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Verbacine and Verballocine, Novel Macrocyclic Spermine Alkaloids from Verbascum pseudonobile Stoj. et Stef. (Scrophulariaceae)

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Abstract: The isolation and structural elucidation of the novel 17-membered lactam alkaloids verbacine (1) and verballocine (2) containing spermine, E- (or Z-) cinnamoyl and phenylpropionyl precursory units, is reported. Evidence for the artificial origin of the previously reported verbaskine is presented.

The species *Verbascum pseudonobile* Stoj. et Stef. (*Scrophulariaceae*) is endemic for Bulgaria.¹ From its total alkaloid-containing extract (0,6% in dry leaves) fractions verbasine and verbaskine have been isolated² but only the structure of the crystalline substance verbaskine has been elucidated³ so far. In the course of our present study it has been found out that verbasine is not chemically individual but a mixture of four compounds, named verbacine (1, about 50% of the total alkaloid extract), verballocine (2, about 40%), verbasitrine¹¹ (about 1%) and isoverbasitrine¹¹ (about 10%). This paper is devoted to the structure determination of 1 and 2.

In the course of the present study it was also found out that verbaskine (6) is not a natural substance, but an artefact produced by the phosgene in the chloroform used as extractant². Verbaskine (6) was not chromatographically (TLC) observed in the fresh prepared^{2,12} extracts of the plant material, while the main alkaloid (verbacine, 1) yielded verbaskine quantitatively by treatment with chloroformic solution of phosgene¹⁸.

With acetic anhydride (one equivalent in chloroform at room temperature), 1 initially reacts at N-13 and gives a product completely identical¹⁴ with the alkaloid verbascenine (4) isolated and structurally characterised earlier⁴ from *Verbascum phoeniceum L*. and *Verbascum nigrum L*. With an excess of acetic anhydride the previously reported⁴ N-9,N-13-diacetylated product **5** was prepared from 1.

This evidence suggested the structure 1 for verbacine. Its spectral properties are in accordance with this deduction.¹⁵ Furthermore, since the absolute configuration of verbascenine (4) is known $(S)^4$, it follows that the absolute configuration of the verbacine (1) is also confirmed to be S.

The substance, named verballocine (2)¹⁶, exhibits the same molecular weight (M⁺ 462) and MS fragmentation as verbacine (1). Its IR (amide C=O at 1647 cm⁻¹, lack of a band for conjugated *trans*-double bond at about 970 cm⁻¹), UV (λ_{max} 256 nm, log ϵ 4,22) and ¹H NMR spectra (2 olefinic protons at 6.64+6.62 (dd) and 6.12+6.05 ppm (dd) (J = 12,7 Hz) suggested the presence of an amidically bounded Z-cinnamoyl residue.⁵ By 16 hours irradiation of its 5% alcoholic solution (in quartz cuvette) by UV light⁶ (a Camag 254 UV lamp placed at 10 cm above the cuvette, no filter) verballocine was converted to verbacine (1) in about 50% yield. In addition, catalytic hydrogenation of verballocine and verbacine (10% Pd/C, 2 hours, normal pressure, ethanol) yielded one and the same saturated product (3)¹⁷. This evidence con-

firms the structure 2 for verballocine and establishes that its absolute configuration is also S.

By treatment with phosgene verballocine (2) formed a hexahydropyrimidinone derivative $(7)^{18}$ which was also identified, along with verbaskine (6), as artefact. The product 8 (mentioned earlier³) was prepared¹⁸ from 3 and phosgene, or by catalytic hydrogenation of 6 or 7.



With formaldehyde 1 and 2 yielded quantitatively cyclic aminals 9^{19} and 10^{20} (between N-9 and N-13), which also were observed in considerable quantity as artificial products in the alkaloid extracts. In the same manner 11^{21} was prepared from 3. The formation of related perhydropyrimidine products as artefacts from natural substances, containing 1,3-propandiamine units, in contact with aldehydes as solvent impurities, has been reported earlier.7 The availability of the significant amide II band (-CONH-, at 1545 cm⁻¹) in the IR spectra of 9, 10 and 11 excluded the alternative cyclization between N-5 and N-9 with formaldehyde, which was earlier reported for the spermidine alkaloid lunarine and its derivatives LBX and LBZ^{8,9}. Further support for this conclusion is the presence of a peak at m/z 139 (C₈H₁₅N₂, 15%) in MS of 9, 10 and 11 which is evidently due to

the initial cleavage of the C-8---N-9 bond followed by split of the N-1--C-17 bond and hydrogen transfer. The analogous fragments in MS of 1-3 and 6-8 are at m/z 127 ($C_7H_{15}N_2$, 10%) and 153 ($C_8H_{13}N_2O$, 12%) respectively.

Compounds 1-3 do not react with acetaldehyde. This can be explained by the larger effective volume of ethylidene ($CH_3CH=$) group than the methylene ($CH_2=$) group and by the higher intraannular repulsion.

By mild acidic hydrolysis 9, 10 and 11 can easily be reconverted to 1, 2 and 3, respectively.¹³ Preparative quantities of either 1 and 2 were best obtained in this way, since 9 and 10 showed better chromatographic (TLC) resolution.

The conversion of 1-3 into 9-11 by formation of hexahydropyrimidine cycle with formaldehyde reverses the sign of the optical rotation (- to +) in contrast to hexahydropyrimidinone derivatives 6-8 which showed the same sign (-) at the D-band of Na (589 nm) as the starting 1-3. Obviously, this is a corollary of a sharp conformational change around the chiral C-8 of 9-11 towards 1-8.

By irradiation of alcoholic solutions of 7 and 10, in the same conditions as described above for 2, the products 6 and 9 could be obtained, respectively. Compounds 1, 6 and 9 also photoisomerized to 2, 7 and



10, but in low yields.

As can be seen, a structural relationship between verbacine (1), verballocine (2) and E/Z-isomeric pair of spermidine alkaloids celacinnine (12) and celallocinnine (13) (isolated from *Maytenus arbutifolia* and *Tripterygium wilfordii* - *Celastraceae*¹⁰) is present. This suggested a related biogenetic origin for these natural compounds.

The above-mentioned minor alkaloids of *Verb. pseudonobile* verbasitrine and isoverbasitrine are 3',4'-dimethoxy derivatives of verbacine (1)

and verballocine (2) respectively. Publication of more detailed information about this conclusion is to follow.¹¹

References and Notes

- 1. Stefanova, B.; Ninova, P. Farmatsiya (Sofia) 1973, 5, 39.
- 2. Ninova, P.; Abdusamatov, A.; Yunusov, S. Yu. Khim. Prir. Soedin. 1971, 540.
- 3. Koblicová, Z.; Turecek, F.; Ninova, P.; Trojánek, J.; Bláha, K. Tetrahedron Lett. 1983, 24, 4381.
- 4. Seifert, K.; Johne, S.; Hesse, M. Helv. Chim. Acta 1982, 65, 2540.
- 5. Lloyd, H. A. Tetrahedron Lett. 1965, 50, 4537.
- 6. Stoermer, R. Ber. Deut. Chem. Ges. 1909, 42, 4865.
- 7. Kühne, H.; Hesse, M. Helv. Chim. Acta 1982, 65, 1470.
- Poupat, C.; Husson, H.-P.; Rodrigues, B.; Husson, A.; Potier, P.; Janot, M.-M. Tetrahedron 1972, 28, 3087.
- 9. Doskotch, R. W.; Fairchild, E. H.; Kubelka, W. Experientia 1972, 28, 383.
- Kupchan, S. M.; Hintz, H. P. J.; Smith, R. M.; Karim, A.; Cass, M. W.; Court, W. A.; Yatagai, M. J. Chem. Soc. Chem. Comm. 1974, 329.
- 11. Drandarov, K., in preparation.
- 12. The plant material has been extracted by 5% aqueous solution of $NH_4H_2PO_4$. After alkalisation with 25% NH_3 , extraction with chloroform and evaporation, the total alkaloid-containing extract was obtained.
- 13. The crude alkaloid extract was treated with excess of formaldehyde in EtOH (30 min at room temperature) and the formed derivatives 9¹⁹ and 10²⁰ were separated by preparative TLC on silica gel with toluene-96% EtOH (90:10, v/v) and diethylamine in the gas phase (S1). The isolated 9 and 10 were reconverted in quantitative yield to 1 and 2 respectively by treatment with 1% HCl, in the presence of 10 equivalents of NH₂OH·HCl, for 1 h at 90° C.
- 14. The N-13-monoacetylated product (4) of verbacine was compared with an authentic sample of verbascenine, isolated from *Verbascum phoeniceum L*. in our laboratory (in accordance to ref.⁴).

- 15. Verbacine (1): Colourless glass-like solid; $|\alpha|_D^{26} = -14.4^{\circ}$ (c 3.7, CHCl₃); $R_F 0.42$ (S1¹³, on alumina); UV (EtOH): λ_{max} (log ϵ) 280 nm (4.31); IR (CHCl₃): 3447 (NH free), 3300 (NH assoc.), 1643 (C=O, amide I), 1599 (C=C conjug.), 1545 (lactam *trans*-CONH-, amide II), 970 cm⁻¹ (conjug. *trans* CH=CH); ¹H NMR (250 MHz) (CDCl₃), δ : 7.71 (d, J = 15.3, 1H, H-C(7⁺)), 6.88 (d, J = 15.5, 1H, H-C(8⁺)); MS: 462 (23, M⁺, C₂₈H₃₈N₄O₂), 419 (33), 390 (2), 331 (29), 288 (52), 259 (8), 257 (10), 245 (7), 231 (7), 217 (7), 205 (5), 188 (17), 146 (22), 131 (100), 127 (10), 103 (58), 98 (27), 91 (32), 84 (87), 72 (7).
- 16. Verballocine (2): Colourless glass-like solid; $[\alpha]_D^{26} = -18^{\circ}$ (c 2.37, CHCl₃); R_F 0.38 (S1¹³, on alumina); UV (EtOH): λ_{max} (log ϵ): 256 nm (4.22); IR (CHCl₃): 3448 (NH free), 3300 (NH assoc.), 1647 (C=O, amide I), 1608 (C=C), 1545 cm⁻¹ (amide II); ¹H NMR (250 MHz) (CDCl₃), δ : 6.64 (d, J = 12.7, \approx 0.5 H, H-C(7')), 6.62 (d, J = 12.7, \approx 0.5 H, H-C(7')), 6.12 (d, J = 12.7, \approx 0.5 H, H-C(8')); MS: 462 (M⁺, C₂₈H₃₈N₄O₅).
- 17. **3**: Colourless glass like solid; $[\alpha]_D^{26} = -22^\circ$ (c 3.7, CHCl₃); R_F 0.44 (S1¹³, on alumina); UV (EtOH): λ_{max} (logɛ): 258 nm (2.49); IR (CHCl₃): 1633 (C=O), 1545 cm⁻¹ (-CONH-); MS: 464 (28, M⁺, C₂₈H₄₀N₄O₂), 421 (70), 392 (2), 330 (4), 288 (11), 259 (11), 247 (8), 217 (5), 205 (4), 190 (6), 146 (16), 131 (16), 127 (6), 112 (25), 91 (45), 85 (59), 83 (100).
- 18. The crude alkaloid extract was treated with excess of phosgen (as CHCl₃ solution) in the presence of 0.5 M sodium phosphate buffer (pH 7) at 0°C for 30 min. After evaporation of the organic layer a small quantity of 70% EtOH was added. The verbaskine (6) crystallized as colourless plates with m.p. 120-122°C (lit. 120-123°C³), R_F 0.56 (S1¹³, on alumina); [α]_D²⁵ = -24° (c 1.92, CHCl₃) (lit. [α]_D²⁰ = -26°, CHCl₃³). From the mother liquor on silica gel with S1¹³ the compound 7 was isolated: colourless glass-like solid; [α]_D²⁵ = -58.5° (c 1.92, CHCl₃); R_F 0.52 (S1¹³, on alumina); UV (EtOH) λ_{max} (logε): 254 nm (4.11); IR (CHCl₃): 1650-1583 (C=O), 1556 cm⁻¹ (amide II); MS: 488 (M⁺, C₂₉H₃₆N₄O₃). Dihydroderivative 8: colourless glass-like solid; [α]_D²⁵ = -45.2° (c 1.92, CHCl₃); R_F 0.57 (S1¹³, on alumina); UV (EtOH) λ_{max} (logε): 258 nm (3.28); IR (CHCl₃): 1650 1590 (C=O), 1557 cm⁻¹ (amide II).
- 19. 9: Colourless glass like solid; $[\alpha]_D^{24} = +13.2^\circ$ (c 1.3, CHCl₃); R_F 0.58 (S1¹³, on alumina); UV (EtOH): λ_{max} (logɛ): 280 nm (4.33); IR (CHCl₃): 3300-3120 (NH assoc.), 1647 (C=O), 1595 (C=C), 1540 (-CONH-), 970 cm⁻¹ (CH=CH conjug.); MS: 474 (88, M⁺, C₂₉H₃₈N₄O₂), 473 (100), 388 (2), 369 (7), 343 (22), 229 (4), 188 (11), 146 (9), 139 (15), 131 (74), 103 (38), 98 (21), 91 (21), 84 (44).
- 20. **10:** Colourless glass-like solid; $[\alpha]_D^{25} = +6.38^{\circ}$ (c 1.6, CHCl₃); R_F 0.54 (S1¹³, on alumina); UV (EtOH): λ_{max} (log ϵ): 254 nm (4.05); IR (CHCl₃): 3300-3120 (NH assoc.), 1645 (C=O), 1607 (C=C), 1542 cm⁻¹(-CONH-); MS: 474 (M⁺, C₂₉H₃₈N₄O₂).
- 21. 11: Colourless glass-like solid; $[\alpha]_D^{25} = +8.64^\circ$; $R_F 0.59$ (S1¹³, on alumina); UV (EtOH) λ_{max} (log ϵ): 258 nm (2.58); IR (CHCl₃): 1638 (C=O), 1543 cm⁻¹ (amide II).

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