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# TWO OLEANANE TRITERPENES FROM *ANAGALLIS ARVENSIS*

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**Key Word Index**—*Anagallis arvensis*; Primulaceae; triterpenes.

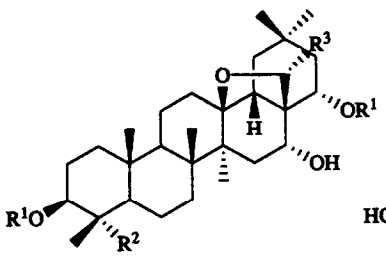
**Abstract**—Two new triterpenes, have been isolated from the weed *Anagallis arvensis*. Their structures, strictly related to that of an oleanane metabolite previously isolated from the same source, have been established on the basis of spectral analyses and chemical correlation with camelliagenin C, a triterpene from *Camellia* sp.

## INTRODUCTION

*Anagallis arvensis* L. (scarlet pimpernel) is a weed of world-wide distribution. Previous studies led to the isolation from this source of triterpenes and triterpenoid saponins [1–3]. In a recent paper [1], we reported the structure determination of a new oleanane triterpene (**1**) isolated from the chloroform extract of this plant. A careful reexamination of the lipid extract of *A. arvensis* has now led to the isolation of the two new triterpene metabolites (**2** and **3**), closely related to **1**.

## RESULTS AND DISCUSSION

Compound **2** was an optically active solid. The IR spectrum showed hydroxyl absorption at 3400 $cm^{-1}$. The FAB mass spectrum contained no molecular ion peak. The molecular formula, $C_{30}H_{50}O_6$, was inferred from the FAB mass, $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) as follows. The FAB mass spectrum showed significant ion peaks at *m/z* 489, 471, 453 and 435 with the *m/z* 489 mass peak as the highest one observed in the spectrum. However, the latter peak was not ascribable to the molecular ion as suggested by the NMR data. The $^1H$ NMR spectrum of **2** indicated the presence of five hydroxyl groups in the molecule displaying five low-field resonances due to exchangeable protons. The $^{13}C$ NMR spectrum exhibited resonances at $\delta$ 98.6, 87.5, 73.6, 69.7, 68.2 and 68.1 for oxygen-bearing carbon atoms. Comparison of the $^1H$ and $^{13}C$ NMR spectra of **2** with those of **1** indicated the presence of a hemiacetal function in compound **2** as well [$^1H$ NMR: $\delta$ 5.45 (1H, *s*, H-28); $^{13}C$ NMR: $\delta$ 98.6 (CH-28) and 87.5 (C-13)]. These data and the carbon and proton count from broad-band $^{13}C$ NMR and $^{13}C$ DEPT spectra established a $C_{30}H_{50}O_6$ molecular formula for the new compound. Thus, the FAB mass spectral ion peaks at *m/z* 489, 471, 453 and 435 were considered to arise from the quasi-molecular ion through the sequential losses of four molecules of water. The absence of resonances due to $sp^2$ and sp carbon atoms in the $^{13}C$ NMR spectrum of **2** indicated that a polycyclic had to account for all six unsaturation equivalents re-

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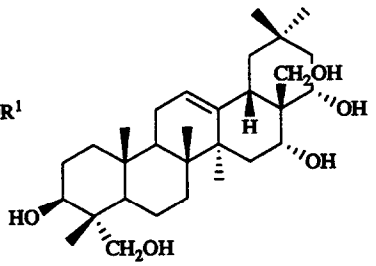
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 1 | H | Me | OH |
| 2 | H | $CH_2OH$ | OH |
| 3 | H | $CH_2OH$ | H |
| 4 | Ac | $CH_2Ac$ | OAc |

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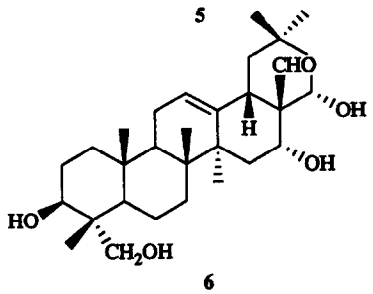


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Table 1. Selected $^1$H NMR spectral data for compounds **2** and **3** (in pyridine-$d_5$, 400 MHz)*

| H | 2 | 3† |
|---|---|---|
| 3 | 4.21 *dd* (11.0, 4.9) | 4.23 *dd* (11.0, 4.9) |
| 12 | $H_{ax}$ 2.14 *ddd* (13.4, 13.4, 5.5) | 2.13 *ddd* (13.2, 13.2, 4.7) |
| | $H_{eq}$ | 1.55 *m* |
| 15 | $H_{ax}$ 2.22 *dd* (14.6, 5.5) | 2.29 *dd* (14.1, 5.5) |
| | $H_{eq}$ | 1.55 d (14.1) |
| 16 | 5.05 *d* (5.5) | 5.02 *d* (5.5) |
| 18 | 2.19 *dd* (11.6, 3.7) | 1.35 *m* |
| 19 | $H_{ax}$ 2.87 *dd* (12.4, 14.6) | 2.91 *dd* (14.5, 12.4) |
| | $H_{eq}$ | 1.75 *m* |
| 21 | $H_{ax}$ 2.89 *dd* (12.2, 12.2) | 2.88 *dd* (11.9, 11.9) |
| | $H_{eq}$ | 1.75 *m* |
| 22 | 5.03 *dd* (12.8, 6.1) | 4.18 *m*§ |
| 23 | $H_a$ 4.17 *d* (10.4) | 4.18 *dd* (9.8, 4.3) |
| | $H_b$ 3.71 *d* (10.4) | 3.72 *dd* (9.8, 3.4) |
| 24 | 1.58 *s*‡ | 1.08 *s* |
| 25 | 1.13 s‡ | 1.02 s |
| 26 | 1.13 *s*‡ | 1.41 *s* |
| 27 | 1.11 *s*‡ | 1.57 *s* |
| 28 | 5.45 *s*‡ | $H_a$ 4.02 *d* (7.3) |
| | | $H_b$ 3.68 *d* (7.3) |
| 29 | 1.08 *s*‡ | 1.09 *s* |
| 30 | 1.02 *s*‡ | 1.04 *s* |

* Residual protonated pyridine as internal reference = 8.71 ppm.
†Assignments based on $^1$H–$^1$H COSY-45 spectrum.
‡ Not assigned.
§ Overlapped with $H_a$-23 and H-3 signals.

quired by the molecular formula. The $^1$H and $^{13}$C NMR spectra of **2** contained one less methyl resonance in comparison with those of **1** and exhibited new signals at $\delta$ 4.17 and 3.71 (AB system, $J_{AB} = 10.4$ Hz) and at $\delta$ 68.1 ($-CH_2-$) attributable to an oxygenated methylene group. These data strongly suggested that in **2** a hydroxymethyl group had replaced one of the methyls present in the structure of **1**. A careful examination of the $^{13}$C NMR spectra of **1** and **2** revealed that the resonance of C-23 of **1** was absent in the $^{13}$C NMR spectrum of **2** while the resonances of C-3, C-5 and C-24 were shifted upfield by 4.5, 7.0 and 3.5 ppm, respectively, and the signal of C-4 was downfield shifted by 3.4 ppm. Moreover, the $^1$H NMR spectrum of **2** showed that the signals for H-3 and H-5 both experienced a downfield shift (H-3: $\Delta\delta = 0.74$ ppm; H-5 $\Delta\delta = 0.23$ ppm) when compared with the corresponding signals in **1**. This evidence was in agreement with the placement of the new hydroxymethyl group on C-4 with $\alpha$ configuration. This was also supported by a positive NOE between H-3 and the H-23 proton at $\delta$ 3.71. Thus, structure **2** was assigned to the metabolite under investigation.

Further support for this structure came from the acetylation of **2** with acetic anhydride–pyridine (room temp., 18 hr) which afforded the tetraacetyl derivative **4**. The analysis of $^1$H NMR data of **4** (see Experimental) indicated, as expected, that the C-16 hydroxyl group was unaffected, as reported for other 16$\alpha$-hydroxy oleanane triterpenes including **1** [4, 5]. With this structural assignment at hand and with the aim of definitively confirming the proposed structure, we attempted the conversion of compound **2** into **1** via the selective tosylation of OH-23 group followed by borohydride reduction. However, treatment with either *p*-toluensulphonylchloride in pyridine or with methansulphonylchloride and mesitilensulphonyl-3-nitro-triazole under the same experimental conditions yielded no ester derivative ever on prolonged treatment (48 hr). Thus, we turned our attention to the chemical correlation of **2** with camelliagenin C (**5**), a triterpene previously isolated from *Camellia japonica*, *C. sasangua* and *C. singensis* [5–9]. Acid hydrolysis of **2** with HCl–dioxane at 60° for 1 hr gave the aldehyde **6** which was reduced with sodium borohydride affording in 65% yield a product which showed $[\alpha]_D$ and spectral data identical to those reported for camelliagenin C [5–9]. This result allowed a partial assignment of the relative stereochemistry of **2**. In fact, the configurations at C-13 and C-28 remained undetermined due to the loss of chirality at these centres during the opening of the oxygenated ring and the subsequent formation of a double bond. Neverthless, since the $CH_2OH$–17 group in camelliagenin C is axial, the closure of the penta-atomic hemiacetal-containing ring in **2** requires that the C–13O bond be $\alpha$ as well. The configuration of C-28 was established through a NOE experiment. Irradiation on the H-28 signal resulted in the enhancement of the $H_{ax}$-15 signal at $\delta$ 2.22, which indicated that the chirality of C-28 is the same as that exhibited by **1**.

Compound **3** analysed for $C_{30}H_{50}O_5$ (FAB-MS and $^{13}$C NMR). The IR spectrum exhibited hydroxyl absorption at 3460 cm$^{-1}$. Data from $^1$H and $^{13}$C NMR (Tables 1 and 2) indicated for this metabolite a structure very similar to that of **2**. In particular, the signal of the hemiacetal proton present in the $^1$H NMR spectrum of **2**

Table 2. $^{13}C$ NMR spectral data for compounds 1–3 (in pyridine-$d_5$, 100.1 MHz)*

| C | 1 | 2† | 3‡ |
|---|---|---|---|
| 1 | 39.6 | 39.4 | 39.4 |
| 2 | 28.4 | 27.9 | 27.9 |
| 3 | 78.2 | 73.6 | 73.6 |
| 4 | 39.6 | 43.0 | 43.0 |
| 5 | 55.7 | 48.7 | 48.7 |
| 6 | 18.3 | 18.1 | 18.1 |
| 7 | 34.5 | 34.2 | 34.2 |
| 8 | 42.8[a] | 42.8[a] | 42.8[a] |
| 9 | 50.6 | 50.6 | 50.7 |
| 10 | 33.2 | 33.2 | 33.2 |
| 11 | 19.4 | 19.5 | 19.4 |
| 12 | 33.5 | 33.6 | 33.4 |
| 13 | 87.5 | 87.5 | 87.6 |
| 14 | 44.2[a] | 44.2[a] | 45.5[a] |
| 15 | 36.8 | 35.9 | 37.0 |
| 16 | 69.8 | 69.8 | 70.5 |
| 17 | 52.9 | 52.9 | 51.3 |
| 18 | 47.4 | 47.4 | 51.6 |
| 19 | 38.7 | 38.6 | 38.4 |
| 20 | 37.3 | 37.2 | 37.2 |
| 21 | 46.7 | 46.7 | 46.5 |
| 22 | 68.1 | 68.1 | 74.6 |
| 23 | 28.7 | 68.2 | 68.1 |
| 24 | 16.4 | 12.9 | 13.0 |
| 25 | 16.6 | 17.0 | 17.0 |
| 26 | 18.8 | 18.8 | 18.7 |
| 27 | 19.8 | 19.7 | 19.9 |
| 28 | 98.6 | 98.6 | 78.0 |
| 29 | 33.8[b] | 33.8[b] | 33.7[b] |
| 30 | 26.0[b] | 26.0[b] | 26.1[b] |

*Pyridine-$d_5$ as internal reference = 135.5 ppm.

†Assignments based on DEPT experiments and comparison with data for compound 1.

‡Assignments based on DEPT and heterocorrelation $^{13}C$–$^{1}H$ experiments and comparison with data for compounds 1 and 2.

[a,b]Assignments may be reversed.

was absent in that of 3 which showed additional signals for an AB system at $\delta$ 4.02 and 3.68 ($J_{AB}$ = 7.3 Hz). The $^{13}C$ NMR spectrum of 3 gave similar information. It lacked the hemiacetal carbon resonance at $\delta$98.6 while it contained one more -$CH_2O$- signal at $\delta$68.1. These data suggested that the difference between 2 and 3 was confined to the degree of oxidation of C-28 which in 3 had to be involved in a hetereal bridge. Further corroboration for structure 3 came from its mass spectrum which exhibited a quasi-molecular ion peak at *m/z* 491 and significant ion peaks at *m/z* 473 $[MH-H_2O]^+$, 455 $[M-2H_2O]^+$, 437 $[MH-3H_2O]$ and 419 $[MH-4H_2O]^+$. The ion peak at *m/z* 231 was ascribable to a fragment ion comprising the D and E rings formed by cleavage of the 9–11 and 8–14 bonds and loss of two molecules of water. Final confirmation for structure 3 was obtained by converting it into camelliagenin C (5) by treatment with dioxane–HCl at 60° for 1 hr.

## EXPERIMENTAL

*General.* Mps: uncorr; FT-IR: films; $^{1}H$ NMR: 400 and 270 MHz; $^{13}C$ NMR: 100.1 MHz. Proton chemical shifts are referenced to the residual MeOH, $CHCl_3$ or pyridine signals (MeOH: 3.34 ppm; $CHCl_3$ 7.26 ppm; pyridine: 8.71 ppm). $^{13}C$ chemical shifts are referenced in pyridine-$d_5$ and $CDCl_3$ to the solvents (149.9 and 77.0 ppm; respectively). NOE spectra: 400 MHz in degassed pyridine-$d_5$ soln; FAB-MS: double focusing mass spectrometer (ZAB 2SE); HPLC: Beckman 340 pump equipped with a differential refractometer. Analytical TLC: silica gel (Merck, Kieselgel 60 $F_{254}$, 0.25 mm); CC: silica gel (Merck, Kieselgel 60, 0.063–0.20 mm).

*Plant material. Anagallis arvensis* was collected in April 1991 in the Naples Botanic Garden. A voucher specimen of the plant is on file at the herbarium of the Dipartimento di Biologia Vegetale dell 'Universita' di Napoli.

*Extraction and isolation.* Fresh plants of *A. arvensis* (1.45 kg, dry wt after extraction) were air-dried in the dark, cut into pieces and extracted with $CHCl_3$, in a Soxhlet extractor. The extract (17 g) was chromatographed on an open column of silica gel (450 g, 5 cm diameter) using eluents of increasing polarity from $CHCl_3$ to $CHCl_3$–MeOH (1:1). Frs of 250 ml were collected and those exhibiting similar TLC profiles were combined.

Frs eluted with $CHCl_3$–MeOH (47:3, mg 340) were rechromatographed on an open column of silica gel (30 g, 1.5 cm diameter) eluted with increasing amounts of MeOH in $CHCl_3$ (from 0 to 50%). Frs. of 25 ml were collected. Frs. 46–52 (80 mg), eluted with $CHCl_3$–MeOH (19:1), were combined and further purified by normal and reverse-phase HPLC using Hibar LiChrosorb Si-60 (250 × 10 mm) and $\mu$-Bondapak RP-18 (250 × 10 mm) columns eluted with $CHCl_3$–MeOH (23:2) and MeOH–$H_2O$ (17:3), respectively, to give 6 mg of pure 3.

The fr. (1.1 g) eluted from the original column with $CHCl_3$–MeOH (9:1) was rechromatographed on an open silica gel column (100 g, 2.5 cm diameter) using eluents of increasing polarity from $CHCl_3$ to $CHCl_3$–MeOH (1:1). Frs. of 75 ml were taken. Frs. 37–39 (19 mg), eluted with $CHCl_3$–MeOH (9:1), were combined and further purified by normal-phase HPLC using a Hibar LiChrosorb Si-60 (250 × 10 mm) column eluted with $CHCl_3$–MeOH (47:3) to give 13 mg of pure 2.

*Compound* 2. Mp 153–155° (MeOH). $[\alpha]_D$ −5.3 (MeOH; *c* 1.0). FTIR $\nu_{max}$ 3400 $cm^{-1}$: $^{1}H$ and $^{13}C$ NMR: see Tables 1 and 2; FAB-MS *m/z*: 489 $[MH-H_2O]^+$, 471 $[MH-2H_2O]^+$, 453 $[MH-3H_2O]^+$, 435 $[MH-4H_2O]^+$.

*Compound* 3. Mp 191–193° (MeOH). $[\alpha]_D$ −107.0 (MeOH; *c* 1.4). FTIR $\nu_{max}$ 3460 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR: see Tables 1 and 2); FAB-MS *m/z* 491 $[MH]^+$, 473 $[MH-H_2O]^+$, 455 $[MH-2H_2O]^+$, 437 $[MH-H_2O]^+$, 419 $[MH-H_2O]^+$, 231 (fragment ion comprising D and E rings deriving from the quasi-molecular ion through the scission of the 9–11 and 8–14 bonds).

*Acetylation of compound* 2. Compound 2 (4.0 mg) was acetylated overnight at room temp. with pyridine–$Ac_2O$ (2:1). Usual work-up and purification by silica gel TLC (petrol–$Et_2O$, 1:1) gave 3.8 mg of pure 4. $^{1}H$ NMR (pyridine-$d_5$, 270 M Hz): $\delta$ 6.80 (1H, *d*, *J* = 4.9 Hz, OH-16), 6.26 (1H, *s*, H-28), 5.67 (1H, *dd*, *J* = 12.1 and (6.0 Hz, H-21), 5.07 (1H, *dd*, *J* = 12.1 and 4.9 Hz, H-3), 4.69 (1H, *br dd*, *J* = 4.9 and 4.9 Hz, H-16), 4.01 (2H, AB system, $J_{AB}$ = 11.5 Hz, $H_2$-23), 2.14, 2.04, 2.03, 1.98 (3H each, acetates), 1.53 (3H, *s*), 1.29 (3H, *s*), 1.14 (3H, *s*), 1.08 (3H, *s*), 0.85 (3H, *s*), 0.82 (3H, *s*).

*Conversion of compound* 2 *into camelliagenin C* (5). To a soln of 2 (6 mg) in dioxane (1 ml), 2 M HCl (two drops) was added and

the soln kept at 60° until the starting material disappeared (*ca* 1 hr, TLC monitoring). A few drops of a satd aq. $NaHCO_3$ soln were added and the resulting mixt. taken to dryness. The crude product was partitioned between $CHCl_3$ and $H_2O$ and the organic layer dried ($Na_2SO_4$) and evapd under red. pres. to give an almost pure aldehyde derivative (**6**): $^1H$ NMR (pyridine-$d_5$, 270 MHz) $\delta$ 9.81 (1H, *s*, H-28), 5.51 (1H, *dd* $J = 3.8$ and 3.8 Hz, H-12), 1.75 (3H, *s*), 1.11 (3H, *s*), 1.06 (3H, *s*), 1.02 (3H, *s*), 1.01 (3H, *s*), 0.89 (3H, *s*).

The crude aldehyde **6** (5 mg) obtained as above was dissolved in EtOH (3 ml) and excess $NaBH_4$ added. The mixture was stirred at room temp for 20 min. Excess reagent was destroyed by dropwise addition of HOAc and the mixture taken to dryness. The residue was partitioned between $CHCl_3$ and $H_2O$ and the organic phase dried ($Na_2SO_4$) and evapd *in vacuo*. Purification by silica gel TLC ($CHCl_3$–MeOH, 17:3) afforded 3.5 mg of a pure compound which had $[\alpha]_D$ and spectral data identical to those reported for camelliagenin C [5–9].

*Acid hydrolysis of compound* **3**. Compound **3** (4 mg) was hydrolysed (*ca* 1 hr) under the same conditions as those used for **2**. Usual work-up and purification by silica gel TLC ($CHCl_3$–MeOH, 17:3) gave 2.5 mg of **6**.

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