Pergamon

0031-9422(95)00525-0

*Phytochemistry*, Vol. 41, No. 1, pp. 289–292, 1996

0031-9422/96 $15.00 + 0.00

# AN ACETOPHENONE GLYCOSIDE FROM *EXACUM AFFINE*

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(*Received in revised form* 5 *June* 1995)

**Key Word Index**—*Exacum affine*; Gentianaceae; acetophenone glycoside; affinoside; glucopaeonol; iridoid glucoside; gentiopicroside; 2′-*O*-*p*-coumaroylloganin.

**Abstract**—A new acetophenone glycoside, affinoside, was isolated from the aerial parts of *Exacum affine* and its structure was determined as 2-*O*-primeverosylpaeonol. The known glucosides, gentiopicroside, 2′-*O*-*E*/*Z*-*p*-coumaroylloganin and glucopaeonol, were also identified.

## INTRODUCTION

The genus *Exacum* comprises about 40 species [1]. Among these plants, *E. tetragonium* [2], *E. bicolor* [3], *E. pedunculatum* [3], *E. macranthum* [4] and *E. affine* have been chemically investigated, although the only known constituent of *E. affine* that has been reported is *p*-coumaric acid [5]. We have reinvestigated the constituents of the aerial parts of this plant cultivated as an ornamental plant in Japan and isolated a new acetophenone glycoside, affinoside (**1**), in addition to glucopaeonol (**2**) [6] and two iridoid glucosides, gentiopicroside (**3**) [7] and 2′-*O*-*p*-coumaroylloganin (**4**) [8].

## RESULTS AND DISCUSSION

The water-soluble parts of the methanolic extract of the fresh aerial parts of *E. affine* (see Experimental) afforded two paenol glycosides, namely, the known glucopaeonol (**2**) and a new one which we have named affinoside (**1**). In addition, the known secoiridoid glucosides gentiopicroside (**3**) and 2′-*O*-*p*-coumaroylloganin (**4**), the latter as an *E*/*Z* mixture, were also isolated.

Glucoside **1**, $C_{20}H_{28}O_{12}$, was obtained as needles, mp 150°, $[\alpha]_D$ − 71.9° (methanol). It showed UV maxima at 226, 267, 291 (sh) nm and IR bands at 3375, 1656 and 1600 $cm^{-1}$. Its $^1H$ NMR spectrum exhibited an aromatic ABX spin system at $\delta$7.75 (*d*, $J$ = 8.8 Hz), 6.87 (*d*, $J$ = 2.3 Hz) and 6.67 (*dd*, $J$ = 8.8 and 2.3 Hz), and two methyl protons at $\delta$3.87 (*s*) and 2.64 (*s*). Furthermore, the $^{13}C$ NMR spectrum of **1** (Table 1) showed two oxygenated aromatic carbon signals at $\delta$166.3 and 160.4, besides a carbonyl carbon signal at $\delta$200.6. These findings suggested the presence of an acetophenone skeleton possessing a methoxyl group and a phenolic oxygen atom on its benzene nucleus. These positions of attachment were corroborated by the NOESY experiments as follows: the doublet proton signal at $\delta$6.87, attributable to H-3,

Table 1. $^{13}C$ NMR data for compounds **1**, **2** and **4**–**6** in $CD_3OD$

| | | | 4 | | | |
|---|---|---|---|---|---|---|
| C | **1** | **2** | *E* | *Z* | **5** | **6** |
| 1 | 122.7 | 122.5 | 97.7 | 97.9 | 115.1 | 97.7 |
| 2 | 160.4 | 160.7 | | | 167.7 | |
| 3 | 103.3 | 102.6 | 150.5 | 151.0 | 101.8 | 152.1 |
| 4 | 166.3 | 166.3 | 115.0 | 115.0 | 166.2 | 114.0 |
| 5 | 109.1 | 109.5 | 30.8 | 31.1 | 108.3 | 32.1 |
| 6 | 133.1 | 133.0 | 42.0 | 42.4 | 134.0 | 42.7 |
| 7 | | | 75.1 | 75.1 | | 75.0 |
| 8 | | | 41.2 | 41.6 | | 42.1 |
| 9 | | | 46.5 | 46.5 | | 46.5 |
| 10 | | | 12.5 | 12.8 | | 13.4 |
| 11 | | | 169.2 | 169.2 | | 169.5 |
| COOMe | | | 51.4 | 51.5 | | 51.6 |
| OMe | 56.4 | 56.2 | | | 56.1 | |
| COMe | 32.2 | 32.1 | | | 26.3 | |
| CO | 200.6 | 200.6 | 168.2 | 168.9 | 204.5 | |
| 1′ | 102.5 | 102.6 | 96.1 | 96.8 | | 100.0 |
| 2′ | 74.8 | 74.9 | 74.6 | 74.3 | | 74.7 |
| 3′ | 78.2 | 78.6 | 75.9 | 75.9 | | 78.3 |
| 4′ | 71.4 | 71.4 | 71.7 | 71.7 | | 71.5 |
| 5′ | 77.4 | 78.4 | 78.5 | 78.5 | | 78.0 |
| 6′ | 70.5 | 64.3 | 62.7 | 62.7 | | 62.7 |
| 1″ | 105.7 | | 126.5 | 127.2 | | |
| 2″ | 74.9 | | 131.4 | 134.1 | | |
| 3″ | 77.7 | | 117.3 | 117.3 | | |
| 4″ | 71.1 | | 162.9 | 160.7 | | |
| 5″ | 66.9 | | 117.3 | 117.3 | | |
| 6″ | | | 131.4 | 134.1 | | |
| α | | | 114.3 | 115.9 | | |
| β | | | 146.8 | 145.8 | | |

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showed a correlation with the aromatic methoxyl, that in turn was correlated with H-5. Furthermore, H-6 revealed cross-peaks with H-5 and the acetyl methyl, respectively, but not with the aromatic methoxyl. This methoxyl, therefore, should be placed at the *para*-position to the acetyl group of acetophenone, and the phenolic oxygen atom at the *ortho*-position (Fig. 1). This was also supported by the $^{13}C$–$^{1}H$ long range COSY spectrum of **1**.

The $^{1}H$ NMR spectrum of **1** showed two anomeric protons at $\delta$5.03 (*d*, $J$ = 7.7 Hz) and 4.28 (*d*, $J$ = 7.5 Hz), and $^{1}H$–$^{1}H$ and $^{1}H$–$^{13}C$ COSY experiments on **1** allowed us to assign all $^{1}H$ and $^{13}C$ resonances of glucose and xylose. The chemical shift value of the anomeric proton of glucose at $\delta$5.03 strongly suggested that C-1′ of glucose was linked to a phenolic oxygen atom rather than an alcoholic one, i.e. a xylosyl moiety. The glucosidic linkage of the xylose to the glucose moiety was additionally verified by the NOESY spectrum experiments, which showed cross-peaks between the anomeric proton of xylose and C-6′ methylene protons of glucose. This was also supported by the glycosidation shift of C-6′ in the $^{13}C$ NMR spectrum. The anomeric configuration of the glucosyl and the xylosyl linkages were determined to be $\beta$ from the coupling constants of the anomeric protons, respectively. Finally, acid hydrolysis of **1** with trifluoroacetic acid yielded glucose, xylose and paenol (**5**) along with a trace of **2**. Thus, the structure of affinoside (**1**) was characterized as 2-*O*-primeverosylpaeonol.

Glucoside **2** was obtained as a powder, $[\alpha]_D$ − 62.5°, (methanol), and was assigned the molecular formula $C_{15}H_{20}O_8$, from its mass spectrum. Its $^{1}H$ and $^{13}C$ NMR spectra closely resembled those of **1** except for the lack of signals arising from the xylose unit, which indicated that **2** was glucosylpaeonol. This was further substantiated by the fact that its negative ion FAB mass spectrum showed a quasimolecular ion peak, $[M - H]^-$, at *m/z* 327 indicating a decrease of 132 mass units in comparison with that of the $[M - H]^-$ of **1**. Glucoside **2**, therefore, was assumed to be glucopaeonol [6].

Compounds **3** and **4** were identified by comparison with published data [7, 8]. The *E*/*Z* mixture of **4** was subjected to preparative HPLC in an attempt to separate each isomer. However, of the two peaks, the more mobile one gave an *E*/*Z* mixture **4** in a ratio of 6:1, whereas the less mobile one furnished an *E*/*Z* mixture **4** in a ratio of 1:1, suggesting that the former peak was the more stable *E* isomer, whereas the latter was the less stable *Z* form. These findings were also in accord with the report by Damtoft *et al.* [9] that *E*/*Z* isomers of a *p*-coumaroyl ester rapidly isomerize to give an unseparable mixture.

Although **3** [7] and 2′-*O*-*E*- and *Z*-*p*-coumaroylloganin (**4**) [8] have been found in only the family Gentianaceae, **5** occurs in a wide variety of families including Rubiaceae, Asclepiadaceae, Betulaceae, Moraceae, Primulaceae and Xanthorrhoea besides Paeoniaceae [10]. Ours is the first example of the paeonol glycosides, **1** and **2**, having been isolated from Gentianaceae along with iridoids.

## EXPERIMENTAL

*General.* Mps: uncorr; NMR: $^{1}H$, 500 MHz, $^{13}C$, 125 Mz, TMS as int. standard; TLC: precoated Silica gel $GF_{254}$, spots visualized by irradiation under UV light (254 nm) and by exposure to $I_2$ vapour; prep. TLC: Silica gel $PF_{254}$, bands detected under UV light; HPLC: YMC D-ODS-AM S-5 (10 × 250 mm) (Yamamura Chemical Laboratories), detection, UV 254 nm; flow rate 6 ml $min^{-1}$; FID-GC: 3% SE-52 (200 × 3 mm), column temp. 180°, $N_2$ 30 ml $min^{-1}$; FABMS: glycerol as the matrix.

*Plant material. Exacum affine* grown at the Research Centre for Plant Seed, Kinki University, were collected in October 1994. A voucher specimen (KW-2), which was identified by Mr Junji Nakanishi (the above Research Centre), is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Kinki University, Higashiosaka 577, Japan.

*Isolation of glucosides.* Fresh aerial parts of *E. affine* (586.6 g) were extracted with hot MeOH. After concn, the extract was triturated with $H_2O$ and the insoluble material was filtered off through a Celite layer. The filtrate and washings were combined and lyophilized to give 18.39 g residue, which was extracted successively with EtOAc

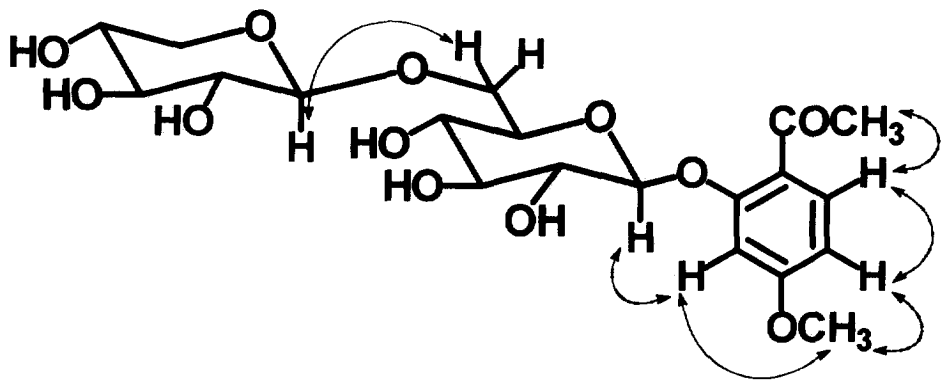
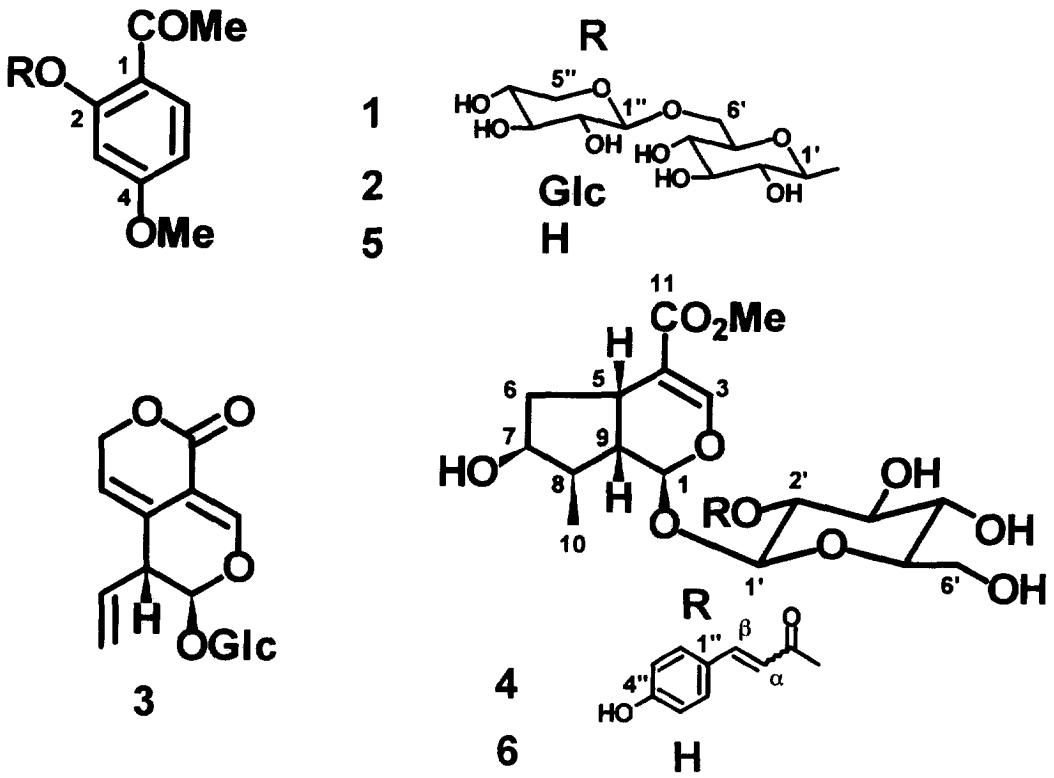



Fig. 1. NOESY correlations of **1**.




and *n*-BuOH. The EtOAc layer (610 mg), after evapn of the solvent, was subjected to prep. TLC ($C_6H_6$–EtOAc–EtOH–$H_2O$, 1:4:1:0.3) to give frs 1–5 in order of decreasing polarity. Fr. 2 (53.8 mg) was subjected to prep. TLC ($CHCl_3$–MeOH–$H_2O$–HOAc, 47:18:3:0.1). The major band was further purified by HPLC (23% MeCN–$H_2O$) to give 7.1 mg (*E*/*Z*; 6:1) of **4** ($R_t$ 9.2 min) as well as the latter 6.6 mg (*E*/*Z*; 1:1) of **4** ($R_t$ 14.6 min), respectively. Fr. 4 furnished **3** (119.3 mg) as a powder. An aliquot (1.63 g) of the residue (4.06 g) obtained through concn *in vacuo* of the *n*-BuOH layer was subjected to prep. TLC ($C_6H_6$–EtOAc–EtOH–$H_2O$, 1:4:2:0.3) to give frs 1–4 in order of decreasing polarity. Fr. 2 was purified by a combination of prep. TLC ($CHCl_3$–MeOH–$H_2O$–HOAc, 47:18:3:0.1) and HPLC (15% MeCN–$H_2O$, $R_t$ 10 min) to yield **2** (2.9 mg). Fr. 3 furnished **3** (419.1 mg) and fr. 4 was recrystallized from EtOH to give **1** (125.3 mg).

*Glucoside* **1**. Needles, mp 150°, $[\alpha]_D^{20}$ − 71.9° (MeOH; *c* 1.03). UV $\lambda_{max}^{MeOH}$ nm (log ε): 226 (3.80), 267 (3.77), 291 sh (3.58); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3375, 1650, 1600; $^1$H NMR ($CD_3OD$): δ2.64 (3H, *s*, COMe), 3.15 (1H, *dd*, *J* = 11.4, 10.2 Hz, H-5″a), 3.20 (1H, *dd*, *J* = 9.0 and 7.5 Hz, H-2″), 3.30 (1H, *t*, *J* = 8.9 Hz, H-3″), 3.39 (1H, *dd*, *J* = 9.7, 8.9 Hz, H-4′), 3.49 (1H, *t*, *J* = 8.9 Hz, H-3′), 3.71 (1H, *dd*, *J* = 9.1, 7.7 Hz, H-2′), 3.72 (1H, *ddd*, *J* = 9.7, 6.6, 2.0 Hz, H-5′), 3.81 (1H, *dd*, *J* = 11.6, 6.6 Hz, H-6′a), 3.85 (1H, *dd*, *J* = 11.4, 5.4 Hz, H-5″b), 3.87 (3H, *s*, OMe), 4.11 (1H, *dd*, *J* = 11.6, 2.0 Hz, H-6′b), 4.28 (1H, *d*, *J* = 7.5 Hz, H-1″), 5.03 (1H, *d*, *J* = 7.7 Hz, H-1′), 6.67 (1H, *dd*, *J* = 8.8, 2.3 Hz, H-5), 6.87 (1H, *d*, *J* = 2.3 Hz, H-3), 7.75 (1H, *d*, *J* = 8.8 Hz, H-6); $^{13}$C NMR: Table 1; FABMS *m*/*z* 459 $[M - H]^-$. (Found: C, 52.26; H, 6.09. $C_{20}H_{28}O_{12}$ requires: C, 52.15; H, 6.13%).

*Glucoside* **2**. Powder, $[\alpha]_D^{20}$ − 62.5° (MeOH; *c* 0.24). UV $\lambda_{max}^{MeOH}$ nm (log ε): 226 (3.69), 267 (3.60), 291 sh (3.35); $^1$H NMR ($CD_3OD$): δ2.65 (3H, *s*, COMe), 3.37 (1H, *dd*, *J* = 9.0, 8.5 Hz, H-4′), 3.48 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.49 (1H, *ddd*, *J* = 9.0, 6.0, 2.0 Hz, H-5′), 3.54 (1H, *dd*, *J* = 9.0, 7.5 Hz, H-2′), 3.68 (1H, *dd*, *J* = 12.0, 6.0 Hz, H-6′a), 3.85 (3H, *s*, OMe), 3.91 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′b), 5.05 (1H, *d*, *J* = 7.5 Hz, H-1′), 6.65 (1H, *dd*, *J* = 9.0, 2.0 Hz, H-5), 6.87 (1H, *d*, *J* = 2.0 Hz, H-3), 7.74 (1H, *d*, *J* = 9.0 Hz, H-6); $^{13}$C NMR: Table 1; FABMS *m*/*z* 327 $[M - H]^-$.

*Glucoside* **4**. Powder, $[\alpha]_D^{20}$ − 95.8° (MeOH; *c* 0.95). UV $\lambda_{max}^{MeOH}$ nm (log ε): 230 (3.93), 302 sh (3.91), 313 (3.94); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3340, 1690, 1618. *E* isomer: $^1$H NMR ($CD_3OD$): δ1.04 (3H, *d*, *J* = 6.5 Hz, H-10), 1.60–1.71 (1H, *m*, H-6a), 1.70–1.79 (1H, *m*, H-8), 2.04–2.14 (2H, *m*, H-6b, H-9), 2.90 (1H, *br dtd*, *J* = 9.0, 8.0, 5.0 Hz, H-5), 3.13 (3H, *s*, $CO_2Me$), 3.70 (1H, *dd*, *J* = 12.0, 5.5 Hz, H-6′a), 3.92 (1H, *dd*, *J* = 12.0, 1.0 Hz, H-6′b), 3.96 (1H, *br td*, *J* = 5.0, 1.5 Hz, H-7), 4.78 (1H, *dd*, *J* = 9.0, 8.0 Hz, H-2′), 4.85 (1H, *d*, *J* = 8.0 Hz, H-1′), 5.40 (1H, *d*, *J* = 2.5 Hz, H-1), 6.22 (1H, *d*, *J* = 16.0 Hz, H-α), 6.80 (2H, AA′BB′ pattern, $J_{ortho}$ = 8.5 Hz, H-3″, H-5″), 7.19 (1H, *d*, *J* = 1.5 Hz, H-3), 7.45 (2H, AA′BB′ pattern, $J_{ortho}$ = 8.5 Hz, H-2″, H-6″), 7.54 (1H, *d*, *J* = 16.0 Hz, H-β). *Z* isomer: $^1$H NMR ($CD_3OD$): δ1.06 (3H, *d*, *J* = 6.5 Hz, H-10),

1.60–1.71 (1H, *m*, H-6a), 1.70–1.79 (1H, *m*, H-8), 2.04–2.14 (2H, *m*, H-6b, H-9), 2.95 (1H, *br td*, $J = 9.0$, 8.0, 5.0 Hz, H-5), 3.44 (3H, *s*, $CO_2Me$), 3.68 (1H, *dd*, $J = 12.0$, 5.5 Hz, H-6′a), 3.91 1H, *dd*, $J = 12.0$, 1.0 Hz, H-6′b), 3.98 (1H, *br td*, $J = 5.0$, 1.5 Hz, H-7), 4.77 (1H, *dd*, $J = 9.0$, 8.0 Hz, H-2′), 4.82 (1H, *d*, $J = 8.0$ Hz, H-1′), 5.35 (1H, *d*, $J = 2.5$ Hz, H-1), 5.65 (1H, *d*, $J = 13.0$ Hz, H-α), 6.73 (2H, AA′BB′ pattern, $J_{ortho} = 8.5$ Hz, H-3″, H-5″), 6.80 (1H, *d*, $J = 13.0$ Hz, H-β), 7.65 (2H, AA′BB′ pattern, $J_{ortho} = 8.5$ Hz, H-2″, H-6″), 7.25 (1H, *d*, $J = 1.5$ Hz, H-3). $^{13}C$ NMR: Table 1; FABMS *m/z* 537 $[M + H]^+$.

*Acid hydrolysis of* **1**. A soln of **1** (30 mg) in TFA (2 ml) was stirred for 1 hr at 80° and concd *in vacuo*. An aq. soln of the resulting residue was extracted with $Et_2O$ (2 ml × 3) and the dried $Et_2O$ layer was concd *in vacuo*. The residue (8.7 mg) was purified by prep. TLC ($CHCl_3$–MeOH, 100:1) to give 7.9 mg **5**, which was identical with an authentic sample (TLC, $^1H$ and $^{13}C$ NMR). The aq. layer (15.1 mg), on concn *in vacuo*, was subjected to prep. TLC ($CHCl_3$–MeOH–$H_2O$–HOAc, 47:18:3:0.1) to give glucose and xylose, which were identified by GC (as TMSi derivatives) ($R_t$: α-xylose, 5.2 min; β-xylose, 7.2 min; α-glucose, 10.8 min; β-glucose, 16.8 min), along with 0.7 mg **2** and 10.5 mg of starting material (**1**), which were identified with authentic samples by TLC.

*Paeonol* (**5**). $^1H$ NMR ($CDCl_3$): δ2.56 (3H, *s*, COMe), 3.84 (3H, *s*, OMe), 6.42 (1H, *d*, $J = 2.5$ Hz, H-3), 6.44 (1H, *dd*, $J = 9.0$, 2.5 Hz, H-5), 7.63 (1H, *d*, $J = 9.0$ Hz, H-6), 12.75 (1H, *s*, OH); $^{13}C$ NMR: Table 1.

*Acknowledgments*—We are grateful to Mr M. Morita (Research Institute for Technology and Science, Kinki University) for FABMS measurements.

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