Phytochemistry, Vol. 27, No. 3, pp. 948–950, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

TAXIFOLIN 3-ARABINOSIDE FROM *TRACHELOSPERMUM JASMINOIDES* VAR. *PUBESCENS*

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(*Revised received* 25 *May* 1987)

Key Word Index—*Trachelospermum jasminoies* var. *pubescens*; Apocynaceae; dihydroflavonol; taxifolin 3-arabinoside; taxifolin 3-glucoside; EIMS; structural elucidation; biologically active substance.

Abstract—A new dihydroflavonol glycoside, taxifolin 3-arabinoside, together with the known substances: taxifolin 3-glucoside, quercetin 3-arabinoside, taxifolin and chlorogenic acid were identified from the leaves of *Trachelospermum jasminoides* var. *pubescens*. Structural elucidation was carried out by comparison of mass spectra with those of their methylated and acetylated derivatives. Taxifolin 3-glycosides were found to be *Aphanomyces cochlioides* zoospore attractants.

INTRODUCTION

In a previous paper [1], we reported the isolation of the new flavone glycoside, apigenin 7-gentiobioside, and several known flavone glycosides from the leaves of *Trachelosperum jasminoides* var. *pubescens* and the inhibitory effect of these compounds on xanthine oxidase. In this paper, we report the structural elucidation of a new dihydroflavonol, taxifolin 3-arabinoside (**2**), three known flavonoids: taxifolin (**1**), taxifolin 3-glucoside (**3**) and quercetin 3-arabinoside (**4**) and chlorogenic acid (**5**) obtained from the same source. Mass spectrometry was shown to be a useful technique for the identification and location of sugars in these flavonoids.

RESULTS AND DISCUSSION

Extraction was carried out as described in the previous paper [1]. The ethyl acetate soluble fraction of the extract was subjected to silica gel and Sephadex LH-20 column chromatography to afford compounds **1**–**5**.

Compound **2** had the following physical properties, mp 122–124°, FABMS: *m/z* 437. (Found: $[M+H]^+$ at 437.1069; $C_{20}H_{20}O_{11}+H$, request: 437.1082). Its IR spectrum showed absorption bands at 3372 (OH), 2924 (CH), 1642 (C=O), 1284 and 1084 (C–O) cm^{-1}. Its UV spectrum showed maxima at 292 and 340 (sh) nm. The bathochromic shifts to 280 (sh) and 312 nm on addition of aluminum chloride indicated the presence of hydroxylated aromatic rings. The mass spectra of **2** and its tetramethyl ether (**2a**) showed significant peaks at *m/z* 304 (aglycone) and ions at *m/z* 286, 265, 150, 152, 153 and 123 and the relative intensity was similar to that of **1**. Amongst these ions, the presence of an *m/z* 123 ion in the spectrum of **2** indicated that the aglycone was a flavanol [2]. Ions at *m/z* 152 and 153, being a protonated ions of a typical fragment from retro-Diels–Alder cleavage were shifted 28 mass numbers higher to *m/z* 180 and 181 in **2a**, suggesting the presence of two hydroxyl groups in the A ring [2].

Information about the B ring was obtained from the *m/z* 123 ion, a typical fragment ion of the flavonol (base peak), which was present in **2**, arising from a retro-Diels–Alder process [3] and the loss of a [H + CO] group. It was shifted 28 mass units higher to *m/z* 151 in the spectrum of **2a**. The increase of mass numbers indicated the presence of two methoxyl groups in the B ring of **2a**. These results revealed the incorporation of four methyl groups into **2** to give **2a**. This structure was supported by an increase of 56 mass numbers in the aglycone (*m/z* 360) ion of the spectrum of **2a**. Losses of carbon monoxide and hydrogen radicals and water from the aglycone ion produced ions at *m/z* 275 and *m/z* 286 of **2**. These were shifted 56 mass numbers higher to *m/z* 331 and *m/z* 342 in **2a**. Therefore, the location of *O*-glycosyl substitution in **2** was established as C-3 by correlation of the shifts of mass numbers in the spectrum of **2** with those in **2a**. Thus, **2** must be a taxifolin 3-*O*-glycoside. The ^1H NMR spectrum gave signals at δ3.16–4,96, 4.67, 5.37, 5.88 and 6.79–6.84. The signal of the C-2 and C-3 protons in the ^1H NMR spectrum supported a C-3 glycosylated structure.

Acetylation of **2** with acetic anhydride–pyridine gave the peracetate **2b**. The mass spectrum of **2b** showed the presence of an acetylated pentopyranoside moiety (*m/z* 259, base peak and the fragment ions *m/z* 199, 169, 157 and 139), which was identified by the presence of metastable peaks at *m/z* 152.9, 97 and 58.9. Thus, the sugar moiety of **2** was established as a pentopyranose type [4]. Acid hydrolysis of **2** gave **1** and arabinose.

The absolute configurations at C-2 and C-3 in **2** were deduced from CD and ^1H NMR spectra studies [5, 6]. The CD curve showed positive Cotton effects at 223, 253 and 329 nm and a negative Cotton effect at 292 nm as shown in Table 1. The absolute stereochemistry of **2** has been deduced as 2R and the structure was established by comparing the CD curve and ^1H NMR spectrum (*ca* $J_{2,3}$ = 8.4 Hz, acetate $J_{2,3}$ = 11.4 Hz) with those of 2*R*, 3*R* taxifolin 3-*O*-glucoside and a alpinone [7], which possess *trans*-diaxially related C-2 and C-3 protons (^1H NMR spectrum evidence). This result shows the same general form as those of lecontine, fustin 3-glucoside and astilbin which were established by comparing the ^1H NMR data with those of 2*R*, 3*R* taxifolin 3-rhamnoside [8–10].

Consequently, **2** is 2*R*,3*R* taxifolin 3-*O*-arabinopyranoside, which to the best of our knowledge is a new natural product.

Compounds **1** and **5** were identified by standard produces and comparison with literature values as taxifolin and chlorogenic acid [11, 12]. Compounds **3** and **4** were characterized as 2*R*,3*R* taxifolin 3-*O*-β-D-glucopyranoside (glucodistilin) [13] and quercetin 3-arabinoside [14], respectively, on the basis of ^{1}H NMR, IR, UV, MS and CD and hydrolysis in a manner similar to that described for **2** [15].

In addition, the effects of compounds **1**–**5** on zoospores of the plant pathogenic fungus, *Aphanomyces cochlioides* which attack sugar beet and spinach, was investigated. The activity was measured by a method described in the Experimental [16]. Thus, compounds **2** and **3** were identified as *A. cochlioides* zoospore attractants (Table 2). Detailed results will be presented in a subsequent paper.

EXPERIMENTAL

^{1}H NMR spectra were run on a 90 MHz instrument and ^{13}C NMR spectra on a 15 MHz instrument in DMSO-d_6 with TMS as int. standard. MS were obtained by direct inlet, electron energy 70 eV, ion source temp. 320°.

Plants of *Trachelosperum jasminoides* Lem. var. *pubescens* were collected at Kamikumamoto, Japan, in August 1970, crushed, extracted with hot MeOH and treated as described previously [1]. The EtOAc extracts were subjected to CC over silica gel with a $CHCl_3$–MeOH gradient. The fraction eluted with 15% MeOH in $CHCl_3$ was rechromatographed using Sephadex LH-20 with H_2O–EtOH gradient. The high polarity fractions 1–10, eluted with EtOH–H_2O (3:97), were rechromatographed using Sephadex LH-20 eluted with EtOH–H_2O (1:19). Fractions 10–20 were purified by rechromatography to give 112.5 mg of **3**, 45.5 mg of **4** and 50.5 mg of **5**. Fractions 21–25, eluted with 4% EtOH in H_2O, were rechromatographed using Sephadex LH-20 to afford 49.8 mg of **2**. Fractions 26–35, cluted with 4% EtOH in H_2O, were rechromatographed using Sephadex LH-20 to afford 30.5 mg of **1**.

Table 1. CD spectra of 2*R*,3*R* taxifolin 3-*O*-arabinoside (**2**), 2*R*,3*R* taxifolin 3-*O*-glucosides (**3**) and alpinone

Compounds	$n\rightarrow\pi^*$	$\pi\rightarrow\pi^*$
Taxifolin 3-*O*-arabinoside (**2**)	$[\theta]_{329}$ 14000	$[\theta]_{292}$ −31800
Taxifolin 3-*O*-glucoside (**3**)	$[\theta]_{329}$ 14500	$[\theta]_{292}$ −30000
Alpinone	$[\theta]_{324}$ 10200	$[\theta]_{282}$ −32000

All measurements were obtained in methanol.

Table 2. Response of *A. cochlioides* zoospores to isolated compounds from *T. jasminoides* var. *pubescens*

Compounds	Concentration (10^{-3} mol) 1	1/10	1/100	1/1000
Taxifolin (**1**)	—	—	—	—
Taxifolin 3-*O*-arabinoside (**2**)	++	+	—	—
Taxifolij 3-*O*-glucoside (**3**)	+++	+	—	
Quercetin 3-*O*-arabinoside (**4**)	—	—	—	—
Chrologenic acid (5)	—	—	—	—

+ Attracted within 5 min, ++ attracted within 10 min, +++ attracted within 15 min, — not attracted.

Taxifolin (**1**) was identified by comparing the IR, ^{1}H NMR and MS spectra with those of an authentic sample.

Taxifolin 3-O-arabinopyranoside (**2**). Colourless powder, $[\alpha]_D^{24}$ = −25.7 (EtOH; *c* 0.9). UV λ_{max}^{EtOH} nm 291, 340; +NaOAc 240, 289, 330 + $AlCl_3$ 280 (sh), 312; +NaOMe 246, 280, 329, ^{1}H NMR δ (in DMSO-d_6): 4.91–3.16 (*m*, gly-H), 4.67 (1H, *d*, *J* = 8.4 Hz, H-3), 5.37 (1H, *d*, *J* = 8.4 Hz, H-2), 5.88 (2H, *d*, *J* = 2.0 Hz, aromatic 6, 8-H), 6.79–6.84 (3H, *m*, aromatic 2′,5′, 6′-H). ^{13}C NMR δ (in DMSO-d_6): 193.3 (C-4), 167.0 (C-7), 163.3 (C-5), 161.9 (C-8a), 145.7 (C-3′), 145.1 (C-4′), 126.6 (C-1′), 118.6 (C-6′), 115.3 (C-5′), 114.5 (C-2′), 95.9 (C-6), 95.0 (C-8), 81.0 (C-2), 74.9 (C-3), sugar moeity; 100.4, 71.5, 69.7, 65.0, 62.2. EIMS *m/z* (rel. int.): 304 [M: $C_{15}H_{12}O_7]^{+\cdot}$ (16.3), 302 $[C_{15}H_{10}O_7]^{+\cdot}$, 286 $[C_{15}H_{10}O_6]^{+\cdot}$, 275 $[C_{14}H_{11}O_6]^+$, 153 $[C_7H_5O_4]^+$ (100), 152 $[C_7H_4O_4]^{+\cdot}$, 123 $[C_7H_7O_2]^+$.

Methylation of taxifolin 3-arabinoside (**2**). Compound **2** (10 mg) was treated with excess CH_2N_2 to give a methylated product (**2a**) as a colourless powder. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1645 (C=O, C=C), 1380 (Me), 1170, 1080 (C–O). UV λ_{max}^{EtOH} nm 291, 340 (sh), ^{1}H NMR δ (in CD_3OD): 2.80–5.20 (*m*, gly-H), 3.80–4.00 (12H, *s*, aromatic-Me), 4.77 (1H, *d*, *J* = 8.8 Hz, H-3), 6.13 (1H, *d*, *J* = 2.2 Hz, aromatic 8-H), 6.19 (1H, *d*, *J* = 2.2 Hz, aromatic 6-H), 6.91–6.99 (3H, *m*, aromatic 2′,5′,6′-H). EIMS *m/z* (rel. int.): 360 [Aglycone: $C_{19}H_{20}O_7]^{+\cdot}$ (6.8), 209 $[C_{10}H_9O_5]^+$ (56.3). 181 $[C_9H_9O_4]^+$ (56.3), 180 $[C_{10}H_{12}O_3]^{+\cdot}$, 151 $[C_9H_{11}O_2]^+$ (100). The crude product was hydrolysed with 3% H_2SO_4 and the reaction mixture extracted with EtOAc. The EtOAc extract was washed with H_2O and evapd to dryness. Prep. TLC ($CHCl_3$) gave tetramethyl taxifolin, a colourless powder. mp 185–187°, IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1645 (C=O, C=C), 1380 (Me), 1170. UV λ_{max}^{EtOH} nm 291, 340 (sh), ^{1}H NMR δ (in $CDCl_3$): 3.80–4.00 (12H, *s*, aromatic Me), 4.77 (1H, *d*, *J* = 8.8 Hz, H-3), 6.13 (1H, *d*, *J* = 2.2 Hz, aromatic 8-H), 6.19 (1H, *d*, *J* = 2.2 Hz, aromatic 6-H), 6.91–6.99 (3H, *m*, aromatic 2′,5′,6′-H).

Acetylation of taxifolin 3-arabinoside (**2**). Compound (**2**) (10 mg) was treated with Ac_2O and pyridine. The product was purified by prep. TLC ($CHCl_3$) to give the peracetylated derivative **2b**. mp 147–151° as a colourless powder. IR ν_{max}^{KBr} cm^{-1}: 2910 (CH), 1760 (MeCO), 1640 (C=O), 1605 (C=C), 1230, 1080 (C–O), ^{1}H NMR δ (in $CDCl_3$): 1.98, 2.04, 2.08 (9H, each *s*, aliphatic MeCO), 2.30 (9H, *s*, aromatic MeCO), 2.38 (3H, *s*, aromatic MeCO), 2.80–5.60 (*m*, gly-H), 4.52 (1H, *d*, *J* = 11.4 Hz, aliphatic 2-H), 5.33 (1H, *d*, *J* = 11.4 Hz, aliphatic 3-H), 6.71 (1H, *d*, *J* = 2.2 Hz, aromatic 8-H), 6.75 (1H, *d*, *J* = 2.2 Hz, aromatic 6-H), 7.26–7.40 (3H, *m*, aromatic 2′,5′,6′-H), EIMS *m/z* (%) 259 $[C_{11}H_{15}O_7]^+$ (100), 199 $[C_{11}H_{15}O_7]^+$ (22.2), 157 $[C_7H_9O_4]^+$ (62.5), 139 $[C_7H_7O_3]^+$ (95.3), 115 $[C_5H_7O_3]^+$ (13.7), 97 $[C_5H_5O_2]^+$ (71.3).

Taxifolin 3-O-β-D-glucopyranoside (**3**). Colourless powder, mp 170–172°, $[\alpha]_D^{24}$ = 26.5 (EtOH; *c* 0.9). Properties of agreed with lit. data.

Methylation of taxifolin 3-glucoside (**3**). Compound **3** (10 mg) was treated with excess CH_2N_2 as described above to give a colourless powder, UV λ_{max}^{EtOH} nm 292, 340 (sh). ^{1}H NMR δ (in CD_3OD): 2.50–4.90 (*m*, gly-H), 3.70–4.00 (12H, *s*, aromatic Me), 4.90 (1H, *d*, *J* = 8.8 Hz, 3-H), 6.13 (1H, *d*, *J* = 2.0 Hz, aromatic 8-H), 6.18 (1H, *d*, *J* = 2.0 Hz, aromatic 6-H), 6.91–7.10 (3H, *m*, aromatic 2′,5′,6′-H). The crude product was hydrolysed with 3% H_2SO_4 to give an aglycone, colourless powder, mp 186–188° whose IR, UV, EIMS and ^{1}H NMR data agreed with those of methylated **2**.

Acetylation of taxifolin 3-glucoside (**3**). Acetylation of **3** (10 mg) with Ac_2O and pyridine gave a colourless powder. IR ν_{max}^{KBr} cm^{-1}: 2915 (CH), 1760 (MeCO), 1640 (C=O, C=C), 1235, 1050 (C–O). ^1H NMR δ (in $CDCl_3$): 1.96, 1.98, 2.04 (9H, each *s*, alphatic MeCO), 2.31 (9H, *brs*, aromatic MeCO), 2.38 (3H, *s*, aromatic MeCO), 2.80–5.87 (*m*, gly-H), 4.58 (1H, *d*, $J = 11.0$ Hz, 2-H), 5.38 (1H, *d*, $J = 11.0$ Hz, 3-H), 6.57 (1H, *d*, $J = 1.8$ Hz, aromatic 8-H), 6.74 (1H, *d*, $J = 1.8$ Hz, aromatic 6-H), 7.26–7.46 (3H, *m*, aromatic 2′,5′,6′-H). The EIMS data of the sugar moiety is similar to that of **2a**.

Zoospore formation. Fungal isolates were grown for 3–4 days on corn meal agar plates at 25°. Each agar plate bearing mycelium was transferred to a Petri dish, 15 cm diameter, containing 400 ml of dist. H_2O and was rinsed with four changes of dist. H_2O within 2 hr to remove nutrient material inhibitory to zoospore formation. The material thus rinsed was then incubated for 12–20 hr at 25°. Zoospore concn was adjusted to 5000/ml with dist. H_2O.

Attraction test. A glass capillary tube (2.5 cm long, 0.8 mm o.d.) was filled with a MeOH soln of the test sample and the solvent spontaneously evapd. The capillary tube was then filled with dist. H_2O and its one end sealed with vaseline. The unsealed end of the capillary tube was stood to a depth of 2 cm in a zoospore suspension in a small plastic vessel (44 mm long, 22 mm wide and 5 mm high) set on a microscope stage and the behaviour of zoospores to the capillary tube observed at a magnification × 40.

Acknowledgement—We are deeply indebted to Professor Dr R. Yokozawa of Higashi Nippon Gakuen University, for his kind help in the measurement of the activity of compounds as zoospore attractants.

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