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HYDROXYCINNAMOYL ESTER GLYCOSIDES AND SAPONINS FROM FLOWERS OF *VERBASCUM PHLOMOIDES*

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Key Word Index—*Verbascum phlomoides*; Scrophulariaceae; iridoids; hydroxycinnamoyl esters; phlomoidoside; saponins; desrhamnoverbascosaponin.

Abstract—A new iridoid ester glycoside acylated with *p*-coumaric acid was isolated from the flowers of Verbascum *phlomoides*, together with one known one, specioside. Caffeic acid esters, verbascoside and forsythoside B were found as minor constituents. A new saponin was also obtained and identified as desrhamnosylverbascosaponin. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Verbascum phlomoides is one of two species yielding the official drug *Flores Verbasci* (*Flos Verbasci*, woolflower) [1]. The drug is used as expectorans and mucilaginosum to prepare anticough teas [2, 3]. The activity of *Decoctum Verbasci* against influenza viruses A_2 and B has recently been described [4]. The following compounds have been reported previously from the flowers of *V. phlomoides*: verbascosaponin [5, 6] verbascosaponins A and B [6], polysaccharides [7, 8], hesperidin [9], diosmine [9, 10], 7-*O*-rutinoside- and 7-*O*-glucoside of tamarixetin [9, 10]. Luteolin and apigenin [10], phenolic acids (*p*-coumaric, caffeic, ferulic, *p*-hydroxybenzoic and vanillic) [11], aucubin, catalpol and their 6-*O*-xylosides [12] were also detected by chromatographic analysis.

The present paper deals with the isolation of a new ester glycoside of aucubin, along with three known glycosides acylated with hydroxycinnamic acids; and a new saponin, in addition to two known ones.

RESULTS AND DISCUSSION

Air-dried flowers of *V. phlomoides* were extracted successively with petrol, chloroform and ethanol. The ethanol extract was fractionated between diethyl ether, *n*-butanol and water. The *n*-butanol-soluble part was subjected to repeated column chromatography on polyamide, followed by silica gel and Sephadex to obtain compounds **1**–**7**.

Compound **1**, an amorphous solid, revealed by LSI-mass spectrometry (negative ion mode) a $[M-H]^-$ at *m/z* 623, in agreement with the molecular formula $C_{29}H_{36}O_{15}$, as well as a fragment ion at *m/z* 295, which was assigned to coumaroylpentose. Ions recorded at *m/z* 163, 147 and 133 (positive ion mode) indicated the presence of glucosyl, coumaroyl and pentosyl units, respectively.

The ^{13}C NMR spectrum of **1** (Table 1) showed the presence of eleven low-field resonances, of which seven were assigned to a coumaroyl moiety and four to the double bonds of aucubin. The remaining signals also indicated the presence of an aucubin, except for the signal of C-6, which was shifted downfield by *ca* 9 ppm as a result of glycosidation [13]. Chemical shifts of the anomeric carbon and C-2 of the second sugar were in accordance with those for methyl-β-D-xylopyranoside [13]: β-D-xylofuranoside gives a C-1 signal at 109.6 ppm and α-D-xylofuranoside at 103.2 ppm [14]. The downfield shift of the signal of C-4 from xylose by *ca* 4.7 ppm and the upfield shift of C-3 and C-5, as compared with the ^{13}C NMR spectral data of xylosylaucubin (Table 2), suggested acylation on C-4 hydroxyl of xylose.

The ^1H NMR spectrum of compound **1** showed two anomeric sugar proton signals, both with a large coupling constant, which suggested the β-configuration of glucose and xylose. ^1H–^1H COSY experiment allowed the assignment of all higher field proton signals and confirmed the acylation on the C-4 oxygen of xylose, because the signal of the H-4 of xylose was shifted downfield to 4.8 ppm. Also, chemical shifts of the other proton signals were in agreement with the structure of compound **1** as 6-*O*-(4″-*O*-*E*-*p*-coumaroyl)-β-D-xylopyranosylaucubin, for which the name phlomoidoside is proposed. The ^1H and ^{13}C NMR spectra also showed minor signals corresponding to the *cis*-isomer (see Experimental). Phlomoidoside (**1**) is one of the major constituents of the flowers of *V phlomoides*. However, 6-*O*-xylosylaucubin has previously been reported from some *Verbascum* species

Table 1. ^{1}H NMR spectral data of compound **1*** (in CD_3OD)

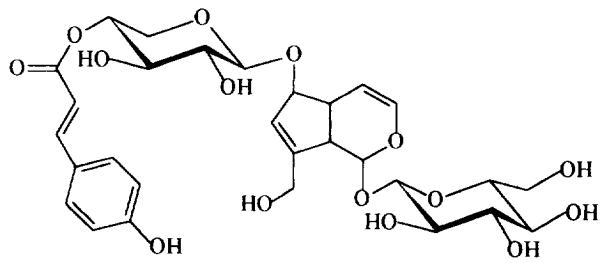
H	Aglycone	Glucose	Xylose	*p*-Coumaroyl (*trans*)
1	4.91 *d* (6.0)	4.68 *d* (7.8)	4.38 *d* (7.5)	–
2	–	3.25 *dd* (7.0, 9.0)	3.20 *dd* (9.0, 7.8)	7.47 *d* (8.5)
3	6.31 *dd* (6.0, 1.4)	3.38 *t* (9.0)	3.65 *t* (9.0)	6.81 *d* (8.5)
4	5.14 *dd* (4.0, 6.0)	3.27 *t* (9.0)	4.81 *ddd* (9.1, 9.5, 5.5)	–
5	2.89 *s* (*br*)	3.30 *m*	A 3.25 *dd* (9.0, 12.1) B 4.05 *dd* (5.4, 11.3)	6.81 *d* (8.5)
6	4.51 *s* (*br*)	A 3.85 *dd* (1.5, 12.5) B 3.64 *dd* (4.0, 12.0)	–	7.47 *d* (8.5)
7	5.90 *s* (*br*)	–	–	7.68 *d* (16.0)
8	–	–	–	6.36 *d* (16.0)
9	2.89 *s* (*br*)	–	–	–
10	A 4.18 *d* (15.4) B 4.38 *d* (15.4)	–	–	–

*Signal assignments based on ^{1}H–^{1}H COSY experiment. Coupling constants (J) in Hz are given in parentheses.

Table 2. ^{13}C NMR chemical shifts of compound **1** in comparison with β-D-xylosylaucubin and methyl-β-D-xylosides

C	1*	6-*O*-β-D-Xylosyl-aucubin [13]†	Methyl-β-D-xylo-pyranoside [13]†	Methyl-β-D-xylo-furanoside [14]
1	97.9	94.8		
3	141.6	139.0		
4	104.6	103.9		
5	44.4	40.0		
6	91.2	88.7		
7	127.1	125.3		
8	149.4	147.7		
9	50.3	45.7		
10	61.4	59.8		
Glucose				
1′	99.9	97.5		
2′	75.1	73.6		
3′	78.2	77.1		
4′	71.5	70.5		
5′	77.8	76.6		
6′	62.6	61.7		
Xylose				
1″	105.7	101.6	105	109.6
2″	73.1	74.0	73.9	80.9
3″	75.1	76.7	76.9	76.0
4″	74.8	70.1	70.5	83.5
5″	63.7	64.0	65.9	62.1
trans-*p*-Coumaroyl				
1‴	127.6			
2‴	131.2			
3‴	116.9			
4‴	161.3			
5‴	116.9			
6‴	131.2			
7‴	147.1			
8‴	114.7			
C = 0	168.6			

*In CD_3OD.
†In D_2O.



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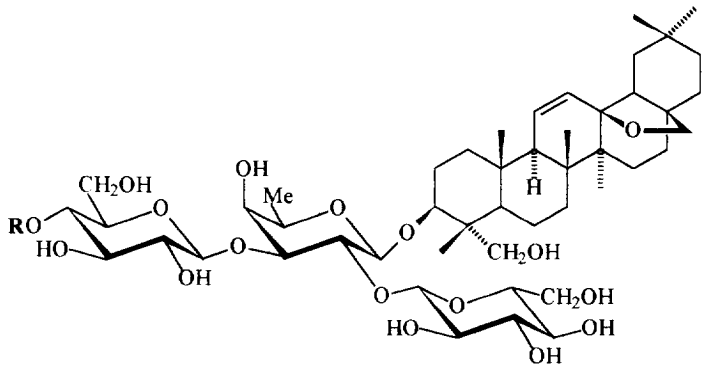

5 : R = Rha

7 : R = H

[13,15], but the *p*-coumaroyl ester of this iridoid acylated on xylose was not described.

Compound **2** was identified as specioside [16], compound **3** as verbascoside [17], and compound **4** as forsythoside B [18] by comparison (TLC, IR, LSI mass spectrometry, ^{1}H NMR) with authentic substances obtained earlier from the inflorescences of *V. lychnitis* [19–21]. Caffeoyl esters of phenylethanoid glycosides occur in the flowers of *V. phlomoides* in relatively very small amounts. Considerably higher concentrations of phenylethanoids have previously been found in *V. lychnitis*, *V. nigrum* and *V. thapsiforme* [20,21].

In addition to the hydroxycinnamoyl esters, saponins **5–7** were obtained from the ethanol extract. Compounds **5** and **6** were identified (LSI-mass spectrometry, ^{1}H NMR, TLC) as verbascosaponin [5, 6, 22] and verbascosaponin A [6, 22], respectively.

Compound **7** showed a higher R_f value than compounds **5** and **6**, and gave glucose upon partial acid hydrolysis. Its M_r determined by LSI-mass spectrometry was lower than that for compound **5** (verbascosaponin) by 146 mu, indicating the lack of rhamnose in the sugar chain. The NMR data of compound **7** were almost identical to those of compound **5** except for the rhamnose unit. Therefore, compound **7** is desrhamnosylverbascosaponin [=3β-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl (1 → 3)-β-D-fucopyranosyl]-13β, 28-epoxy-olean-11-ene, 23 α-ol]. Desrhamnosylverbascosaponin and also verbascosaponin A are detectable by TLC in a freshly prepared ethanol extract from the flowers of *V. phlomoides*. Therefore, they can not be considered as extraction and isolation artefacts. An enzymatic process taking place during drying and storage of the plant material seems to be more probable.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR and ^{13}C NMR were recorded at 300 and 75 MHz, respectively (TMS). LSI MS: Cs^+, glycerol matrix (13 KeV). CC: polyamide MN, $CHCl_3$–MeOH–H_2O (6:3:1, lower layer) for saponins. EtOAc–MeOH–H_2O (100:16.5:13.5) and $CHCl_3$–MeOH–H_2O (4:1:0.1) for esters, *n*-BuOH–pyridine–H_2O (6:4:3, for sugars); spray reagents: H_2O and Liebermann–Burchard reagent for saponins, NH_3 vapour and UV light (365 nm) for hydroxycinnamoyl esters, aniline phthalate for sugars. Partial hydrolysis of saponins was performed on TLC plates, which were exposed to HCl vapour for 18 hr at 18°. After removal of HCl, TLC analysis was performed on the same plates.

Plant material. Verbascum phlomoides L. was cultivated in the Garden of Medicinal Plants in Łódź, Poland and originated from seeds from the Botanical Garden in Stuttgart. Morphological features of the

plants were consistent with those described in ref. [23]. A voucher specimen No 253/90 is deposited at the Department of Pharmacognosy, Medical University of Łódź, Poland.

Isolation. Air-dried and powdered petals (170 g) were Soxhlet-extracted with petrol (60–80°) followed by $CHCl_3$ and then refluxed with EtOH (5 × 11). The EtOH extract was evapd *in vacuo*, the residue suspended in H_2O and extracted with Et_2O and then with *n*-BuOH, successively. The *n*-BuOH-sol. part was subjected to CC over polyamide eluted with a H_2O–MeOH gradient. Frs 24–31 eluted with 10% MeOH were further chromatographed (CC) on silica gel with $CHCl_3$–MeOH–H_2O (4:1:0.1) to obtain compound **1** (30 mg) from frs 20–26 and compounds **2** (1 mg) from frs 30–31, and from frs 58–64 by chromatography on Sephadex LH-20, compounds **3** (*ca* 0.2 mg) and **4** (*ca* 0.5 mg). Frs 32–103 from the polyamide column contained saponins (TLC). They were subjected to CC on silica gel with $CHCl_3$–MeOH:H_2O (6:3:1, lower layer) to obtain 12 mg of compound **5**, 30 mg of compound **6** and 5 mg of compound **7**.

6-O-(4″-O-p(*coumaroyl*-β-D-*xylopyranosyl*)-*aucubin (phlomoidoside)* (**1**). LSI-MS (rel. int.) *m/z* (negative) 623 (62) $[M-H]^-$ (calc. for $C_{29}H_{36}O_{15}$, 624.5884), 295 (12) [coumaroylxylose − H]$^-$; positive: 279 (19) [coumaroylxylose − H_2O + H]$^+$, 163 (12)[gluc + H]$^+$, 147(77) [coumaroyl + H]$^+$, 133(92)[xyl + H]$^+$. UV λ_{max}^{MeOH} nm: 225 and 312 + 5% sol. KOH 362. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1650, 1620, 1605, 1510. ^{1}H and ^{13}C NMR (CD_3OD): Tables 1 and 2. *Cis*-isomer: ^{1}H NMR: δ 5.79 *d* (*J* = 13 Hz H-8‴, 6.76 *d* (*J* = 8.6 Hz) H-3‴H-5‴, 6.89 *d* (*J* = 13 Hz) H-7‴, 7.65 *d* (*J* = 8.6 Hz) H-2‴, H-6‴, other signals identical for *cis*- and *trans*-isomer (Table 1). ^{13}C NMR: δ 167.7 (C = 0), 159.6 (C-4), 145.6 (C-7‴), 133.5 (C-2‴ and C-6‴), 127.5 (C-1), 115.8 (C-8‴), 115.6 (C-3‴ and C-5‴); other signals as for *trans*-isomer (Table 2).

Verbascosaponin (**5**) (=*saponin* **1** [22]). Mp 252–255°. $C_{54}H_{88}O_{21}$ M_r 1073.226 LSI-MS (negative) (rel. int) *m/z*: 1071.8 $[M-H]^-$ (39), 925.5 [M − 146 − H]$^-$ (8), 763.4 [M − H − 146 − 162] (11), 601.0 [M − H − 146 − 2 × 162]$^-$ (2). ^{1}H NMR (CD_3OD): δ 5.93 *d* (*J* = 10.8 Hz) H-12, 5.33 *dd* (*J* = 10.2, 2.8 Hz) H-11, 4.92 *d* (*J* = 1.7 Hz) H-1 rham, 4.9 *d* (*J* = 8.0 Hz) H-1 gluc, 4.62 *d* (*J* = 7.7 Hz) H-1 gluc, 4.47 *d* (*J* = 7.8 Hz) H-1 fuc, 3.75 *d* (*J* = 11.0 Hz) H-23a, 3.55 *d* (*J* = 8.5 Hz) H-28a, 3.32 *d* (*J* = 11.0 Hz) H-23b, 3.21 *d* (*J* = 8.5 Hz) H-28b, 1.26 2H *d* (*J* = 6.2 Hz) CH_3 rham; singlets of CH_3 groups from aglycone: 1.08, 0.99, 0.97, 0.95, 0.89, 0.72. TLC: R_f 0.44.

Verbascosaponin A (**6**) (=*saponin* 2 [22]). Mp 258–262°. $C_{55}H_{92}O_{22}$ M_r 1105.267 LSI MS (negative) (rel. int) *m/z*: 1104.1(100) $[M-H]^-$, 957.8(20) [M − H − 146]$^-$, 941.8(14) [M − H − 162]$^-$, 795.6(30) [M − H − 146 − 162]$^-$, 633.5(6) [M − H − 146 − 2 × 162]$^-$, 455.4(2) [agl.-MeOH − H]$^-$. ^{1}H NMR (CD_3OD): δ 5.38 *d* (*J* = 3.6 Hz) H-12, 4.85 *d* (*J* = 1.8 Hz) H-1 rham, 4.82 *d* (*J* = 8.0 Hz) H-1 gluc, 4.62 *d* (*J* = 7.8 Hz) H-1 gluc, 4.48 *d* (*J* = 7.8 Hz) H-1 fuc, 3.85 *d* (*J* = 10.2 Hz) H-11, 3.25 3H *s* OCH_3, 1.26 6H *d* (*J* = 6.2 Hz) CH_3 rham and CH_3 fuc; singlets of CH_3 groups from aglycone: 1.25, 1.1, 1.0, 0.91, 0.90, 0.74. TLC: R_f 0.40.

Desrhamnoverbascosaponin (**7**). Mp 226–230°. $C_{48}H_{77}O_{17}$ M_r 926.08 LSI-MS (negative): 925(100)[M − H]$^-$, 763(52) [M − 162 − H]$^-$, 601(13) [M − 2 × 162 − H]$^-$, 471(24) [sugar chain]$^-$, 307(85) [gluc + fuc − H]$^-$. ^{1}H NMR (CD_3OD): δ 5.93 *d* (*J* = 10.4 Hz) H-12, 5.33 *dd* (*J* = 10.3, 3.0 Hz) H-11, 4.88 *d* (*J* = 8.0 Hz) H-1 gluc, 4.63 *d* (*J* = 7.7 Hz) H-1 gluc, 4.47 *d* (*J* = 7.8 Hz) H-1 fuc, 1.26 3H *d* (*J* = 6.2 Hz) CH_3-fuc; singlets of CH_3 groups from aglycone: 1.08, 0.99, 0.97, 0.95, 0.89, 0.72. TLC: R_f 0.48. Partial acid hydrolysis gave glucose (TLC).

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