Pergamon

PII: S0031-9422(98)00411-7

Phytochemistry Vol. 49, No. 8, pp. 2603–2605, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

CHALCONES AND ECDYSTEROIDS FROM *VITEX LEPTOBOTRYS*

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(*Received* 23 *February* 1998)

Key Word Index—*Vitex leptobotrys*; Verbenaceae; aerial parts; chalcones; 2′,4′-dihydroxy-4,6′-dimethoxychalcone; 4′-hydroxy-4,2′,6′-trimethoxychalcone; 4,2′,4′,β-tetrahydroxy-6′-methoxy-α,β-dihydrochalcone; ecdysteroids.

Abstract—In addition to some known chalcones and ecdysteroids three new chalcones have been isolated from aerial parts of *Vitex leptobotrys*, the structures of which have been identified as 2′,4′-dihydroxy-4,6′-dimethoxychalcone, 4′-hydroxy-4,2′,6′-trimethoxychalcone and 4,2′,4′,β-tetrahydroxy-6′-methoxy-α,β-dihydrochalcone, respectively. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In continuation of our phytochemical studies on bioactive constituents of Vietnamese plants [1] we have examined the hitherto non-investigated species *Vitex leptobotrys*, growing as a 4–6 m high shrub in North Vietnam [2]. From the aerial parts of this plant besides a series of known chalcones and ecdysteroids the three new chalcones **3**, **5** and **6** were isolated and their structures assigned by MS and especially NMR techniques.

RESULTS AND DISCUSSIONS

From the ethyl acetate extract, upon chromatographic separation, the chalcones cardamomin (**1** [3], yield 0.002%), helichrysetin (**2** [4], yield 0.007%) and 4,4′-dihydroxy-2′,6′dimethoxychalcone (**4** [5], yield 0.0004%), the ecdysteroids ecdysterone ([6], yield 0.031%, in addition 0.27% from the *n*-butanol extract), 24(28)-dehydromakisterone A ([7], yield 0.0004%), makisterone A ([7], yield 0.003%), 24-epi-makisterone A ([7], yield 0.005%), ajugasterone C ([8], yield 0.003%), deoxycrustecdysone (2-deoxy-20-hydroxyecdysone [9], yield 0.002%) and *N-trans*-feruloyl tyramine ([10], yield 0.001%) have been isolated. As new chalcones we identified 2′,4′-dihydroxy-4,6′-dimethoxychalcone (**3**, yield 0.004%), 4′-hydroxy-4,2′,6′-trimethoxychalcone (**5**, yield 0.013%) and 4,2′,4′,β-tetrahydroxy-6′-methoxy-α,β-dihydrochalcone (**6**, yield 0.003%), respectively. From the *n*-butanol extract the ecdysteroid pinnatasterone ([11], yield 0.003%) has been isolated.

The elemental composition of compounds **3** and **5** were shown to be $C_{17}H_{16}O_5$ and $C_{18}H_{18}O_5$, respectively, by high-resolution mass spectrometry. The dihydrochalcone **6** displayed an $[M-H_2O]^+$ peak ($C_{16}H_{14}O_5$).

The ^1H NMR signals were assigned by their coupling pattern (Table 1), the ^{13}C NMR signals by means of gradient-selected HSQC and, concerning the quaternary carbon atoms, gradient-selected HMBC spectra (Table 2). Coupling of C-β with H-2 and H-6 recognized in the gradient-selected HMBC spectra of the chalcones **3**, **5** and **6** indicated a bond between C-β and the disubstituted phenyl ring. In the NOE difference spectrum of **3** irradiation at δ 3.82 (signal of 6′-methoxy group) gave positive enhancement for the signal of H-5′, irradiation at δ 3.70 (signal of 4-methoxy group) gave positive enhancement for the signals of H-3 and H-5 in agreement with the positions of the methoxy groups.

In the NOE difference spectrum of **5** irradiation at δ 3.63 (signals of 2′-methoxy and 6′-methoxy groups) gave positive enhancement for the signals of H-3′ and H-5′, irradiation at δ 3.78 (signal of 4-

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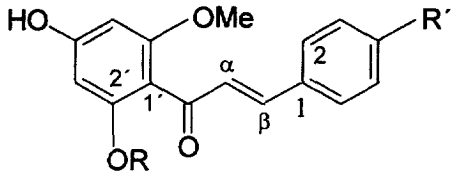
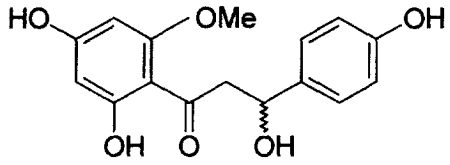


1 R = H, R′ = H
2 R = H, R′ = OH
3 R = H, R′ = OMe
4 R = Me, R′ = OH
5 R = Me, R′ = OMe



6

methoxy group) gave enhancements for the signals of H-3 and H-5 in accordance with the positions of the methoxy groups. Compound **5** proved to be identical (mp, ^{1}H NMR) with a product obtained by partial synthesis [12]. In the NOE difference spectrum of **6** irradiation at δ 3.80 (signal of the 6′-methoxy group) gave positive enhancement for the signal of H-5′ indicating again the position of the methoxy group. The known constituents were mainly identified by their NMR data.

Table 2. ^{13}C NMR data of compounds **3** (pyridine-d_5), **5** (DMSO-d_6) and **6** (pyridine-d_5) (76 MHz, TMS)

C	3	5	6
1	128.7	127.0	130.3
2, 6	130.6	130.3	128.7
3, 5	114.9	114.5	116.3
4	161.9	161.2	159.3
1′	106.1	109.9	105.7
2′	169.0	158.2	165.5
3′	97.3	92.1	96.8
4′	166.9	160.1	166.1
5′	92.6	92.1	94.4
6′	163.8	158.2	163.4
C=O	192.7	193.4	188.4
α	126.0	127.1	46.1
β	142.5	143.3	79.5
4-OMe	55.3	55.3	–
2′-OMe	–	55.5	–
6′-OMe	55.8	55.5	55.8

EXPERIMENTAL

Leaves and stems of *Vitex leptobotrys* Hall. were collected in the National Park Cuc phuong, Ninh binh, Vietnam in September 1996. The species was identified by Dr Tran Dinh Dai, Hanoi. A voucher specimen was deposited in the Herbarium of the Institute of Ecology and Natural Resources, National Center for Natural Science and Technology of Vietnam, Hanoi. The plant material (1.2 kg) was dried at 45°, ground and extracted with 95% MeOH at room temp. MeOH was removed by distillation *in vacuo*, and the aq. soln was extracted with *n*-hexane followed by EtOAc and *n*-BuOH. The EtOAc was evaporated *in vacuo*, the residue chromatographed over silica gel with $CHCl_3$–MeOH (19:1, followed by 4:1) and finally by $CHCl_3$–MeOH–H_2O (13:7:1). For 2′,4′-dihydroxy-4,6′-dimethoxychalcone (**3**) a further chromatography over silica gel using *n*-hexane–EtOAc (4:1) was necessary.

2′,4′-Dihydroxy-4,6′-dimethoxychalcone (**3**)

Yellow crystals from Me_2CO, mp 170–172°C, yield 0.004%. R_f 0.66 [silica gel, $CHCl_3$–MeOH

Table 1. ^{1}H NMR data of compounds **3** (pyridine-d_5), **5** (DMSO-d_6) and **6** (pyridine-d_5) (500 MHz, TMS, *J* Hz)

H	3		5		6	
	δ	*J* (H,H)	δ	*J* (H,H)	δ	*J* (H,H)
2,6	7.72	8.8 (2, 3)	7.59	8.8 (2, 3)	7.52	8.5 (2, 3)
3,5	7.00		6.94		7.19	
3′	6.57	2.2 (3′, 5′)	6.12		6.51	2.1 (3′, 5′)
5′	6.36		6.12		6.47	
α	8.14	15.6 (α, β)*	6.79	16.1 (α, β)	2.93	16.3 (α_A, α_B) 2.9 (α_A, β)
					3.27	12.8 (α_B, β)
β	8.14		7.15		5.52	
4-OH	–		–		†	
4-OMe	3.70		3.78		–	
2′-OH	15.12		–		†	
2′-OMe	–		3.64		–	
4′-OH	13.08		9.84		†	
6′-OMe	3.82		3.64		3.80	

*Measured in Me_2CO-d_6; H-α: δ 7.76, H-β: δ 7.92.
†Only two broad hydroxy signals at δ 11.72 and 12.90 were observed, which were not assigned.

(9:1)]. EI-MS (70 eV) *m/z* (rel. int.): 300.0985 $[M]^+$ ($C_{17}H_{16}O_5$, calcd 300.0998) (100).

4'-Hydroxy-4,2',6'-trimethoxychalcone (**5**)

Yellow needles from MeOH, mp 208–210°C, Ref. [12]: 208–209.5°C, yield 0.013%. R_f 0.76 [silica gel, $CHCl_3$–MeOH (9:1)]. EI-MS (70 eV) *m/z* (rel. int.): 314.1166 $[M]^+$ ($C_{18}H_{18}O_5$, calcd 314.1154) (48), 299.0920 $[M-Me]^+$ ($C_{17}H_{15}O_5$, calcd 299.0919) (22), 286.1207 $[M-CO]^+$ ($C_{17}H_{18}O_4$, calcd 286.1205) (100).

4,2',4',β-Tetrahydroxy-6'-methoxy-α,β-dihydrochalcone (**6**)

Crystals from $CHCl_3$–MeOH, mp 262–267°C, yield 0.003%. R_f 0.59 [silica gel, $CHCl_3$–MeOH (9:1)]. $[\alpha]_D^{21}$-33.7° (pyridine; *c* 0.20). EI-MS (70 eV) *m/z* (rel. int.): 286.0823 $[M-H_2O]^+$ ($C_{16}H_{14}O_5$, calcd 286.0841) (100), 167.0347 ($C_8H_7O_4$, calcd 167.0344) (87).

Acknowledgements—We thank the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, Bonn, for financial support, Dr Tran Dinh Dai, Hanoi, for the identification of the plant material and Dr J. Schmidt, Halle, for the mass spectral measurements. T. T. T. is indebted to the DAAD for a grant.

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