Pergamon

PII: S0031-9422(97)00778-4

Phytochemistry, Vol. 47, No. 8, pp. 1657–1659, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

IRIDOIDS FROM *HEDYOTIS HEDYOTIDEA*†

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(*Received in revised form* 28 *July* 1997)

Key Word Index—*Hedyotis hedyotidea*; Rubiaceae; iridoids; deacetylasperulosidic acid ethyl ester; hedyotoside.

Abstract—Two new iridoids, deacetylasperulosidic acid ethyl ester and hedyotoside and three known iridoids, asperulosidic acid, asperuloside and deacetyl asperuloside, were isolated from *Hedyotis hedyotidea*. Their structures were elucidated by spectroscopic means. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our investigation on the chemical constituents of the genus *Hedyotis*, we have isolated a new alkaloid, chrystoricine, two new iridoids, 6′-acetyl asperuloside and hedyotiside, and eight known iridoid glycosides from *H. chrysotricha*, and methoxyl flavonoids from *H. lindleyana* [1–4]. In the present paper, we report on the iridoid components from *H. hedyotidea*, one of the medicinal herbs in this genus. It is used in the treatment of colds, stamatitis and various inflammations in Chinese folk medicine [5]. Pharmacological studies revealed that alcoholic extracts of whole plants of *H. hedyotidea* had an antiulcer effect, inhibition rates being 40% and 74% on restraint and water-immersion stress-included ulcer rats at dosages of 500 mg/kg (p.o.) and 200 mg/kg (i.o.), respectively. Fraction HHE2 from alcoholic extracts showed analgesic and enterocinesia inhibitory activities in mice. Of the 15 compounds obtained thus far, we report here on the isolation and identification of two new iridoids deacetylasperulosidic acid ethyl ester (**1**) and hedyotoside (**2**), and three known iridoids, asperulosidic acid (**3**), asperuloside (**4**) and deacetyl asperuloside (**5**).

RESULTS AND DISCUSSION

Alcoholic extracts of whole plants of *H. hedyotidea* were subjected to preliminary separation on a macroreticular resin column. The fractions, HHE2 and HHE3, eluted by 25% and 35% EtOH, respectively, were further chromatographed on silica gel (VLC), Sephadex LH-20, polyamide and preparative TLC. Five iridoids and ten other kinds of compounds were eventually obtained.

Deacetylasperulosidic acid ethyl ester (**1**) was obtained as white powder. Its molecular formula $C_{18}H_{20}O_{11}$ was deducted from the FAB mass spectrum ($[M+H]^+$ *m/z* 419) and NMR spectra. Its IR spectrum indicated the presence of hydroxyl (3425 cm^{-1}) and α,β-unsaturated ester (1695 cm^{-1}) groups. UV absorption at λ_{max} 233 nm supprted the presence of a α,β-unsaturated ester. The ^1H and ^{13}C NMR of **1** are similar to those of deacetylasperulosidic acid methyl ester (**6**) [6], the only difference being the ethyl ester signals at δ 4.07 and 1.14 in ^1H NMR of **1** instead of the methyl ester signal at δ 3.47 ppm in **6**. Thus, the structure of **1** must be deacetylasperulosidic acid ethyl ester, which could be an artefact of the extraction process.

Hedyotoside (**2**) had UV absorption at 325 nm, which is different from that of common iridoids at near 235 nm, indicating a multi-conjugated system in the structure. The ^1H NMR spectrum exhibited signals for iridoid glucosides possessing a C-4 methoxycarbonyl group [7]. Signals at δ 7.15, 5.64 and 3.85 can be ascribed to H-3, H-1 and $COOCH_3$, respectively. The resonance of H-3 at δ 7.15 and C-3 at δ 147.8, at higher field than normal for C-4 methoxycarbonyl iridoids, suggested a double bond between C-5 and C-6 in conjugation with the double bond at C-3. Two coupled olefinic Hs at δ 6.21 and 7.55 must be H-6 and H-7, suggesting another double bond at C-7. The CH_2 signals at δ 4.41 and 4.14 in the ^1H NMR and δ 64.7 ppm in the ^{13}C NMR indicated a 10-CH_2OH. Signals at δ 2.82 and 47.1 can be ascribed to H-9 and C-9 reasonably. Signals for a β-glucoside were evident in the remaining ^1H and ^{13}C NMR spec-

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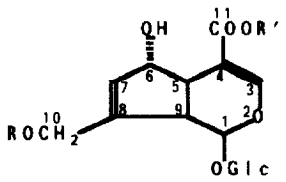
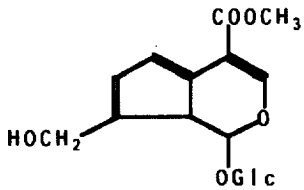
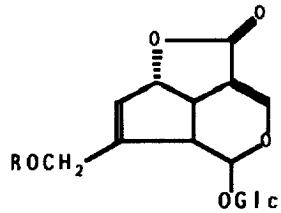
† Part [5] in the series 'The Chemical Investigation of Genus *Hedyotis*.' For part [4] see Ref.



1. R=H, R'=CH_2CH_3
3. R=Ac, R'=H
6. R=H, R'=CH_3



2



4: R=Ac
5: R=H

tra. Thus, the structure of hedyotoside was deduced as **2**. Hedyotoside is the first iridoid glycoside with three conjugated double bonds.

The structures of the known compounds, asperulosidic acid (**3**), asperuloside (**4**) and deacetyl asperuloside (**5**), were identified by IR, ^1H and ^{13}C NMR spectra and comparison with the literature [8].

EXPERIMENTAL

Mps were uncorr. UV were taken in MeOH, IR in KBr. ^1H NMR were obtained at 500 MHz, ^{13}C NMR at 125 MHz, using TPS as int. standard. FAB-MS were measured using glycerol as matrix. Silica gel H and GF254 were used for VLC and TLC, respectively.

Plant material

Whole plants of *H. hedyotidea* were collected from Guangxi province (China) in August 1993. A voucher specimen (9307291) is deposited in the Herbarium of Guangxi Botanic Institute.

Extraction and isolation

Air-dried and finely-cut whole plants (8 kg) were extracted with 95% EtOH under reflux. The extracts were concd *in vacuo* and the residue (520 g) obtained dissolved in 3 l H_2O. After filtration, the filtrate was fractionated in a macroreticular resin column eluting successively with H_2O, 25% EtOH, 35% EtOH and 95% EtOH, to give frs HHE1, HHE2, HHE3 and HHE4, respectively. HHE2 (6.5 g) was separated by VLC on silica gel eluting with mixts of $CHCl_3$ and MeOH of increasing polarity. Fr.1 eluted by $CHCl_3$–MeOH (9:1) was rechromatographed on Sephadex LH-20 eluting with MeOH to give asperuloside (**4**, 62 mg). Fr.2 eluted by $CHCl_3$–MeOH (8.5:1.5) was purified by Sephadex LH-20 CC and further separation by prep. TLC (silica gel, $CHCl_3$–MeOH–H_2O 6:3:1) yielded deacetylasperulosidic acid ethyl ester (**1**, 11 mg) and asperulosidic acid (**3**, 9 mg). Fr.3 eluted by $CHCl_3$–MeOH (4:1) gave deacetyl asperuloside (**5**, 10 mg) after further separation by silica gel prep. TLC. HHE3 was subjected to polyamide CC eluting with 10% and 30% EtOH. The 30% EtOH fr. yielded hedyotoside (**2**, 6 mg) by further separation with reverse phase chromatography on a Lobar Column (Lichroprep RP8, 20% MeOH as mobile phase).

Deacetylasperulosidic acid ethyl ester (**1**). White powder. UV λ max nm: 233. IR ν(KBr) cm^{-1}: 3425, 1695, 1633, 1385, 1078. FAB-MS *m/z*: 511 $[M+glycerol]^+$, 419 $[M+H-H_2O]^+$, 239 $[M-glucosyl-H_2O]^+$. ^1H NMR (D_2O) δ 4.83 (*d*, J = 9.0 Hz, H-1), 7.57 (*d*, J = 1.5 Hz, H-3), 3.0 (*ddd*, J = 1.6, 6.3, 7.6 Hz, H-5), 4.72 (*m*, H-6), 5.89 (*d*, J = 2.0 Hz, H-7), 2.52 (*t*, J = 8.1 Hz, H-9), 4.12 (*d*, J = 15.6 Hz, H-10), 4.30 (*dd*, J = 1.6, 15.6 Hz, H-10), 4.07 (2H, *q*, J = 7.1 Hz, CH_2CH_3), 1.14 (3H, *t*, J = 7.1 Hz, CH_2CH_3), 4.69 (*d*, J = 8.0 Hz, H-1′), 3.54 (*dd*, J = 5.4, 12.4 Hz, H-6′), 3.70 (*dd*, J = 1.7, 12.4 Hz, H-6′), 3.18–3.40 (4H, *m*, H-2′, 3′, 4′, 5′). ^{13}C NMR (D_2O) δ: 103.4 (C-1), 157.6 (C-3), 109.8 (C-4), 43.1 (C-5), 76.9 (C-6), 131.6 (C-7), 151.9 (C-8), 47.1(C-9), 62.8 (C-10), 172.3 (C-11), 64.1 (CH_2CH_3), 16.1 (CH_2CH_3), 101.8 (C-1′), 75.6 (C-2′), 78.4 (C-3′), 72.2 (C-4′), 78.9 (C-5′), 63.4 (C-6′). Acid hydrolysis reaction on TLC [9] revealed that **1** only contained glucose.

Hedyotoside (**2**). Pale yellow powder. UV λ max nm: 325. ^1H NMR (D_2O–DMSO-d_6): δ 5.64 (*br*, H-1), 7.15 (*br*, H-3), 7.55 (*d*, H-6), 6.21 (*d*, H-7), 2.82 (*d*, H-9), 4.41 (*d*, H-10), 4.14 (*d*, H-10), 3.85 (3H, *s*, $COOCH_3$), 4.82 (*d*, H-1′), 3.2-3.9 (6H, *m*, H-2′, 3′, 4′, 5′, 6′). ^{13}C NMR (D_2O–DMSO-d_6): δ 102.4 (C-1), 147.8 (C-3), 111.8 (C-4), 115.8 (C-5), 130.6 (C-6), 125.1 (C-7), 147.0 (C-8), 47.1 (C-9), 64.7 (C-10), 166.9 (C-11), 57.3 ($COOCH_3$), 100.2 (C-1′), 74.2 (C-2′), 75.3 (C-3′), 71.7 (C-4′), 77.2 (C-5′), 61.3 (C-6′). Acid hydrolysis reaction on TLC [9] revealed that **2** only contained glucose.

Acknowledgements—The authors thank Prof. Wei-Kun Xu and Li-Shan Meng from the Guangxi Botanic Institute for the collection of plant materials and the Instrumental Analysis Department of our Institute for measurement of spectra.

REFERENCES

1. Peng, J.-N., Feng, X.-Z. and Liang, X.-T., *Chin. Chem. Letts*, 1995, **6**, 965.
2. Peng, J.-N., Feng, X.-Z. and Liang, X.-T., *Zhongcaoyao*, 1997, **28**(10), 581.

3. Peng, J.-N., Feng, X.-Z. and Liang, X.-T., *Yaoxue Xuebao*, 1997, **32**(12), 908.
4. Peng, J.-N., Feng, X.-Z., Zheng, Q.-T. and Liang, X.-T., *Phytochemistry*, 1997, **46**(16), 1119.
5. *Xin Hua Beng Cao Gang Yao*, Vol.2. Shanghai Science and Technology Press, Shanghai, 1991, p. 42.
6. Ishiguro, K., Yamaki, M. and Takagi, S., *Lloydia*, 1983, **46**, 532.
7. Damtoft, S. Jensen, S. R. and Nielsen, B. J., *Phytochemistry*, 1981, **20**, 2717.
8. El-nagger, L. J. and Beal, J. L., *Lloydia*, 1980, **43**, 649.
9. Zhaeo, P.-P., Li. B.-M. and He, L.-Y., *Yaoxue Xuebao*, 1987, **22**, 70.