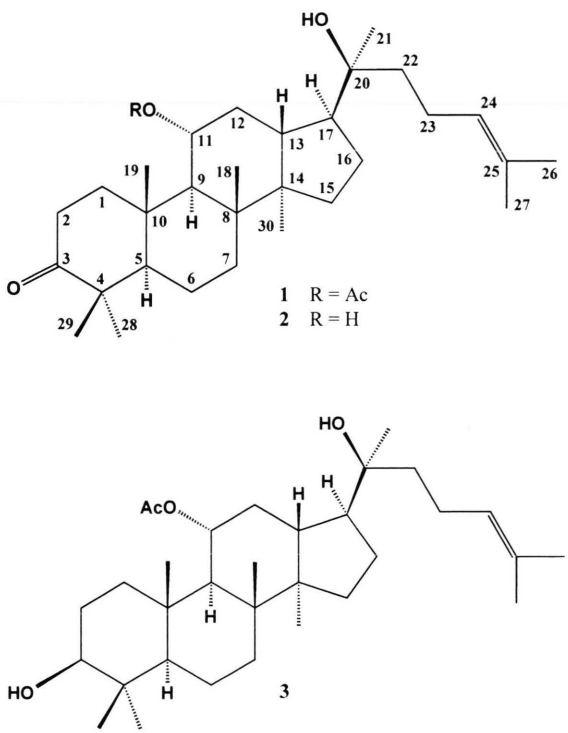

Fig. 1. Compounds isolated from *Argyreia capitata.*

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A. capitata from the greenhouse but also in plant material collected in the wild. After the isolation from roots of *A. capitata* their structures were established on the basis of EIMS, HRMS, FABMS, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HETCOR, HMBC as well as NOE experiments. Each compound gave a positive Liebermann-Burchard-reaction, indicating a triterpenoid structure.

The FAB mass spectrum (negative mode) of arcapitin A (**1**) showed a quasi-molecular ion peak at *m/z* 499 $[M - H]^-$. The EI mass spectrum showed significant peaks at *m/z* 482 and at *m/z* 422. The formulae of these peaks were determined as $C_{32}H_{50}O_3$ $[M\text{-}H_2O]^+$ and as $C_{30}H_{46}O$ $[M - H_2O - CH_3COOH]^+$ by HRMS. The presence of an acetoxy group was confirmed by the signal at δ 1.99 (3 H, s) in the ^{1}H NMR spectrum and by signals at δ 22.0 ($\mathbf{C}H_3CO$) and at δ 170.0 ($CH_3\mathbf{C}O$) in the ^{13}C NMR spectrum.

The ^{1}H NMR, ^{13}C NMR and DEPT spectra revealed the presence of eight further tertiary methyl groups and nine methylene groups (Table I). Six methine groups, including an oxygenated (δ 73.4) and an olefinic methine group (δ 124.5), as well as seven quaternary carbons, including one olefinic (δ 131.8), one oxygenated (δ 75.0) and a keto group (δ 218.2) could also be observed. These structural elements indicated the presence of a tetracyclic triterpene with three six-membered rings and one five-membered ring.

The ^{1}H NMR and the ^{1}H-^{1}H COSY spectra showed that a dimethylallyl group was present, a structural feature e. g. of dammaranes. Since no signal for a secondary methyl group appeared in the spectra, an oxygenated C-20 had to be assumed (δ 75.0 in the ^{13}C NMR spectrum). The location of the carbon of the keto group in position 3 was indicated by the chemical shifts of a methylene group at low field (δ 2.33, 1 H, m; δ 2.55, 1 H, m) which was coupled with a further methylene group (δ 1.85, 1 H, m; δ 1.95, 1 H, m). The methine group bearing the acetoxy group (δ 5.18, 1 H, td, J = 5.3 and 10.9 Hz) was coupled both to a methylene group (δ 1.41, 1 H, m; δ 2.15, 1 H, m) and a methine group at δ 1.89 (m). While the methine group did not show any other couplings, the methylene group further coupled with a methine group at δ 1.83 (m). Consequently the acetoxy group was situated at C-11.

The methyl singlet at δ 1.05 (H-18) showed HMBC correlations to δ 34.8 (C-7) as well as to δ 52.2 (C-9), while no correlation of any methyl signal and C-12 was observed (Table II). Thus a methyl group could be excluded at C-13. On the basis of these data **1** was characterized as a dammarane-type triterpene. This was confirmed by NOE experiments: NOE interactions were observed between H-18, H-19 and H-29 as well as between H-9 and H-30. No NOE interactions were observable between H-30 and any other methyl group.

The coupling constants J = 5.3 and 10.9 Hz of the ^{1}H NMR signal of H-11 at δ 5.18 required an axial H-11. This was confirmed by NOE interactions between H-11 and H-19. The *S*-configuration at C-20 was deduced by comparing the chemical shifts of C-20, C-21 and C-22 with those of published data (Asakawa *et al.*, 1977; Anjaneyulu *et al.*, 1985; Bianchini *et al.*, 1988). Therefore arcapitin A (**1**) is 11α-acetoxydammar-24-ene-20(*S*)-ol-3-one.

The FAB mass spectrum (positive mode) of arcapitin B (**2**) showed a quasi-molecular ion peak at *m/z* 459 $[M + H]^+$. The EI mass spectrum showed a significant peak at *m/z* 440, which was determined as $C_{30}H_{48}O_2$ $[M\text{-}H_2O]^+$ by HRMS. The ^{1}H NMR and ^{13}C NMR data of **2** lacked the signals for the acetyl moiety of **1**, while the H-11 signal was shifted upfield (^{1}H NMR: δ 3.98, 1 H, td). The coupling constants J = 4.9 and 10.7 Hz of the ^{1}H NMR signal of H-11 at δ 3.98 indicated again that H-11 had an axial position. Therefore arcapitin B (**2**) is dammar-24-ene-11α,20(*S*)-diol-3-one.

The FAB mass spectrum (positive mode) of arcapitin C (**3**) showed a significant peak at *m/z* 485 $[M + H - H_2O]^+$, while the EI mass spectrum showed peaks at *m/z* 484 and at *m/z* 424. The formulae of these peaks were determined as $C_{32}H_{52}O_3$ $[M\text{-}H_2O]^+$ and as $C_{30}H_{48}O$ $[M - H_2O - CH_3COOH]^+$ by HRMS. In the ^{13}C NMR spectrum (Table I) the signal of a keto group was missing. Instead an additional methine signal at δ 78.2 appeared, indicating a C-3 alcohol. This was confirmed by the signal at δ 3.20 in the ^{1}H NMR spectrum. Otherwise the NMR data closely resembled those of **1**. The coupling constants of H-3 (Table I) indicated its axial position which was further confirmed by the chemical shift of C-3 (δ 78.2) (Asakawa *et al.*, 1977; Pedreros *et al.*, 1990). Ar-

Table I. ^{1}H NMR data [400 MHz, $CDCl_3$, δ_H (ppm), J (Hz)] and ^{13}C NMR data [100.6 MHz, δ_C (ppm)] of compounds **1**–**3**.

Proton	Carbon	Compound **1**		Compound **2**		Compound **3**	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
H-1a, H-1b	C-1	1.85, 1 H, m 1.95, 1 H, m	41.9	1.70, 1 H, m 2.68, 1 H, m	42.0	1.45, 2 H, m	39.9
H-2a, H-2b	C-2	2.33, 1 H, m 2.55, 1 H, m	34.1	2.46, 2 H, br m	34.2	1.57, 2 H, m	27.7
H-3	C-3	–	218.2	–	218.6	3.20, 1 H, dd (5.9 and 10.1)	78.2
–	C-4	–	47.7	–	47.7	–	39.4
H-5	C-5	1.56, 1 H, m	54.6	1.55, 1 H, m	54.8	0.79, 1 H, m	55.7
H-6a, H-6b	C-6	1.50, 2 H, m	19.6	1.52, 2 H, m	19.7	1.40, 1 H, m 1.57, 1 H, m	18.1
H-7a, H-7b	C-7	1.33, 1 H, m 1.56, 1 H, m	34.8	1.30, 1 H, m 1.56, 1 H, m	35.1	1.27, 1 H, m 1.54, 1 H, m	35.7
–	C-8	–	40.8	–	40.6	–	40.9
H-9	C-9	1.89, 1 H, m	52.2	1.52, 1 H, m	55.3	1.73, 1 H, m	52.7
–	C-10	–	37.9	–	38.2	–	38.8
H-11	C-11	5.18, 1 H, td (5.3 and 10.9)	73.4	3.98, 1 H, td (4.9 and 10.7)	71.3	5.24, 1 H, td (5.8 and 10.7)	72.7
H-12a, H-12b	C-12	1.41, 1 H, m 2.15, 1 H, m	34.6	1.46, 1 H, m 2.18, 1 H, m	39.9	1.40, 1 H, m 2.15, 1 H, m	34.8
H-13	C-13	1.83, 1 H, m	40.3	1.84, 1 H, m	40.8	1.77, 1 H, m	39.9
–	C-14	–	50.0	–	50.0	–	50.0
H-15a, H-15b	C-15	1.12, 1 H, m 1.39, 1 H, m	30.6	1.14, 1 H, m 1.38, 1 H, m	30.7	1.08, 1 H, m 1.40, 1 H, m	30.7
H-16a, H-16b	C-16	1.54, 1 H, m 1.78, 1 H, m	24.9	1.55, 1 H, m 1.81, 1 H, m	25.0	1.54, 1 H, m 1.77, 1 H, m	25.0
H-17	C-17	1.78, 1 H, m	49.6	1.81, 1 H, m	49.7	1.77, 1 H, m	49.9
H-18	C-18	1.05, 3 H, s	16.1	1.01, 3 H, s	16.4	0.78, 3 H, s[a]	15.3[a]
H-19	C-19	0.94, 3 H, s	17.6	1.08, 3 H, s	16.8	0.97, 3 H, s[a]	16.9[a]
–	C-20	–	75.0	–	75.2	–	75.0
H-21	C-21	1.15, 3 H, s	25.7	1.17, 3 H, s	25.7	1.14, 3 H, s	25.6
H-22a, H-22b	C-22	1.47, 2 H, m	40.2	1.50, 2 H, m	40.2	1.45, 1 H, m 1.77, 1 H, m	40.2
H-23a, H-23b	C-23	2.01, 2 H, m	22.6	2.07, 2 H, m	22.6	2.03, 2 H, m	22.6
H-24	C-24	5.13, 1 H, t (7.0)	124.5	5.14, 1 H, t (7.1)	124.6	5.12, 1 H, t (6.9)	124.6
–	C-25	–	131.8	–	131.8	–	131.8
H-26	C-26	1.69, 3 H, s	25.7	1.69, 3 H, s	25.7	1.69, 3 H, s	25.7
H-27	C-27	1.62, 3 H, s	17.7	1.63, 3 H, s	17.7	1.62, 3 H, s	17.7
H-28	C-28	1.10, 3 H, s	27.7	1.10, 3 H, s	27.5	0.99, 3 H, s	28.2
H-29	C-29	1.06, 3 H, s	20.4	1.07, 3 H, s	20.8	1.01, 3 H, s[a]	16.8[a]
H-30	C-30	0.96, 3 H, s	16.0	0.93, 3 H, s	16.2	0.94, 3 H, s[a]	16.1[a]
C**H**$_3$C=O	**C**H$_3$C=O	1.99, 3 H, s	22.0	–	–	1.99, 3 H, s	22.1
–	CH$_3$**C**=O	–	170.0	–	–	–	170.2

[a] Protons were clearly assigned to the carbons by HETCOR spectrum, but assignment of each proton/carbon pair to the positions 18, 19, 29 and 30 may be interchanged.

Table II. Two- and three-bond ^{1}H-^{13}C correlations observed in the HMBC of **1**.

Signal of proton	Correlation with ^{13}C signal of
H-1a, H-1b	C-2, C-3, C-5, C-10, C-19
H-2a,	C-1, C-3, C-10
H-2b	C-1, C-3, C-10
H-5	C-19, C-29
H-9	C-1, C-8, C-10, C-11, C-12, C-14
H-11	$CH_3\underline{C}O$
H-17	C-21
H-18	C-7, C-8, C-9, C-14
H-19	C-1, C-5, C-9, C-10
H-21	C-17, C-20, C-22
H-22a, H-22b	C-17, C-20, C-21, C-23, C-24
H-26	C-24, C-25, C-27
H-27	C-24, C-25, C-27
H-28	C-3, C-4, C-5, C-29
H-29	C-3, C-4, C-5, C-28
H-30	C-8, C-14, C-15
$C\underline{H}_3CO$	$CH_3\underline{C}O$

capitine C (**3**) was thus characterized as 11α-acetoxydammar-24-ene-3β,20(*S*)-diol.

To the best of our knowledge the compounds **1** and **3** have not been described as natural products. A compound assigned structure **2** but without any stereochemistry at C-20 was described as a constituent of *Astrotrichilia asterotricha* (Radlk.) J. F. Leroy (Meliaceae), before (Mulholland *et al.*, 1994). The placement of the hydroxy group at C-11 appears doubtful. The authors only discuss the possible locations of the hydroxy group at C-6 or at C-11 and leave the third possibility (C-12) out. However, the ^{13}C NMR data published for their compound and its acetylated synthetic derivative are in very good agreement with those of a 12β-hydroxy group and its acetylated derivative (Asakawa *et al.*, 1977; Williams *et al.*, 1992). Therefore, compound **2** can be regarded as a new natural product, as well.

As a number of other studies have shown the selective extraction of those compounds located externally on the plant surface is possible by washing the intact plant material with organic solvents (Wollenweber u. Jay, 1988; Arriaga-Giner *et al.*, 1998). When dry leaves or green stems of *A. capitata* were briefly immersed in chloroform, respectively, significant concentrations of **1**–**3** were detected in the chloroform solution by TLC. Therefore these compounds probably occur externally on the surface of the stems and leaves, as has been shown for triterpenes of e. g. *Betula* spp. Such externally localized triterpenes may play a role as antimicrobial agents or act as a deterrent against herbivores (Brieskorn, 1987; Taipale *et al.*, 1993).

Dammarane derivatives have been found in numerous not related plant families from the lichens and ferns to the Asteraceae (Hegnauer, 1966; Das and Mahato, 1983; Hegnauer, 1986; Mahato *et al.*, 1992). Nevertheless, it is interesting to note that **1** – **3** are the first dammarane derivatives reported from a convolvulaceous species. These compounds do not seem to be common secondary metabolites of the Convolvulaceae, since they could not be detected in a number of other species: *A. mollis* (Burm. f.) Choisy, *A. nervosa* (Burm. f.) Boj., *Bonamia spectabilis* (Choisy) Hall. f., *Falkia repens* L., *Ipomoea alba* L., *I. aquatica* Forsk., *I. meyeri* (Spreng.) G. Don, *I. reticulata* O'Donell, *I. turbinata* Lag., *Merremia umbellata* (L.) Hall. and *Odonellia hirtiflora* (Mart. & Gal.) K. Rob.

Experimental

General

EIMS, HRMS and FABMS spectra were obtained with Varian MAT CH_7A, Finnigan MAT 711 and Finnigan MAT CH_5DF spectrometers, respectively. HMBC spectra were recorded on a Bruker DRX 500 spectrometer, NOE spectra on a Bruker AC 400 spectrometer and all other ^{1}H NMR and ^{13}C NMR spectra on a Bruker DPX 400 spectrometer. TMS was used as internal standard.

TLC and preparative TLC were carried out on silica gel 60 F_{254} with cyclohexane-$CHCl_3$-MeOH (50:45:5, v/v) or Et_2O-petroleum ether (30:10, v/v) as solvent systems (systems I and II). Van Urk's reagent was used for the detection, causing a blue colour of **1**–**3** after heating at 100°.

Plant material

Aerial vegetative parts and roots were obtained from plants cultivated in the greenhouse of the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Germany, where voucher specimens are deposited. The plants were grown from seeds, which were collected in the wild in the following areas: near Chiang Mai/Thailand (*Argyreia capitata*), in Indonesia (*A. mollis*, *A. nervosa*), on Madagascar (*Bonamia spectabilis*), in Thailand (*Ipomoea alba*, *I. aquatica*), in Ecuador (*I. alba*, *I. reticulata*, *I. turbinata*, *M. umbellata*) and in Panama (*I. meyeri*, *Odonellia hirtiflora*).

Falkia repens was obtained from the Botanischer Garten, Freie Universität Berlin/Germany. Aerial vegetative parts of *A. capitata* collected in the wild on Madura Island/Indonesia were also analysed.

Extraction and isolation

Dried ground roots (100 g) were extracted with 1.2 l MeOH at room temperature under protection against daylight. After evaporation the residue was suspended in 400 ml H_2O and extracted with 0.9 l petroleum ether and 0.9 l CH_2Cl_2. The combined extracts were evaporated *in vacuo*. In order to remove other lipophilic constituents to some extent the residue was dissolved in 0.15 l petroleum ether and extracted with 0.3 l MeOH-H_2O (80:20, v/v). The MeOH-H_2O layer, containing **1**–**3**, was evaporated *in vacuo* and the residue was chromatographed on a silica gel column with a petroleum ether-$CHCl_3$ gradient (80:20 → 50:50, v/v), followed by petroleum ether-$CHCl_3$-MeOH (50:45:5, v/v). The combined fractions containing a mixture of **1**–**3** (90 mg) were evaporated in vacuo and the compounds were separated by preparative TLC using solvent system I. All compounds were finally purified by prep. TLC with solvent system II.

For the detection of **1** - **3** in the aerial vegetative parts of *A. capitata* and for the analysis of the other species 50 g dried and ground material were extracted as described above and analysed by TLC, respectively.

Spectroscopic data

Arcapitin A (**1**): oil, 28 mg, R_f 0.43 (I), $[\alpha]_D^{20}$ + 5° ($CHCl_3$, *c* 0.65). EIMS 70 eV, *m/z* (rel. int.): 482 $[M - H_2O]^+$ (8), 422 $[M - H_2O - HAc]^+$ (38), 401 (6), 340 (13), 109 (100). (-)-FABMS *m/z*: 499 $[M - H]^-$. HRMS 80 eV, *m/z*: 482.37643 ($C_{32}H_{50}O_3$, calculated for 482.37600), 422.35441 ($C_{30}H_{46}O$, calculated for 422.35487), 401.30583 ($C_{26}H_{41}O_3$, calculated for 401.30557), 340.27647 ($C_{24}H_{36}O$, calculated for 340.27662), 109.10185 (C_8H_{13}, calculated for 109.10173). ^1H NMR and ^{13}C NMR: see Table I.

Arcapitin B (**2**): oil, 6 mg, R_f 0.15 (I). EIMS 70 eV, *m/z* (rel. int.): 440 $[M - H_2O]^+$ (8), 360 (8), 43 (100). (+)-FABMS *m/z*: 459 $[M + H]^+$. HRMS 80 eV, *m/z*: 440.36550 ($C_{30}H_{48}O_2$, calculated for 440.36543). ^1H NMR and ^{13}C NMR: see Table I.

Arcapitin C (**3**): oil, 6 mg, R_f 0.24 (I). EIMS 70 eV, *m/z* (rel. int.): 484 $[M - H_2O]^+$ (3), 424 $[M - H_2O - HAc]^+$ (30), 342 (9), 109 (68), 43 (100). (+)-FABMS *m/z*: 485 $[M + H - H_2O]^+$. HRMS 80 eV, *m/z*: 484.39179 ($C_{32}H_{52}O_3$, calculated for 484.39165), 424.37093 ($C_{30}H_{48}O$, calculated for 424.37052). ^1H NMR and ^{13}C NMR: see Table I.

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