Pergamon

PII: S0031-9422(98)00448-8

Phytochemistry Vol. 49, No. 7, pp. 2109–2114, 1998

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

ECDYSTEROID CONSTITUENTS OF THE MUSHROOM *TAPINELLA PANUOIDES**

KAREL VOKÁČ, MILOŠ BUDĚŠÍNSKÝ, JURAJ HARMATHA† and JITKA KOHOUTOVÁ

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo 2, 16610 Prague, Czech Republic

(*Received in revised form* 24 *March* 1998)

Key Word Index—*Tapinella panuoides*; *Paxillus*; Paxillaceae; chemotaxonomy; ecdysteroids; ergostanes; panuosterones; paxillosterone; 20-hydroxyecdysone.

Abstract—Ecdysteroids, compounds structurally related to the insect moulting hormone ecdysone, were isolated from the mushroom *Tapinella panuoides*. Paxillosterone and two further new ergostane-type ecdysteroids, panuosterone and 25-hydroxypanuosterone, together with the already known cholestane-type ecdysteroids, 20-hydroxyecdysone, ponasterone A, malacosterone and turkesterone were identified by NMR, IR and mass spectrometric methods. The taxonomic value of ecdysteroids for chemosystematics in fungi and particularly in the genera *Paxillus* and *Tapinella*, is discussed.

INTRODUCTION

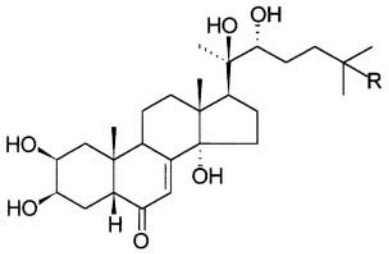
Phytoecdysteroids, plant substances structurally related to the insect moulting hormone ecdysone, are widely distributed in plant species belonging to a large set of families [2]. They are generally considered to have a significance and specific role in the plant–insect chemical interactions. Although a considerable effort has been expended to understand this fact in higher plants, no direct evidence of a clear protection mechanism has been elucidated [3]. Occurrence of ecdysteroid-related compounds has also been reported in fungi (Mycophyta), but no insect hormone activity has been so far investigated [4, 5]. Our discovery of paxillosterone from the mushroom *Paxillus atrotomentosus* (Batsch) Fr. [6, 7] initiated our interest in a more extended investigation, resulting in identification of seven minor ecdysteroids in this mushroom [1, 8] and in testing their insect hormone activity [9]. On the basis of these results, we initiated an extensive chemical prospecting for ecdysteroids in a series of selected mushrooms, particularly in those species which are generally not attacked by insects. The screening of 78 species indicated that only two of them contained ecdysteroids. In this paper we describe the isolation and identification of seven ecdysteroid constituents of *Tapinella panuoides* (Fr. ex Fr.) Gilb.

RESULTS AND DISCUSSION

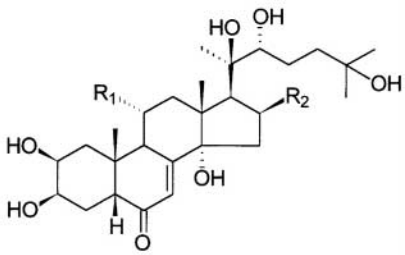
Ecdysteroid constituents of the methanolic extract of *Tapinella panuoides* have been isolated by a separation procedure described in Section 3. The structures of the major ecdysteroid constituent 20-hydroxyecdysone (**1**) and of the minor constituents **2**–**7** were elucidated by IR, mass and NMR spectroscopy (for ^{1}H and ^{13}C NMR data see Tables 1 and 2). The 1D and 2D-COSY spectra at 500 MHz resulted in complete structural assignment of protons. Characteristic NMR data of compounds **2**–**4** (in Tables 1 and 2) corresponded with the data [8] of ponasterone A (**2**), malacosterone (**3**) and turkesterone (**4**), all cholestane-type ecdysteroids. Compounds **1**, **2** and **4** occur frequently in higher plants and were also identified by HPLC analysis using authentic samples for comparison. Data are summarised in Table 3. Malacosterone (**3**) is a rare constituent and so far has been isolated only from the mollusc *Cepea nemoralis* (Gastropoda) [10]. Compounds **5**–**7** were identified as ergostane-type ecdysteroids typical also for *Paxillus atrotomentosus* [1]. One was identical with paxillosterone (**5**), according to NMR data (Tables 1 and 2) and

*Part 58 in the series "Plant Substances". For part 57 see Ref. [1], Vokáč *et al.* (1998) [Vokáč, K., Buděšínský, M., Harmatha, J. and Píš, J., *Tetrahedron*, 1998, **54**, 1657].

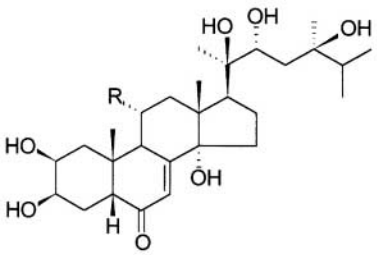
†Author to whom correspondence should be addressed.



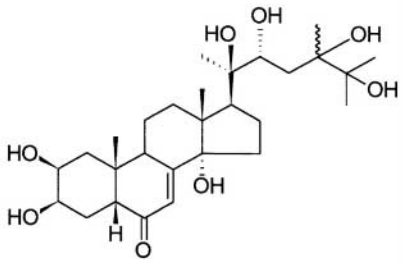
1 R = OH
2 R = H



3 R_1 = H; R_2 = OH
4 R_1 = OH; R_2 = H



5 R = OH
6 R = H



7

HPLC comparison with authentic sample (Table 3). Two compounds were new ecdysteroids: panuosterone (**6**), identified as 11-deoxy derivative of paxillosterone (**5**) and 25-hydroxypanuosterone (**6**), i.e. 11-deoxy-25-hydroxy- derivative of paxillosterone (**5**). The configuration [24*R*] in paxillosterone (**5**) was determined by NMR method (NOE-effects) applied to the 22,24-phenylboronic acid ester of paxillosterone, a cyclic derivative in which carbon C-24 is part of a conformationally fixed system. The method and all observed decisive data are described in our previous paper [1]. The configuration at C-24 in panuosterone (**6**) can be derived by comparison of its ^{1}H NMR data with paxillosterone (**5**). The nearly identical chemical shifts of the side-chain protons in panuosterone (**6**) and paxillosterone (**5**) (see Table 1) indicate the same [24*R*]-configuration also for panuosterone (**6**). The presence of a 25-OH group in 25-hydroxypanuosterone (**7**) influences the side-chain proton chemical shifts and, therefore, does not allow their use for determination of the configuration at C-24. However, from a biogenetic consideration the same configuration as in paxillosterone (**5**) can be assumed. Our attempts to prepare the suitable cyclic 22,24-phenylboronate from 25-hydroxypanuosterone (**7**) for stereochemical analysis were not successful. The 20,22;24,25-phenylboronate diester was the only isolated reaction product, apparently owing to a higher reactivity of vicinal diols compared to the 1,3-diol system and owing to insufficient quantity of compound **7** available for this experiment.

The characteristic structural features of constituents of *Paxillus atrotomentosus*, as presented in our previous paper [1], were not found to be so significant in the case of *Tapinella panuoides*. The major constituent of *P. atrotomentosus*, paxillosterone (**5**), as well as additional six minor constituents [1], contained a characteristic 24-methyl group (ergostane skeleton) and an 11α-OH substituent. Only one of the minor constituents, 20E (**1**), did not contain these two features. The situation in constituents of *T. panuoides* is different. 20E (**1**), which is the major constituent in this case, together with additional three minors **2**–**4**, have the most frequent phytoecdysteroid cholestane skeleton, and only in the case of turkesterone (**4**) the 11α-OH substituent is present. The last three minor constituents **5**–**7** have the ergostane-type structure, but only paxillosterone (**5**) also contains an 11α-OH group. This is additional evidence for the low chemotaxonomic value of the presence of 11α-OH substitution in the case of fungi, as discussed already in Ref. [1], where the absence of 11α-OH in structurally related polyporusterones from *Polyporus umbellatus* [5] was also indicated. More significant for fungi is the presence of the ergostane skeleton (the 24-methyl homology), as was found in the cases of *Paxillus* and *Tapinella* species, as well as in *Lasioshaera nipponica* [4], *Agaricus blazei* [11] or Polyporus *versicolor* [12].

Table 1. Proton NMR data of ecdysteroids **1**–**7** from *Tapinella panuoides* in CD_3OD

Proton	Chemical shifts (ppm)/coupling constants (Hz)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
H-1α	1.79*	1.79 *dd* 13.2; 4.5	1.79 *dd* 13.5; 4.5	2.59 *dd* 13.0; 4.3	2.58 *dd* 12.5; 4.0	1.79 *dd* 13.2; 4.5	1.79 *dd* 13.5; 4.5
H-1β	1.43 *dd* 13.3; 12.3	1.43 *dd* 13.2; 12.4	1.43 *dd* 13.0; 12.3	1.38 *dd* 13.0; 12.3	1.37 *dd* 12.5; 11.8	1.43 *dd* 13.2; 12.4	1.43 *dd* 13.0; 12.0
H-2α	3.84 *ddd* 12.0; 4.0; 3.2	3.84 *ddd* 12.0; 4.2; 3.3	3.82 *ddd* 12.0; 4.0; 3.5	4.01 *ddd* 12.0; 4.0; 3.4	4.00 *ddd* 11.8; 4.0; 2.6	3.83 *ddd* 13.0; 4.0; 3.4	3.84 *ddd* 12.0; 4.5; 3.0
H-3α	3.95 *q* 2.9	3.95 *q* 2.7	3.94 *q* 3.0	3.95 *q* 2.9	3.95 *q* 2.6	3.95 *q* 2.8	3.95 *q* 3.0
H-4α	1.75*	1.74*	1.72*	1.78*	1.78 *ddd* 14.0; 12.8; 2.6	1.75*	1.76*
H-4β	1.70*	1.70*	1.68*	1.68 *dt* 14.2; 3.7; 3.7	1.68 *ddd*	1.69 *dt*	1.69*
H-5	2.38 *dd* 13.0; 4.5	2.38 *dd* 12.8; 4.6	2.40 *dd* 12.6; 4.8	2.33 *dd* 13.2; 3.9	2.3 3 *dd* 12.8; 4.0	2.38 *dd* 13.0; 4.5	2.38 *dd* 13.0; 4.4
H-7	5.81 *d* 2.6	5.81 *d* 2.00	5.81 *d* 2.0	5.80 *dd* 2.6; 1.0	5.80 *dd* 2.7; 0.7	5.81 *d* 2.6	5.82 *d* 2.5
H-9	3.15 *ddd* 11.2; 7.0; 2.6	3.15 *ddd* 10.5; 7.0; 2.6	3.16 *ddd* 10.5; 7.0; 2.5	3.15 *dd* 9.0; 2.6	3.13 *dd* 8.9; 2.7	3.15 *ddd* 10.5; 7.0; 2.6	3.15 *ddd* 10.0; 7.0; 2.5
H-11α	1.69*	1.70*	1.70*	–	–	1.71*	1.70*
H-11β	1.81*	1.81*	1.82*	4.10 *ddd* 10.6; 9.0; 6.2	4.09 *bddd* 10.3; 8.9; 6.3	1.79*	1.79*
H-12α	2.13 *dt* 13.0; 13.0; 4.8	2.12 *dt* 13.0; 13.0; 5.0	2.14 *dt* 12.5; 12.5; 5.2	2.22 *dd* 12.4; 10.6	2.19 *dd* 12.5; 10.3	2.11*	2.13 *dt* 13.0; 13.0; 4.5
H-12β	1.88 *ddd* 12.8; 4.6; 2.3	1.88 *ddd* 12.8; 4.0; 2.0	1.93 *ddd* 12.8; 4.5; 2.5	2.16 *dd* 12.4; 6.2	2.14 *dd* 12.5; 6.3	1.87*	1.87*
H-15α	1.99*	1.96*	2.25 *dd* 13.6; 7.6	1.95*	1.95*	1.95*	1.95*
H-15β	1.60*	1.59*	2.08 *dd* 13.6; 4.7	1.58*	1.56*	1.59*	1.59*
H-16α	1.95*	1.99*	–	2.00*	2.01*	2.02*	2.02*
H-16β	1.73*	1.70*	4.68 *dt* 7.6; 7.6; 4.7	1.75*	1.82*	1.79*	1.88*
H-17	2.39 *dd* 9.5; 8.0	2.37 *t* 8.8; 8.8	2.44 *d* 7.6	2.43*	2.28 *dd* 9.9; 8.4	2.26 *dd* 10.0; 8.5	2.34 *t* 9.0; 9.0
H-22	3.33 *dd* 11.0; 1.7	3.33 *dd* 10.0; 1.7	3.88 *dd* 10.6; 1.5	3.32 *dd* 11.0; 1.8	3.76 *dd* 10.5; 1.7	3.78 *dd* 11.4; 1.7	3.73 *d* 9.2
H-23a	1.66*	1.47*	1.67*	1.66*	1.72 *dd* 14.7; 1.7	1.73*	1.83 *bd* 14.6
H-23b	1.28 *dddd* 13.0; 11.5; 11.0; 4.6	1.23*	1.36*	1.29*	1.44 *dd* 14.7; 10.5	1.44 *dd* 14.7; 10.6	1.53 *dd* 14.6; 9.3
H-24a	1.79*	1.46*	1.86 *ddd* 13.3; 12.0; 5.0	1.80*	–	–	–
H-24b	1.44 *ddd* 13.0; 12.0; 4.0	1.21*	1.50 *ddd* 13.3; 11.5; 4.0	1.43 *ddd* 13.6; 11.6; 4.3	–	–	–
H-25	–	1.56*	–	–	1.89 *h* 6.8	1.90 *h* 6.5	–
Me-18	0.892 *s*	0.890 *s*	1.136 *s*	0.877 *s*	0.879 *s*	0.896 *s*	0.899 *s*
Me-19	0.968 *s*	0.967 *s*	0.990 *s*	1.057 *s*	1.057 *s*	0.968 *s*	0.969 *s*
Me-21	1.204 *s*	1.175 *s*	1.197 *s*	1.193 *s*	1.226 *s*	1.204 *s*	1.207 *s*
Me-26	1.199 *s*	0.924 *d* 6.5	1.197 *s*	1.206 *s*	0.980 *d* 6.8	0.980 *d* 6.5	1.257 *s*
Me-27	1.191 *s*	0.915 *d* 6.5	1.213 *s*	1.220 *s*	0.912 *d* 6.8	0.911 *d* 6.5	1.242 *s*
Me-28	–	–	–	–	1.081 *s*	1.079 *s*	1.199 *s*

*The proton chemical shift was determined from 2D-COSY spectrum.

The predominant presence of ergostane-type ecdysteroids in fungi appeares characteristic, but in higher plants it is rather exceptional and in insects so far totally absent. The only described presence of 24-*epi*-makisterone A as the major ecdysteroid in the leaf-cutting ants was indicated already in the discussion [13] as a fungal component, coming from symbiosis with fungi and taken over by feeding on a diet containing fungi.

Recently, a new survey of classification of the genera *Paxillus* Fr. and *Tapinella* Gilb. in Central Europe was suggested [14]. Differences between these genera were summarised into seven points. Our material has been in part collected and also determined by the author of this new classification proposal. According to this determination, our mushrooms were identified as *Tapinella panuoides* (Fr., Fr.) Gilb. Material used in Ref. [1] according to these criteria and the following new combination of species, was identified as *Tapinella atrotomentosa* (Batsch, Fr.) Šutara comb. nov. The presence of structurally related ecdysteroids in both species supports the new classification proposal and the eventual transference of *T. atrotomentosa* from *Paxillus*

Table 2. Carbon-13 chemical shifts of ecdysteroids **1**–**7** from *Tapinella panuoides* in CD_3OD

Carbon	Chemical shifts (ppm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
C-1	37.36	37.37	37.32	39.09	39.06	37.38	37.37
C-2	68.70	68.73	68.70	68.94	68.92	68.72	68.72
C-3	68.52	68.51	68.50	68.57	68.55	68.52	68.53
C-4	32.86	32.88	32.86	33.28	33.26	32.86	32.84
C-5	51.79	51.80	51.81	52.78	5176	51.80	51.80
C-6	206.45	206.50	206.36	206.66	206.58	205.42	204.46
C-7	122.13	122.14	122.09	122.74	122.28	122.30	122.29
C-8	167.97	168.00	167.00	165.74	165.41	167.60	167.84
C-9	35.09	35.11	34.90	42.94	42.93	34.90	35.13
C-10	39.26	39.26	39.29	39.91	39.93	39.30	39.30
C-11	21.50	21.51	21.39	69.51	69.46	21.54	21.51
C-12	32.51	32.53	32.42	43.79	43.68	32.44	32.41
C-13	*	*	*	*	*	*	*
C-14	85.23	85.25	83.27	84.87	85.40	85.40	85.48
C-15	31.78	31.77	44.95	31.86	31.90	31.83	31.79
C-16	21.50	21.51	73.34	21.52	22.46	21.44	21.32
C-17	50.53	50.48	51.66	50.35	49.97	50.19	50.00
C-18	18.05	18.02	18.41	18.89	18.85	17.98	17.96
C-19	24.40	24.39	24.42	24.62	24.64	24.40	24.39
C-20	77.90	77.86	80.82	77.83	77.72	77.81	77.91
C-21	21.05	20.98	20.64	21.02	20.66	20.69	20.69
C-22	78.42	77.99	77.79	78.42	74.00	74.06	74.02
C-23	27.34	37.66	27.77	27.35	41.20	41.18	39.96
C-24	42.40	30.48	42.57	42.40	76.25	76.26	76.27
C-25	71.29	29.23	71.29	71.29	37.32	37.28	77.51
C-26	29.70	22.74	29.79	29.73	17.32	18.82	25.25
C-27	28.95	23.41	28.89	28.95	18.85	17.30	25.25
C-28	–	–	–	–	22.11	22.16	22.41

*Overlapped with intensive solvent multiplet (*ca.* δ 49).

Fr. to *Tapinella* Gilb.. Following this fact, the only available species belonging to genus *Paxillus*, i.e. *Paxillus involutus* (Batsch, Fr.) Fr. has also been analysed in our laboratory. However, no ecdysteroids were detected in this species. Thus, the presence of ecdysteroids can be considered as an additional criterion for the proposed new combination, however, so far with a limited chemotaxonomic value, resulting from no more then three investigated species.

EXPERIMENTAL

IR spectra were recorded on a Bruker IPS-88 using KBr pellet. NMR spectra were measured on a Varian UNITY-500 instrument (^1H at 500 MHz; ^{13}C at 125.7 MHz) in CD_3OD. Chemical shifts were referenced to the solvent signal at 3.31 ppm (^1H) and 49.00 ppm (^{13}C). Mass spectra were recorded on a ZAB-EQ spectrometer with fast atom bombardment (FAB) ionisation using a glycerol–thioglycerol mixture as a matrix. Relative intensities were calculated in the range of m/z: 300–600. The melting points were determined on a Boëtius apparatus and are uncorrected.

Extraction and isolation of ecdysteroids

Tapinella panuoides mushrooms were collected in forests at Teplice (north Bohemia). Dried and ground mushrooms (40 g) were extracted with

Table 3. Retention times (in minutes) of ecdysteroids from *Tapinella panuoides* under various analytical HPLC conditions

Compound	A	B	C	D
20-Hydroxyecdysone (**1**)	42.7	47.5	31.7	35.2
Ponasterone A (**2**)	54.6	15.1		11.3
Malacosterone (**3**)	40.6	64.2	37.2	
Turkesterone (**4**)	33.9	97.9		
Paxillosterone (**5**)	42.0	47.0	26.2	29.1
Panuosterone (**6**)	48.9	23.5		
25-Hydroxypanuosterone (**7**)	40.6	57.6	40.5	

A: RP; Separon SIX C18; gradient 10–70% methanol in water/50 min; 0.6 ml/min.
B: NP; Silasorb 600; diethylether–hexane–methanol–water (44:43:12:1); 0.8 ml/min.
C: NP; Silasorb 600; diethylether–acetonitrile–water (78:19:3 v/v/v); 0.8 ml/min.
D: NP; Silasorb 600; hexane–isopropanol–water (69:29:25 v/v/v); 0.8 ml/min.

methanol (5×250 ml). The combined extracts were concentrated to 100 ml volume and than diluted with H_2O (100 ml). This soln was extracted with *n*-BuOH (10×50 ml) and the butanolic portion was evaporated to give 5 g of dry residue. This material was separated by column chromatography using neutral Al_2O_3 (100 g, deactivated with 10% of water) and with EtOac–MeOH mixtures (starting from 10% MeOH in EtOAc to 50% MeOH in EtOAc) as a mobile phase. Collected fractions (100 ml) were monitored by RP-HPLC. The fractions containing ecdysteroids were further separated and purified by RP-HPLC using an 8×500 mm column packed with Separon SIX C-18, particle size 5 μm (Tessek, Prague) and MeOH–H_2O as the mobil phase (linear gradient from 18 to 80% of MeOH during 200 min) at a flow rate of 1.2 ml min^{-1} (system I). Compounds **2**, **4** and **6** were directly obtained after evaporation of solvents, pairs of compounds **1**, **5** and **3**, **7** which co-eluted under these conditions were further separated on NP-HPLC using column 8×500 mm packed with Silasorb 600, 5 μm; the mobile phase was either Et_2O–MeCN–H_2O (77:20:3) (system II) or Et_2O–hexane–MeOH–H_2O (44:43:12:1) (system III); the flow rate was 2 ml min^{-1} in both cases. For the chromatographic data obtained with the analytical columns, see Table 3.

20-*Hydroxyecdysone* (**1**)

NP-HPLC separation of the mixture **1** and **5** using system II afforded **1** (16.2 mg), m.p. 230–233°C (Me_2CO). Composition $C_{27}H_{44}O_7$. M: 480. IR ν_{max} cm^{-1}: 3426 (O–H); 1658 (C=O); 1052 (C–O). FAB-MS *m/z* (rel.int.): 503 $[M + Na]^+$ (25), 481 $[M + H]^+$ (85), 463 $[M + H–H_2O]^+$ (40), 445 $[M + H–2H_2O]^+$ (55), 427 $[M + H–3H_2O]^+$ (26), 309 (100). Compound **1** was identified by analytical NP-HPLC in systems A, B, C and D (Table 3) as 20-hydroxyecdysone, using an authentic sample from *Leuzea carthamoides* [15] as internal standard and also by ^1H and ^{13}C NMR spectra (see Tables 1 and 2).

Ponasterone A (**2**)

This compound (1.3 mg) was obtained by RP-HPLC (system I). M.p. 201–203°C (MeOH). Composition $C_{27}H_{44}O_6$. M: 464. IR ν_{max} cm^{-1}: 3554, 3394 (O–H); 1646 (C=O); 1050 (C–O). FAB-MS *m/z* (rel.int.): 487 $[M + Na]^+$ (33), 465 $[M + H]^+$ (97), 447 $[M + H–H_2O]^+$ (73), 429 $[M + H–2H_2O]^+$ (25), 303 (100). HR-MS for $C_{27}H_{45}O_6$ $[M + H]^+$ calculated 465.3220, found 465.3221. For ^1H and ^{13}C NMR see Tables 1 and 2.

Malacosterone (**3**)

NP-HPLC separation of mixture **3** and **7** using system III afforded **3** (1.5 mg) as an amorphous solid. Composition $C_{27}H_{44}O_8$. M: 496. IR ν_{max} cm^{-1}: 3397 (O–H); 1653 (C=O). FAB-MS *m/z* (rel.int.): 519 $[M + Na]^+$ (47), 497 $[M + H]^+$ (51), 479 $[M + H–H_2O]^+$ (20), 461 $[M + H–2H_2O]^+$ (65), 443 $[M + H–3H_2O]^+$ (64), 340 (100). HR-MS for $C_{27}H_{45}O_8$ $[M + H]^+$ calculated 497.3114, found 497.2990. For ^1H and ^{13}C NMR see Tables 1 and 2.

Turkesterone (**4**)

This compound (2.0 mg) was obtained by RP-HPLC (system I) as an amorphous solid. Composition $C_{27}H_{44}O_8$. M: 496. IR ν_{max} cm^{-1}: 3433 (O–H); 1657 (C=O); 1052 (C–O). FAB-MS *m/z* (rel.int.): 519 $[M + Na]^+$ (100), 497 $[M + H]^+$ (66), 479 $[M + H–H_2O]^+$ (84), 461 $[M + H–2H_2O]^+$ (89), 443 $[M + H–3H_2O]^+$ (78), 425 $[M + H–4H_2O]^+$ (36). HR-MS for $C_{27}H_{45}O_8$ $[M + H]^+$ calculated 497.3114, found 497.3101. For ^1H and ^{13}C NMR see Tables 1 and 2.

Paxillosterone (**5**): 2β,3β,11α,14α,20R,22R,24R-*heptahydroxy*-5β-*ergost*-7-*en*-6-*one*

This compound (1.9 mg) was obtained as an amorphous solid after separation from **1** by NP-HPLC (system II). Composition $C_{28}H_{46}O_8$, M: 510. IR ν_{max} cm^{-1}: 3420 (O–H), 1650 (C=O). FAB-MS *m/z* (rel.int.): 533 $[M + Na]^+$ (3), 511 $[M + H]^+$ (96), 493 $[M + H–H_2O]^+$ (100), 475 $[M + H–2H_2O]^+$ (55), 457 $[M + H–3H_2O]^+$ (39); HR-MS for $C_{28}H_{47}O_8$ $[M + H]^+$ calculated 511.3271, found 511.3135. Compound **5** was identified by analytical NP-HPLC in systems A, B, C and D (Table 3) as paxillosterone, using an authentic sample from *Paxillus atrotomentosus* [1] as internal standard and also by ^1H and ^{13}C NMR (see Tables 1 and 2).

Panuosterone (**6**): 2β,3β,14α,20R,22R,24R-*hexahydroxy*-5β-*ergost*-7-*en*-6-*one*

This compound (2.0 mg) was obtained by RP-HPLC (system I), m.p. 207–214°C (acetone). Composition $C_{28}H_{46}O_7$. M: 494. IR ν_{max} cm^{-1}: 3416 (O–H); 1653 (C=O). FAB-MS *m/z* (rel.int.): 517 $[M + Na]^+$ (100), 495 $[M + H]^+$ (94), 477 $[M + H–H_2O]^+$ (85), 459 $[M + H–2H_2O]^+$ (54), 441 $[M + H–3H_2O]^+$ (35); HP-MS for $C_{28}H_{47}O_7$ $[M + H]^+$ calculated 495.3320, found 495.3312. For ^1H and ^{13}C NMR see Tables 1 and 2.

25-*Hydroxypanuosterone* (**7**): 2β,3β,14α,20R,22R,24ξ,25-*heptahydroxy*-5β-*ergost*-7-*en*-6-*one*

This compound (1.1 mg) was obtained after separation from **3** by NP-HPLC (system III), m.p. 248–256°C (MeOH). Composition $C_{28}H_{46}O_8$. M: 510. IR ν_{max} cm^{-1}: 3406 (O–H); 1653 (C=O). FAB-MS *m/z* (rel.int.): 533$[M + Na]^+$ (60), 511 $[M + H]^+$ (27), 493 $[M + H–H_2O]^+$ (23), 457 $[M + H–3H_2O]^+$ (42), 311 (100); HR-MS for $C_{28}H_{47}O_8$ $[M + H]^+$ calculated 511.3271, found 511.3131. For ^1H and ^{13}C NMR see Tables 1 and 2.

Acknowledgements—We are grateful to Dr F. Kotlaba (Institute of Botany ASCR, 25243 Průhonice) and Dr J. Šutara [14] for their help with collection and identification of mushrooms and Dr S. Vašíčková for IR spectra. This work was financially supported by the Grant Agency of the Czech Republic, grant No. 203/98/0451.

REFERENCES

1. Vokáč, K., Budějínský, M., Harmatha, J. and Píš, J., *Tetrahedron*, 1998, **54,** 1657.
2. Lafont, R. and Horn, D. H. S., in *Ecdysone from Chemistry to Mode of Action*, ed. J. Koolman. Georg Thieme, Stuttgart, 1989, pp. 39–64.
3. Camps, F., in *Ecological Chemistry and Biochemistry of Plant Terpenoids*, eds. J. B. Harborne and F. A. Tomas-Barberan. Clarendon Press, Oxford, 1991, pp. 331–376.
4. Takaishi, Y., Adachi, R., Murakami, Y., Ohashi, T., Nakano, K. and Tomimatsu, T., *Phytochemistry*, 1992, **31,** 243.
5. Oisawa, T., Yukawa, M., Takao, Ch., Murayama, M. and Bando, H., *Chem. Pharm. Bull.*, 1992, **40,** 143.
6. Vokáč, K., Budějínský, M. and Harmatha, J., in *Abstracts of the Xth Ecdysone Workshop*. Liverpool, 1992, p. 125.
7. Vokáč, K., Budějínský, M. and Harmatha, J., in *The Ecdysone Handbook*, eds. R. D. Lafont and I. D. Wilson. The Chromatographic Society, Nottingham, 1992, pp. 301–302.
8. Lafont, R. and Wilson, I. (eds.), in *The Ecdysone Handbook*, 2nd edn. The Chromatography Society, Nottingham, 1996.
9. Harmatha, J. and Dinan, L., *Arch. Insect Biochem. Physiol.*, 1997, **35,** 219.
10. Garcia, M., Girault, J. P. and Lafont, R., *Int. J. Invert. Develop.*, 1986, **9,** 43.
11. Kawagishi, H., Katsumi, R., Sazawa, T., Mizuno, T., Hagiwara, T. and Nakamura, T., *Phytochemistry*, 1988, **27,** 2777.
12. Valisolalao, J., Luu, B. and Ourisson, G., *Tetrahedron*, 1983, **39,** 2779.
13. Maurer, P., Girault, J.-P., Larcheveque, M. and Lafont, R., *Arch. Insect. Biochem. Physiol.*, 1993, **23,** 29.
14. Šutara, J., *Česká mykologie*, 1992, **46,** 50 (in English).
15. Píš, J., Budějínský, M., Vokáč, K., Laudová, V. and Harmatha, J., *Phytochemistry*, 1994, **37,** 707.