TENUILOBINE - A NEW POLYAMINE ALKALOID FROM *ONCINOTIS TENUILOBA*

Martin K.-H. Doll, Armin Guggisberg, and Manfred Hesse*

Dedicated in memory of the late professor Yoshio Ban

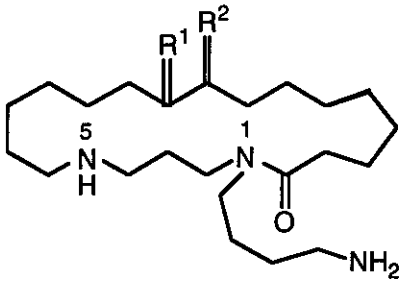
Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

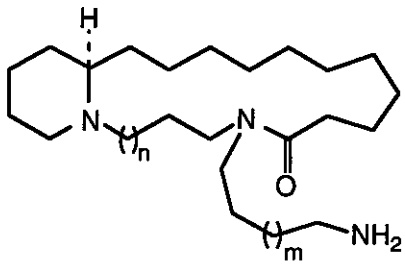
Abstract— A new cross-linked polyamine alkaloid, named tenuilobine (**1**), was isolated from the leaves of *Oncinotis tenuiloba* (Apocynaceae), the structure was established by spectroscopic means. It is the first natural alkaloid containing spermine and spermidine as polyamine parts. Presumably, compound (**1**) is involved in the biosynthesis of the macrocyclic spermidine alkaloids with lactam groups.

Our previous studies on the Apocynaceae *Oncinotis tenuiloba* Stapf have resulted in the isolation and structure elucidation of the macrocyclic lactam alkaloids inandenin-12-one (**2**), inandenin-13-one (**3**), and their corresponding reduction products containing secondary alcohol groups.[1, 2] The closely related bicyclic alkaloid oncinotine (**4**), together with its isomer neooncinotine (**5**), could be identified by co-hplc with authentic samples.[3]

Furthermore, another side alkaloid was isolated from the leaves of *O. tenuiloba* and could be identified as N^4-benzoylspermidine.[4]

Further investigation of the extracts lead to the isolation of tenuilobine (**1**), an unusual cross-linked polyamine alkaloid, containing spermidine as well as spermine. This paper deals with the isolation and structure elucidation of **1** and its biogenetic relation to the macrocyclic alkaloids in the plant.





2 $R^1 = O$ $R^2 = H_2$

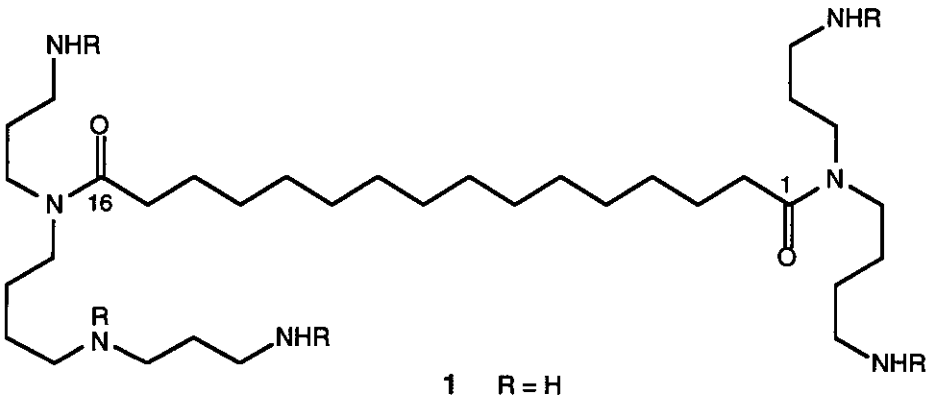
3 $R^1 = H_2$ $R^2 = O$

4 n = 1 m = 1

5 n = 2 m = 0

Tenuilobine (**1**) was isolated as a colorless oil. In ESIms[5] a methanolic solution of the pentahydrochloride showed $[M+1]^+$ at m/z 599.2, indicating a molecular weight of 598.2.[6] Substitution of the solvent by CD_3OD-D_2O (9:1) for ESIms examination revealed the exchange of nine hydrogen atoms by deuterium. Peracetylation (acetic anhydride/sodium acetate) of **1** lead to the introduction of five acetyl groups (ESIms: m/z 831.3 for $[M+Na]^+$) and the acetylation product (**6**) still exchanged four hydrogen atoms by deuterium, when ESIms was performed in the above deuterated solvent mixture. Since no acetyl group could be removed by treating **6** in CH_3OH-HCl (saturated) at room temperature, one has to exclude the presence of hydroxyl groups in the original molecule; consequently acetylation proceeded exclusively at amino groups. Tertiary amino functions are absent as demonstrated by the neutral character of the acetylation product. Furthermore, four of the five introduced CH_3CO substituents gave rise to one single signal (δ 1.92 ppm) in the ^{1}H-nmr spectrum of the acetyl derivative (**6**). Therefore we proposed the presence of four primary and one secondary amino groups in **1**, being consistent with the above findings and the high polarity of the alkaloid on tlc ($CHCl_3$-CH_3OH-25% aq. NH_4OH, 7:4:2; R_f 0.35). This was further supported by the ^{1}H-nmr spectrum of **1**, showing twelve protons in α–position to amino groups (-C*H*$_2$-NR_2), whereof eight protons evoked one single multiplet (δ 2.79 ppm), indicating a similar chemical environment of the methylene groups.

Additionally, only the presence of *N,N*-disubstituted amide is registered from the ir spectrum of **1** (ν 1625 cm^{-1}) which excludes other types of amido or keto groups. This observation is in agreement with the ^{1}H-nmr spectrum of **1**, showing two signals for four -C*H*$_2$-CO-NR_2 protons



1 R = H

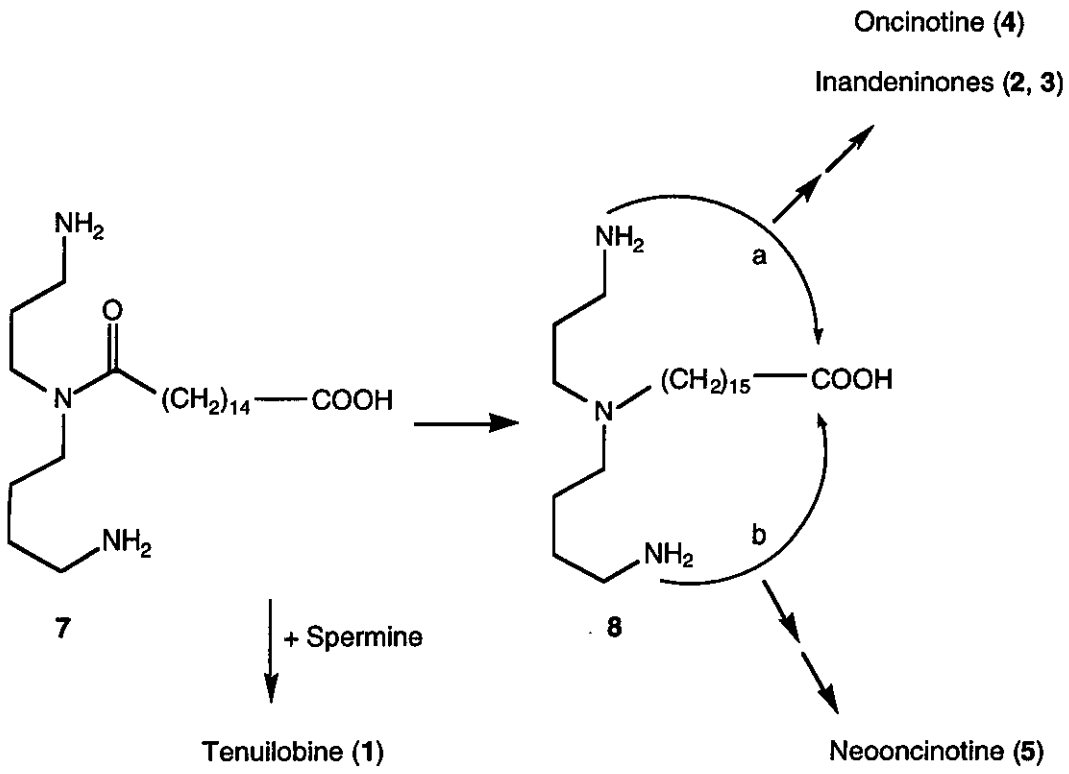
6 R = CH_3CO

(δ 2.50 and 2.45 ppm) and eight R-CO-N(CH_2-)$_2$ protons (δ 3.64 - 3.29 ppm) indicating the presence of totally two tertiary amido functions in the natural product. Including four primary and one secondary amino groups mentioned above, we propose that the alkaloid consists of one spermine and one spermidine unit, being connected at the secondary amino groups of each molecule by an alkanedioic acid. Since there were no other additional signals in the ^{1}H-nmr spectrum of **1** except for 38 protons at δ<1.90 ppm, we suggested the fatty acid to be 1,16-hexadecanedioic acid (the proton account is: 24 -CH_2-CH_2-CH_2-, 8 R-CO-N(CH_2-CH_2-)$_2$, and 6 R_2N-CH_2-CH_2-).

Therefore, tenuilobine (**1**) was identified as *N*^{1}-(4-aminobutyl)-*N*^{1},*N*^{16}-bis(3-aminopropyl)-*N*^{16}-{4-[(3-aminopropyl)amino]butyl}hexadecanediamide. For unambiguous confirmation of the proposed structure, we have synthesised this compound.[7] Recently a different procedure for the synthesis of symmetrically cross-linked polyamine derivatives has been published elsewhere.[8] The synthetic product was found to be identical with the natural alkaloid (**1**) in all respects, including spectroscopic and chromatographic properties. The pentaacetyl derivative of the natural product (**6**) and the corresponding synthetic sample were identical too.

Since tenuilobine (**1**), as well as the macrocyclic inandeninones (**2**) and (**3**), consists of a C_{16}-chain being linked with the corresponding polyamine portion, a biogenetic relationship can be taken into consideration. A common precursor, like the monoamide (**7**), may be reduced enzymatically to give the intermediate (**8**). Now, cyclisation (pathway a) and appropriate oxidation of the carbon-chain (introduction of the keto group) would result directly in the

Scheme: A possible biogenetic pathway for the major alkaloids of *O. tenuiloba*.



formation of **2** and **3**, respectively, bearing an exocyclic 4-aminobutyl group. In the case of oncinotine (**4**), the assumed oxidation process would lead to inandenin-10-one, which could be transformed into **4** *via* a second cyclisation process involving N^5 and the C-10 keto group. Analogously, the generation of neooncinotine (**5**) could be explained. Here, cyclisation would occur at the 4-aminobutyl group of the precurser (**8**) (pathway b), which is presumed to be a minor process, since oncinotine (**4**) is accompanied by neooncinotine (**5**) in only small amounts.[3, 9] It is also possible, that the dicarboxylic acid in **7** is unsaturated, allowing the formation of the keto group *via* an intermediate epoxide.

EXPERIMENTAL

General: Finnigan TSQ 700 mass spectrometer (ESIms); for ESImsms -25 eV and Ar (collision gas, 2.5 mtorr) were used. ^{1}H nmr: Bruker AMX 600 and Bruker ARX 300 with δ in ppm.

Isolation: In addition to the isolation procedure already described,[1] some modifications are given below. The crude extract (7.12 g) was dissolved in 600 ml of *tert*-amyl alcohol saturated with H_2O, and the solution was extracted with 1 N aq. NaOH (5 x 60 ml) to remove flavonoids; phase separation was improved by centrifugation. After evaporation of the alcohol *in vacuo*, the residue was co-evaporated with diluted CH_3OH-HCl leaving the crude hydrochlorides (1.28 g).

Separation: Chromatography of the crude hydrochlorides (silica gel; 100 g, 40-60 μ) afforded seven fractions of increasing polarity: F 1 (113.3 mg, compare fr. A[1]), F 2 (10.3 mg), F 3 (178.7 mg, fr. B[1]), F 4 (70.5 mg), F 5 (172.3 mg, fr. C[1]), F 6 (69.4 mg, fr. D[1]), F 7 (122.4 mg). Elution was performed using $CHCl_3$-CH_3OH-25% aq. NH_4OH (50:12:3) and polarity was successively increased until the ratio of solvents was 7:4:2. All materials were converted into their hydrochlorides as described above.

Tenuilobine = N^1-*(4-Aminobutyl)*-N^1,N^{16}-*bis(3-aminopropyl)*-N^{16}-*{4-[(3-aminopropyl)-amino]butyl}hexadecanediamide}* (1), $C_{33}H_{71}N_7O_2$: Chromatography of fraction F 6 (silica gel; 5 g, 15-25 μ) using $CHCl_3$-CH_3OH-25% aq. NH_4OH (7:3:0.85) resulted finally in 32 mg of tenuilobine pentahydrochloride (**1** · 5 HCl) as colorless oil. ESIms (CH_3OH) m/z: 599.2 ($[M+1]^+$); ESIms (CD_3OD-D_2O, 9:1) m/z: 608.6 ($[M_D+2]^+$); ESImsms (of m/z 599.2) m/z (%): 599.2 ($[M+1]^+$, 100), 581.0 (65), 525.1 (44), 509.9 (29), 453.7 (38), 436.6 (51), 382.3 (30), 365.7 (41), 307.3 (26), 129.0 (23), 112.4 (32), 72.2 (21); ir ν (free base, $CHCl_3$, cm^{-1}): 2990*m*, 2925*s*, 2850*s*, 1625*s*, 1460*m*; ^{1}H-nmr δ (free base, pyridine-d_5, 600 MHz, ppm): 4.21 (br, -NH_2), 3.64 (2 H, *q-like m*), 3.51 (2 H, q-like m), 3.44 (2 H, q-like m), 3.29 (2 H, quint-like m), 2.95 (2 H, m), 2.79 (8 H, m), 2.66 (2 H, m), 2.50 (2 H, t, J=6.5 Hz), 2.45 (2 H, q-like m), 1.85 - 1.62 (14 H, m), 1.58 - 1.27 (24 H, m).

N^1-*(4-Acetamidobutyl)*-N^1,N^{16}-*bis(3-acetamidopropyl)*-N^{16}-*{4-[(3-acetamidopropyl)-(acetyl)amino]butyl}hexadecanediamide}* (6): A mixture of **1** · 5 HCl (15 mg), Ac_2O (10 ml) and NaOAc (250 mg, freshly molten and powdered) was stirred at room temperature for 42 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in H_2O (5 ml). After basification with 5 % aq. $NaHCO_3$, the solution was extracted with $CHCl_3$. Evaporation of the organic layer gave 13.1 mg of **6**. ESIms (CH_3OH) m/z: 831.3 ($[M+Na]^+$);

ESIms (CD_3OD-D_2O, 9:1) m/z: 835.4 ($[M_D+Na]^+$); ir ν ($CHCl_3$, cm^{-1}): 3450*w*, 2990*m*, 2920*s*, 2850*m*, 1660*s*, 1625*s*, 1525*m*, 1435*m*, 1370*m*; ^{1}H-nmr δ ($CDCl_3$, 300 MHz, ppm) : 3.35 - 3.05

(20 H, m), 2.26 - 2.18 (4 H, m), 2.06 - 2.02 (3 H, m, CH_3CO-), 1.92 (12 H, s, 4 x CH_3CO-), 1.71 (2 H, m), 1.61 - 1.47 (16 H, m), 1.22 - 1.19 (20 H, m). Hplc (MN Nucleosil® 100 - 7 C_{18}, CH_3OH-H_2O 6:4, 1.5 ml/min; detection at 225 nm): R_t 14.8 min.

Attempted Solvolysis of 6: A solution of **6** (1 mg) in abs. CH_3OH (10 ml) was saturated with HCl gas. After stirring over night at room temperature, the solvent was evaporated *in vacuo* and the residue was taken up in H_2O (3 ml). The solution was basified (saturated aq. $NaHCO_3$), extracted with $CHCl_3$, and the organic layer was evaporated *in vacuo*. ESIms (CH_3OH+NaBr) m/z (%): 831.3 ($[M+Na]^+$, 51), 415.8 ($[M+Na+1]^{2+}$, 100).

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the *Swiss National Science Foundation*. Thanks are extended to Mr. Laurant Bigler for measuring ESIms, Mr. Daniel Rentsch and Dr. Daniel Nanz for recording ^{1}H-nmr spectra (600 MHz).

REFERENCES AND NOTES

1. M. K.-H. Doll, A. Guggisberg, and M. Hesse, *Phytochemistry*, 1995, in press.
2. Inandeninones from *Oncinotis inandensis* Wood et Evans: H. J. Veith, M. Hesse, and H. Schmid, *Helv. Chim. Acta*, 1970, **53**, 1355.
3. M. K.-H. Doll, A. Guggisberg, and M. Hesse, unpublished results.
4. M. K.-H. Doll, A. Guggisberg, and M. Hesse, *Helv. Chim. Acta*, 1994, **77**, 1229.
5. All attempts to measure compound (**1**) under EIms or CIms (NH_3) conditions were unsuccessfull.
6. Because of its atomic composition ($C_{33}H_{71}N_7O_2$) an odd mass number of the molecular ion (m/z 597) is expected. The most intense signal in the molecular ion region is found at m/z 599.2, representing the first isotope peak of the protonated molecular ion.
7. M. K.-H. Doll, A. Guggisberg, and M. Hesse, *Tetrahedron*, in preparation.
8. G. Sosnovsky and J. Lukszo, *Z. Naturforsch.*, 1994, **49b**, 1580.
9. A. Guggisberg. M. M. Badawi, M. Hesse, and H. Schmid, *Helv. Chim. Acta*, 1974, **57**, 414.

Received, 27th March, 1995