103. Tetrahydrolipstatin: Thermal and Hydrolytic Degradation

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Dedicated to Dr. *O. Isler* on the occasion of his 80th birthday

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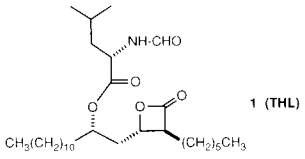
The thermal and hydrolytic degradation of tetrahydrolipstatin (THL, **1**) was investigated. All main degradation products were isolated, characterized, and synthesized. Labile intermediates unavailable to isolation were detected and identified by GC/MS analysis of their silylated derivatives, and whenever possible, compared with independently prepared reference compounds. The identified degradation products represent at least 97% of the total degradation mixture. Two main reaction pathways are proposed. Pharmacological data are reported for the degradation mixture and the main degradation products.

1. Introduction. – Tetrahydrolipstatin (THL; **1**; *N*-formyl-L-leucine (*S*)-1-{[(2S,3*S*)-3-hexyl-4-oxooxetan-2-yl]methyl}dodecyl ester), the hydrogenated derivative of lipstatin, a naturally occurring lipase inhibitor isolated from *Streptomyces toxytricini* [1] [2], is a potent and irreversible inhibitor of pancreatic lipase from several species, including man [3] [4]. It is in phase II of clinical development as an antiobesity- and cholesterol-lowering agent. This demands *inter alia* a close investigation of the stability of **1** on storage over longer periods of time at various temperatures under dry and humid conditions.



1 (THL)

THL (**1**) is characterized by an aliphatic hydrocarbon back-bone bearing a β-lactone moiety and a *N*-formyl-L-leucyl side chain in δ-position to the lactone C=O C-atom and was suspected to be unstable at higher temperature and prone to hydrolysis. Although it is stable at ambient temperature for years ($\sim 0.2\%$ **1** lost after 4 years), it proved to decompose at temperatures above its melting point of 42–44°. We report in this paper the structures of the products formed and propose pathways for the degradation process.

2. Stability Tests. – The lability of **1** when stored *above* its melting point became obvious with the stability tests carried out at 45° and 65°. After 1 month at 45°, the

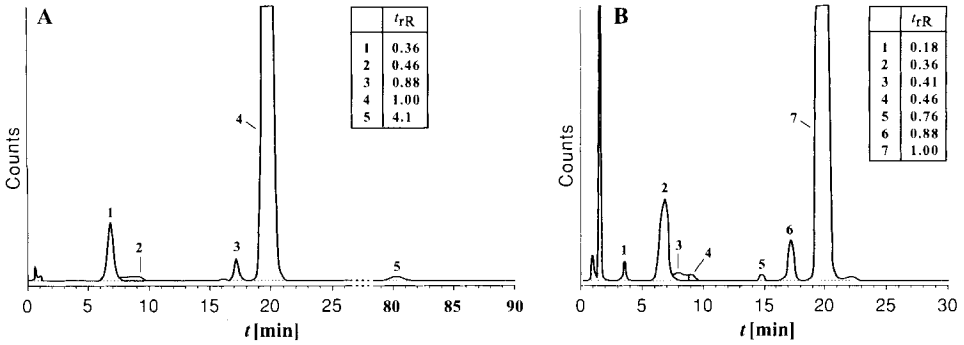


Fig. 1. *HPLC chromatograms of THL* (**1**) *samples stored at 45° for 1 month. A:* dry sealed (laboratory atmosphere without further precautions); *B:* 85% rel. humidity.

Table 1. *Degradation Products and Amount after 1 Month at 45°*[a])

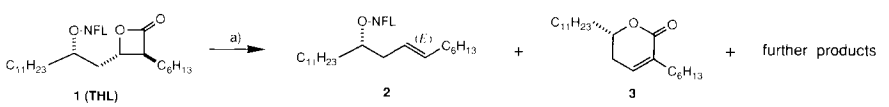
t_R [min]	t_{rR}[b])	Storage[c])	
		Dry[d]) [%]	85% r.h.[e]) [%]
3.6.	0.18	–	0.3
7.0	0.36	4.3	7.9
8.1	0.41	–	0.9
9.0	0.46	0.8	0.4
14.9	0.76	–	0.3
17.4	0.88	1.1	2.6
19.6	*1.00*	(92.8)	(87.4)
80	4.1	0.7	–

[a]) Analyzed by HPLC, all values are by area-%; degradation products of less than 0.2% are omitted.
[b]) t_{rR}: *Relative* retention time, calculated with t_R of THL (**1**) as standard.
[c]) Sample kept at the temp. stated in an exsiccator or drying pistol.
[d]) Laboratory atmosphere without further precautions.
[e]) 85% Relative humidity obtained by equilibration of the gas phase with a sat. aq. KCl soln.

degradation was up to 7% under dry conditions and up to 13% with 85% relative humidity (*cf. Fig. 1* and *Table 1*). The HPLC analysis on a C_{18} reverse-phase column of the degradation mixture revealed that, under dry conditions, highly unpolar products ($t_{rR} = 2.1$ (traces) and 4.1) were formed which could not be detected in the mixture generated with high humidity. This observation prompted us to look at the products formed from **1** under both thermal and hydrolytic conditions.

3. Results. – 3.1. *Thermal Degradation.* Heating neat THL (**1**) to 200° under water pump vacuum for 1 h provided one main product, namely leucyloxy-alkene **2** (≥ 92%

Scheme 1



1 (THL) 2 3

NFL = *N*-formylleucyl
[a]) 120°, neat, 3 d, Ar; or 200°, neat, water pump vacuum, 1 h.

yield), and, in *ca.* 1% yield, α,β-unsaturated δ-lactone **3** *(cf. Scheme 1)*. Along with these products, representing more than 93% of the degradation mixture, we observed at least ten further products (TLC), most of them with higher polarity than **1**.

3.2. *Hydrolytic Degradation.* The hydrolytic degradation of **1** provided a more complex mixture of products than the thermal decomposition. The HPLC chromatogram showed, among the signals for the olefin **2** and the α,β-unsaturated lactone **3**, only signals corresponding to products with a higher polarity than **1**, *e.g.* relative retention time (t_{rR}) < 1 on a C_{18} reverse-phase column *(cf. Table 2)*. Both HPLC and GC of the silylated

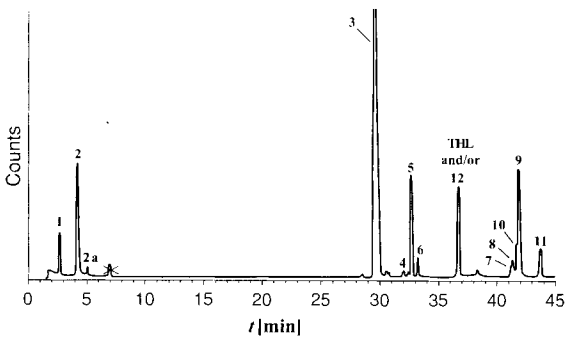


Fig. 2. *GC of a silylated THL* (**1**) *sample stored at 85° with 85% rel. humidity for 2 days.* For correlation of peak number with structure, *cf. Table 4.*

Table 2. *Degradation of THL* (**1**) *on Storage: Product Distribution*[a])

t_{rR}[b])	Dry[c]) [%]				85% r.h.[d]) [%]			Identified as
	45° 3 months	45° 5 months	65°[e]) 3 months	85° 1 week	45°[e]) 3 months	45° 6 months	85°[e]) 3 d	
0.36	10	17	3	7	21	18	23	**10**
0.41	–	–	–	–	4	2	1	**12**[f])
0.46	3	2	2	3	1	2	1	**11**[f])
0.51	–	–	4	–	4	0.4	0.5	**8**
0.76	0.4	–	1	3	4	4	3	**6**
0.81	–	0.5	3	1	–	–	–	–
0.88	11	27	16	26	21	22	26	**9**
1.00	(73)	(49)	(3)	(24)	(41)	(7)	(37)	**1**
2.1	0.2	2	28	4	4	43	5	**3**
4.1	2	2	40	32	–	0.8	3	**2**
< 0.1								**7**[g])

[a]) Analyzed by HPLC, all values are by area-%; degradation products of less than 0.2% are omitted.
[b]) *Relative* retention time, t_R (**1**) = 1.00.
[c]) Laboratory atmosphere without further precautions.
[d]) 85% Relative humidity obtained by equilibration of the gas phase with a sat. aq. KCl soln.
[e]) Preparative run of 1–2 g.
[f]) Isomers of **10** *(cf. Scheme 3)*; characterized by IR, NMR, GC/MS.
[g]) *N*-formyl-L-leucine (**7**) was isolated, but amount not estimated by HPLC (t_{rR} < 0.1).

degradation products revealed the presence of at least 12 compounds, five of which represent more than 80% of the total (*cf. Fig. 2*, and *Table 2*).

The isomeric structures of the products were elucidated by GC/MS of their silylated derivatives, whereas their configuration was determined either by comparison on GC/MS and/or HPLC with independently synthesized reference compounds or by isolation and physico-chemical characterization of the degradation products.

Column Chromatography. As shown in *Table 2*, the degradation of THL at 85° with 85% relative humidity for 3 days provides a similar distribution of products as at 45° for 3 months. This observation gave us the opportunity to produce within a few days larger amounts of degradation mixtures which could be separated in part by column chromatography. On silica gel with CH_2Cl_2/AcOEt, three products **A–C** (9%) were isolated in a fairly pure state, and two mixtures **D** (45%) and **E** (7%) were obtained. Finally, the polar compounds were eluted with MeOH yielding mixture **F** (36%; *cf. Table 3*).

Table 3. *Column Chromatography of Degradation Mixture*

Fraction	R_f[a])	Eluent (*v*/*v*)	[%][b])	Identified as
A	0.78	CH_2Cl_2/AcOEt 95:5	3	**3**
B	0.47	CH_2Cl_2/AcOEt 95:5	1	**4**
C	0.29	CH_2Cl_2/AcOEt 95:5	5	**2**
D	0.23	CH_2Cl_2/AcOEt 90:10	45	**1, 6**
E	0.11	CH_2Cl_2/AcOEt 90:10	7	mixture
F	< 0.1	MeOH	36	**7**, mixture

[a]) Eluent: CH_2Cl_2/AcOEt 95:5.
[b]) By weight of amount submitted to chromatography.

Product **A** was identified as α,β-unsaturated δ-lactone **3**, the absolute configuration being established by comparison of the optical rotation of **3**, *e.g.* $[\alpha]_D^{20} = +54.2$ ($c = 1$, $CHCl_3$) with m.p. 50–51°, with the value found for the reference compound, $[\alpha]_D^{20} = +57.2$ ($c = 1$, $CHCl_3$) with m.p. 51–53°. The physico-chemical data of product **B** proved to be in accordance with those of racemic 4-hydroxy-δ-lactone (3*R**,4*R**,6*R**)-**4** *(cf. Scheme 3)*, a compound described by *Barbier* and *Schneider* [5]. Acid-catalyzed dehydration of product **B** provided **3**, establishing the absolute configuration of **4** as (3*S*,4*S*,6*S*). Product **C** was shown to be the decarboxylation product of **1**, namely alkene **2**. The configuration at the carbinol C-atom was established by saponification of product **C** which yielded *N*-formyl-L-leucine and (*S*,*E*)-henicos-7-en-10-ol (**5**) [2]. Product **D** was a mixture of **1** with a trisubstituted δ-lactone bearing a *N*-formylleucine moiety. By ^{1}H-NMR analysis and comparison with a reference compound prepared from **4** with *N*-formyl-L-leucine, the δ-lactone was identified as (3*S*,4*S*,6*S*)-4-leucyloxy-lactone **6**.

Products **E** and **F** were mixtures of isomeric mono- and dihydroxy acids, and other compounds. Only one compound from **F** could be isolated and characterized, namely *N*-formyl-L-leucine (**7**). Attempts to isolate further compounds from **E** and **F** by chromatography either on silica gel or on *RP-8* reverse-phase columns (*Lobar* columns, *Merck*) failed due to instability of the degradation products.

Fortunately, the *GC/MS analysis* of the silylated degradation mixture permitted structures for all components to be proposed. The configuration was attributed by

comparison of the retention times on HPLC and GC of reference compounds of known configuration with those of the products from the degradation mixture. For correlation in doubtful cases, the products were isolated from the crude mixture by HPLC and then submitted to GC/MS analysis. This was done for the compounds with t_{rR} 0.36, 0.41, 0.46, 0.51, 0.76, and 0.88.

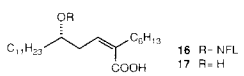
The two peaks 5 and 6 in GC *(cf. Fig. 2)* showed in the MS analysis the same $M^{+\cdot}$ peak and also a similar fragmentation pattern. This was the first evidence that hydrolysis of **1** gave stereoisomers. We believe that the formation of the isomers must result from cleavage of the β-lactone ring which is known to proceed by attack of a nucleophile at either the acyl C-atom with retention of configuration or at the C(β)-atom cleaving the O–alkyl bond with inversion of configuration [6]. By comparison with reference compounds of known configuration, we were able to attribute to peak 6 structure **4** and to peak 5 structure **8** which is the C(4) epimer of **4**. Furthermore, the main product detected with HPLC (*cf. Table 2*, t_{rR} 0.88) and shown to cause peak 11 of the GC was identified as *N*-formylleucine ester of **8**, namely **9**. The latter is an epimer of **6** which was isolated by chromatography but could not be found in the GC/MS analysis.

Table 4. *Degradation Products by Hydrolysis of THL* (**1**)[a])[b])

No.[c])	t_{rR}[d])	Compound[e])	Comment
1	–	L-leucine	Artefact
2	0.1	**7** (NFL-OH)	Monosilylated **7**
2a		**7**	Disilylated **7**
3	2.1	**3**	α,β-Unsaturated δ-lactone
4	0.21	**15**	Dihydroxy acid
5	0.51	**8**	Epimer of **4**
6	0.67	**4**	Epimer of **8**
7	0.41	**12**	C(3): *R*; for attribution of configuration *cf. Discussion*
8	0.51?	**16**[i])	Artefact; dehydration of **10** and/or **11**
9	0.36	**10**	Epimer of **11**
10	0.46	**11**	Epimer of **10**
11	0.88	**9**	Epimer of **6**
12	4.1	**2**	Decarboxylation product
–	0.76	**6**	Epimer of **9**; not detected but isolated
–	< 0.2	**14**[f])	Detected?
–	2.6	**5**[f])	Artefact?[g])
–	[h])	**17**[f])[j])	Artefact; hydrolysis of **16** *(vide supra)*
–	[h])	**18**[f])[j])	(2 *E*/*Z*)-isomers

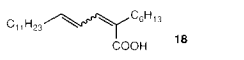
[a]) 85° with 85% rel. humidity for 2 d.
[b]) Silylated for GC/MS.
[c]) Referring to peak numbers in *Fig. 2*.
[d]) *Relative* retention time; HPLC conditions stated in *Exper. Part.*
[e]) For structures *cf. Schemes 2* and *3*; unsilylated structures shown.
[f]) Traces.
[g]) Positively detected only by GC/MS.
[h]) Not determined.

[i])



16 R = NFL
17 R = H

[j])



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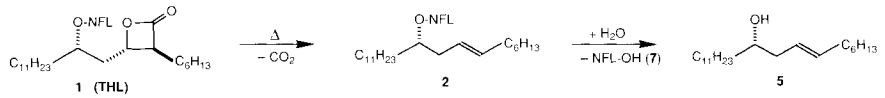
Peaks 7, 9, and 10 proved to correspond to both position and stereoisomers. Peak 9 was identified as corresponding to δ-leucyloxy-β-hydroxy acid **10** with retained configuration (attribution by comparison with a synthesized reference sample), whereas peak 10 is assigned to the C(3) epimer of **10**, namely **11**. Peak 7 proved to correspond to a δ-hydroxy acid **12** (or **13**) with the *N*-formylleucyloxy group in β-position. The characteristic MS peak of *m/z* 257 $[C_{11}H_{23}CH(OSiMe_3)^+]$ allowed the latter structure to be ascertained. The configuration at C(3) could not be established directly by comparison with a reference sample, since we were not able to synthesize a compound of this type in acceptable purity. Together with these compounds, we also identified products which were isolated in pure form by column chromatography from either thermally degraded or hydrolyzed THL (**1**) samples. A compilation of the degradation products is given in *Table 4*.

4. Discussion. – As shown in *Table 2*, the main degradation of THL at temperatures below 85° is caused by hydrolysis of **1**. The products resulting form thermal decomposition represent only a minor part of the degradation mixture.

4.1. *Thermal Degradation.* The decarboxylation of β-lactones to alkenes is a well known reaction and is often used to prepare alkenes stereospecifically [2] [6]. In the reaction mixture after thermolysis, and also hydrolysis, we only found the expected (*E*)-configurated alkene **2** with the configuration at the two remaining chiral centers not being affected during the degradation [2] [7] (*Scheme 2*). Alkene **2** proved to be prone to

Scheme 2



1 (THL) **2** **5**

NFL = *N*-formylleucyl

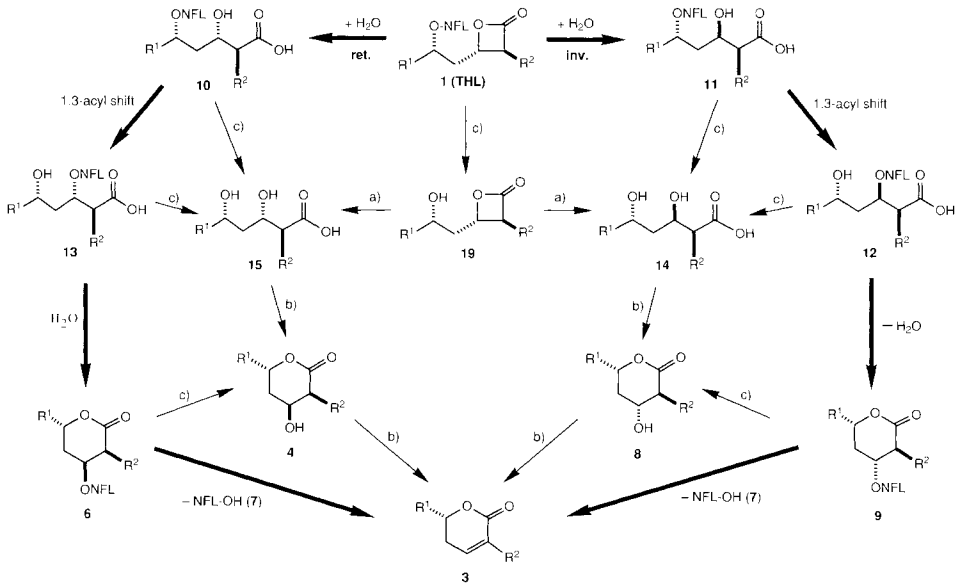
hydrolysis yielding the homoallylic alcohol **5** by cleavage of the ester link. The alcohol was found in low amount in the degradation mixture and was identified by comparison with a reference sample obtained from **2** by saponification [2]. The formation of the δ-lactone **3** is less obvious. The lactone cannot arise from a thermal process (decarboxylation), because the C-frame is still intact. A possible pathway is proposed in *Scheme 3*. In the first step, the β-lactone is hydrolyzed to the β-hydroxy acid **10** which rearranges through a 1,3-acyl shift to δ-hydroxy acid **13**. The latter forms δ-lactone **6** which subsequently eliminates *N*-formyl-L-leucine (**7**) to yield **3**. To go through this reaction sequence only a catalytic amount of H_2O must be present. All THL (**1**) samples used for our decomposition experiments contained traces of H_2O (max. 0.5% H_2O, THL is slightly hygroscopic!).

Thermolysis of **1** on a preparative scale was done several times at temperatures between 120° and 200°. The ratio olefin/δ-lactone ranged from 88:12 (0.45% H_2O) to 99:1 (0.1% H_2O). We suppose that the different amounts of H_2O in the samples are responsible for this variation of product ratio.

Therefore, we can summarize as follows: *1*) alkene **2** is the sole thermal degradation product, and *2*) the further degradation products, most of them of higher polarity than **1**, arise from a hydrolytic process.

4.2. *Hydrolytic Degradation.* The epimers found in the degradation mixture of **1** (*vide supra*) suggest two hydrolytic degradation pathways, one with *retention* and one with

Scheme 3. *Degradation of THL* (**1**) *under Hydrolytic Conditions*



NFL = *N*-formylleucyl; $R^1 = C_{11}H_{23}$; $R^2 = C_6H_{11}$

[a]) $+H_2O$. [b]) $-H_2O$. [c]) $+H_2O$, –NFL-OH.

inversion of configuration at C(3), the former C(β)-atom of the β-lactone moiety. The compounds with conserved configuration, namely **4**, **6**, **10**, **13**, and **15**, and their epimers, *e.g.* **8**, **9**, **11**, and **14**, together with α,β-unsaturated lactone **3**, and β-(*N*-formylleucyl)oxy-δ-hydroxy carboxylic acid **12** (for attribution of configuration *vide infra*) permit us to propose the degradation pathways depicted in *Scheme 3*.

Degradation Pathway with Retention of Configuration at C(3). The rearranged product **13**, which obviously results from **10** by a 1,3-acyl shift, is beside **15** the sole product in this series which could not be isolated. But the observation that **10** neat, or in solution, rearranges quantitatively (TLC) at ambient temperature within a few hours to days to δ-lactone **6** gives us an explanation for our fruitless attempts to detect or even to isolate **13**. We also were not able to isolate dihydroxycarboxylic acid **15**, but we detected it at its silylated derivative in the GC/MS analysis. The acid is a hydrolysis product formed either from **10** or **13**, or from THL (**1**) *via* hydroxy-β-lactone **19** [8]. The latter was detected in very low amount (< 0.2%) in the degradation mixture. Dihydroxy acid **15** was synthesized by saponification of **4** (KOH in aqueous dioxane, r.t., 4 h) and proved to be very reactive. It cyclizes at a temperature above 0° (neat or in solution in CH_2Cl_2 or MeOH within hours to a few days) quantitatively (TLC) to hydroxy-δ-lactone **4**, a compound which also was found in low amount (< 0.2%) in degradation samples.

The detection of reactive intermediates as **10**, **15**, and also **11**, **12**, and **14** (*vide infra*) as their silylated derivatives in the degradation mixture by GC/MS suggests a stabilization of these compounds by **1** and/or the degradation products. The stabilizing effect is lost by diluting the reaction mixture giving rise to formation of δ-lactones **4** and **6**, and their C(4) epimers **8** and **9** at a higher rate. δ-Lactone **6** could not be observed by GC/MS in the

degradation mixture, due to the antiperiplanar arrangement of the substituents permitting easy elimination of *N*-formylleucine (**7**). The latter takes place either under the conditions of derivatization applied to the degradation mixture or in the GC or MS system. The instability of leucyloxy-δ-lactone **6** is also documented by the MS spectrum of the pure compound which provides no $M^{+\cdot}$ peak but a signal of m/z 336 corresponding to $[M - (\text{OCHLeuOH}]^{+\cdot}$.

The quantitative analysis of the degradation mixture (*cf. Table 2*) allows us to propose the following main reaction pathway for the hydrolysis of **1** with retention of configuration (bold arrows on the left half of *Scheme 3*): In the first step, the β-lactone is hydrolyzed to the β-hydroxy acid **10** which, after rearrangement, gives the δ-lactone **6**. The latter eliminates readily *N*-formylleucine (**7**) yielding the stable unsaturated δ-lactone **3**.

Further reaction pathways depicted on the left part of *Scheme 3* are also observed but contribute only modestly to the formation of **3**.

Degradation Pathway with Inversion of Configuration at C(3). Running the degradation of THL in a dioxane/buffer solution of pH 6.8 yielded δ-lactones **8** and **9** together with four further degradation products of higher polarity than the starting material. The *trans*-relationship of the hexyl chain and the OH or leucyloxy group in **8** and **9** was elucidated by ^{1}H-NMR spectroscopy as well as the relative configuration of the two δ-lactones. The absolute configuration was shown by dehydration of the two compounds to **3** and comparison of the optical rotation value with independently prepared samples of **3** and (*R*)-**3**.

GC/MS Analysis of the polar fraction *(cf. Exper. Part)* showed that, in addition to the compound with known configuration, two further products, namely **11** and **12**, were present. These two products have already been found in the degradation mixtures of neat THL stored under dry or humid conditions. Since, so far, we had isolated only compounds with conserved and inverted configuration at C(3), we attributed to **11** and **12** the structures shown in *Scheme 3* with (*R*)-configuration at C(3). Structure **13**, an epimer of **12**, was excluded, because we were not able to detect the intermediate **13** in samples of **10** which partially had turned into δ-lactone **6**. Obviously, it cyclizes to **6** immediately after its formation.

Therefore, we propose for the formation of the compounds with inverted configuration at C(3) the degradation pathway depicted on the right half of *Scheme 3*. In a first step (bold arrows) the β-lactone moiety is opened by attack of H_2O at the C(β)-atom yielding β-hydroxy acid **11** with inverted configuration at C(3). Following the reaction pathway already discussed for the reaction with retention of configuration at the C(β)-atom, we are able to explain the formation of the δ-lactone **9** *via* rearranged product **12** as well as the formation of **8**, both are precursors of **3**, the final degradation product of the solvolysis of **1**. Hydroxy-β-lactone **19** and its ring-opened product **14**[1]), both postulated precursors of **8**, were not positively detected in the degradation mixture obtained in the dioxane/buffer solution. However, **19** was found in traces (< 0.2%) in the degradation mixture of neat THL (**1**). The main or even sole degradation pathway followed is, therefore, the reaction sequence indicated by bold arrows.

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[1]) Tests with **14** (silylation conditions applied on its potassium salt) gave rise to GC/MS of trisilylated **14** and of its cyclization product **8** as monosilylated derivative.

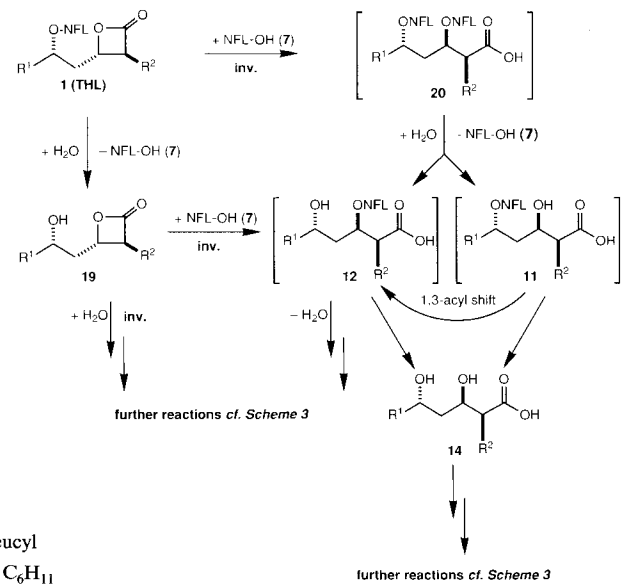
The opening of β-lactones by H_2O with inversion of the configuration at the C(β)-atom is reported for β-butyrolactones [9] [10]. *Olson* and *Miller* observed also a pH-dependence of the course of hydrolysis and were able to demonstrate that in a pH range of 2 to 8 predominantly inversion of configuration occurs, whereas at higher or lower pH values also cleavage with retention of configuration at the C(β)-atom is found [9]. They and others [11–13] deduced from kinetic experiments that the reaction in aqueous solution below pH 9 is H_2O-catalyzed and independent of pH. An acid-catalyzed hydrolysis of the lactone moiety occurs exclusively in concentrated (> 2M) aqueous solutions of strong acids [9] [14].

The relatively high stability of THL (**1**) to hydrolysis compared with *e.g.* propiolactone or β-butyrolactone is due to the substitution of the β-lactone moiety with two long alkyl chains which render the attack by a nucleophile, *e.g.* H_2O, more difficult. This steric and protecting effect of the substituents may also explain the fact that cleavage of the β-lactone moiety in **1** takes place by attack at the C=O C-atom *and* at C(3) and not exclusively at C(3) as observed with simple β-lactones.

So far, we described the degradation of THL (**1**) as an *inter*molecular process beginning with the attack of a H_2O molecule at the β-lactone moiety. However, especially when neat **1** is decomposed, we cannot exclude the attack of a nucleophile other than H_2O, *e.g.* *N*-formylleucine (**7**; *cf. Scheme 4*). Cleavage of the β-lactone ring (**1** or **19**) by addition of **7** to the C(β)-atom would yield diester **20** or hydroxy ester **12** with inverted configuration at C(3). Hydrolysis of one or two of the ester groups in **20** would give hydroxycarboxylic acids **11** and **12**, or dihydroxycarboxylic acid **14**, all three of which could continue to follow the reaction sequence already shown in *Scheme 3*.

In dilute solutions, often used to synthesize our reference compounds, an attack of **7** is not very likely: firstly, since its concentration is very low compared to that of H_2O, and

Scheme 4. *Ring Cleavage with Inversion at C(3) by Attack of NFL-OH* (**7**)



NFL = *N*-formylleucyl

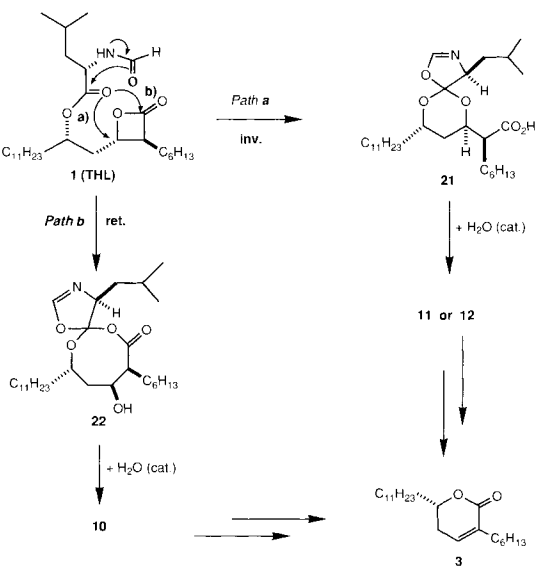
$R^1 = C_{11}H_{23}$, $R^2 = C_6H_{11}$

secondly, the rate constant for the reaction of a nucleophile, like a carboxylic acid, with the β-lactone moiety, is not very likely to be very large (*e.g.* the rate constant for acetate, which can give us an idea of that of a deprotonated α-formylamino acid, is only six times larger than that of H_2O [12] [13]). Attempts to detect by GC/MS or even to isolate a di-*N*-formylleucyl ester like **20** in degraded THL (**1**) samples failed. Also in degradation experiments in dioxane/0.1M acetate buffer (3:1) at 100° for 3 d, we could not detect acetylated products (GC/MS).

Under neat conditions, a nucleophilic attack by **7** seems to be more likely. To check this possibility, we heated a mixture of **1** and *N*-formyl-L-phenylalanine neat (amino acid dissolved only in part) or in DMSO solution with 85% relative humidity to 85° for 4 days. In both samples, we were not able to detect by spectroscopic methods (NMR, MS) products bearing a phenylalanine moiety. Therefore, under our decomposition conditions, the attack of a nucleophile other than H_2O obviously does not occur.

Hitherto, the results were interpreted as reaction sequences beginning with an *inter*-molecular attack of a nucleophile on THL (**1**). However, they do not permit us to strictly exclude a degradation pathway which starts with an *intra*molecular attack of the *N*-formylleucyl moiety at the C(β)-atom (with inversion of configuration at C(3), *Path a* in *Scheme 5*) or at the acyl C-atom (with retention of configuration at C(3), *Path b*)[2]). After

Scheme 5. *Intramolecular Attack at C(3)*



hydrolysis, both intermediates **21** and **22** would give the same reaction products shown in *Scheme 3*. The ortho-esters **21** and **22** were not found and not specially sought for, because this type of compound is not very stable under the slightly acidic reaction conditions (liberation of **7**) [17] [18].

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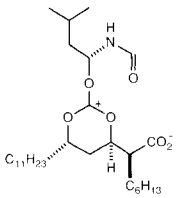
[2]) Neighboring group participation of amides under neutral or acidic conditions occurs *via* nucleophilic attack by the O-atom on either positively charged or neutral reagents [15] [16].



23

An intermediate in the formation of **21** is the 1,3-dioxan-2-ylium ion **23**. *Mestdagh* and *Pancrazi* have shown that 1,3-dioxolan-2-yl cations are intramolecularly attacked by amide groups forming [4.4]-spiro compounds, ring contracted analogues of [4.5]-spiro-ortho-ester **21** [19]. Spiro-ortho-esters are hydrolyzed very rapidly [19]. 1,3-Dioxan-2-yl cations like **23** are synthesized under strongly acidic conditions or in the presence of *Lewis* acids under nonaqueous conditions [20]. Rings with more than six members, necessary for the formation of **22**, are not formed under these conditions [20]. Hence, a cleavage of the β-lactone moiety in THL (**1**) with neighboring group participation can be excluded for *Path b* in *Scheme 5* but not for *Path a* which might contribute to the formation of hydroxy acids **11** and **12**.

5. Pharmacological Data of THL (1) and Degradation Products. – Results for **1** and some of its degradation products were obtained from two *in vitro* tests: inhibition of

Table 5. *Degradation Products of THL* (**1**): *Pharmacological Data* [22] (n.t.: not tested)

Compound	IC_{50} (PPL)[a]	IC_{50} (PHP)[b]	$\frac{IC_{50}\text{ (PHP)}}{IC_{50}\text{ (PPL)}}$	Prel. toxicity[c]
	[μg/ml]	[μg/ml]		[mg/kg]
THL (**1**)	0.2	0.16	0.8	> 400 *i.v.* 2000–4000 *i.p.* > 4000 *p.o.*
24[d])	0.31	0.22	0.7	125–250 *i.v.* > 5000 *p.o.*
19	51	0.038	0.0007	
2	225	180	0.8	250–500 *i.v.* > 4000 *p.o.*
3	> 100	> 85		250–500 *i.v.* > 4000 *p.o.*
4	653	60	0.09	
5	> 1000	> 850		
6	~ 3500	n.t.		250–500 *i.v.* > 4000 *p.o.*
7[e])	n.t.	n.t.		250–500 *i.v.* > 4000 *p.o.*
8	≫ 880	> 857	< 1	
9	≫ 900	> 860	< 1	
10	n.t.	n.t.		

[a]) PPL: porcine pancreatic lipase, *in vitro*.
[b]) PHP: rat post-heparin plasma, *in vitro* (systemic lipases).
[c]) Preliminary toxicity in mice.
[d]) Mixture of degradation products; THL (**1**) content: 47%!
[e]) *N*-Formyl-L-leucine (**7**).

porcine pancreatic lipase (PPL) [3]; and inhibition of rat postheparin plasma lipolytic activity (PHP), a mixture of two systemic lipases, *e.g.* lipoprotein lipase and hepatic triglyceride lipase [21]. These and the preliminary toxicity values (mice, lethal dose) are compiled in *Table 5*. The results can be summarized as follows: *1*) δ-Lactones are less active, by a factor of at least 450, than **1** as inhibitors of PPL and PHP. *2*) The decarboxylation product **2** is only a very weak inhibitor of PPL and PHP. *3*) The δ-hydroxy-β-lactone **19** showed a weak inhibitory activity in the PPL test ($IC_{50} = 51$ µg/ml) but proved to be a very potent inhibitor of the PHP activity ($IC_{50} = 0.038$ *vs.* 0.16 µg/ml for THL). *4*) The degradation mixture **24** obtained by hydrolysis of THL at 85° with 85% relative humidity for 3 d, exhibited the activity expected from the remaining content of **1** (47%). The degradation products do not contribute significantly to the overall inhibitory activity[3]). *5*) Prel. toxicity (mice): the lethal dose for *p.o.* administration is very high (> 4000 mg/kg). For *i.v.* administration the lethal dose ranged between 125 and 500 mg/kg.

6. Conclusion. – As above its melting point (42–44°), THL (**1**) is prone to both hydrolysis and thermolysis, storage over longer periods of time must exclude these conditions. The galenical formulation(s) should also reflect the hydrolytic instability which suggests the use of desiccating and/or stabilizing excipients as well as the utilization of an appropriate packaging. At least 97% of the products formed from **1** were identified. The main degradation products as well as the degradation mixture have no significant inhibitory activity on either the pancreatic or the systemic lipases and display no adverse effects.

The skillful technical assistance of Ms. *C. Haenggi* and Mr. *E. Schertenleib* is gratefully acknowledged. We wish to thank our colleagues from the Central Research Units for determination of the physical and analytical data: Dr. *W. Arnold* (NMR), Dr. *A. Dirscherl* (microanalyses), Dr. *M. Grosjean* (IR), Mr. *W. Meister* (MS). We also thank Dr. *N. Gains* for reviewing the manuscript and Ms. *M. Steffan* for her secretarial work.

Experimental Part

General. Org. extracts were dried (Na_2SO_4), filtered, and evaporated on a rotary evaporator at 40–50°/15–20 Torr. TLC: *DC-Fertigplatten Kieselgel F_{254} (Merck)*. Column chromatography: *Merck* silica gel *60* (230–400 mesh ASTM). M.p.: *Tottoli* cap. melting-point apparatus; uncorrected. IR (cm^{-1}): *Nicolet 7199 FT-IR*; in KBr or neat. ^{1}H-NMR (δ[ppm] rel. to TMS as internal standard; *J* in Hz): *Bruker AC-250*. MS: *MS-9-ZAB*, data system *SS 200* (*Finnigan-MAT*); EI, 70 eV (*m/z*, % relative intensity, fragment). The samples of N-*formyl*-L-*leucine (S)-1-{[(2S,3S)-3-hexyl-4-oxooxetan-2-yl]methyl}dodecyl ester* (THL; **1**) used for degradation experiments contained 0.1 to 0.5% H_2O. The degradation experiments were performed in a sealed, thermostated exsiccator or drying pistol. Dry: the sample was heated in normal laboratory atmosphere without further precautions; humid: ~ 85% rel. humidity was generated by constant equilibration of the gas phase in the sealed container over a sat., aq. KCl soln. HPLC: column: *NovaPak C_{18} 4µ* 125 × 3.9 mm (*Waters*); mobile phase: MeCN/H_2O 80:20 (MeCN from *Rathburn*, HPLC quality, and deionized H_2O); flow: 1.0 ml/min for 25 min, then 1.5 ml/min for 65 min; detection: UV 195 nm, *Uvikon 735 LC* (*Kontron*); injected amount: 20 µg in 20 µl; integration: all values are by area-percent and integrated with the *ACCESS. CHROM* software of *PE Nelson* on a *DEC µVAX*. GC/MS: For silylation the samples were dissolved in a 1:1 mixture of pyridine/*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). After at least 2 h, an aliquot of the reaction soln. was submitted to GC/MS. The apparatus was composed of a *Hewlett-Packard* gas chromatograph model *5890A*, coupled directly to a mass spectrometer, model *7070F*, of *VG Instruments*, *via* a 60-cm fused-silica capillary with an internal diameter of 0.15 mm. The temp. of this

[3]) The low amount (generally $\ll 0.2\%$) of **19** detected in the degradation mixture cannot be perceptible in the IC_{50} (PHP) value!

interface was kept at 300°. The chromatography column consisted in a 15-m fused-silica capillary of 0.3 mm internal diameter coated with the methyl silicon gum DB-1. After injection the column temp. was increased from 100 to 330° at a heating rate of 4°/min. Temp. of the injection part was kept at 200°. EI-MS: at 70 eV at a source temp. of *ca.* 250°. The data acquisition was carried out with the data system *SS 300* (*Finnigan-MAT*). The EI-MS data are compiled in *Table 6*.

1. *Synthesis of Reference Compounds.* 1.1. N-*Formyl*-L-*leucine* (S,E)-*1-(Non-2-enyl)dodecyl Ester* (**2**). Neat 3.13 g (6.3 mmol) **1** were heated to 200° under water pump vacuum for 1 h. FC of the mixture on silica gel yielded 2.29 g (81%) of **2** as a colorless oil. $[\alpha]_D^{20} = -14.7$ ($c = 1$, $CHCl_3$). IR: 3302 (NH), 1739 (ester), 1667, 1529 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*, $J = 7$, 2 CH_3CH_2); 0.94 (*d*, $J = 6$) and 0.96 (*d*, $J = 6$) ($(CH_3)_2CH$); 1.25 (br. *s*, 13 CH_2); 1.44–1.78 (*m*, CH_2(11), $(CH_3)_2$ $CHCH_2$); 1.92–2.04 (*m*, CH_2(6)); 2.17–2.36 (*m*, CH_2(9)); 4.71 (*dt*, $J = 4.5$, 9, H–C–N); 4.90 (*quint.*, $J = 6$, CHO); 5.24–5.38 (*dt*, $J = 6$, 15) and 5.42–5.56 (*dt*, $J = 6$, 15) (*AB* system, HC=CH); 6.02 (*d*, $J = 9$, NH); 8.21 (*s*, CHO). MS: no M^+, 292 (60, $[M - OCHLeuOH]^+$), 160 (22, $[OCHLeuOH + H]^+$). Anal. calc. for $C_{28}H_{53}NO_3$ (451.74): C 74.45, H 11.83, N 3.10; found: C 74.16, H 11.96, N 3.04.

1.2. (3S,4S,6S)-*3-Hexyl-3,4,5,6-tetrahydro-4-hydroxy-6-undecyl-2*H-*pyran-2-one* (**4**). A soln. of 31.68 g (64 mmol) of **1** in 3.4 l of dioxane and 128 ml of 1N KOH was stirred at r.t. for 2.5 h. Then 8 ml of 1N KOH in 1.4 l of dioxane were added and stirring continued for additional 1.5 h. The soln. was acidified with 140 ml of 1N HCl and evaporated. The residue was dissolved in Et_2O, washed with brine, the org. phase dried, and evaporated. Recrystallization of the residue from AcOEt yielded 16.20 g (71%) of **4**. M.p. 105–107° ([7]: 107.5°). $[\alpha]_D^{20} = -3.67$ ($c = 0.3$, $CHCl_3$). IR: 3425 (OH), 1695 (lactone). ^{1}H-NMR and MS: *cf.* [5]. Anal. calc. for $C_{22}H_{42}O_3$ (354.58): C 74.52, H 11.94; found: C 74.48, H 11.80.

1.3. (S)-*3-Hexyl-5,6-dihydro-6-undecyl-2*H-*pyran-2-one* (**3**). A soln. of 250 mg (0.7 mmol) of **4** and 100 mg of TsOH in 15 ml of $CHCl_3$ was refluxed for 18 h and then evaporated. The residue was dissolved in CH_2Cl_2, washed with H_2O, dried, and evaporated. FC on silica gel with hexane/AcOEt 80:20 gave 180 mg (76%) of **3** as a solid product. Crystallization from hexane/AcOEt yielded colorless crystals. M.p. 51–53°. $[\alpha]_D^{20} = +57.2$ ($c = 1$, $CHCl_3$). IR: 1720 (lactone). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 1.10–1.85 (*m*, 14 CH_2); 2.15–2.45 (*m*, 2 CH_2C=); 4.35 (*q*-like, $J = 6$, H–C(6)); 6.53 (*t*-like, $J = 5$, CH=). MS: 336 (20, M^+), 181 (45, $[M - C_{11}H_{23}]^+$), 155 (100, $C_{11}H_{23}^+$). Anal. calc. for $C_{22}H_{40}O_2$ (336.56): C 78.51, H 11.98; found: C 78.40, H 12.00.

1.4. (R)-*3-Hexyl-5,6-dihydro-6-undecyl-2*H-*pyran-2-one* ((*R*)-**3**). Prepared in analogy to **3** from (3S,4S,6R)-*3-hexyl-3,4,5,6-tetrahydro-4-hydroxy-6-undecyl-2*H-*pyran-2-one* [8] in 92% yield. M.p. 50–52°. $[\alpha]_D^{20} = -57.1$ ($c = 1$, $CHCl_3$).

1.5. (S,E)-*Henicos-7-en-10-ol* (**5**). Compound **1** (2.00 g, 4 mmol) was decarboxylated as stated in *1.1*. The crude product was dissolved in 30 ml of THF and 10 ml of 1N NaOH and refluxed for 24 h. Then, the soln. was evaporated, the residue dissolved in H_2O and extracted with Et_2O. The combined org. phases were washed with H_2O, dried and evaporated. FC on silica gel with hexane/AcOEt 95:5 yielded 0.90 g (72%) of **5** as colorless crystals. M.p. 42–44° ([7]: 40.5–41°). $[\alpha]_D^{20} = +0.6$ ($c = 1$, $CHCl_3$). ($[\alpha]_D^{20} = +1$ ($c = 1$, $CHCl_3$) [7].) IR: 3350 (OH), 1630, 962 (olefin). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 1.18–1.53 (*m*, 14 CH_2); 1.60 (*s*, OH); 1.96–2.14 (*m*, 3 H) and 2.19–2.32 (*m*, 1 H) (CH_2–C=); 3.52–3.65 (br., CHO); 5.32–5.46 (~ *dt*, $J = 7$, 16) and 5.48–5.62 (~ *dt*, $J = 7$, 16) (*AB* system, HC=CH). MS: no M^+, 185 (4.3, $[C_{11}H_{23}CH{=}OH]^+$). GC/MS (monosilylated): 382 (0.1, M^+), 367 (2.2, $[M - CH_3]^+$), 257 (100, $[C_{11}H_{23}CH{=}OSiMe_3]^+$), 227 (6, $[C_6H_{13}CH{=}CHCH_2CH = OSiMe_3]^+$). Anal. calc. for $C_{21}H_{42}O$ (310.57): C 81.22, H 13.63; found: C 80.97, H 13.66.

1.6. N-*Formyl*-L-*leucine* (3S,4S,6S)-*3-Hexyl-3,4,5,6-tetrahydro-2-oxo-6-undecyl-2*H-*pyran-4-yl Ester* (**6**). A stirred soln. of 708 mg (2 mmol) of **4**, 20 mg (0.16 mmol) 4-(dimethylamino)pyridine, and 318 mg (2 mmol) of N-*formyl*-L-*leucine* (**7**) in 3 ml of abs. DMF was cooled to 5°, and 454 mg (2.2 mmol) of *N*,*N*′-dicyclohexylcarbodiimide were added. After 18 h at r.t., the mixture was poured into H_2O and extracted with Et_2O. The combined org. extracts were washed with H_2O, dried, and evaporated. FC of the residue on silica gel with hexane/AcOEt 2:1 yielded 260 mg of **6**. M.p. 98–100°. $[\alpha]_D^{20} = +14.2$ ($c = 1$, $CHCl_3$). IR: 3361 (NH), 1712 (ester, lactone), 1680, 1654, 1518 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 0.96 (*d*, $J = 5.7$, $(CH_3)_2CH$); 1.18–1.86 (*m*, 15 CH_2, $(CH_3)_2CHCH_2$); 2.0–2.19 (*m*, 1 H) and 2.28 (*dt*, $J = 2$, 14, 1 H) (CH_2(5)); 2.32–2.50 (*dt*, $J = 5$, 10, H–C(3)); 4.52–4.70 (*m*, H–C(6), NH); 5.32–5.38 (*m*, H_{eq}–C(4)); 5.97 (br. *d*, $J = 7$, NH); 8.21 (*s*, CHO). MS: no M^+, 336 (15, $[M - OCHLeuOH]^+$), 160 (16, $[OCHLeuOH + H]^+$). CI: 513 (39, $[M + NH_4]^+$]. Anal. calc. for $C_{29}H_{53}NO_5$ (495.74): C 70.26, H 10.78, N 2.83; found: C 70.30, H 10.56, N 2.83.

1.7. (3S,4R,6S)-*3-Hexyl-3,4,5,6-tetrahydro-4-hydroxy-6-undecyl-2*H-*pyran-2-one* (**8**) *and* N-*Formyl*-L-*leucine* (3S,4R,6S)-*3-Hexyl-3,4,5,6-tetrahydro-2-oxo-6-undecyl-2*H-*pyran-4-yl Ester* (**9**). A soln. of 5 g (10.1 mmol) of **1** in 100 ml of dioxane and 40 ml of 0.1M phosphate buffer pH 6.8 was refluxed for 5 d. The mixture was evaporated, the residue suspended in H_2O and extracted with CH_2Cl_2. The combined org. phases were washed with H_2O, sat.

$NaHCO_3$ soln., and brine, dried, and evaporated yielding 4.65 g of a yellowish, sticky oil. FC with hexane/AcOEt 80:20 gave 0.32 g (9%) of **3** (m.p. 51–53°. $[\alpha]_D^{20} = +56.8$ ($c = 1$, $CHCl_3$)), 0.49 g (14%) of **8**, and 0.13 g (3%) of **9**. With MeOH, 2.90 g of a mixture of more polar compounds were eluted.

Data of **8**: M.p. 69–70° (hexane/AcOEt). $[\alpha]_D^{20} = -33.0$ ($c = 0.9$, $CHCl_3$). IR: 3438 (OH), 1687 (lactone). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 1.17–1.79 (*m*, 15 CH_2); 1.79–2.00 (*m*, H_{ax}–C(5)); 1.86 (*d*, $J = 4.5$, OH); 2.21 (*ddd*, $J = 3.5$, 4.5, 14, H_{eq}–C(5)); 2.41 (*dt*, $J = 4.5$, 10, H_{ax}–C(3)); 3.88–4.03 (*m*, H–C(4)); 4.13–4.25 (*m*, H–C(6)). MS: 354 (1.5, $M^{+\cdot}$), 292 (8, $[M - (H_2O + CO_2)]^{+\cdot}$). Anal. calc. for $C_{22}H_{42}O_3$ (354.58): C 74.52, H 11.94; found: C 74.57, H 11.93.

Data of **9**: Colorless oil. $[\alpha]_D^{20} = -57.2$ ($c = 0.9$, $CHCl_3$). IR: 3313 (NH), 1740 (ester, lactone), 1675, 1525 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 0.97 (*d*, $J = 5$, $(CH_3)_2CH$); 1.18–1.95 (*m*, 15 CH_2, H–C(4), $(CH_3)_2CHCH_2$); 2.30 (*ddd*, $J = 2.5$, 5, 13, H–C(5)); 2.67 (*dt*, $J = 5$, 8.5, H–C(3)); 4.17–4.31 (*m*, H–C(6)); 4.63–4.76 (*m*, H–C–N); 5.03–5.15 (*m*, H–C(4)); 5.90 (*d*, $J = 9$, NH); 8.24 (*s*, CHO). MS: no $M^{+\cdot}$, 338 (28, $[M-OCHLeuOH]^{+\cdot}$), 160 (12, $[OCHLeuOH + H]^{+\cdot}$). Anal. calc. for $C_{29}H_{53}NO_5$ (495.75): C 70.26, H 10.78, N 2.83; found: C 69.98, H 10.82, N 2.78.

1.8. *N-Formyl-L-leucine (S)-1-{(2S,3S)-2-(Benzyloxy)-3-[(benzyloxy)carbonyl]-3-hexylpropyl}dodecyl Ester* (**25**). Prepared in analogy to **6** from benzyl (2*S*,3*S*,5*R*)-3-(benzyloxy)-2-hexyl-5-hydroxyhexadecanoate [8] in 52% yield: colorless liquid. IR: 3310 (NH), 1736 (ester), 1691, 1515 (amide). ^{1}H-NMR ($CDCl_3$): 0.8–1.0 (*m*, 2 CH_3CH_2, $(CH_3)_2CH$); 1.1–1.9 (*m*, 15 CH_2, CH_2CHOCH_2, $(CH_3)_2CHCH_2$); 2.71–2.84 (*m*, CHC_6H_{13}); 3.63–3.76 (*m*, $CHOCH_2$); 4.46 (*d*) and 4.50 (*d*), (*AB* system, $J = 11$, $PhCH_2OCH$); 4.60–4.73 (*m*, H–C–N); 4.94–5.09 (*m*, H–C(1)); 5.11 (*d*) and 5.13 (*d*), (*AB* system, $J = 12$, $PhCH_2OCO$); 5.89 (*d*, $J = 10$, NH); 7.17–7.40 (*m*, 10 arom. H); 8.15 (*s*, CHO). MS: 694 (20, $[M + H]^{+\cdot}$).

1.9. *(2S,3S,5S)-5-{[(S)-2-(Formylamino)-4-methylpentanoyl]oxy}-2-hexyl-3-hydroxyhexadecanoic Acid* (**10**). Hydrogenolysis of 1.0 g (1.44 mmol) of **25** dissolved in 40 ml of abs. THF in presence of 250 mg of 5% Pd/C at r.t. for 2 h provided, after evaporation at r.t., a yellow oil which was purified by FC on silica gel. With hexane/AcOEt 50:50, 0.15 g (21%) of **6**, and with MeOH, 0.22 g of **10** were eluted. The latter was dissolved in pentane/Et_2O 50:50, washed with aq. buffer pH 3, dried, and evaporated at r.t.: 0.14 g (19%) of **10** as a yellowish, sticky oil. IR: 3345 (OH), 1711 (acid), 1668, 1526 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like $J = 7$, 2 CH_3CH_2); 0.96 (*d*, $J = 5.6$, $(CH_3)_2CH$); 1.17–1.88 (*m*, 15 CH_2, $(CH_3)_2CHCH_2$, $CH_2(4)$); 2.40–2.52 (*m*, H–C(2)); 3.78–3.87 (*m*, H–C(3)); 4.59–4.75 (*m*, H–C–N); 4.5–5.5 (br., 2 OH); 5.03–5.15 (*m*, H–C(5)); 6.33 (*d*, $J = 10$, NH); 8.22 (*s*, CHO). MS: 514 (8, $[M + H]^{+\cdot}$), 496 (17, $[M - H_2O]^{+\cdot}$).

1.10. *Potassium (2S,3R,5S)-2-Hexyl-3,5-dihydroxyhexadecanoate* (**14**). A soln. of 50 mg (0.14 mmol) of **8** in 1.5 ml of dioxane and 0.17 ml of 1N KOH was stirred at r.t. for 19 h. The solvent was evaporated, the residue

Table 6. *EI-MS Data*

Compound	$M^{+\cdot}$	Characteristic fragment ions [*m*/*z*] (rel. intensities)
3	336 (15)	321 (3), 293 (8), 265 (4), 181 (55), 155 (100)
4 (*O*-TMS deriv.)	426 (0.5)	411 (5), 342 (6), 336 (5), 283 (100), 252 (13), 185 (37), 161 (68), 155 (42), 145 (50), 129 (70)
5 (*O*-TMS deriv.)	382 (< 0.1)	367 (2), 257 (100)
7 (*O*-TMS deriv.)	231 (< 0.1)	216 (40), 144 (100), 129 (12), 114 (50), 102 (22)
8 (*O*-TMS deriv.)	426 (0.1)	411 (5), 342 (3), 336 (6), 283 (100), 252 (4), 185 (34), 161 (25), 155 (40), 145 (29), 129 (47)
9	495 (< 0.1)	338 (15), 336 (22), 253 (20), 181 (50), 155 (100), 114 (80)
10 (*O*,*O*-di-TMS deriv.)	657 (< 0.1)	642 (1), 499 (4), 483 (5), 442 (4), 409 (8), 317 (12), 283 (100), 232 (62), 216 (25), 129 (45), 114 (30)
11 (*O*,*O*-di-TMS deriv.)	657 (< 0.1)	499 (1), 483 (3), 409 (6), 317 (15), 283 (100), 232 (24), 216 (22), 129 (49), 114 (25)
12 (*O*,*O*-di-TMS deriv.)	657 (< 0.1)	642 (8), 498 (2), 483 (6), 409 (15), 343 (80), 257 (83), 232 (100), 216 (73), 129 (41), 114 (45)
15 (*O*,*O*,*O*-tri-TMS deriv.)	588 (< 0.1)	573 (1), 498 (1), 483 (2), 343 (35), 288 (22), 283 (18), 257 (100), 217 (30), 124 (27)
16 (*O*-TMS deriv.)	567 (0.5)	552 (10), 408 (67), 337 (14), 253 (100), 242 (32), 142 (28), 114 (80)
17 (*O*,*O*-di-TMS deriv.)	498 (< 0.1)	483 (4), 343 (5), 314 (28), 257 (100), 155 (8), 129 (13), 103 (24)
18 (*O*-TMS deriv.)	408 (12)	393 (10), 337 (5), 253 (100)

dissolved in Et_2O, washed with brine, dried, and evaporated: 50 mg (99%) of **14** as yellowish oil. IR: 3320 (OH), 1705, 1670, 1460 (COO^-). GC/MS of its trisilylated derivative (M.W. 588): no $M^{+\cdot}$, 573 (0.7, $[M - CH_3]^{+}$), 498 (1.1, $[M - Me_3SiOH]^{+}$).

1.11. *(2S,3S,5S)-2-Hexyl-3,5-dihydroxyhexadecanoic Acid* (**15**). A soln. of 0.5 g (1.4 mmol) of **4** in 15 ml of dioxane and 2.8 ml of 1N KOH was stirred for 1 h at r.t. Then, the pH was adjusted to 3 by addition of 1N HCl, the mixture diluted with H_2O and extracted with Et_2O. The combined org. extracts were washed with H_2O, dried, and evaporated at r.t.; **15** as a colorless oil which crystallized in the freezer. M.p. 54–57°. IR: 3364 (OH), 1697 (acid). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 CH_3CH_2); 1.19–1.88 (*m*, 15 CH_2, CH_2(4)); 2.41 (*quint.*-like, $J = 4$, H–C(2)); 3.88–4.05 (*m*, 2 CH(OH)); 4.3–5.5 (br., 2 OH, COOH). MS: 354 (1.5, $[M - H_2O]^{+\cdot}$), 336 (2.5, $[M - 2\ H_2O]^{+\cdot}$).

2. *Degradation of THL* (**1**) *in Presence of OCHPheOH.* A mixture of 99 mg (0.2 mmol) of **1** and 39 mg (0.2 mmol) of N-*formyl*-L-*phenylalanine* neat (**A**) or in soln. (**B**) in 0.5 ml of DMSO (*Fluka, p.p.a*) was heated to 85° with 85% rel. humidity in a drying pistol for 96 h. Then, the samples were taken up in Et_2O and sat. aq. $NaHCO_3$ soln., the org. layer separated, and the aq. phase extracted twice with Et_2O. The pooled org. solns. were washed with brine, dried, and evaporated. The procedure was repeated with hexane to strip off the remaining traces of OCHPheOH. The residues were dissolved in Et_2O, washed once with aq. buffer of pH 2 and twice with brine, dried, and a CH_2N_2 soln. in Et_2O was added. After 30 min, the yellow solns. were evaporated and the resulting yellowish oils, *e.g.* **A**: 88mg, **B**: 76 mg, were analyzed. The TLC plates showed the normal pattern of degradation products. Both samples gave nearly identical spectra. IR: 1824 (lactone, *ca.* 30% of the starting material still present). ^{1}H-NMR ($CDCl_3$): no aromatic H-atoms. MS: no significant peaks of m/z 91 ($[C_7H_7]^+$), and /or 77 ($[C_6H_5]^+$) or 65 ($[C_5H_5]^+$).

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