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A new phenylethanoid glycoside from *Clerodendrum inerme*

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A new phenylethanoid glycoside, 2-(3-methoxy-4-hydroxylphenyl) ethyl-*O*-2″,3″-diacetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-(*E*)-feruloyl-β-D-glucopyranoside, was isolated from the aerial parts of *Clerodendrum inerme* (L.) Gaertn, together with monomelittoside, melittoside, inerminoside A1, verbascoside, isoverbascoside, campneoside I. Their structures were determined by spectroscopic methods.

Clerodendrum inerme (L.) Gaertn. is a medicinal plant that grows in South and Southeast Asia, Australia and Pacific islands, both in the wild and as a garden hedge. In southern China, it grows as a satellite plant in mangrove forest (Lin et al. 2001). In traditional medicine, the fresh leaves are used for treating skin diseases. And the leaf contains components responsible for induction of systemic resistance against viruses (Prasad et al. 1995). The previous research on *Clerodendrum inerme* yielded iridoid glucosides (Kanchanaooom et al. 2001), sterols (Pandey et al. 2003, Akihisa et al. 1990), neo-clerodane diterpenes (Achari et al. 1990, Achari et al. 1992, Jagan et al. 1993), flavonoids (Achari et al. 1990, Vendantham et al. 1977), phenylethanoid glycosides and phenylalcohoid glycosides (Kanchanaooom et al. 2001). We have investigated the chemical constituents of the aerial parts of *Clerodendrum inerme*, and report here the isolation and characterization of a new phenylethanoid glycoside with other six compounds.

Compound **1,** yellow amorphous powder, possesses the molecular formula $C_{35}H_{44}O_{17}$ from ESI-MS (m/z 735 $[M-H]^-$). Its ^1H and ^{13}C NMR spectra showed similarity to that of Velutinoside IV reported in the literature (Karioti et al. 2005). In the ^1H NMR spectrum, three aromatic protons resonating at δ 6.83, 7.11 and 7.22 as an ABX system, two protons of *trans* configured double bond at δ 7.68,6.38 J = 16.0 Hz and a methoxy group at δ 3.90 exhibited proton signals characteristic of a *E*-feruloyl group. Three aromatic protons resonating at δ 6.71, 6.75 and 6.85 as an ABX system, some multiplet signals observed at δ 2.85 due to a β-methylene and two protons at δ 4.08 and 3.76 exhibited a 3-methoxy-4-hydroxy-phenylethanol moiety. Additionally, two signals assignable to anomeric protons, a doublet at δ 4.37 (J = 7.4 Hz, H-1′ of β-glucose) and a doublet at δ 5.20 (J = 1.6 Hz, H-1″ of α-rhamnose), indicated the presence of two sugar moieties in **1**. These findings were confirmed by ^{13}C NMR and HSQC spectral data. Two anomeric carbons resonated at δ 104.3 and 100.4, respectively. The downfield shift of C-3′ indicated that this position is a glycosylation site. This was confirmed by HMBC experiments, where two crosspeaks between H-1″/C-3′ and H-3′/C-1″ were observed. That indicated the usual linkage between glucose and rhamnose (Rha $^{1\rightarrow3}$ Glu). In the HMBC spectrum, a crosspeak between H-2″ and the C resonated at δ 171.7 and a crosspeak between H-3″ and another C resonated at δ 172.3 indicated C-2″ linked with an acetyl group, and C-3″ with another. Some HMBC correlations are shown in the Fig. All protons and carbons were assigned with the help of interpretation of $^1H-^1H$ COSY, HSQC and HMBC spectra.

Experimental

1. Apparatus

The NMR spectra were obtained on a Bruker AV-500 spectrometer (500 MHz for ^1H NMR, 125 MHz for ^{13}C NMR). ESI-MS spectrum was recorded on a Bruker Esquire-LC ion trap mass spectrometer operated in negative ion mode. The compounds were isolated with a Waters Delta 600 HPLC system.

2. Plant material

Clerodendrum inerme (L.) Gaertn, the aerial parts were collected from Sanya of Hainan Province, Southern China, in October 2002 and identified by Prof. Si Zhang. A voucher sample (No. GLMMM008) is kept in the Herbarium of the South China Sea Institute of Oceanology.

3. Extraction

The dry powdered aerial parts (4.5 kg) of *Clerodendrum inerme* were extracted with 95% EtOH at 80 °C three times. After evaporation of the solvent under reduced pressure, the residue (0.6 kg) was suspended in H_2O and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively.

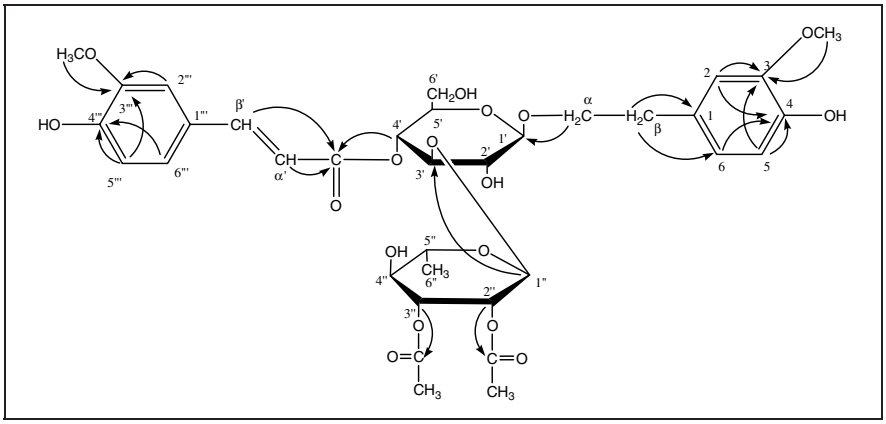

Fig.: HMBC correlations of compound **1**

4. Isolation and characterization of 1

The *n*-butanol extracts (80 g) was subjected to macroporous resin CC with elution of CH_3OH-H_2O, the fraction (30% CH_3OH) was chromatographed repeatedly on reverse phase silica gel CC and sephadex LH-20 CC eluted with CH_3OH-H_2O. Then the compounds were prepared with the help of HPLC. Compound **1**: ^1H NMR (500 MHz, CD_3OD): δ 1.15 (3 H, d, J = 6.2 Hz, H-6″), 1.98 (3 H, s, 3″-OAc), 2.07 (3 H, s, 2″- OAc), 2.85 (2 H, m, H-β), 3.43 (1 H, dd, J = 9.7, 1.0 Hz, H-4″), 3.47 (1 H, m, H-5′), 3.55 (1 H, H-2′), 3.57 (1 H, H-6′a), 3.67 (1 H, H-6′b), 3.76 (1 H, m, H-α), 3.78 (1 H, m, H-5″), 3.84 (3 H, s, 3-OCH_3), 3.86 (1 H, H-3′), 3.90 (3 H, s, 3‴- OCH_3), 4.08 (1 H, m, H-α), 4.37 (1 H, d, J = 7.4 Hz, H-1′), 4.97 (1 H, H-3″), 5.01 (1 H, H-4′), 5.20 (1 H, d, J = 1.6 Hz, H-1″), 5.36 (1 H, dd, J = 2,2 Hz, H-2″), 6.38 (1 H, d, J = 15.9 Hz, H-α ′), 6.71 (1 H, dd, J = 8.3, 2 Hz, H-6), 6.75 (1 H, d, J = 2 Hz, H-2), 6.83 (1 H, d, J = 8 Hz, H-5‴), 6.85 (1 H, d, J = 8 Hz, H-5), 7.11 (1 H, dd, J = 8, 2 Hz, H-6‴), 7.22 (1 H, d, J = 2 Hz, H-2‴), 7.68 (1 H, d, J = 16 Hz, H-β′); ^{13}C NMR (125 MHz, CD_3OD): 18.4 (C-6″), 20.6,171.7 (2″-OAc), 20.8, 172.3 (3″-OAc), 36.6 (C-β), 56.6 (3‴-OCH_3), 56.6 (3-OCH_3), 62.5 (C-6′), 70.5 (C-5″), 70.7 (C-4′), 71.2 (C-4″), 71.5 (C-2″), 72.1 (C-α), 73.3 (C-3″), 76.0 (C-5′), 76.1 (C-2′), 82.1 (C-3′), 100.4 (C-1″), 104.3 (C-1′), 112.1 (C-2‴), 113.1 (C-2), 115.1 (C-α′), 116.6 (C-5‴), 117.1 (C-5), 121.2 (C-6), 124.3 (C-6‴), 127.7 (C-1‴), 133.1 (C-1), 147.5 (C-3), 147.6 (C-4), 148.0 (C-β′), 149.5 (C-3‴), 150.9 (C-4‴), 168.2 (CO).

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