Two New Iridoids from *Hedyotis chrysotricha*‡

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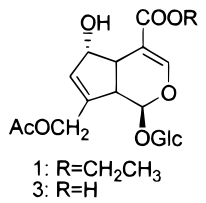
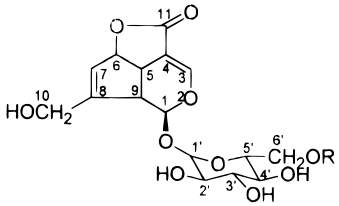
Two new iridoid glycosides, asperulosidic acid ethyl ester (**1**) and 6′-acetyl deacetylasperuloside (**2**), were isolated from *Hedyotis chrysotricha* (Palib) Merr. Their structures were elucidated by spectroscopic means.

Many species of the genus *Hedyotis* (Rubiacae) are used as Chinese folk medicine. In our investigation on the chemical constituents of the genus *Hedyotis*, we have isolated and identified iridoid glycosides from *H. hedyotidea* and methoxyl flavonoids from *H. lindleyana*.[1−3] *Hedyotis chrysotricha* (Palib) Merr. is another medicinal herb in this genus called "Shidachuan", which has been used for treatment of cancer, nephritis, hepatitis, rheumatic arthritis, and inflammations.[4] Pharmacological screening revealed that the alcoholic extract of the whole plant of *H. chrysotricha* exhibited a hepatoprotective effect in rats. In previous studies, we isolated an alkaloid[5] and iridoid glycosides from this species.[6,7] In this paper, we report two additional new iridoid glycosides, asperulosidic acid ethyl ester (**1**) and 6′-acetyl deacetylasperuloside (**2**), from this plant.



1: R=CH_2CH_3
3: R=H



2: R=Ac
4: R=H

The alcoholic extract of the whole plant of *H. chrysotricha* was subjected to preliminary separation on a macroreticular resin column. The fractions HC_1 and HC_2 eluted by 25% and 95% EtOH, respectively, were further separated by Si gel vacuum−liquid chromatography (VLC), Sephadex LH-20, polyamide, and preparative TLC on Si gel to afford asperulosidic acid ethyl ester (**1**) and 6′-acetyl deacetylasperuloside (**2**).

Asperulosidic acid ethyl ester (**1**) was obtained as a white powder. Its molecular weight (460) was based on the ions at *m/z* 921 $[2M + H]^+$ and 443 $[M + H - H_2O]^+$ in the FABMS. Its IR spectrum indicated the presence of hydroxyl (3427 cm^{-1}) and α,*β*-unsaturated ester (1709 cm^{-1}) groups. The UV absorption at λ_{max} 234 nm supported the presence of an α,*β*-unsaturated ester. The ^1H and ^{13}C NMR of **1** are nearly identical with those of asperulosidic acid (**3**).[7] The only difference is that **1** has signals at 4.25, 1.32 ppm in ^1H NMR and 64.4, 16.4 ppm in ^{13}C NMR for an ethyl ester group. The configuration of C6−OH was confirmed as α by $J_{H1,9} = 9.0$ Hz, $\Delta\delta$ $C_3 - C_4 = 47.8 > 47$ ppm, and $\delta_{C1} = 103 > 99$ ppm.[8] Thus, the structure of **1** must be asperulosidic acid ethyl ester.

That **1** is a rare ethyl ester raises the possibility of its being an artifact of the extraction process. It is possible to be produced from **3** or even from asperuloside, which have also been isolated from this plant.[7] To resolve the question, HPLC analysis of the MeOH extract of *H. chrysotricha* was performed; **1** was detected in the MeOH extract, so **1** must be a natural product.

6′-Acetyl deacetylasperuloside (**2**) was obtained as a white powder, $[\alpha]^{25}_D$ −156.6° (*c* 0.06, MeOH). Its UV absorption at λ_{max} 233 nm and IR bands at 3429 (OH), 1738 (COO) cm^{-1} were indication of iridoid glycosides. The molecular formula of $C_{18}H_{22}O_{21}$ was deduced from the FABMS at *m/z* 415 $[M + H]^+$ and confirmed by the ^{13}C NMR spectrum. The ^1H and ^{13}C NMR spectra of **2** were similar to those of deacetylasperuloside (**4**)[7] previously isolated from this plant. The differences appeared in the glycone moiety. Compound **2** showed the presence of an acetyl group at δ 1.99 in the ^1H NMR and at δ 176.8, 22.9 in the ^{13}C NMR. The position of this acetyl group was determined to be at the 6′-OH of the glucosyl moiety by the fact that the chemical shifts of H-6′ at 4.17 and 4.29 appeared downfield, respectively, by 0.51 and 0.61 from those of deacetylasperuloside (**4**). Thus, **2** must be 6′-acetyl deacetylasperuloside.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer, and IR spectra were recorded on a Perkin−Elmer 683 spectrophotometer. ^1H and ^{13}C NMR were obtained on a Bruker AM-500 spectrometer, TSP [3-(trimethylsilyl) propiopic acid-d_4 sodium salt] as internal standard. FABMS were recorded on a Zabspec spectrometer. Optical rotations were measured on a Perkin−Elmer 241 polarimeter. Si gel H and GF_{254} were used for VLC and TLC, respectively.

Plant Material. The whole plant of *H. chrysotricha* (Palib) Merr. (Rubiacea) was collected from Anhui Province, China, in August 1993, and authenticated by Prof. Xiao-Long Liu of the Wuhu College of Traditional Chinese Medicine. A voucher specimen (9307292) was deposited in Herbarium of Wuhu College of Traditional Chinese Medicine.

Extraction and Isolation. Air-dried and finely sliced whole plant of *H. chrysotricha* (38 kg) was extracted with 95% EtOH under reflux. The extracts were pooled and concentrated in vacuo, the residue dissolved in H_2O, filtered, and the filtrate fractionated over a macroreticular resin column eluting successively with H_2O, 25% EtOH, and 95% EtOH to give fractions HC0, HC1, and HC2. Fraction HC1 (35 g) was separated by VLC on Si gel, eluting with mixtures of $CHCl_3$ and MeOH of sequentially increasing polarity. The fraction eluted by $CHCl_3$−MeOH (95:5) was rechromatographed on a Sephadex LH-20 column, eluting with MeOH followed by purification on preparative TLC to give asperulosidic acid ethyl ester (**1**, 40 mg). Fraction HC2 (180 g) was subjected to Celite

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2**

	1		**2**	
number	^{1}H NMR (δ)	^{13}C NMR (δ)	^{1}H NMR (δ)	^{13}C NMR (δ)
1	5.03 (d, 9.0)	103.9	5.73 (d, 2.0)	95.7
3	7.75 (d, 1.0)	157.8	7.25 (d, 2.0)	152.6
4		110.0		107.6
5	3.18 (br t, 6.1)	43.2	3.54 (m)	38.6
6	4.88 (m)	76.7	5.54 (m)	89.4
7	6.13 (br)	134.2	5.56 (m)	127.6
8		146.9		149.9
9	2.78 (t, 8.2)	47.6	3.21 (m)	45.9
10	4.96 (d, 14.7)	66.0	4.08 (br, 2H)	61.3
	4.90 (d, 14.7)			
11		172.4		176.5
10-AcO		177.0		
10-AcO	2.18 (s, 3H)	23.3		
CH_2	4.25 (q, 7.2)	64.4		
CH_3	1.32 (t, 7.2)	16.4		
1′	4.87 (d, 9.0)	102.0	4.73 (d, 9.1)	101.3
2′		75.9	3.15 (t, 8.5)	75.2
3′	3.36−3.53 (m, 4H)	79.2		78.0
4′		72.6	3.36−3.56 (m, 3H)	72.1
5′		78.4		76.5
6′	3.90 (dd, 1.7, 12.4)	63.9	4.29 (dd, 2.1, 12.3)	65.8
	3.71 (dd, 6.0, 12.4)		4.17 (dd, 5.0, 12.3)	
6′-AcO				176.8
6′-AcO			1.99 (s, 3H)	22.9

[a] ^{1}H NMR was measured at 500 MHz and ^{13}C NMR at 125 MHz in D_2O, TSP as internal standard.

(500 g) column chromatography. The fraction (35 g) eluted by $CHCl_3$−MeOH (94:6) was further separated on Si gel VLC, eluting with succeedingly increased volumes of MeOH in $CHCl_3$. The fraction eluted by $CHCl_3$−MeOH (88:12), after purification on a polyamide column, was rechromatographed on Si gel preparative TLC, and compound **2** (12 mg) was obtained.

Asperulosidic acid ethyl ester (1): white powder, $[\alpha]^{25}_D$ −25.0° (*c* 0.06, MeOH); UV (MeOH) λ_{max} 234 nm; IR (KBr) ν_{max} 3427, 1727, 1709, 1633, 1267, 1078 cm^{-1}; FABMS *m*/*z* 921 [2M + H]$^+$, 443 [M + H − H_2O]$^+$, 281 [M − glucosyl − OH]$^+$; ^{1}H and ^{13}C NMR, see Table 1. Acid hydrolysis reaction TLC[9] revealed compound **1** contained only glucose by co-chromatography with glucose.

HPLC Analysis. Instruments: Waters 600 Pump; Waters 996 photodiode array detector, wavelength 230 nm. Column: Nova-pak C_{18} (3.9 × 150 mm), 4 μ. Mobilephase: 20% MeOH, flow rate 0.8 mL/min. Sample preparation: 50 g of *H. chrysotricha* was refluxed with MeOH for 2 h. The extract was purified on a macroreticular resin column eluted with 5% and 30% MeOH. The elution of 30% MeOH was concentrated to 10 mL, and 20 μL of this solution was injected to the column. The retention time of **1** was 18.16 min.

6′-Acetyl deacetylasperuloside (2): white powder, $[\alpha]^{25}_D$ −156.6° (*c* 0.06, MeOH); UV (MeOH) λ_{max} 233 nm; IR (KBr) ν_{max} 3446, 1742, 1658, 1246, 1018 cm^{-1}; FABMS *m*/*z* 507 [M + glycerol]$^+$, 415 [M + H]$^+$, 210 [aglycon]$^+$, 205 [6′ − AcO − glc]$^+$; ^{1}H and ^{13}C NMR, see Table 1. Acid hydrolysis reaction on TLC[9] revealed compound **2** contained only glucose by co-chromatography with glucose.

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