was used, while **6**–**9** were separated using Et_2O–petrol (1:10, five developments). The roots (100 g) afforded 5 mg **1**, 10 mg **2**, 3 mg **3**, 2 mg **4**, 1 mg **5**, 2 mg **6**, 2 mg **7**, 3 mg **8** and 3 mg **9**, while the aerial parts (210 mg) gave 30 mg squalene, 3 mg caryophyllene, 5 mg germacrene D, 2 mg bicyclogermacrene, 2 mg **1** and 1 mg **2**.

2-Methoxy-9-tigloyloxy-8, 10-epoxythymol tiglate (**7**). Colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730 (PhOCOC=C), 1710 (C=CCO$_2$R), 1650 (C=C); MS *m/z* (rel. int.): 374.173 $[M]^+$ (2) ($C_{21}H_{26}O_6$), 274 $[M-RCO_2H]^+$ (2), 192 $[274-O=C=C(Me)CH=CH_2]^+$ (14), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (71).

2-Methoxy-9-(tigloyloxy and isobutyryloxy)-8,10-epoxythymol (isobutyrate and tiglate, respectively) (**8** *and* **9**). Inseparable colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750 (PhOCOR), 1735 (PhOCOC=C), 1715 (C=CCO$_2$R), 1650 (C=C); MS *m/z* (rel. int.): 362.173 $[M]^+$ (6) ($C_{20}H_{26}O_6$), 274 $[M-C_3H_7CO_2H]^+$ (5), 262 $[M-C_4H_7CO_2H]^+$ (8), 192 $[274-O=C(Me)CH=CH_2]^+$ and $[262-O=C-CMe_2]^+$ (78), 83 $[C_4H_7CO]^+$ (100), 71 $[C_3H_7CO]^+$ (20), 55 $[83-CO]^+$ (60).

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6′-*p*-HYDROXYBENZOYLMUSSAENOSIDIC ACID–AN IRIDOID GLUCOSIDE FROM *VITEX NEGUNDO**

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Key Word Index—*Vitex negundo*; Verbenaceae; iridoid; 6′-*p*-hydroxybenzoylmussaenosidic acid; ^{13}C NMR.

Abstract—Further chromatography of an ethanolic extract of *Vitex negundo* resulted in the isolation of another new iridoid glucoside which was characterized as 6′-*p*-hydroxybenzoylmussaenosidic acid.

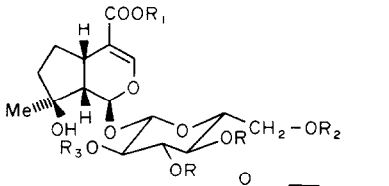
In a previous communication [1], we reported on the isolation and characterization of a new iridoid, 2′-*p*-hydroxybenzolymussaenosidic acid (**1b**), from the ethanolic extract of the leaves of *Vitex negundo* L. We now report on the characterization of another minor iridoid from the same extract, which has been assigned the structure 6′-*p*-hydroxybenzoylmussaenosidic acid (**1a**).

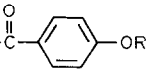
Compound **1a** was isolated as a viscous solid. Its mass spectrum showed $[M]^+$ at 496, which corresponded to the molecular formula $C_{23}H_{28}O_{12}$. As in the case of **1b**, hydrolysis of **1a** resulted in the formation of *p*-hydroxybenzoic acid. The ^1H NMR spectrum of **1a** in DMSO-d_6 displayed signals at δ1.20 (3H) for a C-8 methyl group. A C-3 proton was located at δ7.40 as a sharp singlet and four protons of the aromatic moiety were observed as an AA′BB′ pattern at δ6.93 and 7.90 (J = 8.5 Hz), respectively. Other signals were located at the usual positions.

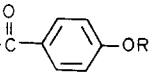
Acetylation at room temperature resulted in the formation of the tetra-acetate **2**, mp 91–92°. ^1H NMR signals for three acetate methyls were observed at δ1.93–2.10. A fourth signal appeared at δ2.33 and was assigned to the aromatic acetoxyl methyl. This clearly indicated that the *p*-hydroxybenzoyl moiety was attached to the glucose part of the molecule.

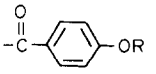
Methylation of **2** with diazomethane gave the methyl ester **3** as a viscous mass. The signal for a carbomethoxy group appeared at δ3.73. Alkaline hydrolysis of **1a** again resulted in the formation of two compounds. One of them

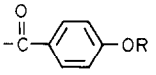
*RRL Communication No. RRL/81/024.



1a $R=R_1=R_3=H$, $R_2=$ 

1b $R=R_1=R_2=H$, $R_3=$ 

2 $R_1=H$, $R=R_3=-COMe$, $R_2=$ 

3 $R_1=-Me$, $R=R_3=-COMe$, $R_2=$ 

was identified as *p*-hydroxybenzoic acid. The second compound, an amorphous powder, was converted into a methyl ester on treatment with diazomethane. The elemental analysis of the ester corresponded to the molecular formula $C_{17}H_{26}O_{10}$. The ester matched well in all respects with the compound obtained on alkaline hydrolysis of 2′-*p*-hydroxybenzoylmussaenosidic acid (**1b**) and subsequent methylation (co-TLC, superimposable IR, ^{1}H NMR, etc). Therefore, it was the *p*-hydroxybenzoyl ester of mussaenosidic acid [2]. However, the position of the *p*-hydroxybenzoyl moiety was different in **1a**. The mass spectrum of both compounds under similar conditions gave almost the same fragmentation pattern.

Table 1. ^{13}C NMR data of 6′-*p*-hydroxybenzoylmussaenosidic acid (**1a**) and 2′-*p*-hydroxybenzoylmussaenosidic acid (**1b**) (90 MHz, DMSO-d_6, TMS as int. standard)

C No.	1a	1b
1	94.4	93.5
3	150.4	148.6
4	112.0	112.2
5	31.2	29.7
6	29.6	28.9
7	41.4	41.2
8	78.4	77.7
9	50.4	50.5
10	24.0	24.1
11	168.0*	167.1*
1′	98.4	95.9
2′	74.0	77.3
3′	76.8	74.1†
4′	70.4	70.1
5′	73.2	73.1†
6′	63.2	60.8
1″	120.8	120.5
2″	131.2	131.2
3″	115.2	114.9
4″	162.4	161.5
5″	115.2	114.9
6″	131.2	131.2
C=O	165.6*	164.6*

*, † Values are interchangeable within each column.

The position of the *p*-hydroxybenzoyl moiety in **1a** was finally established by comparison of its ^{13}C NMR spectrum in DMSO-d_6 with that of **1b** (Table 1). The downfield shift of C-6′ clearly indicated that this position was esterified. Moreover, the chemical shift for C-1′, which in the case of **1b** was observed at δ95.9, was shifted to δ98.4 in the case of **1a**. This clearly indicated that the 2′ position was unoccupied in **1a**. The other carbons of the glucose moiety were also observed at their expected position [3]. The chemical shift values for C-8–C-10 were more or less unchanged, as in **1b**, indicating the same stereochemistry at C-8. It is interesting to note that the positions of the methyl of the carbomethoxy group in the ^{1}H NMR spectrum of the methyl ester (**3**) of the acetate and the similar derivative of **1b** differ considerably (cf. δ3.73 and 3.33) [1], as does the chemical shift value of H-3 (cf. δ7.40 and 7.07). In the case of the methyl ester of the acetate of **1b** the signals were observed upfield of their expected positions. It appears that in the latter case, the ring A protons somehow fall in the shielding zone of the aromatic system attached to the sugar moiety at C-2′. In the case of a closely related iridoid (*p*-hydroxyphenylpropionyl group attached to C-2′) isolated from *Galium verum* L., X-ray crystallography has shown that ring A is indeed clouded by the *p*-hydroxyphenylpropionyl moiety [4].

EXPERIMENTAL

Mps are uncorr.

The leaves of *Vitex negundo* L. (Herbarium No. 11607) were collected locally. The air-dried leaves were first defatted and then extracted with $CHCl_3$ followed by EtOH. The EtOH extract was dried and subjected to CC over Si gel (2.3 kg) eluted with different EtOAc–MeOH mixtures.

Compound **1a** was obtained from the EtOAc–MeOH (93:7) fraction. It analysed for $C_{23}H_{28}O_{12}$ (calcd for C, 55.64; H, 5.52%; observed C, 55.52; H, 5.80%). $[\alpha]_D^{25}$ −120° (MeOH; *c* 3%); UV λ_{max}^{MeOH} nm: 258; IR ν_{max}^{KBr} cm^{-1}: 3300, 1695, 1680, 1640, 1610, 1590, 1510, 1450, 1425, 1375, 1310, 1260, 1065, 1020, 980, 940, 920, 860, 770, 680; ^{1}H NMR (DMSO-d_6, TMS as int. standard); δ1.20 (3H, *s*, Me-8), 2.13 (1H, *dd*, *J* = 10, 3.3 Hz, H-9), 5.16 (1H, *d*, *J* = 3.3 Hz, H-1), 6.93 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 7.40 (1H, *d*, *J* = 1.0 Hz, H-3), 7.90 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″).

Acetylation of **1a**. Acetylation with Ac_2O–C_5H_5N gave the tetra-acetate **2** mp 91–92° (from MeOH). Analysed for $C_{31}H_{36}O_{16}$ (calcd for C, 55.02; H, 5.42%; observed C, 55.95, H, 5.62%). ^{1}H NMR ($CDCl_3$, TMS as int. standard): δ1.26 (3H, *s*, Me-8), 1.93–2.10 (9H, 3*s*, 3×–OCOC<u>H</u>$_3$), 2.33 (3H, *s*, Ar–OCOC<u>H</u>$_3$), 3.50–5.10 (*m*, –CH_2 and –CH–OAc of glucose moiety), 5.25 (1H, *d*, *J* = 3.3 Hz, H-1), 7.66 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 7.40 (1H, *s*, H-3), 8.06 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″).

Methylation of **2**. Compound **2** with CH_2N_2 gave a viscous mass **3** which analysed for $C_{32}H_{38}O_{16}$ (calcd for C, 56.63; H, 5.60%; observed C, 56.53; H, 5.40%). ^{1}H NMR ($CDCl_3$, TMS as int. standard): δ1.33 (3H, *s*, Me-8), 2.0–2.16 (9H, 3*s*, 3×–OCOC<u>H</u>$_3$), 2.36 (3H, *s*, Ar–OCOC<u>H</u>$_3$), 3.73 (3H, *s*, –COC<u>H</u>$_3$), 5.30 (1H, *d*, *J* = 3.3, Hz, H-1), 7.16 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 7.26 (1H, *s*, H-3), 8.06 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″).

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STRESS COMPOUNDS IN TOBACCO CALLUS INFILTRATED BY *PSEUDOMONAS SOLANACEARUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; callus; stress compounds; phytuberin; phytuberol; *Pseudomonas solanacearum*.

Abstract—Two sesquiterpenoids, phytuberin and phytuberol, have been identified in tobacco callus infiltrated by *Pseudomonas solanacearum*.

Seven sesquiterpenoidal stress compounds, solavetivone, 3-hydroxysolavetivone, solanascone, phytuberin, phytuberol, glutinosone and capsidiol, have been isolated from *Nicotiana* species [1]. Of these seven, phytuberin and phytuberol have been obtained in tobacco leaves treated with ethrel [2]. Phytuberin has also been isolated from tobacco leaves infiltrated with the bacterium *Pseudomonas lachrymans*, a nonpathogen of tobacco [3].

In this paper, we report the occurrence of two of these compounds in the tobacco callus challenged by *Pseudomonas solanacearum* U-7. Strain U-7 was highly pathogenic to tobacco plants. The methylene chloride extract from the callus contained phytuberin (**1**) and phytuberol (**2**). Both **1** and **2** were absent from healthy callus tissues.

EXPERIMENTAL

Callus tissues were induced from the pith of tobacco plants (*Nicotiana tabacum* cv Burley 21) by standard procedures on Linsmaier–Skoog agar medium [4], containing 3 mg indoleacetic acid, 3 mg naphthaleneacetic acid and 0.1 mg kinetin per l. Callus tissues were subcultured every 4 weeks on 20 ml medium for five or six times before use.

Pseudomonas solanacearum U-7 was grown on Kelman's TZC medium [5] for 2 days at 30°. Inoculums were prepared by suspending the bacteria in H_2O (10^7 cells/ml).

Well-grown callus tissues (7 g fr. wt/flask) were infiltrated with 1 ml/flask of the bacteria suspension. The infected callus exhibited a necrotic response within 2 days of infiltration. The callus tissues, incubated for 2 days after infiltration of *P. solanacearum*, were harvested and freeze-dried. The dried material (3.90 g dry wt) was extracted with CH_2Cl_2. The extract was evaporated to dryness to give 57.2 mg of yellow oil. The oil was analysed with capillary GC (OV-101, 0.2 mm × 50 m, 100–230°, 2°/min and PEG 20 M 0.2 mm × 25 m, 100–210°, 2°/min) and capillary GC/MS (OV-101 0.27 mm × 50 m, 100–230°, 2°/min). The presence of **1** and **2** was suggested by GC/MS and these compounds were identified with authentic samples by the MS and retention times (**1**: OV-101 45.0 min and PEG 20 M 39.0 min; **2**: OV-101 38.9 min and PEG 20 M 41.6 min). MS of **1**: *m/z* (rel. int.) 294 $[M]^+$ (8), 249 (10), 234 (14), 205 (100), 189 (61), 149 (41), 107 (46), 95 (38), 93 (39) 91 (37) and 67 (29). MS of **2**: *m/z* (rel. int.) 252 $[M]^+$ (12), 237 (6), 234 (3), 205 (41), 149 (36), 107 (37), 95 (36), 77 (35), 59 (51), 55 (37), 43 (100) and 41 (75). Up to 38.3 μg **1** and 3.83 μg **2** per g dry wt of callus tissues was recognized by capillary GC analysis.

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