Pergamon

0031-9422(95)00030-5

*Phytochemistry*, Vol. 39, No. 3, pp. 689–694, 1995

0031-9422/95 $9.50 + 0.00

# SPERMIDINE ALKALOIDS TYPE INANDENINE FROM *ONCINOTIS TENUILOBA*

MARTIN K.-H. DOLL, ARMIN GUGGISBERG and MANFRED HESSE*

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

(*Received* 11 *November* 1994)

**Key Word Index**—*Oncinotis tenuiloba*; Apocynaceae; leaves; spermidine alkaloids; inandenin-12-one; inandenin-13-one; inandenin-12-ol; inandenin-13-ol; Schmidt degradation.

**Abstract**—From the leaves of *Oncinotis tenuiloba*, four spermidine alkaloids, inandenin-13-one, inandenin-12-one and their corresponding alcohols, have been isolated. Structural elucidation was done by chemical degradation, as well as correlations with authentic samples.

## INTRODUCTION

During the search for polyamine alkaloids, several species of *Oncinotis* have been investigated. First, *O. nitida* was analysed, containing mainly oncinotine, a bicyclic macrolactam, together with its isomers and monohydroxy derivatives. Furthermore, inandeninones have been isolated as minor constituents of this species, consisting of an inseparable mixture of isomeric macrocyclic keto lactams [1–3]. In a later study, these inandeninones were found to be the major alkaloids of *O. inandensis* and their structures determined by Schmidt degradation of the mixture followed by GC, TLC and mass spectral analyses of the cleavage products [4, 5]. Preliminary examinations of *O. nigra*, indicated mainly an inseparable mixture of inandeninols (here the keto groups of the inandeninones are reduced to secondary alcohols) and isomers, beside small amounts of inandeninones [6].

In a continuation of this work we investigated *O. tenuiloba* and report herein a modified isolation procedure for the inandenin-12/13-one† mixture (**1/2**) and their first separation via the *N,N'*-diacetyl derivatives **3** and **4**, allowing characterization of the pure compounds. Structures were confirmed by Schmidt degradation and identification of the cleavage products was further extended by HPLC analysis. As a minor constituent, a mixture of inandenin-12/13-ol (**7/8**) was isolated. For the first time, their isolation will be reported as well as the separation of their *N,N'*-diacetyl derivatives, **9** and **10**. The structures of the alcohols **7** and **8** were confirmed by comparison (HPLC) of their *N,N'*-diacetyl derivatives, **9** and **10**, with those prepared from **3** and **4** (reduction and acetylation).

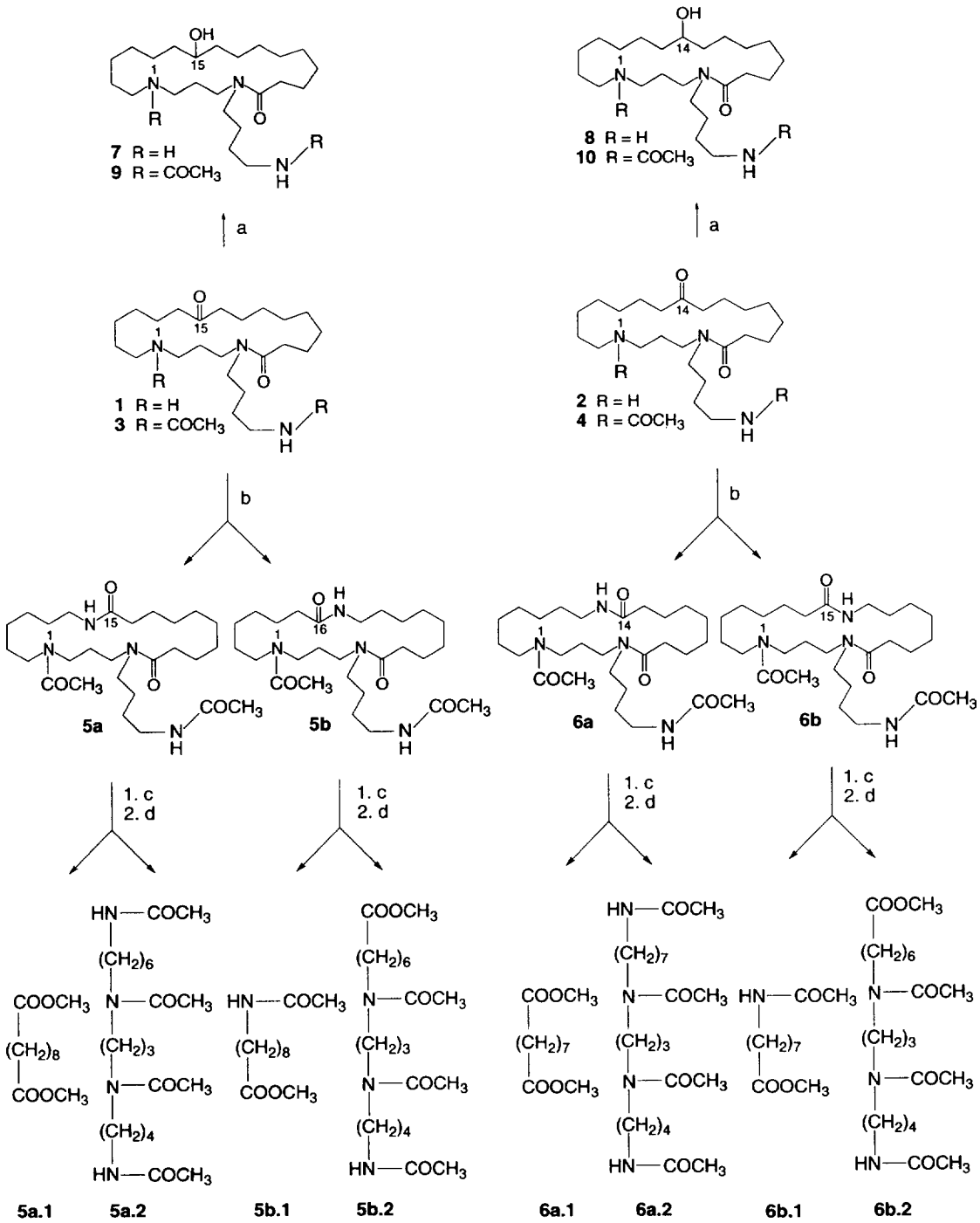
*Author to whom correspondence should be addressed.

†Numberings and names used in the text are in accordance with earlier publications; for systematic names consult Experimental and Scheme 1.

## RESULTS AND DISCUSSION

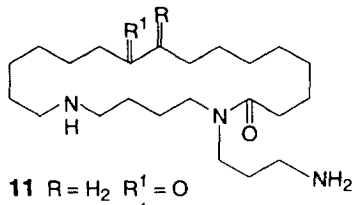
Extraction of dried leaves of *O. tenuiloba* with a mixture of methanol and water containing 3% acetic acid gave a resinous material, which was defatted by partitioning between 1N HCl and diethyl ether. The remaining acidic solution was made alkaline and adsorbed on a weakly acidic ion-exchange resin. After rinsing and desorption, a mixture of crude bases was obtained, which was subdivided by column chromatography leading to four fractions of increasing polarity (fractions A–D). Further purifications were done by HPLC as indicated in the Experimental. Out of fraction B, we isolated a mixture of inandenin-12/13-one (**1/2**), as confirmed by ESI mass spectrometry and co-TLC with an authentic sample [4]. In addition, a small amount of a mixture of the corresponding alcohols, inandenin-12/13-ol, was obtained (10%, with respect to the ketones).

The isomeric keto lactam mixture, **1/2**, was transformed to the *N,N'*-diacetyl derivatives **3/4** ($Ac_2O$/NaOAc), which were readily separable by semipreparative HPLC; the ratio of isolated **3** and **4** was *ca* 1.5:1. Structures were determined by chemical degradation of the pure compounds (Scheme 1). First a nitrogen atom was introduced in an α-position to the keto group by the Schmidt reaction [7]. The resulting mixtures of the two isomeric macrocyclic lactams, **5a/5b** (from **3**) and **6a/6b** (from **4**), were hydrolysed with aqueous HCl at high temperature to give four degradation products from each. Extraction of the acidic reaction mixtures with diethyl ether, followed by esterification with ethereal diazomethane, provided the ethereal fractions E-1 (degradation of **3**) and E-2 (degradation of **4**). The remaining aqueous solutions were evaporated and the residues treated with saturated methanolic HCl, followed by acetylation and usual work-up to give the corresponding fractions W-1 and W-2.

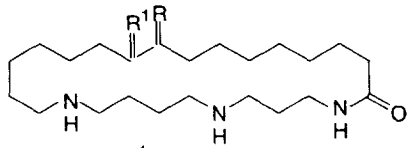



Scheme 1. Reduction and Schmidt degradation of inandenin-12-one [= 5-(4-aminobutyl)-1,5-diazacyclohenicosan-6,15-dione] (**1**) and inandenin-13-one [= 5-(4-aminobutyl)-1,5-diazacyclohenicosan-6,14-dione] (**2**). (a) $NaBH_4$/MeOH. (b) $NaN_3/H_2SO_4$. (c) 2 N HCl, 150°. (d) Organic layer: $CH_2N_2/Et_2O$. Aq. layer: 1. HCl/MeOH, reflux; 2. $Ac_2O$/NaOAc.

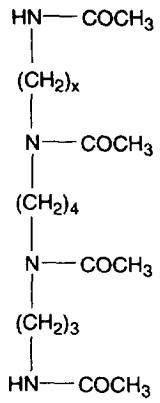
GC analysis of fraction E-1 showed 85% of dimethyl 1,10-decanedioate (**5a.1**) and 10% of dimethyl 1,11-undecanedioate (**7a.1**). Considering the major constituent, the distance between the two carbonyl groups in the original compound **1** has to be eight methylene groups. In the case of fraction E-2, only dimethyl 1,9-nonanedioate (**6a.1**) was found, indicating seven methylene groups between the carbonyl carbon atoms in **2**. Furthermore, the detection of the above mentioned **7a.1** gives evidence for the presence of a further isomeric alkaloid, inandenin-



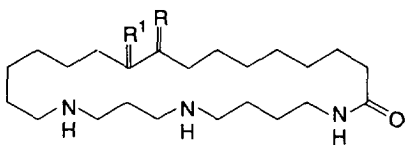
**11** R = $H_2$ $R^1$ = O
**12** R = O $R^1$ = $H_2$



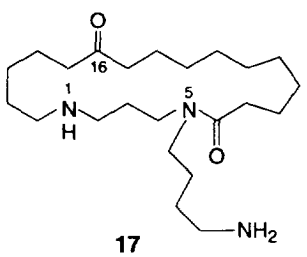
**13** R = $H_2$ $R^1$ = $H_2$
**14** R = O $R^1$ = O



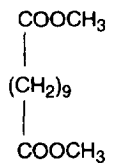
**5a.3** x=6, expected from **11** and **13**
**6a.3** x=7, expected from **12** and **14**



**15** R = $H_2$ $R^1$ = O
**16** R = O $R^1$ = $H_2$



**17**



**7a.1**

11-one [ = 5-(4-aminobutyl)-1,5-diazacyclohenicosan-6,16-dione] (**17**), as a minor product in the original fraction B.

HPLC analysis of fractions W-1 and W-2 revealed first, methyl 9-acetamidononanoate (**5b.1**) and methyl 8-acetamidooctanoate (**6b.1**), respectively, supporting the above findings of the carbonyl to carbonyl distances in the original substances. Concerning the orientation of the polyamine part in structure **1**, the identification of 1,15-diacetamido-5,9-diacetyl-5,9-diazapentadecane (**5a.2**) in fraction W-1 was most important, indicating a terminal 4-aminobutyl group. In the same way, the structure of **2** was supported by the presence of 1,16-diacetamido-5,9-diacetyl-5,9-diazahexadecane (**6a.2**) in fraction W-2. Additionally, **5a.2** indicates a distance of six methylene groups between N-1 and the keto group in **1**; analogously, this distance is seven methylene units in **2** (cf. **6a.2**). In both cases, the absence of the isomers, **5a.3** and **6a.3**, was shown by co-HPLC with appropriate compounds. In this way, the isomeric structures **11** and **12** (neoinandeninones)‡, as well as their ring enlarged counterparts **13** and **14** (isoinandeninones), could be definitely excluded to be present in the plant. Furthermore, the isomeric inandeninone structures, **15** and **16** (pseudoinandeninones), could be excluded as well, due to a different EI mass spectral fragmentation pattern which would be expected of their *N,N'*-diacetyl derivatives, e.g. the loss of a 3-acetamidopropyl radical by α-cleavage at the N-5 position would not be observed (rel. int. of $[M - 100]^+ > 10\%$ for **3** and **4**).

A sample of the inandenin-12/13-ol mixture (**7/8**) was peracetylated to obtain the *N,N',O*-triacetyl derivatives. Afterwards, the *O*-acetyl group was selectively removed by treatment with saturated methanolic HCl to obtain a mixture of *N,N'*-diacetyl inandeninols, **9/10**. These derivatives could be separated by analytical HPLC, revealing a ratio between **9** and **10** of 3.3:1. The structures of **9** and **10** were assigned by comparison with semi-synthetic samples of known structures obtained by treatment of the pure *N,N'*-diacetyl inandeninones **3** and **4** with sodium borohydride. Co-chromatography of these semi-synthetic samples and the *N,N'*-diacetylated mixture **9/10** of natural origin resulted in a clear identification of the compounds according to the structures given in Scheme 1.

Because of the small amounts of the alcohols **7** and **8** available we were unable to get information about their optical rotation.

‡Trivial names of isomeric structures given in brackets are analogous to those defined in the oncinotine series [8].

## EXPERIMENTAL

*General.* Leaves of *O. tenuiloba* Stapf were collected in Kenya by G. M. Mungai (E. A. Herbarium Nairobi) in 1989. CC: Merck silica gel 60 (15–40 μm). TLC: precoated silica gel 60 $F_{254}$, 0.2 mm (Merck). Amines were detected by spraying with Schlittler reagent [9]; for amides $Ce^{4+}$–$H_2SO_4$ was used additionally. $^1H$ NMR: 300 MHz with δ in ppm using the appropriate solvent as int. standard. CIMS: chemical ionization with $NH_3$. EIMS (probe): 70 eV. $NH_4OH$ is a 25% aq. soln of $NH_3$ (Merck). The HPLC system was attached to an UV detector (225 nm).

*Extraction.* Air-dried leaves (1.3 kg) were powdered and extracted with MeOH–$H_2O$–HOAc (9:1:0.3; ×4, total 8.5 l). The combined extracts were evapd to yield 162 g of crude material which was dissolved in 0.5 N HCl (1 l) and extracted with $Et_2O$ (4 × 800 ml). The aq. layer was adjusted to pH 8 (1 N NaOH) and adsorbed on ion-exchange resin (Amberlite IRC-50, 280 g). After rinsing with MeOH–$H_2O$, 2:3 (0.5 l), the resin was transfered into an Erlenmeyer flask and washed with $H_2O$ (2 × 0.8 l), MeOH–$H_2O$, 2:3 (5 × 0.8 l) and left in $H_2O$ overnight. Desorption with 0.1 N HCl (6 l) and evapn of the solvent yielded a residue, which was extracted with EtOH (100 ml) and MeOH (80 ml). Removal of alcohol and drying *in vacuo* gave 7.12 g of crude extract.

*Collected frs of different polarities.* The crude extract (3.5 g) was sepd by CC using $CHCl_3$–MeOH–$NH_4OH$, 50:12:3 (a) and 7:3:1 (b) as eluents. When the $R_f$ values reached *ca* 0.3 (TLC monitoring with solvent b), elution was continued with b to give fr. A [78 mg; mainly 1 substance: $R_f$ (b) 0.73], fr. B [279 mg; 2 substances: $R_f$ (b) 0.57 and 0.48], fr. C [188 mg; mixt.: $R_f$ (b) 0.45–0.25] and fr. D [360 mg; mixt.: $R_f$ (b) ⩽ 0.25]. All frs were evapd with diluted MeOH–HCl to obtain the hydrochlorides as brownish amorphous solids or oils.

*Sepn and purification.* Frs A–D were subjected to prep. HPLC (Bischoff, Spherisorb Si 5 μm, 250/12/20) as free bases. Frs A–C were filtered over silica gel (b). The free base of fr. D was obtained by CC ($CHCl_3$–MeOH–$NH_4OH$, 70:35:13); application of 770 mg of fr. D resulted in 527 mg of fr. D′, pure enough for HPLC. All resulting materials were converted to their hydrochlorides as described above. Frs B-1 and B-2: $CH_2Cl_2$–MeOH–$NH_4OH$, 80:15:3.3 (8 ml $min^{-1}$); from 229 mg of fr. B were obtained fr. B-1 (79.3 mg; resin; $R_t$ 20–40 min) and fr. B-2 (7.9 mg; oil; $R_t$ ⩾ 47 min). Fr. C-1: from 188 mg of fr. C only fr. C-1 (18 mg; oil; $R_t$ 24 min) could be isolated. The solvent was the same as described for fr. B, 12 ml $min^{-1}$. Fr. C-1 was chromatographed twice resulting finally in 6 mg of $N^4$-benzoylspermidine [10]. Fr. D-1: $CH_2Cl_2$–MeOH–$NH_4OH$, 70:35:12 (9 ml $min^{-1}$); application of 585 mg of fr. D′ yielded fr. D-1 (17.5 mg; oil; $R_t$ 32–42 min). Fr. A has not been further evaluated and the constituents of fr. D-1 are not elaborated as yet.

*Fr. B-1 (mixture of* 5-(4-*aminobutyl*)-1,5-*diazacyclohenicosan*-6,15-*dione* (**1**)·2 *HCl and* 5-(4-*aminobutyl*)-1,5-*diazacyclohenicosan*-6,14-*dione* (**2**)·2 *HCl*). ESIMS *m/z* (rel. int.): 396 $[M + 1]^+$ (100), 378 $[M + 1 - H_2O]^+$ (**9**). CIMS *m/z* (rel. int.): 396 $[M + 1]^+$ (< 5), 378 $[M + 1 - H_2O]^+$ (100). EIMS *m/z* (rel. int.): 394 $[M - 1]^+$ (< 5), 379 (7), 378 (27), 377 (26), 334 (6), 320 (4), 279 (9), 265 (7), 112 (26), 111 (38), 110 (31), 99 (11), 98 (59), 97 (28), 96 (30), 95 (13), 91 (15), 86 (10), 85 (22), 84 (82), 83 (30), 82 (26), 81 (18), 79 (11), 72 (27), 71 (23), 70 (65), 69 (35), 68 (18), 67 (19), 58 (26), 57 (33), 56 (58), 55 (100), 45 (12), 44 (88), 43 (61), 42 (63), 41 (80).

*Fr. B-2 (mixture of* 5-(4-*aminobutyl*)-15-*hydroxy*-1,5-*diazacyclohenicosan*-6-*one* (**7**)·2 *HCl and* 5-(4-*aminobutyl*)-14-*hydroxy*-1,5-*diazacyclohenicosan*-6-*one* (**8**)·2 *HCl*). CIMS *m/z*: 398 $[M + 1]^+$.

*Fr. C-1* (N-4-*aminobutyl*)-N-(3-*aminopropyl*)*benzamide*·2*HCl* = $N^4$-*benzoylspermidine*·2 *HCl*). [10].

*Acetylation of fr. B-1. Mixture of* 5-(4-*acetamidobutyl*)-1-*acetyl*-1,5-*diazacyclohenicosan*-6,15-*dione* (**3**) *and* 5-(4-*acetamidobutyl*)-1-*acetyl*-1,5-*diazacyclohenicosan*-6,14-*dione* (**4**). A mixt. of $Ac_2O$ (20 ml), NaOAc (200 mg, freshly molten and powdered) and 47 mg of fr. B-1 was stirred overnight at room temp. Usual work-up gave 49.5 mg (87%, oil) of the mixt. **3**/**4**. CIMS *m/z* (rel. int.): 480 $[M + 1]^+$ (100), 438 $[M + 1 - C_2H_2O]^+$ (9).

*Sepn of* **3** *and* **4**. Semiprep. HPLC (MN, VarioPrep Nucleosil 100-7$C_{18}$, 250/10; MeOH–$H_2O$, 11:9, 6 ml $min^{-1}$). From the mixt. **3**/**4** (42 mg), **4** (10.9 mg, $R_t$ 17 min) and **3** (15.7 mg, $R_t$ 20 min) were isolated as oils.

*Compound* **3**. $^1H$ NMR ($CDCl_3$): δ 3.33–3.16 (10H, *m*), 2.43–2.33 (4H, *m*), 2.27–2.18 (2H, *m*), 2.07–2.04 (3H, *m*, MeCO), 1.96–1.95 (3H, *d*-like *m*, MeCO), 1.83–1.68 (2H, *m*), 1.59–1.42 (2H, *m*), 1.26–1.22 (12H, *m*). CIMS *m/z* (rel. int.): 480 $[M + 1]^+$ (100), 438 (10). EIMS *m/z* (rel. int.): 479 $[M]^+$ (12), 437 (30), 436 (100), 379 (14), 351 (16), 337 (17), 323 (10), 169 (42), 168 (11), 157 (14), 143 (11), 112 (22), 84 (11), 70 (22), 69 (11), 57 (11), 55 (14), 43 (14), 41 (12).

*Compound* **4**. $^1H$ NMR ($CDCl_3$): δ 3.36–3.14 (10H, *m*), 2.41–2.33 (4H, *m*), 2.29–2.20 (2H, *m*), 2.06–2.03 (3H, *m*, MeCO), 1.96–1.95 (3H, *d*-like *m*, MeCO), 1.81–1.69 (2H, *m*), 1.60–1.43 (12H, *m*), 1.27–1.22 (12H, *m*). CIMS *m/z* (rel. int.): 480 $[M + 1]^+$ (100), 438 (9). EIMS *m/z* (rel. int.): 479 $[M]^+$ (12), 437 (31), 436 (100), 379 (13), 351 (14), 337 (16), 324 (11), 323 (10), 169 (38), 168 (10), 157 (13), 143 (11), 112 (20), 70 (17), 55 (13), 43 (12).

*Schmidt degradation—transformation of* **3** *and* **4** *to their dilactam mixtures* 5-(4-*acetamidobutyl*)-1-*acetyl*-1,5,16-*triazadocosan*-6,15-*dione*/5-(4-*acetamidobutyl*)-1-*acetyl*-1,5,15-*triazadocosan*-6,16-*dione* (**5a**/**5b**) *and* 5-(4-*acetamidobutyl*)-1-*acetyl*-1,5,15-*triazadocosan*-6,14-*dione*/5-(4-*acetamidobutyl*)-1-*acetyl*-1,5,14-*triazadocosan*-6,15-*dione* (**6a**/**6b**), *respectively*. To a stirred soln of **4** (5.4 mg) in $CHCl_3$ (1 ml) and conc. $H_2SO_4$ (0.3 ml) was added $NaN_3$ (1 mg) at room temp. After 30 min, another 0.5 mg of $NaN_3$ was added and stirring continued for 1 hr. Small amounts of ice were added and the mixt. extracted repeatedly with small portions of $CHCl_3$. The combined $CHCl_3$ layers were extracted once with 5% $NaHCO_3$ followed by reextraction (×4) of the $NaHCO_3$ layer with $CHCl_3$. The combined organic layers were dried ($Na_2SO_4$) and evapd leaving the mixt. **6a**/**6b** (4.9 mg; oil). Analogously **5a**/**5b** (5.8 mg) was obtained from **3** (5.8 mg).

*Dilactam mixt.* **6a/6b**. EIMS *m/z* (rel. int.): 494 [M + 1]$^+$ (25), 452 [M + 1 − $C_2H_2O$]$^+$ (100), 423 (12), 394 (19), 366 (18), 352 (20), 338 (12), 169 (49), 168 (11), 157 (24), 143 (18), 114 (16), 112 (34), 100 (10), 98 (13), 97 (11), 84 (18), 83 (12), 72 (15), 71 (10), 70 (34), 69 (16), 57 (16), 56 (16), 55 (29), 44 (16), 43 (17), 41 (19).

*Acid-catalysed hydrolysis of dilactam mixts* **5a/5b, 6a/6b** *and derivatization of degradation products.* The mixt. **6a/6b** was heated with 2N HCl (1.5 ml) for 16 hr at 150° in a sealed tube. After extraction (×4) with $Et_2O$, the combined organic layers were treated with $CH_2N_2$–$Et_2O$ to give a soln (fr. E-2) of the expected diMe dioate (**6a.1**). The aq. layer was evapd and the residue dissolved in MeOH (18 ml). The soln was satd with HCl gas and refluxed for 1 hr. After removing solvent, the mixt. was peracetylated with $Ac_2O$–NaOAc as described above. Usual work-up yielded a solid mixt. (fr. W-2, 6.3 mg). In the same way, **5a/5b** was treated to yield fr. W-1 (7.1 mg), as well as the $Et_2O$ soln (fr. E-1) of the diMe dioate (**5a.1**).

*GC analysis of ethereal solns fr. E-1 and fr. E-2.* Capillary column DB-1, 140°; inlet 240°; FID. E-1: diMe 1,10-decanedioate (**5a.1**) ($R_t$ 7.85 min, 85%) and diMe 1,11-undecanedioate (**7a.1**) ($R_t$ 12.49 min, 10%). E-2: diMe 1,9-nonanedioate (**6a.1**) ($R_t$ 5.01 min, 95%). All compounds were identified by co-chromatography with appropriate refs (commercially available).

*HPLC analysis of frs W-1 and W-2.* $5C_8$ (MN, Nucleosil 100; 200/8/4). W-1: MeOH–$H_2O$ (36:64, 1.5 ml min$^{-1}$). 1,15-Diacetamido-5,9-diacetyl-5,9-diazapentadecane (**5a.2**): $R_t$ 5.30 min. The isomeric compound 1,15-diacetamido-4,9-diacetyl-4,9-diazapentadecane (**5a.3**) could not be detected in the sample ($R_t$ 5.66 min). Me 9-acetamidononanoate (**5b.1**): $R_t \approx 23$ min, broad peak with small intensity. W-2: MeOH–$H_2O$ (1:2, 1.8 ml min$^{-1}$). 1,16-Diacetamido-5,9-diacetyl-5,9-diazahexadecane (**6a.2**): $R_t$ 20.14 min. This compound could be clearly distinguished from its isomer 1,16-diacetamido-4,9-diacetyl-4,9-diazahexadecane (**6a.3**) which is not present in the sample ($R_t$ 21.99 min). Me 8-acetamidooctanoate (**6b.1**): $R_t$ 13.10 min; the third intense peak ($R_t$ 18.59) was suggested to be methyl 17-acetamido-9,13-diacetyl-9,13-diazaheptadecanoate (**6b.2**). Except for Me 16-acetamido-8,12-diacetyl-8,12-diazahexadecanoate (**5b.2**) and Me 17-acetamido-9,13-diacetyl-9,13-diazaheptadecanoate (**6b.2**), all compounds were identified by co-HPLC with ref. compounds. For the synthesis of the isomers **5a.2**, **5a.3** and **6a.2**, **6a.3**, respectively, see ref. [5]. The prepn of **6b.1** is given in ref. [11]; the higher homologue **5b.1** was synthesized as described below.

*Acetylation of fr. B-2. Mixt. of 5-(4-acetamidobutyl)-1-acetyl-15-hydroxy-1,5-diazacyclohenicosan-6-one* (**9**) *and 5-(4-acetamidobutyl)-1-acetyl-14-hydroxy-1,5-diazacyclohenicosan-6-one* (**10**). Fr. B-2 (5.8 mg) was acetylated as described above (fr. **B-1**). The product was dissolved in MeOH (20 ml) and the soln satd with HCl gas. After stirring overnight and removing solvent, the residue was dissolved in $H_2O$ (2 ml), neutralized with aq. $Na_2CO_3$ and extracted with $CHCl_3$ (4 × 2 ml). Evapn of solvent gave the mixt. **9/10** (10.6 mg, resin). ESIMS *m/z*: 504.7 [M + Na]$^+$.

*Treatment of* **3** *with* $NaBH_4$ To a stirred soln of **3** (3.5 mg) in MeOH–$H_2O$ (4:1) was added $NaBH_4$ (20 mg) at room temp. After 1 hr, another 20 mg of $NaBH_4$ was added and stirring continued overnight. After neutralization (2 N HCl) and evapn, $H_2O$ (3 ml) was added and the mixt. extracted ($CHCl_3$). Evapn of the $CHCl_3$ layer yielded **9** (10.9 mg). EIMS *m/z* (rel. int.): 482 [M + 1]$^+$ (< 5), 438 (26), 169 (55), 168 (18), 157 (33), 143 (20), 142 (14), 129 (14), 114 (33), 112 (56), 110 (12), 101 (12), 100 (28), 98 (27), 97 (14), 96 (10), 95 (18), 91 (17), 87 (10), 86 (15), 85 (16), 84 (46), 83 (18), 82 (18), 81 (21), 79 (14), 77 (20), 73 (18), 72 (63), 71 (20), 70 (87), 69 (49), 68 (11), 67 (29).

*Treatment of* **4** *with* $NaBH_4$. From 1.8 mg of **4** we prepd 5.9 mg of **10** as described above for **9**. EIMS *m/z* (rel. int.): 482 [M + 1]$^+$ (< 5), 438 (< 5), 169 (34), 157 (15), 143 (15), 114 (24), 112 (33), 111 (14), 105 (10), 100 (11), 98 (23), 97 (16), 96 (10), 95 (21), 93 (11), 91 (26), 87 (13), 86 (17), 85 (18), 84 (32), 83 (21), 82 (13), 81 (31), 80 (11), 79 (14), 77 (12), 73 (14), 72 (60), 71 (30), 70 (61), 69 (48), 68 (11), 67 (32), 65 (13).

*Identification and quantification of major constituents of acetylated fr. B-2 by HPLC.* System: $7C_{18}$ (MN, Nucleosil 100; 250/8/4), MeOH–$H_2O$, 1:1 (1.5 ml min$^{-1}$). Injection of acetylated fr. B-2 (in MeOH) showed only 2 peaks with $R_t$ > 7.5 min: (1) $R_t$ 23.93 min and (2) $R_t$ 25.61 min. Co-chromatography of acetylated fr. **B-2** and semi-synthetic samples **9** and **10** revealed that the first eluting compound is identical with **10** and the second one corresponds to **9**. Integration showed that the ratio between **10** and **9** in the natural derivative was 1:3.3.

*Azacyclodecan-2-one.* A soln of cyclononanone (250 mg, 1.8 mmol) in $CHCl_3$ (25 ml) was cooled to 0° and conc. $H_2SO_4$ (3 ml) added. After the successive addition of $NaN_3$ (143 mg, 2.2 mmol), stirring was continued for 15 min at 0° and finally for 1.5 hr at room temp. After addition of $H_2O$ (20 ml), the mixt. was extracted with $CHCl_3$. The combined organic layers were washed with 5% $NaHCO_3$, brine and dried ($Na_2SO_4$) to yield the title compound (229 mg, 82%) after evapn. EIMS *m/z* (rel. int.): 155 [M]$^{+\cdot}$ (82), 138 (100), 137 (32), 126 (32), 112 (76), 110 (26), 99 (32), 98 (84), 97 (28), 91 (30), 84 (48), 83 (28), 82 (26), 72 (26), 70 (40), 69 (28), 67 (32), 59 (24), 57 (32), 56 (82), 55 (78), 54 (24), 53 (20), 45 (36), 44 (24), 43 (48), 42 (50).

*Methyl 9-acetamidononanoate* (**5b.1**). A mixt. of azacyclododecan-2-one (212 mg) and 2 N HCl (8 ml) was heated in a sealed glass tube at 150° for 20 hr. After filtration and evapn of solvent, the brownish crystalline solid was dissolved in MeOH (70 ml). The soln was satd with HCl gas and refluxed for 2 hr. After removing solvent, the residue was stirred with a mixt. of $Ac_2O$ (15 ml) and freshly molten NaOAc (735 mg) overnight. Usual work-up and purification of the crude product by CC resulted in pure **5b.1** (194 mg, 57%). EIMS *m/z* (rel. int.): 230 [M + 1]$^+$ (50), 198 (25), 156 (80), 128 (12), 114 (25), 101 (11), 100 (32), 87 (40), 85 (47), 74 (20), 73 (84), 72 (100), 69 (18), 67 (14), 60 (42), 59 (39), 58 (18), 56 (25), 55 (71).

*Acknowledgement*—This work was supported by the Swiss National Science Foundation.

## REFERENCES

1. Badawi, M. M., Guggisberg, A., van den Broek, P., Hesse, M. and Schmid, H. (1968) *Helv. Chim. Acta* **51**, 1813.
2. Guggisberg, A., Badawi, M. M., Hesse, M. and Schmid, H. (1974) *Helv. Chim. Acta* **57**, 414.
3. Badawi, M. M., Bernauer, K., van den Broek, P., Gröger, D., Guggisberg, A., Johne, S., Kompis, I., Schneider, F., Veith, H.-J., Hesse, M. and Schmid, H. (1973) *Pure Appl. Chem.* **33**, 81.
4. Veith, H.-J., Hesse, M. and Schmid, H. (1970) *Helv. Chim. Acta* **53**, 1355.
5. Guggisberg, A., Veith, H.-J., Hesse, M. and Schmid, H. (1976) *Helv. Chim. Acta* **59**, 3026.
6. Guggisberg, A. and Hesse, M. (1983) in *The Alkaloids* (A. Brossi, ed.), Vol. 22, p. 85. Academic Press, New York.
7. Koldobskii, G. I., Ostrovskii, V. A. and Gidaspov, B. V. (1978) *Russ. Chem. Rev.* **47**, 1084.
8. Guggisberg, A., van den Broek, P., Hesse, M., Schmid, H., Schneider, F. and Bernauer, K. (1976) *Helv. Chim. Acta* **59**, 3013.
9. Schlittler, E. and Hohl, J. (1952) *Helv. Chim. Acta* **35**, 29.
10. Doll, M. K.-H., Guggisberg, A. and Hesse, M. (1994) *Helv. Chim. Acta* **77**, 1229.
11. Veith, H. J., Guggisberg, A. and Hesse, M. (1971) *Helv. Chim. Acta* **54**, 653.