83. Oncinotine-Type Spermidine Alkaloids from *Oncinotis tenuiloba*

Transformation of *N*-Acetyloncinotin-12-one to *N,N'*-Diacetylinandenin-12-one

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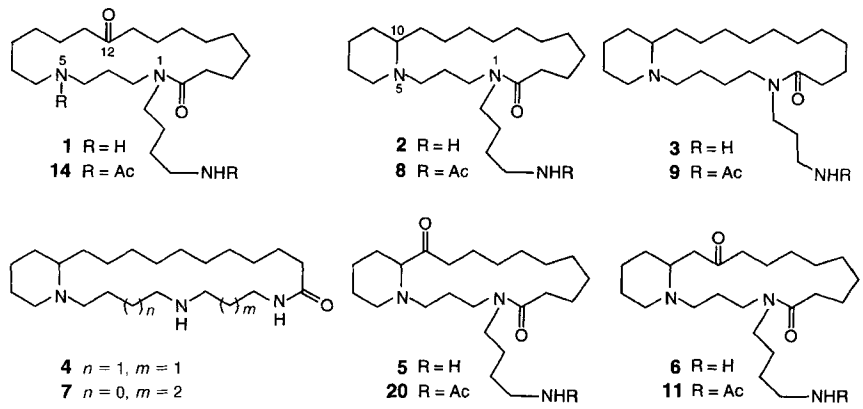
From extracts of *Oncinotis tenuiloba* STAPF, two novel polyamine alkaloids, oncinotin-11-one (**5**) and oncinotin-12-one (**6**), were isolated. Peracetylation of **6** provided the *N*-acetyl derivative **11** as well as *N,N'*-diacetylinandenin-10-en-12-one (**12**) due to a β-elimination-type side reaction resulting in ring enlargement of **11** (*Scheme 1*). Deuteration of **12** yielded **13**, showing the same retention time as *N,N'*-diacetylinandenin-12-one (**14**), when co-HPLC was performed together with different keto-isomeric *N,N'*-diacetylinandeninones. Structure elucidation was extended by *Schmidt* degradation of **6** and *N,N'*-diacetyl(10,11-$^2H_2$)inandenin-12-one (**13**); the degradation products were identified by GC and ESI-MS. The structure of **5** was proposed on the basis of spectroscopic means. Comparison of the spectroscopic data of **5** with those obtained from synthetic material as well as co-HPLC of the *N*-acetyl derivative **20** together with the corresponding synthetic compound revealed the identity of the substances and confirmed the structure of **5**. Additionally, oncinotine (**2**) and neooncinotine (**3**) were isolated, separated, and identified with authentic samples by co-HPLC of their *N*-acetyl derivatives **8** and **9**, respectively.

1. Introduction. – In earlier investigations of plants belonging to the genus *Oncinotis* (Apocynaceae), several polyamine alkaloids have been isolated. The major alkaloids of *O. inandensis* WOOD *et* EVANS [1] and *O. tenuiloba* STAPF [2] have been found to be inandenin-12-one (**1**)[1]) and inandenin-13-one, two isomeric macrocyclic oxo lactams which are also found in the leaves of *O. nitida* BENTH. [3]. In general, the corresponding secondary alcohols (inandeninols) occur as minor constituents [4]; from the leaves *O. nigra* PICH. [5], they have been isolated as the main constituents. A closely related bicyclic macrolactam alkaloid, oncinotine (**2**), has been isolated as the major alkaloid from the stem bark of *O. nitida*, accompanied by small amounts of the isomeric neooncinotine (**3**) and isooncinotine (**4**) [6]. Preliminary investigations revealed evidence for the presence of a monohydroxylated structural analog of **2**, hydroxyoncinotine, but structure elucidation was not complete due to the small amount of the isolated alkaloid [4]. Although the occurrence of acyclic polyamine derivatives has been established, at least for *O. tenuiloba* [7] [8], the predominant alkaloids of *Oncinotis* plants can be divided into two basic structural types of macrolactams: the inandenines and the oncinotines.

To obtain more information about a possible biogenetic relationship between the inandeninones and the oncinotines, the search for further examples of related alkaloids is of considerable importance. In continuation of this research, we investigated the leaves of *O. tenuiloba*. Extraction and distribution of the crude alkaloid mixture into fractions of

[1]) Atom numberings and names used in the text and *Schemes* are in accordance with earlier publications; for systematic names, *cf. Exper. Part* and references given for the compounds.



different polarities resulted in crude fraction *Fl* [7] representing the least polar fraction among others (compared to fraction *A* in [2]). Earlier TLC investigations revealed the presence of at least three compounds.

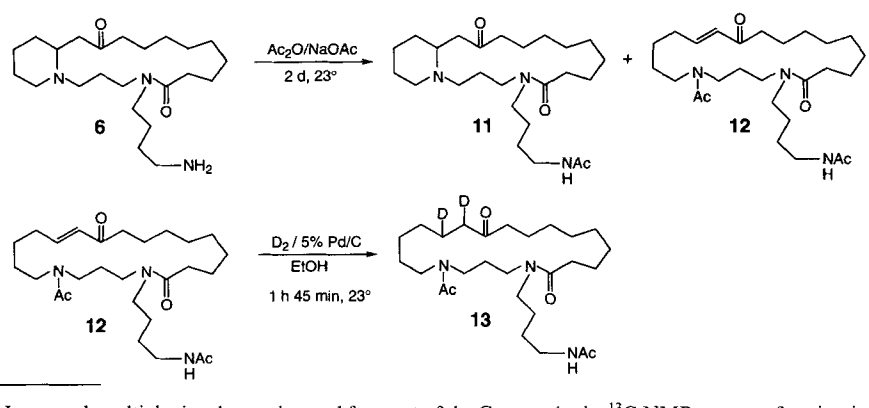
2. Results and Discussion. – Flash chromatography of *Fl* furnished oncinotin-11-one (**5**) and oncinotin-12-one (**6**), two novel polyamine alkaloids combining structural features of the inandenines as well as of the oncinotines. Besides, a third fraction (F_c), consisting of a mixture of oncinotine (**2**) and neooncinotine (**3**), was obtained. In contrast to **5** and **6**, preliminary co-TLC could not differentiate fraction F_c from synthetic oncinotine (**2**). The suggestion of **2** being the major constituent of F_c was further supported by comparison of the corresponding ESI-MS/MS spectra, with the molecular ion peak at *m/z* 380 and an almost identical fragmentation pattern with the base peak at *m/z* 309 ($[(M+1)-C_4H_{10}N]^+$) and a corresponding signal (*m/z* 72, $[C_4H_{10}N]^+$), attributed to radical cleavage of the 4-aminobutyl portion in oncinotine (**2**). An additional signal (*m/z* 58, $[C_3H_8N]^+$) in the spectrum of F_c indicated the presence of the isomeric neooncinotine (**3**), since an analogous fragmentation at N^1 leads to the cleavage of the 3-aminopropyl portion.

To confirm the above findings and to determine the relative amounts of **2** and **3** in the natural extract, F_c was acetylated (Ac_2O/NaOAc) to give a mixture of the expected *N*-Ac derivatives **8** and **9**, respectively, readily separable by HPLC (*RP-18*; MeCN/citrate buffer 3:7, 1.5 ml/min). Subsequent co-chromatography of the acetylated mixture F_c together with the *N*-Ac derivatives **8** and **9** of synthetic oncinotine and neooncinotine [9], respectively, clearly demonstrated the presence of *N*-acetyloncinotine (**8**; t_R 9.64 min) and *N*-acetylneooncinotine (**9**; t_R 7.09 min) in the acetylated natural extract. Integration revealed that the ratio **8**/**9** was *ca.* 2.4:1, reflecting the relative amounts of the alkaloids **2** and **3** in the original fraction F_c. It should be noted that the ratio **2**/**3** in *O. tenuiloba* is about the same as already determined for *O. nitida*, based on EI-MS investigations of chemical degradation products of the corresponding mixture **8**/**9** (7:3) [6]. In contrast to *O. nitida*, isooncinotine (**4**) could not be detected in the leaves of *O. tenuiloba* when co-chromatography of acetylated F_c was performed together with the corresponding *N*-Ac derivative. Analogously, the fully synthetic isomer pseudooncinotine (**7**) [9] could be detected neither.

For the structure elucidation of oncinotin-12-one (**6**), the ^{13}C-NMR spectra of the dihydrochloride were most useful. At higher frequencies, signals were observed at 208 and 174 ppm indicating the presence of a C=O group and a *N*,*N*-disubstituted carboxamide moiety, respectively, in **6**. This was further supported by the IR spectrum of **6**·2 HCl, showing absorptions at 1712 and 1630 cm^{-1}. Further DEPT experiments demonstrated a methine C-signal (58 ppm[2])) along with further resonances indicating CH_2 groups, as observed for oncinotine (**2**)·2 HCl (65 ppm, C(10) [10]). Since the ESI-MS revealed a molecular weight of 393 (equivalent to *M*(**2**) + 14 amu), we considered **6** as an oxo derivative of oncinotine (**2**). Furthermore, an ESI-MS/MS of **6** showed a fragment ion at *m*/*z* 72 (29%), as expected from the cleavage of the terminal 4-aminobutyl portion at N^1-atom. Since no fragment ion with *m*/*z* 58 could be detected, the corresponding *neo*-isomer (analogously to **3**) was excluded to accompany **6** as a minor constituent of the original fraction. The additional presence of the ring-enlarged isomer of **6**, analogously to isooncinotine (**4**), could also be excluded, since no absorptions accounting for *N*-monosubstituted carboxamides were observed in the IR spectrum of **6**·2 HCl.

Acetylation of **6** using Ac_2O/NaOAc led to the formation of the corresponding *N*-Ac derivative **11**, accompanied by a considerable amount of the less polar nonbasic byproduct **12** (*Scheme 1*), which showed the typical color reaction of carboxamides on TLC when sprayed with *Schlittler* reagent [11] and Ce^{4+} [12]. Moreover, it was found that prolongation of the reaction time favored the formation of **12**. The absence of basic N-atoms in **12** was supported by the MS and NMR spectra, demonstrating the introduction of a second Ac group. Furthermore, no methine C-signal was found in the DEPT-NMR spectrum, suggesting the cleavage of the N(5)–C(10) bond in **6** during acetylation and thus leading to an inandeninone-type structure in **12**. The UV spectrum of the byproduct displayed a shoulder at 226 nm, a region where for α,β-unsaturated aliphatic ketones are expected to show an UV maximum. This was in agreement with the ^{13}C-NMR spectrum of **12**, showing signals at 147 and 131 ppm, as well as a shift to higher field for the C=O signal (201 ppm; compared to 210 ppm in **11**).

Scheme 1



[2]) In general, multiple signals are observed for most of the C-atoms in the ^{13}C-NMR spectra of oncinotine-type compounds. This is probably due to s-*cis*/s-*trans*-isomerization of the carboxamide portion. For the discussion in the text, only the most intense signals are selected. Protonation at N(5) results in a downfield shift of the C(10) signal by 3–4 ppm.

Deuteration (D_2, 5% Pd/C) of **12** led to the corresponding saturated ketone **13**, which could not be distinguished from *N,N'*-diacetylinandenin-12-one (**14**) or *N,N'*-diacetylinandenin-13-one on TLC; moreover, the spectroscopic data of **13** closely paralleled those established for **14** and its 13-oxo isomer [2] [13]. Correlations on HPLC of **13** with **14** and the 13-oxo and 10-oxo isomers of *N,N'*-diacetyl-inandeninones [10] resulted in identical retention times only for **13** and **14**. Consequently, **13** can be classified as an isotopomeric *N,N'*-diacetylinandenin-12-one.

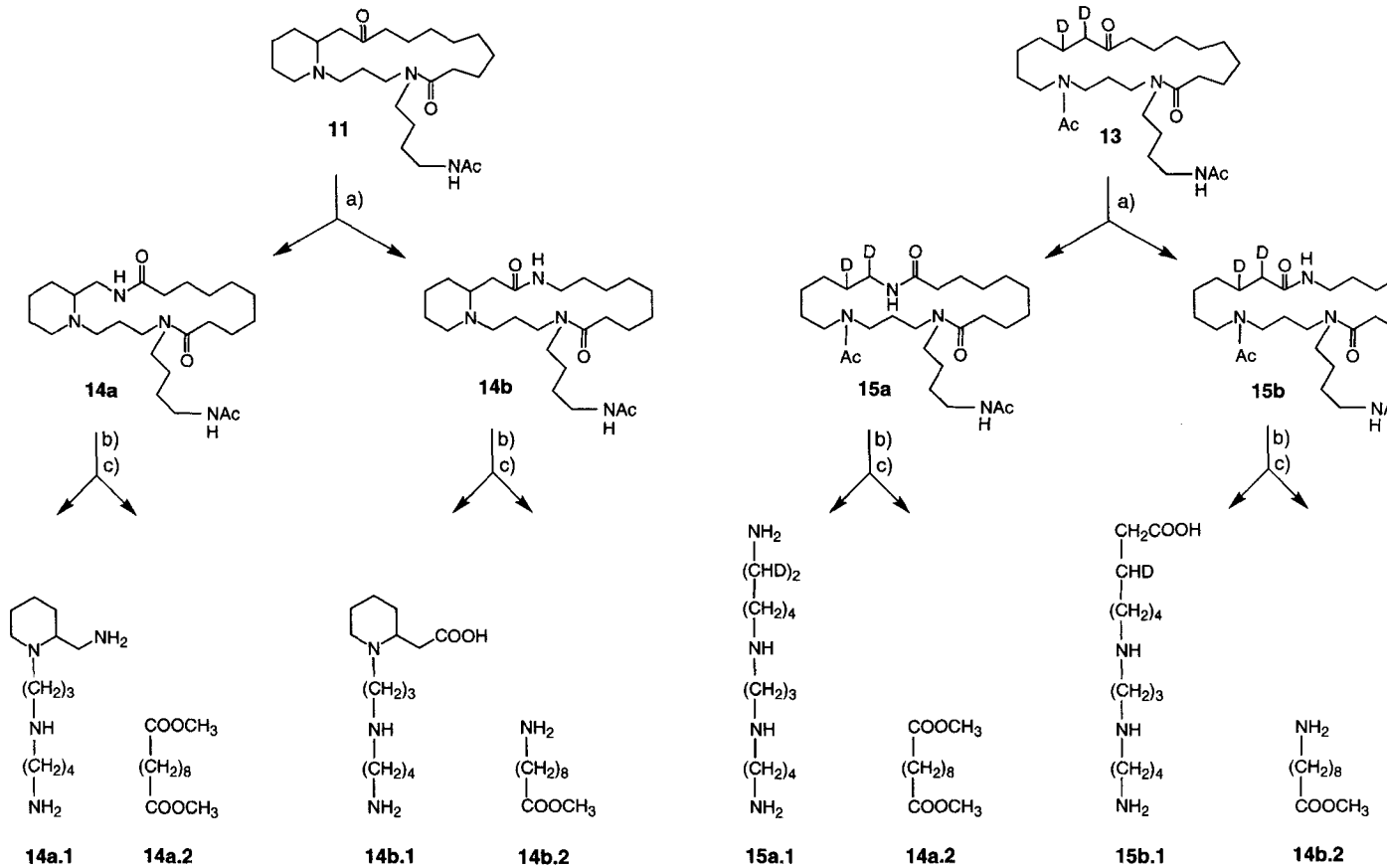
To determine the position of the oxo group in the original alkaloid **6**, the *N*-Ac derivative **11** as well as the deuterated by-product **13** were subjected to a chemical degradation (*Scheme 2*). A *Schmidt* reaction [14] [15], followed by acid-catalyzed hydrolysis of the resulting dilactam mixtures **14a**/**14b** (from **11**) and **15a**/**15b** (from **13**) gave four degradation products for each starting compound. Extraction of the acidic reaction mixtures with Et_2O, followed by treatment of the organic layer with CH_2N_2/Et_2O, provided the ethereal solutions E-1 (degradation of **11**) and E-2 (of **13**), containing the corresponding dimethyl dioates. The N-containing degradation products of **11** and **13** were collected by evaporation of the remaining aqueous layers to give the mixtures W-1 and W-2, respectively.

GC Analysis of E-1 and E-2 revealed dimethyl decanedioate (**14a.2**) to be the only component in both ethereal solutions. Considering the original compounds **11** and **13**, an identical distance of eight CH_2 units between the oxo group and the lactam C=O is encountered, as it has already been expected for **13**. Consequently, the natural alkaloid was identified as oncinotin-12-one (**6**). A CI-MS examination of E-2 demonstrated that no D-atom was incorporated into **14a.2**. Accordingly, the position of the C=C bond in the original acetylation by-product **12** had to be between C(10) and C(11), thus providing *N,N'*-diacetyl(10,11-$^2H_2$)inandenin-12-one (**13**) on reduction with D_2/Pd. This conclusion was supported by the detection of 8-aminononanoic acid (**14b.2**) together with the corresponding deuterated degradation products **15a.1** and **15b.1** in W-2 (ESI-MS). It should be noted that most of the deuterium in α-position to the COO group in **15b.1** was exchanged by H during the acid-catalyzed hydrolysis of the dilactam **15b** as indicated in *Scheme 2*. In addition, the expected degradation products **14a.1**, **14b.1**, and **14b.2** were detected by EI-MS in the extract *W1*.

The formation of *N,N'*-diacetylinandenin-10-en-12-one (**12**) can be considered as a β-elimination-type process (or *retro-Michael*-type reaction) during acetylation of **6**. This ring-enlargement reaction not only provides a link between oncinotines and inandenines, but also seems to be a useful tool in the synthesis of macroheterocycles. Further investigations of this reaction using methyl 10-oxo-11-(1-ethylpiperidin-2-yl)undecanoate (**18**) as a model compound confirmed the above findings (*Scheme 3*; for the synthesis of **16**, *cf.* [9]). Treatment of **18** with Ac_2O/NaOAc at 23° for 1.5 d resulted in *ca.* 50% of the enone **19**, which was obtained in almost quantitative yield by elevation of the temperature (60°) and prolongation of the reaction time. The configuration of the C=C bond in **12** and **19** was found to be (*E*) by ^{1}H-NMR spectroscopy. Interestingly, in a preliminary communication *Trost* and *Cossy* mentioned the corresponding ring-closure reaction of a *N,N'*-diprotected inandenin-10-en-12-one leading to *N*-acetyloncinotin-12-one (**11**) but, unfortunately, they disclosed no experimental details [16].

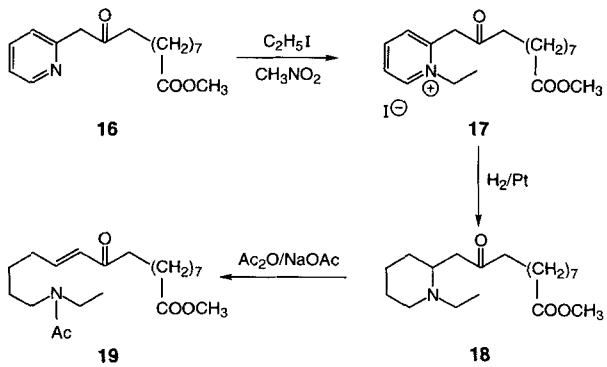
The structure of the isomeric oncinotin-11-one (**5**) was deduced by spectroscopic means. ESI-MS/MS Investigations revealed a molecular weight of 393 and a fragment ion

Scheme 2



a) NaN_3/H_2SO_4. b) 2N aq. HCl, 150°. c) Org. layer: CH_2N_2/Et_2O.

Scheme 3



at *m/z* 72, indicating the loss of a terminal 4-aminobutyl portion. Signals assigned to a keto group (213 ppm), a *N,N*-disubstituted carboxamide moiety (173 ppm) and a methine C-atom (74 ppm) were evident in the ^{13}C-NMR spectrum of the free base, similar as observed for **6**·2 HCl. Due to the shift to higher frequencies, observed for the resonances of the keto group and the methine C-atom, when compared to those in **6**·2 HCl, the keto function in **5** was suggested to be located at C(11). Additional confirmation was provided by the IR spectrum of **5**·2 HCl, presenting absorptions of the *N,N*-disubstituted carboxamide moiety (1630 cm^{-1}) as well as of the keto group (1725; compared to 1712 cm^{-1} for CO in the IR of **6**·2 HCl).

Whereas the small amount of the isolated alkaloid prevented a chemical degradation of **5**, its structure could be corroborated through a total synthesis of the corresponding racemate[3]) (for detailed spectroscopic data *cf.* [17]). Co-chromatography (RP-HPLC) of the *N*-Ac derivative **20** together with the corresponding synthetic compound revealed identical retention times supporting the above finding.

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Experimental Part

General. Flash chromatography (FC): *Merck, LiChroprep Si 60* (15–25 μm). UV (λ_{max} [nm]): *Perkin-Elmer 555* spectrophotometer. Citrate buffer: Citric acid (7.697 g), NaOH (0.840 g), NaCl (3.510 g) *ad* 1000 ml (H_2O); 1:10 concentration was used. The HPLC system was connected with an UV detector (225 nm). IR (cm^{-1}): *Perkin-Elmer 781.* NMR Spectra: *Bruker AMX-600, Bruker AM 400*, and *Bruker ARX 300*; chemical shifts in δ (ppm) and coupling constants *J* in Hz, using the appropriate solvent as internal standard. Unless otherwise noted, ^{1}H-NMR spectra were recorded at 300.1 MHz and ^{13}C-NMR spectra at 75.5 MHz in $CDCl_3$; multiple signals observed for the same C-atom in the ^{13}C-NMR spectra are due to different diastereoisomers resulting from *s-cis*/*s-trans*-isomerization of carboxamide linkages. CI- and EI-MS: *Finnigan MAT 90*; 70 eV (EI), NH_3 (CI). ESI-MS: *Finnigan TSQ 700* mass spectrometer; for MS/MS (–25 eV), Ar (2.5 mtorr) was used as collision gas.

Extraction and Separation. The original isolation procedure has been already described in [2] (for a later modification, see [7]). Reproducing the modified procedure with 1.3 kg of dried leaves of *Oncinotis tenuiloba* yielded 121.5 mg of crude fraction *F1* (equivalent to 113.3 mg of *F1* in [7]). FC of *F1* (silica gel, 10 g; $CHCl_3$/MeOH/25% aq. NH_3 soln. 85:14:1) resulted in three fractions, which were converted into their hydrochlorides by co-evaporation with dil. MeOH/HCl. For TLC monitoring (R_f), $CHCl_3$/MeOH/25% aq. NH_3 soln. (78:19:3) was used.

[3]) Unfortunately, we cannot give details on the optical rotation of the natural alkaloid.

5-(4-Aminobutyl)-1,5-diazabicyclo[15.4.0]henicosane-6,16-dione (= *Oncinotin-11-one*; **5**). Viscous oil (8.4 mg). R_f 0.51. IR ($CHCl_3$; **5**·2 HCl): 3360*m*, 2930*s*, 2860*s*, 1725*m*, 1630*m*, 1460*m*, 1375*w*, 1085*w*. ESI-MS (MeOH; **5**·2 HCl): 394.5 (100, $[M + 1]^+$), 197.8 (12, $½[M + 2]^{2+}$), 188.0 (36). ESI-MS/MS (of *m/z* 394.3): 394.5 (45, $[M + 1]^+$), 377.1 (15), 322.9 (100), 310.3 (14), 305.5 (60), 276.9 (13), 266.5 (18), 248.4 (31), 239.7 (20), 72.5 (67). For additional spectroscopic data, see [17].

5-(4-Aminobutyl)-1,5-diazabicyclo[15.4.0]henicosane-6,15-dione (= *Oncinotin-12-one*; **6**). Viscous oil (22.7 mg). R_f 0.42. IR ($CHCl_3$; **6**·2 HCl): 3370*m*, 2930*s*, 2860*s*, 1712*m*, 1630*m*, 1460*m*, 1375*w*, 1220*w*, 1085*w*, 1015*w*. ^{1}H-NMR (MeOH; **6**·2 HCl): 3.55–2.81 (*m*, 13 H); 2.63–2.18 (*m*, 4 H); 2.13–1.58 (*m*, 16 H); 1.27–1.12 (*m*, 8 H). ^{13}C-NMR (MeOH; **6**·2 HCl; diastereoisomeric carboxamides): 207.94, 207.71, 207.27, 206.84 (4*s*, CO); 174.23, 173.89, 173.63 (3*s*, CON); 58.16, 57.89, 55.82, 54.77 (4*d*, CH); 52.22, 50.03, 49.69, 49.56, 48.96, 48.77, 44.07, 43.83, 43.36, 42.35, 41.37, 40.89, 40.37, 38.31, 38.06, 35.76, 31.72, 30.44, 28.66, 28.30, 28.20, 28.01, 26.45, 26.25, 26.03, 25.75, 25.58, 25.43, 25.16, 25.04, 24.84, 24.50, 24.40, 23.41, 23.11, 22.56, 22.38, 21.89, 21.57, 21.48, 20.90, 20.47, 19.47, 19.22, 17.65, 17.24 (4*t*, 20 CH_2). ESI-MS (MeOH; **6**·2 HCl): 394.2 (100, $[M + 1]^+$), 197.7 (15, $½[M + 2]^{2+}$), 189.1 (8). ESI-MS/MS (of *m/z* 394.2): 393.9 (46, $[M + 1]^+$), 322.7 (5), 311.4 (38), 305.0 (5), 239.9 (100), 182.9 (11), 98.5 (6), 84.6 (8), 72.8 (29).

Fraction F_c*: Natural Mixture of 5-(4-Aminobutyl)-1,5-diazabicyclo[15.4.0]henicosan-6-one* (= *Oncinotine*; **2**) *and 6-(3-Aminopropyl)-1,6-diazabicyclo[16.4.0]docosan-7-one* (= *Neooncinotine*; **3**). Viscous oil (5.2 mg). R_f 0.32. ESI-MS (MeOH; **2**/**3**·2 HCl): 380.4 (100, $[M + 1]^+$), 190.7 (24, $½[M + 2]^{2+}$). ESI-MS/MS (of *m/z* 380.4): 380.2 (71, $[M + 1]^+$), 363.4 (15), 335.4 (4), 309.1 (100), 291.6 (14), 251.5 (9), 130.9 (6), 112.2 (5), 98.6 (9), 83.9 (8), 72.1 (45), 58.2 (7).

Data of **2**·*2 HCl* (synthetic). ESI-MS (MeOH; **2**·2 HCl): 380.3 (100, $[M + 1]^+$), 190.8 (80, $½[M + 2]^{2+}$). ESI-MS/MS (of *m/z* 380.2): 380.1 (49, $[M + 1]^+$), 309.0 (100, $[(M + 1) - C_4H_{10}N]^+$), 291.6 (8), 252.4 (6), 130.5 (5), 98.3 (4), 72.2 (42, $[C_4H_{10}N]^+$). For additional spectroscopic data, see [10].

Acetylation of Fraction F_c*: Mixture of 5-(4-Acetamidobutyl)-1,5-diazabicyclo[15.4.0]henicosan-6-one* (= N-*Acetyloncinotine*; **8**) *and 6-(3-Acetamidopropyl)-1,6-diazabicyclo[16.4.0]docosan-7-one* (= N-*Acetylneooncinotine*; **9**). A mixture of **2**/**3**·2 HCl (4.5 mg) and anh. NaOAc (50 mg; freshly molten and powdered) in Ac_2O (3.0 ml) was stirred for 24 h at 23°. The excess of Ac_2O was evaporated under reduced pressure (water bath, 50°), the residue was taken up in H_2O (1.5 ml) and basified with Na_2CO_3. Extraction with $CHCl_3$, drying (Na_2SO_4), and evaporation left 5.2 mg of **8**/**9**.

HPLC Separation of the Mixture **8**/**9** (*MN Nucleosil 100 7* C_{18}; MeCN/citrate buffer (3:7, 1.5 ml/min)). The compounds were identified by co-injection of the mixture **8**/**9** together with pure, synthetic samples [9]. t_R (**9**) 7.09 min, and t_R (**8**) 9.64 min. Integration revealed a ratio of 1:2.4 for **9**/**8**. Analogously, *isooncinotine* (**4**) could not be detected in the natural sample (t_R 5.86 min for the *N*-Ac derivative of **4**); *pseudooncinotine* (**7**) was also not present (t_R 6.13 min for its *N*-acetyl derivative).

Acetylation of **6**: *Mixture of 5-(4-Acetamidobutyl)-1,5-diazabicyclo[15.4.0]henicosane-6,15-dione* (= N-*Acetyloncinotin-12-one*; **11**) *and (*E*)-5-(4-Acetamidobutyl)-1-acetyl-1,5-diazacyclohenicos-16-ene-6,15-dione* (= N,N′-*Diacetylinandenin-10-en-12-one*; **12**). The reaction was carried out as already described for the mixture **2**/**3**, except that the reaction mixture was stirred for 48 h. From 17.0 mg of **6**·2 HCl, 21.1 mg of crude material were obtained. FC (silica gel, 4.50 g) yielded **12** (4.9 mg, yellowish lac; elution with $CHCl_3$/MeOH 9:1) and **11** (3.3 mg, yellowish lac; elution with $CHCl_3$/MeOH/25% aq. NH_3 soln. 9:1:0.5).

HPLC Separation of the Crude Mixture **11**/**12** (*MN Nucleosil 100 7* C_{18}; MeCN/citrate buffer (35:65, 1.5 ml/min)). t_R (**11**) 2.70 min, t_R (**12**) 6.07 min.

Data of **11**. IR ($CHCl_3$): 3450*w*, 3000*m*, 2940*s*, 2860*m*, 1715*m*, 1670*s*, 1630*s*, 1515*w*, 1460*w*, 1370*w*, 1260*w*, 1100*w*. ^{1}H-NMR (600.1 MHz): 3.26–3.10 (*m*, 3 CH_2NCO); 2.72–2.68 (*m*, CH_2CO); 2.42–2.20 (*m*, 9 H); 1.92–1.91 (*m*, Me); 1.66–1.18 (*m*, 24 H). ^{13}C-NMR (150.9 MHz; diastereoisomeric carboxamides): 210.36 (*s*, CO); 173.29, 173.04 (2*s*, CON); 170.22 (*s*, Me*C*O); 55.04 (*d*, CH); 51.22, 50.44, 47.98, 46.62, 45.02, 44.66, 43.76, 42.99, 42.43, 39.19, 39.10, 32.69, 32.23, 30.73, 29.71, 27.53, 27.50, 27.31, 27.18, 26.87, 26.76, 26.68, 26.06, 25.77, 25.31, 24.81, 24.58, 24.25, 23.51, 23.32, 22.32 (31 signals, 20 CH_2, 1 Me). ESI-MS (MeOH): 894.3 (20, $[2M + Na]^+$), 474.5 (13, $[M + K]^+$), 458.5 (100, $[M + Na]^+$). EI-MS: 435.5 (5, $M^{+\cdot}$), 417.4 (4), 378.5 (7), 363.5 (3), 321.5 (4), 295.6 (8), 252.5 (4), 223.4 (5), 194.3 (4), 180.2 (4), 171.3 (5), 166.3 (6), 152.3 (9), 143.2 (14), 139.2 (17), 138.3 (16), 124.2 (34), 123.2 (100), 114.2 (9), 111.2 (25), 110.2 (75), 97.2 (48), 96.2 (54), 84.2 (48), 82.2 (28), 72.2 (37), 70.2 (65), 56.2 (17), 55.2 (46).

Data of **12**. UV (MeOH): 226 (slight sh). IR ($CHCl_3$): 3450*w*, 3000*m*, 2940*s*, 2865*m*, 1670*s*, 1635*s*, 1520*w*, 1465*m*, 1430*m*, 1370*m*, 1240*m*, 1090*w*. ^{1}H-NMR (600.1 MHz; diastereoisomeric carboxamides): 6.78–6.73 (*m* apparent br. *dt*, CH); 6.15, 6.10, 6.09 (3*d*, *J* = 15.8, CH); 3.40–3.19 (*m*, 5 CH_2NCO); 2.55–2.49 (*m*, 2 H); 2.31–2.22 (*m*, 4 H); 2.09–2.07 (*d*-like *m*, Me); 1.99–1.97 (*m*, Me); 1.81–1.72 (*m*, 4 H); 1.65–1.47 (*m*, 8 H); 1.33–1.25 (*s*-like *m*,

5 CH_2). ^{13}C-NMR (150.9 MHz; diastereoisomeric carboxamides): 201.29 (*s*, CO); 173.14, 172.92, 172.75 (3*s*, CON); 170.19 (*s*, CONH); 146.98, 145.98 (2*d*, CH); 131.77, 131.56, 131.10 (3*d*, CH); 49.64, 49.09, 48.03, 47.81, 47.54, 46.55, 46.20, 45.49, 45.10, 44.89, 43.99, 43.83, 43.35, 39.81, 38.98, 38.88, 38.60, 32.94, 32.57, 32.46, 32.10, 31.92, 31.80, 29.69, 29.51, 28.70, 28.47, 28.43, 28.39, 28.33, 28.25, 28.13, 28.02, 27.71, 27.66, 27.45, 27.37, 27.16, 26.96, 26.77, 26.52, 26.24, 25.54, 25.40, 25.31, 25.19, 24.93, 24.80, 23.91, 23.68, 23.46, 23.30, 23.20, 21.66, 21.57, 21.53 (56 signals, 19 CH_2, 2 Me). ESI-MS (MeOH): 516.6 (15, $[M + K]^+$), 500.5 (100, $[M + Na]^+$). EI-MS: 477.4 (4, $M^{+\cdot}$), 434.4 (6), 349.5 (4), 308.6 (3), 263.4 (5), 230.4 (4), 180.3 (7), 169.3 (54), 157.3 (21), 155.2 (11), 149.2 (5), 143.2 (20), 140.2 (6), 131.2 (5), 129.2 (11), 126.2 (7), 114.2 (18), 112.2 (49), 100.2 (20), 98.2 (21), 91.2 (6), 84.2 (47), 81.2 (21), 77.1 (6), 72.2 (53), 70.1 (100), 69.2 (39), 56.2 (25), 55.2 (47).

5-(4-Acetamidobutyl)-1-acetyl-1,5-diaza(16,17-$^2H_2$)cyclohenicosane-6,15-dione (= N,N′-*Diacetyl(10,11-$^2H_2$)-inandenin-12-one*; **13**). A soln. of **12** (7.0 mg) in EtOH (5 ml) was stirred over 5% Pd/C (8 mg) in a D_2 atmosphere at 23° and normal pressure. After 1 h 45 min, the mixture was removed with by a syringe and passed through a filter (*Acrodisc® LC13 PVDF*, 0.2 µm), which was washed repeatedly with EtOH. Evaporation left **13** (6.9 mg) as colorless oil. R_f 0.65 ($CHCl_3$/MeOH 4:1). IR ($CHCl_3$): 3450*w*, 3000*m*, 2930*s*, 2860*m*, 1710*m*, 1660*s*, 1630*s*, 1520*w*, 1460*m*, 1430*m*, 1370*m*, 1260*m*, 1090*m*. ^{1}H-NMR (600.1 MHz): 3.42–3.15 (*m*, 5 CH_2NCO); 2.44–2.36 (*m*, CH_2COCHD); 2.33–2.24 (*m*, CH_2CON); 2.14–2.08 (*d*-like *m*, MeCO); 2.05–2.02 (*s*-like *m*, MeCO); 1.91–1.73 (br., $NCH_2CH_2CH_2N$); 1.61–1.43 (br., 11 H); 1.31–1.26 (br., 12 H). ^{13}C-NMR (150.9 MHz; diastereoisomeric carboxamides): 211.72 (*s*, CO); 172.74 (*s*, CON); 170.47 (*s*, CONH); 49.48, 49.33, 48.10, 46.47, 46.18, 45.63, 44.44, 43.98, 43.90, 42.38, 41.98, 39.22, 39.13, 35.66, 32.95, 32.80, 32.64, 29.68, 29.05, 28.93, 28.79, 28.70, 28.64, 28.58, 28.44, 28.35, 28.19, 28.01, 27.69, 26.99, 26.69, 26.55, 26.28, 26.00, 25.47, 25.24, 25.14, 23.79, 23.29, 21.52 (40 signals, 19 CH_2, 2 CHD, 2 Me). ESI-MS (MeOH): 520.3 (19, $[M + K]^+$), 504.4 (100, $[M + Na]^+$). EI-MS (MeOD): 481.3 (14, $M^{+\cdot}$), 464.3 (2), 453.4 (8), 439.3 (95, $[M - MeCO]^+$), 438.3 (93), 410.3 (11), 395.3 (9), 381.3 (15), 353.3 (20), 339.3 (20), 326.3 (15), 314.1 (41), 313.1 (74), 297.3 (12), 283.3 (6), 269.3 (8), 255.3 (7), 241.1 (8), 237.1 (14), 228.3 (9), 208.1 (8), 186.2 (6), 170.1 (42), 169.1 (100), 168.1 (25), 157.2 (32), 155.1 (15), 143.1 (27), 129.1 (13), 114.1 (25), 113.1 (18), 112.1 (59), 100.2 (18), 98.2 (17), 84.2 (32), 72.2 (21), 70.2 (51), 55.2 (21), 44.3 (26), 41.3 (12).

HPLC Co-Chromatography of **13** *and the Isomeric* N,N′-*Diacetylinandeninones* (*MN Nucleosil 100 7C_{18}*; MeOH/H_2O (1:1, 1.5 ml/min)). t_R (*N,N′*-diacetylinandenin-13-one) 22.79 min; t_R (*N,N′*-diacetylinandenin-12-one (**14**)) 26.56 min; t_R (*N,N′*-diacetylinandenin-10-one) 38.15 min; co-injecting **13** together with the above mentioned reference compounds [2] [10] revealed identical t_R values only for **13** and **14**.

Schmidt *Degradation: Transformation of* **11** *and* **13** *to the Mixtures 5-(4-Acetamidobutyl)-1,5,16-triazabicyclo-[16.4.0]docosane-6,15-dione/5-(4-Acetamidobutyl)-1,5,15-triazabicyclo[16.4.0]docosane-6,16-dione* (**14a/14b**) *and 5-(4-Acetamidobutyl)-1-acetyl-1,5,16-triaza(17,18-$^2H_2$)cyclododocosane-6,15-dione/5-(4-Acetamidobutyl)-1-acetyl-1,5,15-triaza(17,18-$^2H_2$)cyclododocosane-6,16-dione* (**15a/15b**), *Respectively*. To a stirred mixture of **11** (4.5 mg), $CHCl_3$ (1 ml), and conc. H_2SO_4 (0.3 ml), NaN_3 (1 mg) was added at 23°. After 30 min, further NaN_3 (1 mg) was added and stirring continued for 1 h. Small amounts of ice were added, and the mixture was transferred quantitatively into a separation funnel and adjusted to pH 8–9 (Na_2CO_3). The org. layer was separated and the aq. phase extracted repeatedly with $CHCl_3$. The combined $CHCl_3$ layers were extracted once with brine (1 ml), and evaporated and co-evaporated with abs. EtOH (2 × 2 ml) to yield 5.8 mg of **14a/14b**. In a similar manner, **15a/15b** (3.4 mg) was obtained from **13** (3.6 mg) as already described [2].

Mixture **14a/14b**. ESI-MS: 924.1 (47, $[2M + Na]^+$), 489.6 (11, $[M + K]^+$), 473.7 (100, $[M + Na]^+$), 451.7 (21, $[M + 1]^+$).

Mixture **15a/15b**. ESI-MS: 535.8 (14, $[M + K]^+$), 519.7 (100, $[M + Na]^+$), 497.7 (17, $[M + 1]^+$).

Acid-Catalyzed Hydrolysis of the Mixtures **14a/14b** *and* **15a/15b**, *and Derivatization of the Degradation Products*. The mixture **14a/14b** was heated in a sealed glass tube with 2N aq. HCl (1.5 ml) for 20 h at 150°. After extraction with Et_2O, the combined org. layers were treated with CH_2N_2/Et_2O to give fraction E-1. Evaporation of the aq. layer left the corresponding residue W-1. The analogous fractions E-2 and W-2 were obtained in the same way from **15a/15b**.

GC Analysis of Ethereal Solutions E-1 and E-2 (capillary column *DB-1*, 140°; inlet 240°; FID). E-1: *Dimethyldecanedioate* (**14a.2**), t_R 8.75 min. E-2: **14a.2**, t_R 8.75 min. CI-MS (NH_3): 248.4 (100, $[M + 1 + NH_3]^+$), 231.4 (18, $[M + 1]^+$). Identification was performed by co-chromatography together with commercially available reference compounds.

ESI-MS Examination of the Fractions W-1 and W-2. W-1 (MeOH): 272.5 (9, $[M + 1]^+$) (*1-(8-amino-4-aza-octyl)piperidine-2-acetic acid* (**14b.1**)), 243.5 (86, $[M + 1]^+$) (*1-(8-amino-4-azaoctyl)-2-(aminomethyl)piperidine* (**14a.1**)), 174.4 (50, $[M + 1]^+$) (*methyl 9-aminononanoate* (**14b.2**)), 122.3 (100, ½$[M + 2]^{2+}$) (**14a.1**). W-2 (MeOH): 276.5 (9), 275.5 (17, $[M + 1]^+$) (*16-amino-8,12-diaza[3-^2H]hexadecanoic acid* (**15b.1**)); 174.4 (100, $[M + 1]^+$) (**14b.2**), 124.1 (76, ½$[M + 2]^{2+}$) (*5,9-diaza[14,15-$^2H_2$]pentadecane-1,15-diamine* (**15a.1**)).

N-Ethyl-2-[10-(methoxycarbonyl)-2-oxodecyl]pyridinium Iodide (**17**). A mixture of **16** [9] (981.8 mg, 3.36 mmol), $MeNO_2$ (8 ml), EtI (6 ml), and MeOH (4 drops) was stirred under reflux for 9 h. The solvents were evaporated leaving **17** as a yellow residue (1.41 g, 94%) which crystallized upon standing. IR (KBr): 3450*w*, 3010*s*, 2920*s*, 2850*s*, 1739*s*, 1700*s*, 1625*m*, 1573*m*, 1512*m*, 1475*m*, 1435*m*, 1412*m*, 1382*m*, 1360*m*, 1310*m*, 1250*m*, 1210*m*, 1170*s*, 1160*s*, 1110*m*, 1075*w*, 1030*m*, 970*w*, 880*w*, 785*s*, 665*m*. ESI-MS (MeOH): 320.1 (M^+).

Methyl 11-(1-Ethylpiperidin-2-yl)-10-oxoundecanoate (**18**). To a soln. of **17** (200 mg, 0.45 mmol) in MeOH (20 ml), PtO_2 (50 mg) was added and the mixture stirred at atmospheric pressure under H_2 at 23°, until absorption was complete (45 min). Evaporation of the solvent and FC of the residue (silica gel, 40–60 μm, 8 g; $CHCl_3$/MeOH/25% aq. NH_3 soln. 95:5:0.5) afforded 143.1 mg (92%) of **18** as colorless oil. IR (CCl_4): 2930*s*, 2850*m*, 1740*s*, 1712*s*, 1435*w*, 1360*w*, 1260*w*, 1195*w*, 1170*w*, 1100*w*, 1015*w*. ^{1}H-NMR: 3.66 (*s*, CO_2Me); 2.99–2.96 (br. *m*, 1 H); 2.73–2.55 (*m*, 3 H); 2.45–2.27 (*m*, 7 H); 1.66–1.52 (br. *m*, 8 H); 1.37–1.29 (*s*-like *m*, 10 H); 1.03 (*t*, $J = 7.2$, CH_2Me). ^{13}C-NMR: 210.36 (*s*, CO); 174.23 (*s*, CO_2Me); 55.27 (*d*, CH); 51.41 (*q*, CO_2Me); 50.21, 47.81, 43.92, 43.80, 34.07, 31.37, 29.21, 29.12, 29.07, 25.40, 24.92, 23.72, 22.65 (13*t*, 14 CH_2); 11.34 (*q*, CH_2Me). ESI-MS (MeOH): 326.3 ($[M + 1]^+$).

Methyl (E)-16-(N-Ethylacetamido)-10-oxohexadec-11-enoate (**19**). A mixture of **18** (102 mg, 0.31 mmol), Ac_2O (8 ml), and NaOAc (150 mg, freshly molten and powdered) was stirred under Ar at 23°. After 40 h, a nearly 1:1 distribution of **18** to **19** was found (TLC). Stirring was continued for 1 h at 60°, the solvent evaporated *in vacuo*, and the residue taken up in H_2O. After basifying (K_2CO_3), the aq. layer was extracted with $CHCl_3$. Drying (Na_2CO_4) and evaporation of the org. layer yielded a residue which was purified by FC (silica gel, 40–60 μm, 3 g; CH_2Cl_2/MeOH 95:5) to give 113.4 mg (98%) of **19** as an oil. IR (CCl_4): 2930*s*, 2855*m*, 1740*s*, 1698*w*, 1650*s*, 1435*w*, 1420*w*, 1360*w*, 1260*w*, 1170*w*. ^{1}H-NMR (2 diastereoisomeric acetamides, ratio 2:1): 6.80 (*dt*, $J = 15.8$, 7.0, CH); 6.11, 6.08 (2*d*, $J = 15.8$, 15.8, CH); 3.66 (*s*, CO_2Me); 3.38–3.22 (*m*, 4 H); 2.52 (*t*, $J = 7.4$, CH_2CO); 2.32–2.23 (*m*, 4 H); 2.09, 2.07 (2*s*, MeCO); 1.64–1.47 (*m*, 8 H); 1.30 (*s*-like *m*, 8 H); 1.18, 1.11 (2*t*, $J = 7.2$, 7.1, CH_2Me). ^{13}C-NMR: 200.77, 200.50 (2*s*, CO); 174.24 (*s*, CO_2Me); 170.02, 169.78 (2*s*, COMe); 146.57, 145.71 (2*d*, CH); 130.69, 130.56 (2*d*, CH); 51.40 (*q*, CO_2Me); 48.14, 44.82, 43.24, 40.43, 40.33, 40.09, 38.75, 34.06, 32.16, 32.04, 29.20, 29.06, 28.62, 27.47, 25.52, 25.42, 24.90, 24.20, 23.77, 22.97 (20*t*, 13 CH_2); 21.57, 21.38 (2*q*, COMe); 14.06, 12.96 (2*q*, CH_2Me). CI-MS: 385.4 (20, $[M + 1 + NH_3]^+$), 368.4 (100, $[M + 1]^+$).

5-(4-Acetamidobutyl)-1,5-diazabicyclo[15.4.0]henicosane-6,16-dione (= *N-Acetyloncinotin-11-one*; **20**). The reaction was carried out as described for fraction F_c. From 3.0 mg of **5**, 6.5 mg of crude product were obtained. Filtration over silica gel afforded 3.3 mg of **20**.

HPLC Correlation of N-*Acetyloncinotin-12-one* (**11**) *and* N-*Acetyloncinotin-11-one* (**20**; *MN Nucleosil 100 7C18*; MeCN/citrate buffer (25:75, 1.5 ml/min)): t_R (**11**) 7.55 min, t_R (**20**) 5.99 min. Co-injection of **20** together with the appropriate fully synthetic sample [17] showed identical retention times.

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