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2′-*p*-HYDROXYBENZOYL MUSSAENOSIDIC ACID, A NEW IRIDOID GLUCOSIDE FROM *VITEX NEGUNDO*.

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Key Word Index—*Vitex negundo*; Verbenaceae; iridoid; 2′-*p*-hydroxybenzoyl mussaenosidic acid.

Abstract—Ethanolic extract of the leaves of *Vitex negundo* yielded a new iridoid named 2′-*p*-hydroxybenzoyl mussaenosidic acid. The structure was established on the basis of chemical and spectral data.

INTRODUCTION

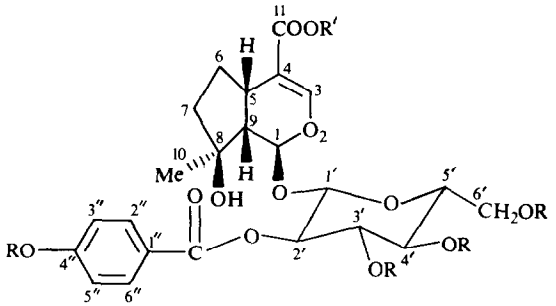
Vitex negundo is well known for its medicinal value [1]. Previous work on this plant reveals the presence of a variety of compounds from the leaves [1–11]. *V. negundo* and *V. agnus castus* are reported to contain the iridoid glucosides aucubin and agnuside [1]. We now report the presence of a new iridoid glycoside in the leaves of *V. negundo* which has been assigned the structure 2′-*p*-hydroxybenzoyl mussaenosidic acid (**1**).

RESULTS AND DISCUSSION

Chromatography of the ethanolic extract of the defatted leaves of *Vitex negundo* resulted in the isolation of a minor component, crystallized from MeOH, mp 160–162°. MS (FD) showed a molecular ion at *m/z* 496 which corresponds to the molecular formula $C_{23}H_{28}O_{12}$. Preliminary chemical tests confirmed its nature as an iridoid glucoside and revealed the presence of a carboxylic function. Acid hydrolysis gave glucose and an acid (mp 213–214°). The latter was identified as *p*-hydroxybenzoic acid. The UV spectrum (λ_{max}^{MeOH}nm:258) also suggests the presence of a *p*-hydroxybenzoic acid moiety in **1**. The ^{1}H NMR spectrum of **1** displayed a singlet at δ 1.20 integrating for three protons due to the methyl group attached to the carbon-bearing oxygen function. A doublet at 5.40 (J = 3.3 Hz) was assigned to the C–1 proton. Two doublets (each 2H, J = 8.5 Hz) centred at 6.87 and 7.76 respectively were assigned to the AB protons of the benzene ring of the *p*-hydroxybenzoyl moiety. A weak doublet at 7.07 (1H, J = 1.0 Hz) was assigned to the C–3 proton which is β to the carbonyl function.

Acetylation of **1** under mild conditions formed a tetra-acetate (**2**) mp 120–121°. The ^{1}H NMR spectrum of the acetate displayed signals between δ2.0–2.20 for three acetyl groups of the glucose moiety and one signal at 2.40 (3H, *s*) for the aromatic acetyl group of the *p*-hydroxybenzoyl moiety. This showed that the *p*-hydroxybenzoyl moiety is attached to the glucose and not to the basic skeleton.

Treatment of **2** with diazomethane gave the methyl ester (**3**) mp 100–101°. ^{1}H NMR spectrum displayed, among other signals, a three proton singlet at δ3.33 for the methyl group of the carbomethoxy moiety.

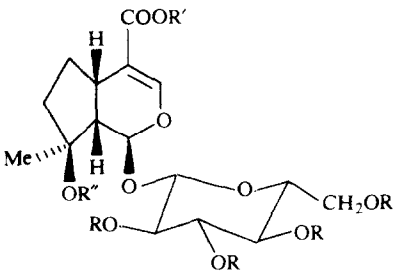


1 R = R′= H
2 R = COMe, R′ = H
3 R = COMe, R′ = Me

Alkaline hydrolysis of the iridoid (**1**) gave two compounds which were separated by CC. One of them was identified as *p*-hydroxybenzoic acid. The other compound (**4**) which was isolated as an amorphous powder was converted into its methyl ester (**5**) by treatment with diazomethane. The elemental analysis of **5** corresponded to molecular formula $C_{17}H_{26}O_{10}$. The ^{1}H NMR spectrum of compound **5** displayed a signal at δ1.20 (3H, *s*) for a tertiary methyl. A double doublet at 2.20 (J = 10 Hz and 3.3 Hz) was assigned to the C–9 proton. A sharp doublet at 5.40 (J = 3.3 Hz) was assigned to the C–1 proton and a singlet for three protons at 3.63 was observed for the methyl protons of the carbomethoxy grouping. A weak doublet at 7.30 (J = 1.0 Hz) was assigned to the C–3 proton.

Compound **5** on acetylation with Ac_2O and pyridine gave a tetra-acetate (**6**) mp 124°. The tetra-acetate (**6**) when further treated with the acetylating mixture in the presence of BF_3, furnished a penta-acetate (**7**) mp 116–118°.

The physical and spectral data for compounds **5**, **6** and **7** matches well with the data for mussaenoside **5** and its acetate **6** (mp 124–126°), an iridoid glucoside isolated from the leaves of *Mussaenda parviflora* by Takeda *et al.* [12]. This identification was confirmed by the comparison of **5** and **6** with the authentic samples of mussaenoside and its acetate which showed complete identity in all respects (TLC, mmp and IR).



4 R = R′ = R″ = H
5 R′ = —— Me, R = R″ = H
6 R′ = —— Me, R = —— COMe, R″ = H
7 R′ = —— Me, R = R″ = —— COMe

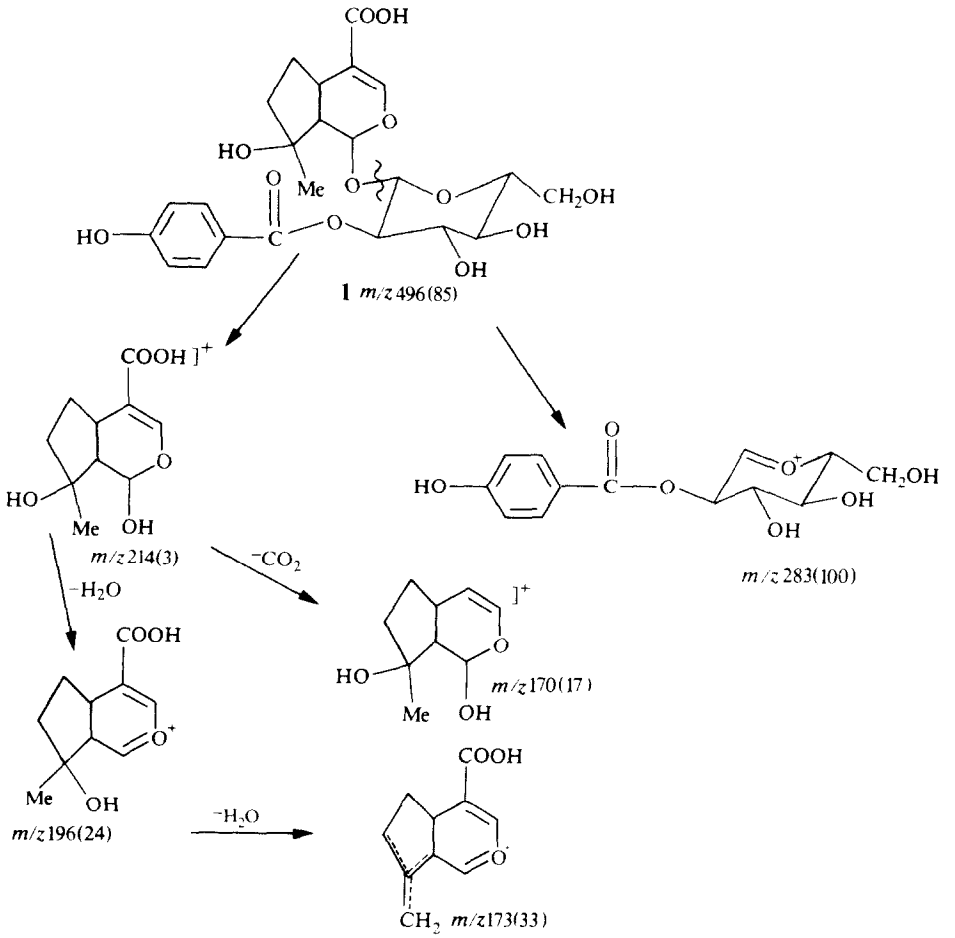
From these studies it is obvious that compound **1** is the *p*-hydroxybenzoyl ester of the mussaenoside and there is a carboxylic acid group instead of carbomethoxy group at C–4. The *p*-hydroxybenzoyl group is linked at C–2′ of the glucose moiety, as shown by the ^{13}C NMR spectra of **1** and its acetate **2**. This was corroborated by the assignments of C–1′ (95.9), C–2′ (77.3) and C–3′ (74.1). The benzoylation effect for C–1′, C–2′ and C–3′, by comparison with the unbenzoylated product, is *ca* −3.5, +3.0, −3.0 respectively. The chemical shift values for the C–8, C–9 and C–10 carbons clearly indicates that the tertiary hydroxyl is β at C–8 when compared with other known compounds of similar nature [13].

The structure has been further confirmed by its mass spectral fragmentation pattern which is given in Scheme 1.

EXPERIMENTAL

All mps are uncorr. The leaves of *Vitex negundo* L. (Herbarium no. 11607) were collected locally. The air-dried leaves were first defatted and extracted with $CHCl_3$ followed by EtOH. The EtOH extract was dried and then subjected to CC over Si gel (2.5 kg) and eluted with EtOAc–MeOH mixtures. Compound **1** was obtained from EtOAc–MeOH (19:1), crystallized from MeOH as white needles, mp 160–162°, $C_{23}H_{28}O_{12}$ $[\alpha]_D^{24}$ −117.6° (MeOH; *c* 3%). UV λ_{max}^{MeOH} nm: 258; IR ν_{max}^{KBr} cm^{-1}: 3300, 1700, 1680, 1640, 1610, 1590, 1430, 1410, 1370, 1310, 1255, 1220, 1065, 1020, 980, 940, 860, 770, 680: ^1H NMR (DMSO–d_6): δ1.20 (3H, *s*, H–10), 2.20 (1H, *dd*, *J* = 10 Hz, 3.3 Hz, H–9), 5.40 (1H, *d*, *J* = 3.3 Hz, H–1), 6.87 (2H, *d*, *J* = 8.5 Hz, ArH–3″ and H–5″), 7.07 (1H, *d*, *J* = 1.0 Hz, H–3), 7.76 (2H, *d*, *J* = 8.5 Hz, ArH–2″ and 6″). MS *m*/3 (rel. int.): 496[M^+] (85), 478 (10), 283 (100), 196 (24), 180 (5), 178 (33), 170 (17), 152 (3), 138 (4).

Wieffering field test for iridoids [14]. Compound **1** (5 mg) was added to 1 ml of reagent, prepared by mixing HOAc (10 ml) $CuSO_4$ (1.0 ml of 0.2% soln) and conc HCl (0.5 ml). The mixture was heated over a small flame. After a few seconds a light blue colour was obtained.



Scheme 1.

Table 1. ^{13}C NMR chemical shifts of 2′-*p*-hydroxybenzoyl mussaenosidic acid (**1**) and its tetra-acetate (**2**)

C-atom	1	2	Multiplicity
1	93.5	92.8	*s*
3	148.6	149.5	*d*
4	112.2	112.6	*s*
5	29.7	29.4	*d*
6	28.9	28.4	*t*
7	41.2	41.5	*t*
8	77.7	78.9	*s*
9	50.5	50.9	*d*
10	24.1	24.0	*q*
11	167.1*	166.9	*s*
1′	95.9	95.1	*d*
2′	77.3	72.3	*d*
3′	74.1†	70.8	*d*
4′	70.1	68.3	*d*
5′	73.1†	72.1	*d*
6′	60.8	61.6	*t*
1″	120.5	127.0	*s*
2″	131.2	131.7	*d*
3″	114.9	122.3	*d*
4″	161.5	154.1	*d*
5″	114.9	122.3	*d*
6″	131.2	131.7	*d*
C=O	164.6*	163.8	*s*
$-OCOCH_3$	—	21.1, 20.7, 20.6	*q*
$-OCOCH_3$	—	170.2, 169.4, 170.7, 171.1	*s*

* and † values are interchangeable.

Acetylation of **1**. Treatment of **1** with Ac_2O–pyridine gave the tetra-acetate (**2**) crystallized from EtOAc-petrol as colourless needles mp 120–121°, analysed for $C_{31}H_{36}O_{16}$. ^1H NMR ($CDCl_3$): δ1.30 (3H, *s*, H-10), 2.0–2.20 (9H, 3*s*, 3× OCOMe), 2.40 (3H, *s*, Ar-OCOMe), 3.8–5.20 (*m*,-CH_2 and CH-OAc of glucose moiety), 5.40 (1H, *d*, *J* = 3.3 Hz, H-1), 7.10 (2H, *d*, *J* = 8.5 Hz, Ar H-3″ and H-5″), 7.30 (1H, *s*, H-3), 8.0 (2H, *d*, *J* = 8.5 Hz, Ar H-2″ and H-6″).

Methylation of **2**. Treatment of **2** with CH_2N_2 gave **3** as fine colourless meedles from EtOAc–petrol, mp 100–101°, analysed for $C_{32}H_{38}O_{16}$. ^1H NMR ($CDCl_3$): δ1.3 (3H, *s*, H-10), 1.90–2.20 (9H, 3*s*, 3×OCOMe), 3.33 (3H, *s*,-COOMe), 5.40 (1H, *d*, *J* = 3.3 Hz, H-1), 7.10 (2H, *d*, *J* = 8.5 Hz, Ar H-3″ and H-5″), 7.3 (1H, *s*, H-3), 7.9 (2H, *d*, *J* = 8.5 Hz, Ar H-2″ and H-6″).

Alkaline hydrolysis of **1**. Compound **1** (80 mg) was refluxed with methanolic KOH for 30 min. After usual work-up it gave a mixture of two compounds. Compound **4** (30 mg) was separated by CC over Si gel as a colourless powder, analysed for $C_{16}H_{24}O_{10}$.

Methylation of **4**. Treatment of **4** with CH_2N_2 gave a white powder (**5**), analysed for $C_{17}H_{26}O_{10}$: $[\alpha]_D^{24}$ −114° (MeOH; *c* 0.9%). UV λ_{max}^{MeOH} nm: 238; IR ν_{max}^{KBr} cm^{-1}: 3400, 1695, 1640; ^1H NMR (DMSO-d_6): δ1.20 (3H, *s*, *ter.*-Me), 2.20 (1H, *dd*, *J* = 10.0 Hz and 3.3 Hz, H-9); 3.63 (3H, *s*, COOMe), 5.40 (1H, *d*, *J* = 3.3 Hz, H-1), 7.30 (1H, *d*, *J* = 1.0 Hz, H-3).

Acetylation of **5**. Compound **5** with Ac_2O–pyridine gave a tetra-acetate (**6**) crystallized from MeOH mp 124–126°, analysed for $C_{25}H_{34}O_{14}$. UV λ_{max}^{MeOH} nm: 237; IR ν_{max}^{KBr} cm^{-1}: 1750, 1705, 1640: ^1H NMR ($CDCl_3$): δ1.33 (3H, *s*, H-10), 1.92–2.09 (12H, 4×OCOMe), 2.31 (1H, *dd*, *J* = 9.5 Hz, and 3.0 Hz, H-9), 3.03 (1H, *m*, H-5), 3.70 (3H, *s*, COOMe), 5.33 (1H, *d*, *J* = 3.0 Hz, H-1), 7.34 (1H, *d*, *J* = 1.0 Hz, H-3).

Treatment of **6** *with* Ac_2O–BF_3. To a soln of **6** (40 mg) in Ac_2O was added BF_3-etherate (5 drops) and the mixture was allowed to stand at room temp. for 2 min. Ice-cold H_2O was added and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4, conc and **7** (40 mg) was crystallized from EtOH, as colourless needles mp 116–118°. UV λ_{max}^{MeOH} nm: 240; IR ν_{max}^{KBr} cm^{-1}: 1745, 1710, 1660; ^1H NMR ($CDCl_3$): δ1.51 (3H, *s*, H-10), 1.90–2.08 (3H, *s*, 5×OCOMe), 2.66 (1H, *dd*, *J* = 8.5 Hz and 2.0 Hz, H-9), 2.95 (1H, *m*, H-5), 3.71 (3H, *s*, COOMe), 5.71 (1H, *d*, *J* = 2.0 Hz, H-1), 7.40 (1H, *d*, *J* = 1.0 Hz, H-3). Analysed for $C_{27}H_{36}O_{15}$.

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