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VERBALLOSCENINE, THE *Z* ISOMER OF VERBASCENINE FROM *VERBASCUM PHOENICEUM*

KONSTANTIN DRANDAROV

Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Sofia, Dunav St. 2, BG-1000 Sofia, Bulgaria

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Key Word Index—*Verbascum phoeniceum*; *Verbascum pseudonobile*; Scrophulariaceae; leaves; *Z*-cinnamic acid amide; macrocyclic lactam alkaloids; polyamine; spermine.

Abstract—The *Z* isomer (13-acetyl-1-(*Z*)-cinnamoyl-6-oxo-8-phenyl-1,5,9,13-tetraazacycloheptadecane; verballoscenine) of the 17-membered macrocyclic lactam alkaloid verbascenine was isolated from *Verbascum phoeniceum* and structurally characterized. The presence in the same plant material of the analogous N-13 non-acetyl alkaloids verbacine and verballocine as precursors in the biogenesis of verbascenine and verballoscenine is reported. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The substance called verbascenine (**3**) has been isolated from *Verbascum phoeniceum* L. and *Verbascum nigrum* L. (Scrophulariaceae) [1]. It has been structurally characterized as a 17-membered macrocyclic lactam alkaloid containing spermine, phenylpropionyl, acetyl and *E*-cinnamoyl precursor units [1]. The analogous N-13 non-acetyl alkaloid verbacine (**1**) and its configurational *Z* isomer verballocine (**2**) have also been isolated from *Verbascum pseudonobile* Stoj. et Stef. (Scrophulariaceae), a species endemic in Bulgaria [2]. Verbacine (**1**) and verballocine (**2**) have been structurally characterized [3]. We report here the isolation (from the leaves of *V. phoeniceum*) and structure elucidation of the *Z* isomer of verbascenine (**3**), here called verballoscenine (**4**). Using the same plant material, **1** and **2** were found to be potential precursors in the biogenesis of **3** and **4**.

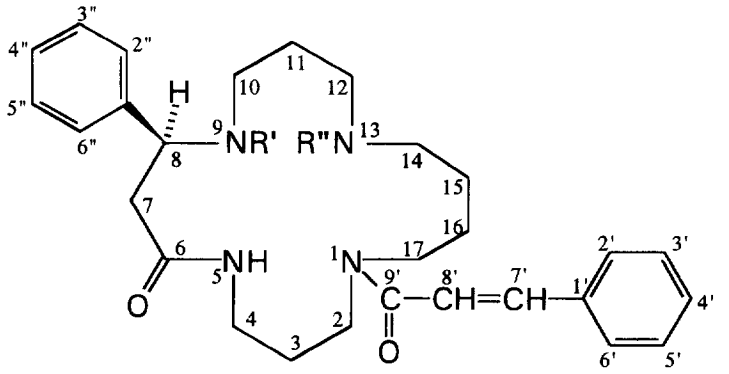
RESULTS AND DISCUSSION

A product fully identical (TLC, $[\alpha]_D$, UV, IR and EI-mass spectroscopy) with verbascenine (**3**) has been prepared by monoacetylation of verbacine (**1**) [3]. In order to compare semisynthetically prepared **3** from *Verbascum phoeniceum* (collected in the summer in western Bulgaria), an authentic sample of verbascenine (**3**) was isolated in our laboratory. In the course of that study, along **3** (1.65×10^{-3}% in dry leaves), an optically active substance ($[\alpha]_D^{25} -19.0°$; $CHCl_3$, *c* 2.6) was observed chromatographically (TLC) and isolated (prep. TLC) in equal quantity (1.65×10^{-3}%) from the same alkaloid extracts. This product showed the same molecular weight ($[M]^+$ at *m/z* 504) and EI-mass spectroscopy fragmentation pattern as verbascenine (**3**). Its ^1H NMR (two olefinic protons at δ 6.64 (*d*) and 6.06+6.04 (*dd*) with $J = 12.7$ Hz) and UV (λ_{max}^{EtOH} nm (log ε): 256 (4.03)) spectra supported the conclusion that it is the *Z* isomer of verbascenine (**3**). This alkaloid was named verballoscenine (**4**). Similarly to the monoacetylation of verbacine (**1**) reported previously [3], the N-13 acetyl derivative was prepared from verballocine (**2**). The derivative showed the same spectral and chromatographic properties as verballoscenine (**4**).

Further acetylation of **3** and **4** gives the N-9–N-13 diacetylated products **5** and **6**, respectively, of which compound **5** has been reported previously [1, 3].

As reported for **1** and **2** and related *E*/*Z* isomeric cinnamamidic compounds [3], verballoscenine (**4**, *Z*) was similarly photoisomerized to verbascenine (**3**, *E*). Since the absolute configurations of verbascenine (**3**), **1** and **2** are known to be (*S*) [1, 3], it follows that the absolute configuration of the verballoscenine (**4**) is also (*S*).

As shown previously [3], with formaldehyde compounds **1** and **2** immediately form the cyclic aminals **7** and **8** in quantitative yield. Compounds **7** and **8** showed good chromatographic (TLC) resolution in comparison with the starting compounds **1** and **2** [4]. Using a special two-dimensional TLC technique (see Experimental) the presence of small quantities of N-13 unsubstituted verbacine (**1**) (0.45×10^{-4}% in dry leaves) and verballocine (**2**) (0.9×10^{-4}%) were also established in the same plant extracts. This suggests that **1** and **2** are precursors of verbascenine (**3**) and verballoscenine (**4**) in the biogenetic pathway.



Compound	R′	R″	C-7′–C-8′ bond
1 (Verbacine)	H	H	*trans*
2 (Verballocine)	H	H	*cis*
3 (Verbascenine)	H	$COCH_3$	*trans*
4 (Verballoscenine)	H	$COCH_3$	*cis*
5	$COCH_3$	$COCH_3$	*trans*
6	$COCH_3$	$COCH_3$	*cis*
	R′+R″		
7	$-CH_2-$		*trans*
8	$-CH_2-$		*cis*

Several alkaloids carrying a cinnamamide moiety have been isolated from different plant species in both *E* and *Z* forms [5–11]. In some cases the presence of *Z* isomers together with their *E* counterparts has been ascribed to an artificial isomerization under the isolation conditions [10, 11]. In the initial paper by Seifert *et al.* [1] no mention is made of the presence of *Z* isomer of verbascenine. By reproducing their method of isolating (MeOH/HOAc; pH < 7) the total alkaloid mixture, the *Z* isomer (**4**) was again detected. The same results were obtained by isolating the alkaloids using MeOH/phosphate buffer (pH = 7) and $CHCl_3$/diluted aqueous NH_3 (pH > 7) from dried or fresh leaves. Treating the pure compounds **1**–**4** with all types of isolation conditions (including the strong acidic medium reported here) showed the absence of *E*-to-*Z* or *Z*-to-*E* isomerization. Moreover, the addition of exogenous verbascenine (**3**) to the extraction mixture (fresh leaves, 1% methanolic CH_3COOH or diluted aqueous H_3PO_4) did not cause a detectable (TLC densitometry, see Experimental) increase in the concentration of the *Z* isomer verballoscenine (**4**). This was true even when the extraction time was increased to 7 days.

The results obtained exclude any possibility of artificial *E*/*Z* isomerization under the isolation conditions and suggest that the *Z* isomers **2** and **4** arise in the intact plant tissue.

Substances **1**–**4** were not observed in plant material collected in the same region but in autumn, and TLC of the extracts showed only traces of unidentified Dragendorff-positive compounds.

EXPERIMENTAL

General. EI-MS: 70 eV, 490 K; ^1H NMR; 100 MHz, int. standard: TMS; IR spectra recorded in $CHCl_3$.

Plant material. Leaves of *V. phoeniceum* were collected in western Bulgaria in July and Sept. A voucher specimen was deposited in 1995 at the Herbarium-SOM, Sofia, Bulgaria (accession number 152718).

Chromatography. TLC glass plates Kieselgel 60 F_{254} (Merck). Prep. TLC on hand-made plates: 5 g Kieselgel G F_{254} + 15 ml 1% aq. $NaHCO_3$ for one plate 20/20, coating thickness *ca* 0.5 mm. Solvent systems: S1, EtOAc–MeOH (8:2, v/v); S2–toluene, 96% (v/v) EtOH (9:1, v/v), Et_2NH in the gas phase. Detection by quenching of background fluorescence or Dragendorff's reagent (D144a [12]).

Extraction procedure. Air-dried leaves of *Verbascum phoeniceum* (250 g) were extracted twice overnight with 3 l of 3% aq. H_3PO_4. The combined resultant extracts were alkalinized with 25% NH_3 and extracted twice with 1 l of $CHCl_3$. The $CHCl_3$ extracts were concd and re-extracted with 3% H_3PO_4. The acidic aq. re-extract was alkalinized (25% NH_3), extracted with $CHCl_3$ and, after evaporation, the total alkaloid extract obtained. Similarly, 50 g minced fresh leaves were extracted twice with 300 ml 3% aq. H_3PO_4 (or 300 ml 1% CH_3COOH in MeOH, by method in ref. [1]).

Control experiments. Fresh leaves, collected in May 1996, several days before flowering. TLC densitometry of alkaloid extract from this material showed presence of 3.28×10^{-4}% (in fresh leaves) ver-

bascenine (**3**) and 1.85×10^{-4}% verballoscenine (**4**). For the control experiments, at the beginning of the extraction time to the mixtures was added 1 mg pure verbascenine (**3**) dissolved in 1 ml EtOH. This is about five times more than the total quantity of endogenous **3** and **4** in 50 g fresh leaves. All extraction procedure were performed under diffuse room light.

Quantitative analysis of **3** *and* **4** *in total alkaloid mixture.* Direct densitometry of chromatograms (two-fold development in S1 on silica gel) at 254 nm. At this wavelength **3** and **4** show nearly the same *A* (log ε: **3**, 4.08; **4**, 4.03). As an external standard 5 and 10 μl of 0.05% soln of **3** in $CHCl_3$ were spotted. A linear relationship spot area/concn in the range 1–10 μg/spot was found. After preliminary analysis, the total alkaloid mixture isolated from fresh leaves was dissolved in $CHCl_3$ to give a 0.05% soln of compounds **3** and **4**. The resulting soln was spotted (5 and 10 μl).

2D TLC of total alkaloid mixture. In the corner of an analytical TLC plate (10/10) were spotted about 200 μg of alkaloid mixture in about 10 μl $CHCl_3$. The chromatogram was developed twice in S2 (first direction). On removal of solvents the plate was treated with vapours of HCHO (a few ml of 40% aq. HCHO in a closed chromatographic tank) for 10 min. Before the developing in the second direction (S2) of *in situ* formed (from **1** and **2**) **7** and **8**, beside the first start position was spotted a $CHCl_3$ soln of standard mixture of compounds **7** and **8**. R_f values in S2: **1**, 0.13; **2**, 0.13; **7**, 0.48; **8**, 0.45 [4].

Group separation of **1**+**2** *and* **3**+**4**. Compounds **1** and **2** are stronger bases than their derivatives **3**+**4** and **7**+**8**. Thus group separation was done by extraction from aq. solns with different pH. The $CHCl_3$ soln of the total alkaloid mixture was extracted with 2% aq. H_3PO_4 with pH corrected to 4 with NaOH. (The $CHCl_3$ was prewashed with 1% aq. NH_2OH–HCl to eliminate any HCHO possibly present as an impurity.) Compounds **3** and **4** remained in the organic layer (extract A). To the aq. layer, containing **1** and **2**, were added several drops of 40% aq. HCHO. After a few min the pH was corrected to 5 with diluted NaOH, and extracted with $CHCl_3$. The formed cyclic aminals **7** and **8** passed to the organic solvent (extract B). Extracts A and B were evaporated and the residues were separated by prep. TLC on silica gel with S1 (two-fold development). The compounds were recovered from the scraped chromatographic zones with EtOH satd with gaseous NH_3. On evaporation of the eluate, the residue was dissolved in a small amount of $CHCl_3$ and passed through a short alumina column (5 g). Elution was done using C_6H_6–MeOH (20:1). From 250 g dry leaves about 4 mg each of **3** and **4** were isolated (gravimetrically) and 100 μg of **7** (resp. **1**) and 205 μg of **8** (resp. **2**) (UV spectrophotometrically). Compounds **7** and **8** can easily be reconverted to **1** and **2**, respectively, as reported in ref. [3].

N-13 Monoacetylated **3** *and* **4** *and N-9,N-13 diacetylated compounds* **5** *and* **6**. Preparation was from verbacine (**1**) and verballocine (**2**) by the method described in ref. [3]. The purification of the products was as described in the previous paragraph. Compound **5**: R_f 0.57 (S1), R_f 0.35 (S2). Compound **6**: R_f 0.42 (S1), R_f 0.32 (S2); EI-MS 70 eV: *m/z* (rel. int.): 546 $[M]^+$ (24), 503 $[M-COCH_3]^+$ (76), 415 $[M-C_6H_6-CH{=}CH-C{\equiv}O]^+$ (52), 131 $[C_6H_6-CH{=}CH-C{\equiv}O]^+$ (100).

Verbascenine (**3**). R_f 0.42 (S1), R_f 0.29 (S2); $[\alpha]_D^{25} = -27.0°$ ($CHCl_3$; c 2.7); $-15.7°$ (MeOH; c 2.38); lit. $[\alpha]_D^{22} = -15.0°$ (MeOH; c 0.43) [1]; EI-MS 70 eV: *m/z* (rel. int.): 504 $[M]^+$ (10) 373 $[M-C_6H_6-CH{=}CH-C{\equiv}O]^+$ (28), 131 $[C_6H_6-CH{=}CH-C{\equiv}O]^+$ (100).

Verballoscenine (**4**). Glass-like solid; $[\alpha]_D^{25} -19.0°$ ($CHCl_3$; c 2.6), $-10.7°$ (MeOH; c 2.38); R_f 0.33 (S1), R_f 0.26 (S2); UV λ_{max}^{EtOH} nm (log ε): 256 (4.03); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH free), 3300 (NH assoc.), 1667–1587 *s* (C=O, amide I+C=C unconjug.), 1542 *m* (lactam *trans*-CONH–, amide II); ^1H NMR (100 MHz, $CDCl_3$, TMS): δ 6.64 (1H, *d*, *J* = 12.7 Hz, H-7′), 6.06 (≈0.5 H, *d*, *J* = 12.7 Hz, H-8′), 6.04 (≈0.5 H, *d*, *J* = 12.7 Hz, H-8′); EI-MS 70 eV, *m/z* (rel. int.): 504 $[M]^+$ (12), 373 $[M-C_6H_6-CH{=}CH-C{\equiv}O]^+$ (31), 131 $[C_6H_6-CH{=}CH-C{\equiv}O]^+$ (100).

REFERENCES

1. Seifert, K., Johne, S. and Hesse, M., *Helvetica Chimica Acta*, 1982, **65**, 2540.
2. Stefanova, B. and Ninova, P., *Farmatsiya (Sofia)*, 1973, **5**, 39.
3. Drandarov, K., *Tetrahedron Letters*, 1995, **36**, 617.
4. Drandarov, K. and Hais, I. M., *Journal of Chromatography*, 1996, **724**, 416.
5. Lloyd, H. A., *Tetrahedron Letters*, 1965, **50**, 4537.
6. Stoessl, A., Rohringer, R. and Samborski, D. J., *Tetrahedron Letters*, 1969, **33**, 2807.
7. Ripperger, H., Schreiber, K. and Budzikiewicz, H., *J. Prakt. Chem.*, 1970, **312**, 449.
8. Ekevåg, U., Elander, M., Gawell, L., Leander, K. and Lüning, B., *Acta Chimica Scandinavica*, 1973, **27**, 1982.
9. Kupchan, S. M., Hintz, H. P. J., Smith, R. M., Karim, A., Cass, M. W., Court, W. A. and Yatagai, M., *Journal of the Chemical Society, Chemical Communications*, 1974, 329.
10. Meurer, B., Wray, V., Grotjahn, L., Wiermann, R. and Strack, D., *Phytochemistry*, 1986, **25**, 433.
11. Meurer, B., Wray, V., Wiermann, R. and Strack, D., *Phytochemistry*, 1988, **27**, 839.
12. Hais, I. M. and Macek, K. (ed.), *Handbuch der Papierchromatographie*, Vol. 1, 2nd edn. G. Fischer, Jena, p. 932.