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Dichotomains A and B: Two New Highly Oxygenated Phenolic Derivatives from *Dicranopteris dichotoma*

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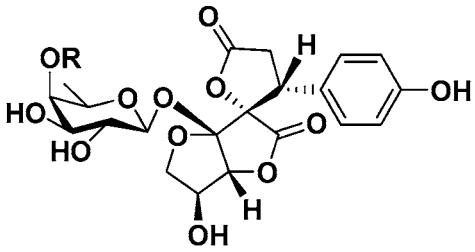
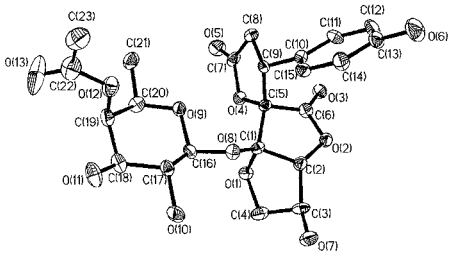
ABSTRACT



1. R = Ac
2. R = H



1

Dichotomains A (1) and B (2), two new highly oxygenated phenolic derivatives that feature a spirodilactone moiety in their structures, were isolated from the fronds of *Dicranopteris dichotoma*. Their structures were elucidated on the basis of NMR and MS spectroscopic data, and the stereochemistry of 1 was finally determined by single-crystal X-ray diffraction. Compound 2 showed weak anti-HIV-1 activity.

Members of the *Dicranopteris* species are very common ferns and grow as a large community containing no other species of plants. Previous studies of the *D.* species have reported flavonoids,[1,2] phenolic,[3] proanthocyanidins,[4] and clerodane-type glycosides.[2,5] Recent research showed that some clerodane-type glycosides isolated from the *D.* species exhibited the activities of accelerating the growth of the stems of lettuce or inhibiting the growth of the roots.[5] Aiming to find potentially bioactive secondary metabolites from this species, we chemically investigated the fronds of *D. dichotoma* Bernb. and isolated two new highly oxygenated

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(1) Kishimoto, Y. *Yakugaku Zasshi* **1955**, *75*, 1437−1439.
(2) Diraviam, P. R.; Visuvasam, S. M.; Alexis, J. B.; Subarayan, G.; Toshiyuki, U.; Masako, S.; Akinobu, T.; Hiroyuki, F.; Nobutoshi, T. *Chem. Pharm. Bull.* **1995**, *43(10)*, 1800−1803.
(3) Kuraishi, T.; Mitadera, Y.; Murakami, T.; Tanaka, N.; Saiki, Y.; Chen, C. M. *Yakugaku Zasshi* **1983**, *103*, 679−682.
(4) Yoshiki, K.; Masafumi, M.; Gen-ichiro, N.; Itsuo, N. *Chem. Pharm. Bull.* **1990**, *38 (4)*, 856−860.
(5) Tadashi, A.; Tadashi, O.; Yoshikazu, H.; Takayuki, S.; Mihoko, U.; Shinji, O. *Phytochemistry* **1997**, *46 (5)*, 839−844.

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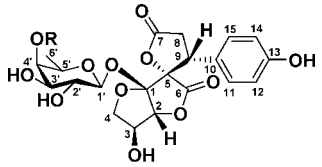
phenolic derivatives, dichotomains A (**1**) and B (**2**), which featured a spirodilactone moiety in their structures. In addition, bioactivity experiments indicated that compound **2** showed weak anti-HIV-1 activity.

The fronds of *D. dichotoma* were collected from Xiaowei Mountain, Nanxi town, Hekou county, Yunnan province, PRC, in March 2005 and were identified by professor Xiao Cheng of Kunming Institute of Botany, Chinese Academy of Sciences. The dry fronds (32 kg) were ground and extracted with acetone (3 × 30 L) at room temperature. The acetone extract was concentrated in vacuo to give a crude extract, which was then suspended in H_2O and subjected to column chromatography over DM 130 eluting with 95% ethanol. The elute from 95% ethanol (1.5 kg) was concentrated in vacuo and was subjected to column chromatography over Si gel, Sephadex LH-20, and Rp-18 repeatedly. Further purification with semipreparation HPLC (Agilent 1100 HPLC system; Zorbax SB−C18, 250 × 9.4 mm; UV detector) yielded two highly oxygenated phenolic derivatives, dichotomains A (**1**, 40 mg) and B (**2**, 25 mg).



Dichotomain A (**1**),[6] $[\alpha]_D^{27} = +2.8$ (*c* 1.58, MeOH), was obtained as colorless sliced crystals. Its HR-ESIMS exhibited a molecular ion peak at *m*/*z* 509.1299 $[M - H]^-$ (calcd 509.1295), corresponding to $C_{23}H_{26}O_{13}$ with 11 degrees of unsaturation. IR absorptions at the presence of 3431, 2935, 1806, and 1727 cm^{-1} implied the existence of hydroxy groups, methylenes, and carbonyl groups. However, initial observation of the ^{13}C NMR spectrum of **1** (Table 1) only found 21 signals, assigned as three ester carbonyl groups, seven oxymethines, three methines, one oxymethylene, one methylene, one dioxygenated quaternary carbon, two monooxygenated quaternary carbons, one quaternary carbon, and two methyls. Careful analysis of the HSQC spectrum of **1** revealed that two relatively high-intensive methine carbon signals at δ_C 131.2 (C-11 and C-15) and δ_C 116.7 (C-12 and C-14) were correlated with the two proton aromatic signals at δ_H 7.19 (2H, d, J = 8.7 Hz, H-11 and H-15) and δ_H 6.75 (2H, d, J = 8.7 Hz, H-12 and H-14), respectively. The chemical shifts and coupling constants of these signals, in combination with the observed $^1H-^1H$ COSY (H-11/H-15 with H-12/H-14) and HMBC correlations,

(6) Dichotomain A (**1**): a colorless sliced crystal, mp 170−171 °C; $[\alpha]_D^{27} = +2.8$ (*c* 1.58, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (0.80) nm; IR (KBr) υ_{max} 3431, 2935, 1806, 1727, 1616, 1519, 1450, 1250, 839 cm^{-1}; NMR can be found in Table 1; negative FABMS *m*/*z* (rel int.) 509 (100, $[M - H]^-$); HR-ESIMS found 509.1299, calcd for $C_{23}H_{26}O_{13}$ 509.1295. Compound **1** (6.59 mg) was hydrolyzed with 3% HCl (7 mL) under reflux for 1 h. The reaction mixture was concentrated under reduced pressure and chromatographed on silica gel using 10% $CHCl_3$ and MeOH as an eluent to yield D-fucose (1.52 mg) with an optical rotation value of $[\alpha]_D^{25} = +73.5$.

Table 1. ^1H and ^{13}C NMR Assignments of **1** and **2**[a]

	1		**2**	
no.	δ_H (mult., J, Hz)	δ_C	δ_H (mult., J, Hz)	δ_C
1	- -	109.0	- -	109.0
2	4.00 (overlap)	89.1	4.00 (overlap)	89.2
3	4.30 (m)	74.4	4.33 (m)	74.8
4α	4.00 (overlap)	77.4	4.02 (overlap)	77.3
4*β*	4.35 (dd, 6.6, 9.0)		4.37 (dd, 6.5, 9.0)	
5	- -	91.0	- -	91.1
6	- -	172.0	- -	172.2
7	- -	175.9	- -	176.1
8α	3.12 (dd, 12.9, 17.6)	34.3	3.12 (dd, 12.9, 17.6)	34.4
8*β*	2.73 (dd, 8.9, 17.6)		2.79 (dd, 8.9, 17.6)	
9	4.67 (dd, 8.9, 12.9)	44.9	4.79 (overlapped)	44.8
10	- -	124.2	- -	124.4
11, 15	7.19 (2H, d, 8.7)	131.2	7.27 (2H, d, 8.5)	131.4
12, 14	6.75 (2H, d, 8.7)	116.7	6.77 (2H, d, 8.5)	116.6
13	- -	159.2	- -	159.8
fucosyl				
1′	4.79 (overlapped by H_2O)	97.5	4.78 (d, 7.8)	97.5
2′	3.64 (dd, 7.8, 9.7)	72.0	3.71 (m)	71.8
3′	3.75 (dd, 3.6, 9.7)	72.9	3.55 (dd, 3.3, 9.8)	74.8
4′	5.12 (brd, 3.0)	74.7	3.64 (overlap)	72.9
5′	3.80 (dd, 6.8, 12.8)	71.2	3.68 (overlap)	72.6
6′	1.06 (3H, d, 6.5)	16.4	1.23 (3H, d, 6.4)	16.5
acetyl				
CH_3	2.12 (3H, s)	20.8	- -	- -
CO	- -	172.8	- -	- -

[a] Data were recorded in MeOD on Bruker AM 400 MHz (^1H, ^{13}C) and Bruker DRX 500 MHz spectrometers (COSY, HMQC, HMBC, ROESY); chemical shifts (δ) are expressed in parts per million with reference to the most downfield signal of MeOD (δ_H = 3.30 ppm) for ^1H and to the center peak of the most downfield signal of MeOD (δ_C = 49.0 ppm) for ^{13}C.

suggested the presence of a para-substituted aromatic ring. Therefore, 23 carbon atoms were present in **1**, which supported the molecular formula obtained from HR-ESIMS. In addition, the HMBC spectrum showed correlations of the proton signal at δ_H 4.63 (dd, J = 8.9, 12.9 Hz, H-9) with C-5, C-6, C-7, C-8, C-10, and C-11. The above evidence, coupled with a proton spin system deduced from $^1H-^1H$ COSY correlation, H-8/H-9, led to the establishment of fragment **1a** (Figure 1).

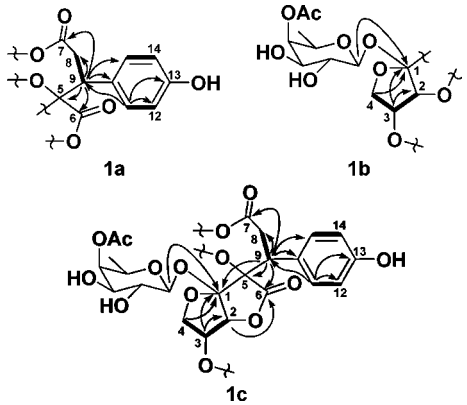


Figure 1. Structural fragments and key COSY (—) and HMBC (→) correlations of **1**.

In addition to fragment **1a**, there are still 12 carbons including one sugar moiety signal (six carbons), one acetyl

group, and four other carbons (C-1, C-2, C-3, and C-4) in the 1D and 2D NMR spectra. The sugar was further deduced to be D-fucose by acid hydrolysis of **1** to provide fucose, identified by TLC comparison with an authentic sample and its optical rotation value ($[\alpha]_D^{25} = +73.5$). The acetyl group was determined to locate at C-4′ (δ_C 74.7) of D-fucose based on the HMBC correlation of H-4′ (δ_H 5.12, brd, $J = 3.0$ Hz) with the acetyl carbonyl carbon (δ_C 172.8). The linkage position of D-fucose was determined to be C-1 (δ_C 109.0) of its aglycone by the HMBC correlation of the anomeric proton at δ_H 4.79 with C-1. In addition, the HMBC spectrum also exhibited correlations from H-3 (δ_H 4.30, m) to C-1 and C-2 (δ_C 89.1) and from H-4 (δ_H 4.00 and 4.35) to C-6. The above analysis, along with ^{1}H−^{1}H COSY correlations, H-2/H-3/H-4, determined the existence of fragment **1b** (Figure 1).

Furthermore, HMBC cross peaks of H-2 at δ_H 4.00 with C-6 and of H-9 at δ_H 4.67 with C-1 established the reasonable connection patterns of C-1 with C-5 and C-2 with C-6 and permitted fragments **1a** and **1b** to be joined together as shown in fragment **1c** (Figure 1).

However, the NMR spectra including 2D NMR spectra did not provide sufficient information to elucidate the connection patterns of C-3, C-5, and C-7, and further solid evidence such as X-ray diffraction was necessary. Fortunately, after many attempts with different solvents, a single crystal of compound **1** was finally obtained from MeOH−H_2O (95:5) and an X-ray crystallographic analysis was realized (Figure 2), which clarified the still uncertain structural details.

The stereochemistry of **1** was also determined by X-ray analysis[7] and acid hydrolysis of **1** to provide D-fucose.[6] According to the IUPAC sequence rule,[8] the chiral center with the lowest locant, the configuration of the five chiral centers, C-1, C-2, C-3, C-5, and C-9, was deduced as *R*, *R*, *S*, *S*, and *R*, respectively, and the D-fucose was determined to be β-configuration.

Dichotomain B (**2**),[9] $[\alpha]_D^{27} = -16.2$ (*c* 0.80, MeOH), a white amorphous powder has the molecular formula $C_{21}H_{24}O_{12}$ as determined by analysis of ^{1}H and ^{13}C NMR spectral data, together with HR-ESIMS (found 467.1187, calcd 467.1189).

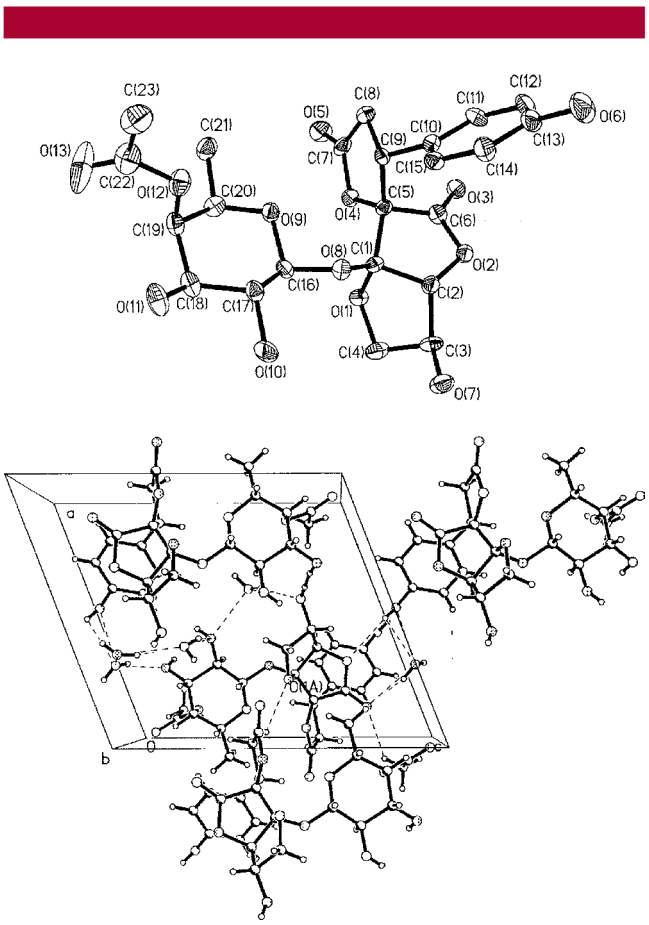


Figure 2. X-ray structure of **1** showing relative configuration.

It has been found that **2** shared most structural features with **1** by detailed comparison of their ^{1}H and ^{13}C NMR spectral data (Table 1) and further analysis of the HMBC spectra of **2**. The only difference between compounds **1** and **2** could be rationalized to an acetyl group, which occurred at C-4′ of **1** whereas it disappeared in **2**. The fucose was confirmed by acid hydrolysis of **2** to provide fucose, identified by TLC comparison with an authentic sample and its optical rotation value ($[\alpha]_D^{27} = +75.0$), and was also deduced to have β-configuration from the coupling constant ($J = 7.8$ Hz) of its anomeric proton.

Compounds **1** and **2** were evaluated for their cytotoxicity toward the A549 cell line, using the same bioassay method as that previously described.[10] Both compounds are nonbioactive with IC_{50} values of more than 100 μg/mL.

In addition, compounds **1** and **2** were tested for the cytotoxicity against C8166 cells (CC_{50}) using the MTT

(7) Crystallographic data for **1**: $C_{23}H_{26}O_{13}$, $M = 510.40$, monoclinic, space group $P2_1$, $a = 11.594$ (2) Å, $b = 9.740$ (2) Å, $c = 13.117$ (3) Å, $\beta = 111.10$ (3), $V = 1381.9$ (5) Å^3, $Z = 2$, $d = 1.400$ g/cm^3, crystal dimensions of 0.02 × 0.15 × 0.20 mm were used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 50.0°$), Mo Kα radiation. The total number of independent reflections measured was 2753, of which 2633 were observed ($|F|^2 \geq 3\sigma|F|^2$). Final indices: $R_f = 0.087$, $Rw = 0.178$ ($w = 1/\sigma\,|F|^2$). The crystal structure (**1**) was solved by the direct method SHELX-86 (Sheldrich, G. M. *SHELX-86*; University of Gottingen: Gottingen, Germany, 1985), expanded using difference Fourier techniques, and refined by the program and method NOMCSDP (Lu, Y.; Wu, B. M. *Chin. Chem. Lett*, **1992**, *3*, 637−640) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 288110). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(8) *IUPAC Nomenclature of Organic Chemistry*, Sections A−H; Pergamon: New York, 1979. Recommendation for section A, Spiro hydrocarbons.

(9) Dichotomain B (**2**): a white amorphous powder, mp 200−201 °C; $[\alpha]_D^{27} = -16.5$ (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (0.63) nm; IR (KBr) υ_{max} 3424, 2925, 1802, 1616, 1519, 1450, 1227, 839 cm^{-1}; NMR can be found in Table 1; negative FABMS *m*/*z* (rel int.) 467 (100, [M − H]$^-$); HR-ESIMS found 467.1187, calcd for $C_{21}H_{24}O_{12}$ 467.1189. Compound **2** (3.62 mg) was hydrolyzed using the same methods as those for **1** to obtain D-fucose (1.03 mg) with an optical rotation value of $[\alpha]_D^{27} = +75.0$.

(10) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. J. *Natl. Cancer Inst.* **1991**, *83*, 757−766.

method as reported previously,[11] and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[12] Compound **1** showed no bioactivity, and compound **2** exerted minimal cytotoxicity against C8166 cells with $CC_{50} > 200$ μg/mL and showed anti-HIV-1 activity with $EC_{50} = 114.8$ μg/mL and a selectivity index (CC_{50}/EC_{50}) of more than 1.74.

Supporting Information Available: 1D and 2D NMR spectral data of dichotomains A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Zheng, Y. T.; Ben, K. L.; Zhang, W. F.; Wang, J. H. *Immunopharmacol. Immunotoxicol*. **1995**, *17*, 69−79.

(12) Zheng, Y. T.; Ben, K. L.; Jin, S. W. *Acta Pharm. Sin*. **1999**, *20*, 239−243.