MS, mp, mmp and co-TLC) with those of an authentic specimen of cycloartenone. Interestingly, contrary to earlier reports [10–12] that cycloartenone occurred exclusively as enol esters of its α-isomer, it has now been obtained simply by boiling the latex of *A. integra* with EtOH without hydrolysing the latex.

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# 8-EPIKINGISIDE AND ITS VANILLATE ESTER, ISOLATED FROM *GENTIANA PYRENAICA*

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**Key Word Index**—*Gentiana pyrenaica*; Gentianaceae; secoiridoid glucoside; 8-epikingiside; 6′-vanilloyl 8-epikingiside.

**Abstract**—8-Epikingiside and 6′-vanilloyl 8-epikingiside, a new natural compound, have been isolated from the aerial parts of *Gentiana pyrenaica*. The structures were elucidated on the basis of spectroscopic data.

## INTRODUCTION

In a recent paper we reported the presence of two iridoid glucosides, loganin and 6′-(2*R*-methyl-3-veratroyloxy propanoyl) loganin, in the aerial parts of *Gentiana pyrenaica* L. (Gentianaceae) [1]. In the course of our investigation on the monoterpenic constituents of the title species we now describe the isolation and structure elucidation of the known 8-epikingiside (**1**) along with 6′-vanilloyl 8-epikingiside (**2**) a new natural secoiridoid glucoside.

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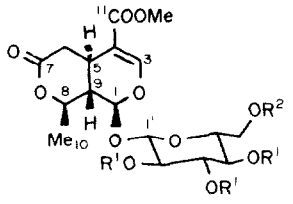
## RESULTS AND DISCUSSION

Aerial parts of *G. pyrenaica* were extracted as described in the experimental. Compounds **1** and **2** were obtained from the chloroform extract by centrifugal TLC and HPLC using ordinary silica gel and RP-18 columns, respectively.

The UV spectrum of compound **1** showed an intense absorption band at 237 nm characteristic of a conjugated carbonyl function. Furthermore, its $^1$H NMR spectrum displayed a methoxycarbonyl singlet at $\delta$3.72 and an olefinic doublet at 7.58 (H-3). These data, together with a positive vannillin reaction, suggested **1** to be a secoiridoid.

Acetylation of **1** with acetic anhydride–pyridine gave a tetraacetate (**1a**) which exhibited $^1$H NMR resonances of



**1** $R^1 = R^2 = H$
**1a** $R^1 = R^2 = Ac$
**2** $R^1 = H$ $R^2$ = vanilloyl

four acetyl groups at $\delta$1.97, 2.02, 2.04 and 2.10 attributed to the glucose moiety. This result was confirmed by CIMS data which showed a peak at *m/z* 331 corresponding with the tetraacetylglucose oxonium ion [2].

The proton–proton connectivity pattern of **1** was determined by two-dimensional homonuclear correlation spectroscopy (COSY DQF). Subsequent to this, a kingiside-type structure [3] was presumed for **1**. However, further analysis of the $^1$H NMR spectrum, completed by spin decoupling experiments, revealed a coupling constant value $J_{8,9} = 6.5$ Hz for **1** different to that of kingiside ($J_{8,9} = 2.6$ Hz) [4] but similar to that of 8-epikingiside [5, 6]. This finding was corroborated by the $^{13}$C NMR chemical shift of the methyl group at C-8 (C-10) which resonated at $\delta$21.7 for **1** against 17.7 for kingiside [7]. From the above data **1** was concluded to be 8-epikingiside, a compound which has recently been isolated from *Syringa vulgaris* (Oleaceae) [8].

The comparison of the $^1$H NMR spectra of **1** and **2** indicated a close structural relationship in the aglyconic signals of the two compounds. The latter exhibited additional resonances at $\delta$3.90 due to a phenolic methoxy group, at $\delta$7.54 (H-6″), 7.52 (H-2″) and 6.82 (H-5″) assigned to a 1,3,4-trisubstituted aromatic ring. Further the presence of NOEs between the methoxy group and H-2″ suggested the presence of a vanilloyl unit which explained the UV absorptions at 260 and 292 nm for **2**. This result was in agreement with FABMS data which showed peaks at *m/z* 151 ($FAB^+$) and 167 ($FAB^-$) corresponding to the vanilloyl unit.

The chemical values of H-6′A and H-6′B at $\delta$4.71 and 4.53 indicated the linkage of the vanilloyl unit to the C-6′ hydroxy group. The deshielding of the C-6′ signal, in the $^{13}$C NMR spectrum, as well as the upfield shift of C-5′ when compared to **1** confirmed the acylation at C-6′ of the glucose. Furthermore, the positive ion FABMS spectrum displayed a fragment at *m/z* 313 attributable to the glucose part esterified with vanillic acid. As for **1**, the coupling constant value $J_{8,9} = 7$ Hz for **2** and the resonance of C-10 at $\delta$21.6 determined its 8$\alpha$-H configuration.

Thus, the structure of **2** was established to be 6′-vanilloyl 8-epikingiside, a new natural compound. This is, with lilacoside and fliederoside [6] previously isolated from *Syringa vulgaris* (Oleaceae), the third 8-epikingiside derivative reported in the literature. Syringalactone A and B isolated from *S. vulgaris* and reported in ref. [8] are identical to fliederoside and lilacoside.

Table 1. $^1$H NMR data (300 MHz) of the secoiridoids

| H | 1* | 1a† | 2* |
|---|---|---|---|
| 1 | 5.49 *d* (7.5) | 5.28 *d* (5.5) | 5.20 *d* (7) |
| 3 | 7.58 *d* (1) | 7.45 *br s* | 7.53 *d* (1) |
| 5 | 3.07 *dddd* (11.5, 7.5, 4.5, 1) | 3.12 *ddd* (8.5, 7.5, 6.5) | 2.94 *dddd* (12, 7, 4, 1) |
| 6A | 2.50 *dd* (16.5, 11.5) | 2.37 *dd* (16.5, 8) | 2.04 *dd* (16, 2) |
| 6B | 2.86 *dd* (16.5, 4.5) | 3.04 *dd* (16.5, 6.5) | 2.71 *dd* (16, 4) |
| 8 | 4.49 *quint* (6.5) | 4.36 *quint* (6.5) | 4.20 *dq* (7, 6) |
| 9 | 2.13 *td* (7.5, 6.5) | 2.08 *m* | 2.04 *t* (7) |
| 10 | 1.51 *d* (6.5) | 1.49 *d* (6.5) | 1.30 *d* (6) |
| 11-OMe | 3.72 *s* | 3.74 *s* | 3.71 *s* |
| 1′ | 4.70 *d* (8) | 4.88 *d* (8) | 4.71 *d* (7.5) |
| 2′ | 3.20 *dd* (9, 8) | 5.02 *dd* (9.5, 8) | 3.21 *dd* (9, 7.5) |
| 3′ | | 5.24 *t* (9.5) | 3.37–3.48 *m* |
| 4′ | 3.30–3.70 *m* | 5.11 *t* (9.5) | |
| 5′ | | 3.75 *ddd* (9.5, 4.5, 2.5) | 3.61 *m* |
| 6′A | 3.91 *dd* (12, 2) | 4.15 *dd* (12.5, 2.5) | 4.71 *dd* (12, 2.5) |
| 6′B | 3.62 *dd* (12, 6) | 4.29 *dd* (12.5, 4.5) | 4.53 *dd* (12, 5.5) |
| 2″ | | | 7.52 *d* (2) |
| 5″ | | | 6.82 *d* (8) |
| 6″ | | | 7.54 *dd* (8, 2) |
| Ar-OMe | | | 3.90 *s* |
| Me-CO | | 1.97–2.10 4*s* | |

*$CD_3OD$.
†$CDCl_3$.
Values in parentheses are coupling constants in Hz.

## EXPERIMENTAL

$^1H$ and $^{13}C$ NMR spectra were recorded with TMS as int. standard.

*Plant material. G. pyrenaica* was collected when in flower at Puymorens pass (2000 m) in France (Pyrénées Orientales). A voucher sample is kept at the Pharmacognosy Laboratory.

*Isolation.* Dried and powdered aerial parts (240 g) were successively extracted with *n*-hexane, $C_6H_6$, $CHCl_3$, $Me_2CO$ and MeOH at room temp. The $CHCl_3$ extract (7 g) was fractionated by centrifugal TLC with $CHCl_3$–MeOH as eluent. Compound **2** (7 mg) was obtained from fractions eluted by $CHCl_3$–MeOH (9:1) and purified on HPLC using, first, a silica gel column ($C_6H_{14}$–*iso*-PrOH–MeOH, 14:3:3) and then a RP-18 column (MeOH–$H_2O$, 9:11). Fractions eluted with $CHCl_3$–MeOH (3:2) afforded compound **1** (1.5 mg) which was successively purified by HPLC on RP-18 (MeOH–$H_2O$ 7:13) and on a silica gel column ($C_6H_{14}$–*iso*-PrOH–MeOH, 14:3:3).

8-*Epikingiside* (**1**). UV $\lambda_{max}^{MeOH}$ nm: 237. FAB$^+$MS *m/z*: 427 $[M+Na]^+$, 405 $[M+H]^+$, 243 $[M-Glc+2H]^+$. FAB$^-$MS *m/z*: 403 $[M-H]^-$, 241 $[M-Glc]^-$. $^1H$ NMR: Table 1. $^{13}C$ NMR (75.46 MHz, $CD_3OD$): $\delta$174.7 (C-7), 168.3 (C-11), 154.5 (C-3), 109.6 (C-4), 100.7 (C-1'), 96.3 (C-1), 78.5 (C-5'), 78.0 (C-3'), 75.8 (C-8), 74.2 (C-2'), 71.7 (C-4'), 62.8 (C-6'), 51.8 (11-OMe), 41.9 (C-9), 34.6 (C-6), 28.2 (C-5), 21.7 (C-10).

*Compound* **2**. UV $\lambda_{max}^{MeOH}$ nm: 227, 235, 260, 292. FAB$^+$MS *m/z*: 577 $[M+NA]^+$, 555 $[M+H]^+$, 313, 151. FAB$^-$MS *m/z*: 553 $[M-H]^-$, 241, 167. $^1H$ NMR: Table 1. $^{13}C$ NMR (75.46 MHz, $CD_3OD$): $\delta$174.5 (C-7), 168.2 (C-11), 167.9 (Ar-CO), 154.4 (C-3), 153.1 (C-4''), 148.9 (C-3''), 125.1 (C-6''), 122.4 (C-1''), 116.0 (C-2''), 113.8 (C-5''), 109.3 (C-4), 100.8 (C-1'), 96.4 (C-1), 77.8 (C-3'), 76.0 (C-5'), 75.6 (C-8), 74.7 (C-2'), 71.7 (C-4'), 63.7 (C-6'), 56.5 (Ar-OMe), 51.9 (11-OMe), 41.9 (C-9), 34.8 (C-6), 28.8 (C-5), 21.6 (C-10).

*Acetylation of* **1**. Compound **1** was treated with $Ac_2O$–pyridine in the usual way to give a tetraacetate (**1a**) which was purified by HPLC on silica gel column ($C_6H_{14}$–*iso*-PrOH–MeOH, 15:2:3). CIMS *m/z*: 590 $[M+NH_4]^+$, 573 $[M+H]^+$, 331. $^1H$ NMR: Table 1. $^{13}C$ NMR (75.46 MHz, $CDCl_3$): $\delta$170.5 (C-7), 170.1–169.1 (Me-CO), 168.1 (C-11), 151.4 (C-3), 110.2 (C-4), 96.5 (C-1'), 93.9 (C-1), 73.2 (C-8), 72.4 (C-3', C-5'), 70.6 (C-2'), 68.2 (C-4'), 61.5 (C-6'), 51.6 (11-OMe), 40.8 (C-9), 33.4 (C-6), 25.2 (C-5), 20.7 (C-10), 20.6–20.3 (Me-CO).

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