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A NEW ALLOSIDE FROM *Neocheiropteris palmatopedata*

Ye-Gao Chen,[1*] Jian-Hong Yang,[1]
Yan Zhang,[2] and Ying Liu[1]

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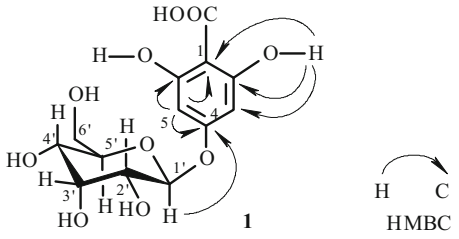
*A new compound, 2,4,6-trihydroxybenzoic acid 4-*O*-β-D-allopyranoside (**1**), together with four known compounds, sucrose (**2**), fructose (**3**), ecdysterone (**4**), and β-sitosterol (**5**) was isolated from the roots of* Neocheiropteris palmatopedata *(Baker) Christ. The new compound was identified based on extensive spectroscopic studies including HR-ESI-MS, FAB-MS, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY spectra, and the known compounds were identified by their spectroscopic data analysis, comparison with reports in the literature, and co-chromatography with authentic standards. Compounds **2**, **3,** and **5** were obtained from the Neocheiropteris genus for the first time, and **4** was originally isolated from the plant.*

Keywords: *Neocheiropteris palmatopedata*, 2,4,6-trihydroxybenzoic acid 4-*O*-*β*-D-allopyranoside, ecdysterone.

Neocheiropteris palmatopedata (Baker) Christ (Polypodiaceae) is a beautiful and ornamental fern endemic to China and is used in folk medicine to treat gasteremphraxis, rheumatism, ovarian dropsy, astriction, laryngopharyngitis, and malnutritional stagnation [1, 2]. Previously investigation on the constituents of the *Neocheiropteris* genus has isolated only one steroid, ecdysterone from *N. ensata* [3]. During our search for new bioactive natural compounds from medicinal plants in Yunnan, China, we investigated the root of *N. palmatopedata* and isolated a new 2,4,6-trihydroxybenzoic acid glycoside, 2,4,6-trihydroxybenzoic acid 4-*O*-*β*-D-allopyranoside (**1**), together with four known compounds, sucrose (**2**), fructose (**3**), ecdysterone (**4**), and *β*-sitosterol (**5**).

Compound **1** has the molecular formula $C_{13}H_{16}O_{10}$ by negative HR-ESI-MS at *m/z* 331.0658 $[M - H]^-$ (calcd for $C_{13}H_{15}O_{10}$, 331.0665). The ^{1}H NMR, ^{13}C NMR, and DEPT spectra suggested the presence of a 1,2,4,6-tetrasubstitued benzene ring [δ 5.74 (2H, s, H-3,5) and δ 93.4 (C-3,5)], six sugar proton signals at δ 3.35–3.90 (H-2′–6′) and δ 61.4–75.0 (C-2′–6′), with an anomeric proton of sugar at δ 5.04 (1H, d, J = 7.9, H-1′) and δ 98.2 (C-1′), and six active hydroxyl groups at δ 14.80 (2H, s, HO-2,6), 5.09, 4.93, 4.68, and 4.55 (sugar hydroxyls). The ^{13}C NMR and DEPT spectra displayed seven carbons including a carboxyl group (δ 175.8) besides a hexose, suggesting that **1** was a hydroxyl-substituted benzoic acid glycoside. The two similar deshielded hydroxyl signals (δ 14.80) were due to the formation of hydrogen bond between carboxyl group at C-1 and H-2,6, and the aglycone was thus determined as 2,4,6-trihydroxybenzoic acid [4], which was supported by correlations in HMBC spectrum from HO-2,6 to C-1 (δ 99.6), C-3,5 and C-2,6 (δ 163.8), and from H-3,5 to C-1, C-2,6 and C-4 (δ 161.2).



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1) Department of Chemistry, Yunnan Normal University, Kunming 650092, P. R. China, fax: +86 871 5516061, e-mail: ygchen48@gmail.com; 2) Department of Chemistry, Kunming Medical College, Kunming 650031, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 149–150, March–April, 2010. Original article submitted November 26, 2008.

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On acid hydrolysis, **1** afforded D-allose, which was detected by direct co-TLC comparison with authentic samples. The β-anomeric configuration for the allose was determined based on the coupling constants of 7.9 Hz for H-1′ [5–8]. Three-bond correlation between the allosyl anomeric proton H-1′ and C-4 in the HMBC spectrum suggested the glycosidation position was at C-4. Finally, the structure of **1** was identified as 2,4,6-trihydroxybenzoic acid 4-*O*-β-D-allopyranoside. Its ^{1}H and ^{13}C NMR spectra were fully assigned by a combination of ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY spectroscopy.

On the basis of spectroscopic data analysis, comparison with reports in the literature, and co-chromatography with authentic standards, compounds **2–5** were identified as sucrose (**2**), fructose (**3**), ecdysterone (**4**) [9], and β-sitosterol (**5**). Compounds **2**, **3,** and **5** were discovered from the *Neocheiropteris* genus for the first time, and **4** was originally isolated from the plant.

EXPERIMENTAL

General Comments. MS were determined on a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer. NMR spectra were measured on a Bruker DRX-500 spectrometer with TMS as internal standard. Silica gel (200–300 mesh, Qingdao Marine Chemical Co., China) and Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.) were used for column chromatography, and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co., China). Solvents were of industrial purity and distilled prior to use.

Isolation of 1–5 from *N. palmatopedata*. The roots of *N. palmatopedata* were collected from Kunming of Yunnan Province, China in March, 2006. The dried roots of *N. palmatopedata* (0.5 kg) were powdered and exhaustively extracted with 95% EtOH at room temperature. The EtOH extract (85 g) was evaporated in vacuo, suspended in H_2O (1.0 L), and then extracted with EtOAc and *n*-BuOH successively. The EtOAc extract (18 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc to obtain six fractions (fractions I–VI). Fraction II (2.3 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc to yield **5** (1.57 g). Fraction VI (5.7 g) was isolated on an MCI gel column with a gradient of 60–90% MeOH in H_2O to afford four subfractions VI-1–VI-4. Fraction VI-1 (580 mg) was purified by repeated CC on Sephadex LH-20 with MeOH to afford **2** (21 mg). Fraction VI-3 (49 mg) was isolated on Sephadex LH-20, eluting with MeOH to obtain **3** (6 mg) and **4** (15 mg). The *n*-BuOH extract (18.5 g) was subjected to CC on silica gel eluting with a gradient of 10–100% MeOH in $CHCl_3$ to obtain four fractions (fractions A–D). Fraction A (0.4 g) was purified by CC on silica gel eluting with chloroform–MeOH 5:1, and then on Sephadex LH-20 with MeOH to provide **1** (6 mg). Fraction B (5.2 g) was isolated on CC (MCI, MeOH–H_2O 6:4) to afford **2** (3.05 g).

2,4,6-Trihydroxybenzoic acid 4-*O*-β-D-allopyranoside (1), yellow syrup. Negative FAB-MS, *m/z* (%): 331 ([M – H]$^-$, 100); ^{1}H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 5.73 (2H, s, H-3,5), 5.04 (1H, d, J = 7.9, H-1′), 3.90 (1H, br.s, H-3′), 3.65, 3.45 (each 1H, m, H-6′), 3.63 (1H, m, H-5′), 3.37 (1H, m, H-4′), 3.35 (1H, m, H-2′); ^{13}C NMR spectrum (DMSO-d_6, δ, ppm): 175.8 (COOH), 163.8 (C-2,6), 161.2 (C-4), 99.6 (C-1), 98.2 (C-1′), 93.4 (C-3,5), 75.0 (C-5′), 71.8 (C-3′), 70.9 (C-2′), 67.4 (C-4′), 61.4 (C-6′); negative HR-ESI-MS: *m/z* 331.0658 [M – H]$^-$, $C_{13}H_{15}O_{10}$ requires 331.0665.

Sucrose (2), colorless crystal. ^{1}H NMR (D_2O, δ, ppm, J/Hz): 5.26 (1H, br.s), 4.07 (1H, d, J = 8.6), 3.90 (1H, t, J = 8.3), 3.74–3.32 (11H, m); ^{13}C NMR (D_2O, δ, ppm): 104.1 (C-2′), 92.6 (C-1), 81.9 (C-5′), 77.1 (C-3′), 74.6 (C-3), 73.2 (C-4′), 72.9 (C-5), 71.6 (C-2), 69.8 (C-4), 62.9 (C-6), 62.0 (C-6′), 60.8 (C-1′); identified by co-chromatography with authentic standard.

Fructose (3), colorless powder. ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 4.04 (1H, d, J = 3.8), 3.92–3.64 (6H, m); ^{13}C NMR (DMSO-d_6, δ, ppm): 109.7 (C-2), 85.2 (C-5), 83.7 (C-3), 79.5 (C-4), 63.3 (C-1), 61.1 (C-6); identified by co-chromatography with authentic standard.

Ecdysterone (4), yellow oil. ^{1}H NMR (CD_3OD, δ, ppm): 5.83 (1H, s), 3.98 (1H, br.s), 3.87 (1H, m), 3.63 (1H, m), 3.37–3.33 (3H, m), 1.23 (3H, s), 1.22 (3H, s), 1.21 (3H, s), 0.99 (3H, s), 0.91 (3H, s); ^{13}C NMR (CD_3OD, δ, ppm): 206.9 (C-6), 168.4 (C-8), 122.6 (C-7), 85.7 (C-14), 78.9 (C-22), 78.4 (C-20), 71.8 (C-25), 69.2 (C-2), 69.0 (C-3), 52.2 (C-5), 51.0 (C-17), 48.9 (C-13), 42.8 (C-24), 39.7 (C-10), 37.8 (C-1), 35.5 (C-9), 33.3 (C-4), 33.0 (C-15), 32.2 (C-12), 30.1 (C-27), 29.5 (C-26), 27.8 (C-16), 24.9 (C-19), 22.0 (C-11), 22.0 (C-23), 21.5 (C-21), 18.5 (C-18).

β-Sitosterol (5), colorless needles, identified by co-chromatography with authentic standard.

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