201. Synthesis of New, Unnatural Macrocyclic Trichothecenes: 3-Isoverrucarin A ((1″-*O*)(3→4)*abeo*-Verrucarin A), Verrucinol, and Verrucene

46th Communication on Verrucarins and Roridins[1])

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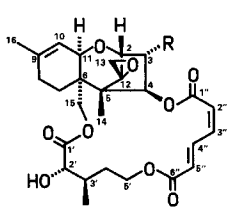
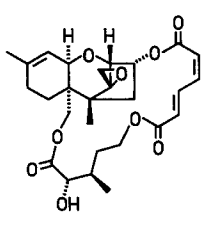
A new unnatural macrocyclic trichothecene, an analogue of verrucarine A (**1**), which was named 3-Isoverrucarin A ((1″-*O*)(3→4)*abeo*-verrucarin A; **3**) was synthesized starting from anguidine (**5**). The two key reactions were the removal of the 4*β*-acetoxy group of anguidine (**5**) by a *Barton* deoxygenation and the final macrolactonization. During the cyclization procedure, two unexpected new macrocyclic by-products, which were named verrucinol (**19**) and verrucene (**20**), were formed. They represent novel types of macrocyclic trichothecenes, the macrolidic moiety of verrucene (**20**) consisting only of the (*Z*,*E*)-muconic-acid residue. The formation of the analogous macrolide **26** of verrucene (**20**) was not possible, probably because the ring strain is too strong.

Introduction. – The trichothecenes belong to a class of sesquiterpenoid secondary metabolites produced by moulds, especially various species of *Fungi imperfecti*, such as *Fusarium*, *Stachybotrys*, *Trichothecium*, *Myrothecium*, and *Cephalosporium*. Many members of the family exhibit interesting biological effects such as antibiotic, antifungal, and cytotoxic activities [2–7]. However, their general toxicity is very high. It is well established that not only the epoxy group in the sesquiterpene moiety but also the



1 R = H
2 R = OH



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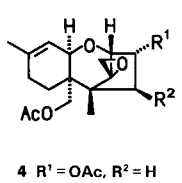
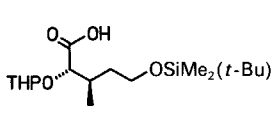
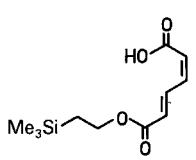


4 R^1 = OAc, R^2 = H
5 R^1 = OH, R^2 = OAc



6 THP = tetrahydro-2*H*-pyranyl



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[1]) 45th Communication: [1].

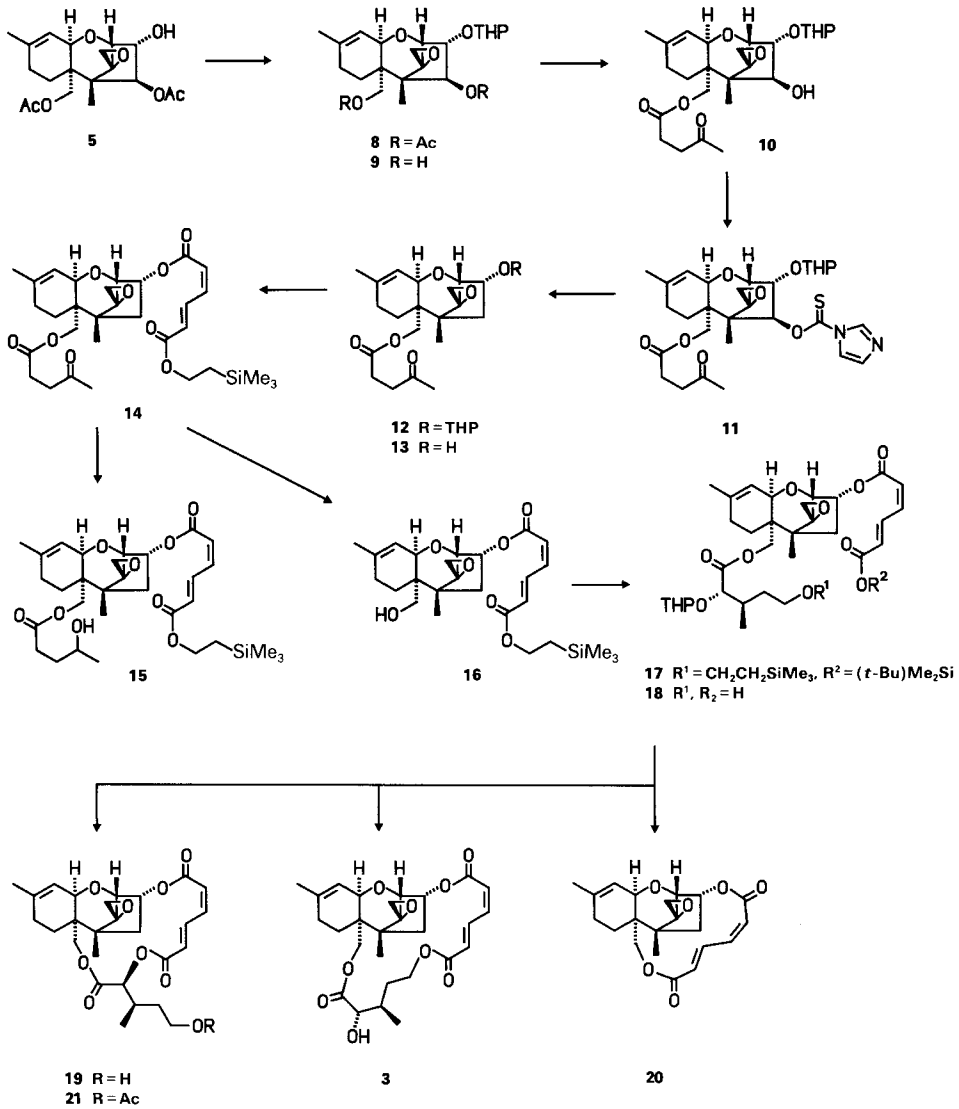
macrocyclic unit of the verrucarins, roridins, and baccharins are essential structural requirements for the biological properties of the naturally occurring metabolites. However, they vary considerably with the individual nature of the macrocyclic system and the oxygenation pattern of the trichothecene part in a manner which is not yet understood. In order to gain more insight into the relationship between the chemical structure and biological activity and possibly to achieve a more favourable ratio between a usefull biological activity and toxicity, we have initiated a programme directed towards the synthesis of unnatural macrocyclic trichothecenes. After the synthesis of two model compounds [8] [9], we completed the synthesis of verrucarin A (**1**) and the unnatural 3α-hydroxyverrucarin A (**2**) [10].

Results. – In all naturally occurring macrocyclic trichothecenes, the macrolidic moiety is attached to the 4β- and 15-OH groups of the trichothecene skeleton. We now have synthesized a verrucarin-A analogue **3** with the 1″-carboxy group attached to the 3α-OH group. This new macrocyclic compound, which bears the trichothecene moiety with the oxygen pattern of the naturally occurring calonectrin (**4**), was named 3-Isoverrucarin A ((1″-*O*)(3→4)*abeo*-verrucarin A). Anguidine (**5**) which can be isolated readily from cultures of various *Fusarium* species served as starting material. The first key reaction, the deoxygenation of anguidine (**5**) at the 4-position, was successfully achieved by making use of the *Barton* procedure [11], which had previously been applied for the conversion of anguidine (**5**) into calonectrin (**4**) [12]. The same transformation has recently been achieved *via* base-catalyzed regiospecific elimination of a 3,4-dimesyloxy derivative [13]. The strategy for the construction of the macrolidic lactone was similar to that which was applied for the synthesis of verrucarin A (**1**) and 3α-hydroxyverrucarin A (**2**). The same verrucarinic- and muconic-acid synthons **6** and **7**, respectively, which had to be selectively attached to the trichothecene skeleton were used. The tetrahydro-2*H*-pyranyl and levulinoyl groups were chosen for the protection of the 3α-OH and 15-OH group, respectively. The synthesis is outlined in *Scheme 1*.

Anguidine (**5**) was transformed into the 3,4,5,6-tetrahydro-2*H*-pyranyl (THP) ether **8**[2]) and hydrolyzed to the diol **9** (96% yield). The 15-OH group was selectively esterified (dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine ((Me$_2$N)Py), Et$_3$N) with levulinic acid according to *Neises* and *Steglich*'s procedure [14] affording the 15-monolevulinate **10** in 52% yield. The remaining 4-OH group was activated by the formation of the imidazolecarbothioate **11**. The deoxygenation procedure was completed by refluxing **11** with Bu$_3$SnH to yield the desired product **12** (96% yield). After the removal of the THP group, the monoprotected (*E*,*Z*)-muconic acid **7** was attached (DCC, (Me$_2$N)Py, Et$_3$N) to the 3α-OH group of **13** without isomerization of the (*Z*)-double bond. On the other hand, condensation of **7** with the 4β-OH group of the trichothecene skeleton in the presence of DCC, (Me$_2$N)Py, and Et$_3$N had afforded the corresponding (*E*,*E*)-isomer in a yield of *ca.* 33% [10]. Surprisingly, the triester **14** was unstable upon drying. After 15 h, more than 50% of the material had polymerized to a gum which proved to be insoluble in common solvents. Therefore, the cleavage of the levulinate moiety had to be carried out immediately after the muconic acid had been attached. The deprotection procedure using NaBH$_4$ proved to be unsuccessful. The hydroxy ester **15** was isolated as the main produced. Similar results were reported by *Roush* and *Blizzard*

[2]) Ether **8** as well as the subsequent THP ethers are mixtures of diastereoisomers.

Scheme 1



[15]. Treatment of **15** with pyridinium *p*-toluenesulfonate (PPTS) in MeOH afforded **16** in only small amounts (TLC). On the other hand, treatment of **14** with hydrazine hydrate furnished **16** in a satisfactory yield (82%). The product was stable upon drying. Esterification of the verrucarinic acid **6** with the 15-OH group of **16** according to [14] (DCC, $(Me_2N)Py$, Et_3N) yielded **17** (72%). The two silyl protecting groups were removed by treatment with Bu_4NF.

The cyclization of the *seco*-acid **18** using the *Yamaguchi*'s mixed-anhydride method (2,4,6-trichlorobenzoyl chloride, Et_3N, CH_2Cl_2, $(Me_2N)Py$, toluene) [16] led to a mixture of products. After the removal of the THP group, the desired 1″-*O* (3→4)*abeo*-verrucarin A (**3**) and the two unexpected macrocyclic products **19** and **20** were isolated. However, the yields of **19** and **20** were difficult to reproduce (see *Table*). They were named verrucinol and verrucene, respectively. Obviously, cleavage of the verrucarinic acid had occurred during the lactonization procedure which led to verrucene (**20**). In a previous synthesis of a macrocyclic model trichothecene, the cleavage of a 15-mevalonate had been observed during storage [9]. The observation that the most polar product verrucinol (**19**) could not be detected on TLC before the cleavage of the THP group suggested the migration of the THP group from the 2′-OH to the 5′-OH group. The formation of verrucinol (**19**) and verrucene (**20**) was suppressed using either the mixed-anhydride method with pivalic acid [17] or the *Mitsunobu* method [18]. The limited amounts of the starting materials prevented us from an optimization of the cyclization conditions and from a more detailed examination of the side reactions.

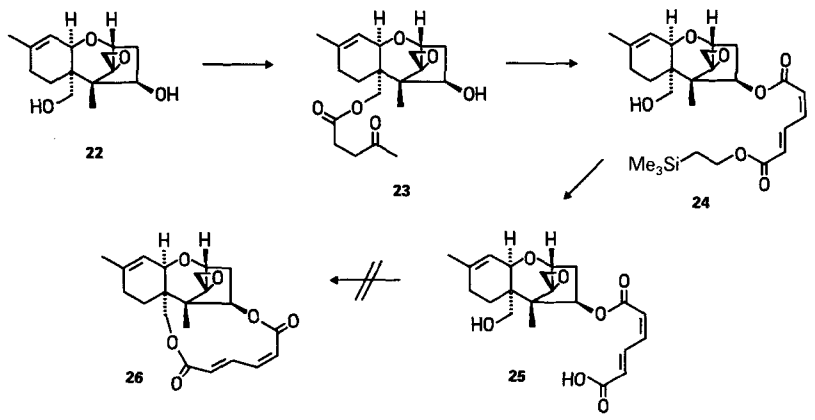
Table. *Macrolactonization of* seco-*Acid* **18**

Method	Conditions	Yields [%]		
		3	**19**	**20**
A	1) 2,4,6-trichlorobenzoyl chloride, Et_3N, CH_2Cl_2	6	22	4
	2) $(Me_2N)Py$, toluene, 110°	9	22	9
		10	6	23
		50	–	16
B	1) pivalic acid, Et_3N, CH_2Cl_2	32	3	6
	2) $(Me_2N)Py$, CH_2Cl_2, 20°	43	–	–
C	diethyl azodicarboxylate, Ph_3P, benzene, 20°	28	–	–

The structure of 1″-*O* (3→4)*abeo*-verrucarin A (**3**) was derived from ^{1}H-NMR and CI-MS (MH^+ at *m/z* 503). The ^{1}H-NMR data of the macrolidic part corresponded to that of verrucarin A (**1**). The proton of the secondary 2′-OH group led to a *d* (J = 6 Hz) at 2.77 ppm. Because it is known that no inversion takes place under the reaction conditions used, it is reasonable to assume that the configuration at C(2′) of **3** and **19** is the same as in **18**. A significant difference in the ^{1}H-NMR of **3** and verrucinol (**19**; CI-MS: MH^+ at *m/z* 503) was found for H–C(2′) and CH_2(5′). The H–C(2′) of **3** was identified as *dd* (J = 2, 6) at 4.35 ppm, whereas H–C(2′) of **19** appeared as *d* (J = 3.5) at 5.61 ppm resulting from the esterification of the 2′-OH group. The ring closure between the 5′-OH and the C(6″)OOH groups gave rise to 2 *m* at 4.10 and 4.68 ppm for CH_2(5′) of **3**, respectively, the CH_2(5′) group of **19** showed a *m* at 3.73 ppm. A further proof for the constitution of **19** resulted from the acetylation of the primary OH group of **19** (→**21**) which resulted in a downfield shift of the CH_2(5′) signal. Surprisingly, in comparison to **3** and **20**, **19** proved to be relatively unstable during storage. The structure of **20** was indicated by CI-MS (MH^+ at *m/z* 373), elemental analysis ($C_{21}H_{24}O_6$), and ^{1}H-NMR data proving the absence of the verrucarinic-acid moiety. The unexpected ring closure between the 15-OH and the C(6″)OOH groups was indicated by the downfield shift of the CH_2(15) signal which appeared as an *AB* system at 3.97 and 4.81 ppm.

Verrucene (**20**) is the first case of a macrocyclic trichothecene in which the macrolidic part consists exclusively of the (*Z*,*E*)-muconic acid. It can be considered as a 'mini-verrucarin'. The structure of **20** was confirmed by a direct synthesis which started from **16**. After the removal of the (trimethylsilyl)ethoxy group, the cyclization procedure according to *Yamaguchi* [16] yielded verrucene (**20**) in 29% yield.

Scheme 2



It was obvious to try a cyclization analogous to **16**→→**20** between the 4β- and 15-OH groups. The synthetic pathway is outlined in *Scheme 2*. Selective protection of the 15-OH group of verrucarol (**22**) was achieved by esterification with levulinic acid leading to the levulinate **23** (68% yield). Condensation of **23** with the muconic acid **7** and the subsequent cleavage of the levulinate group yielded the 4-monomuconate **25** in 50% yield. According to ^{1}H-NMR, the product contained *ca.* 30% of the undesired (*E*,*E*)-muconate which could not be separated by column chromatography. The (trimethylsilyl)ethoxy group was removed by Bu_4NF and the resulting hydroxy acid submitted to the lactonization procedure according to [16] or [17]. Unfortunately, no cyclized product **26** could be isolated or detected by TLC. The acid **25** completely decomposed during the cyclization procedures.

A comparison of *Dreiding* models of the macrolidic compounds verrucene (**20**) and **26** did not obviously contradict the formation of **26** from **25**. Therefore, a more accurate insight into the conformational strain was gained by applying computer-assisted molecular-modelling[3]). A comparison of the energy-minimized conformation of **20**, **26**, and the 4α-epimer of **26** showed that the conformations of **26** (4β-configuration) and the 4α-epimer of **26** are strained (13 and 7 kcal/mol, resp.), whereas a less strained or even unstrained conformations were found for verrucene (**20**) (0–3.5 kcal/mol). These results correspond to experimental observations.

Results on the biological activity of 3-Isoverrucarin A ((1″-*O*)(3→4)*abeo*-verrucarin A; **3**) and verrucene (**20**) will be reported in a subsequent paper.

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[3]) The molecular modelling and energy minimization were performed with proprietary united-atom force field MOLOC developed by the CAMM group, Dr. *K. Müller, F. Hoffmann-La Roche & Co. AG*, Basel. We are very grateful for his generous support.

Experimental Part

General. Water-sensitive reactions were carried out under Ar or N_2. CH_2Cl_2, 1,2-dichlorethane, toluene, and benzene were filtered through an Al_2O_3 column and stored over molecular sieves (4 Å). THF and DMSO were dried by distilling over $LiAlH_4$ or CaH_2, respectively. All org. extracts were dried (Na_2SO_4) and evaporated at < 50°. TLC: silica gel *60 F_{254} (Merck)*; detection with 10% H_2SO_4 soln. in MeOH or $KMnO_4$ soln. Prep. TLC: silica gel *60 F_{254} (Merck)*, 20 × 20 plates, thickness of layer 0.25 or 0.50 mm. Column chromatography (CC): silica gel *60* (60–200 or 35–70 μm; *Merck*). M.p.: *Kofler* block; corrected. $[\alpha]_D$: *Perkin-Elmer-141* polarimeter. IR: *Perkin-Elmer-177* grating spectrometer. NMR: *Varian-EM-360* spectrometer (^{1}H, 60 MHz), *Bruker*-WH-90 spectrometer with *Fourier* transform (^{1}H, 90 MHz; ^{13}C, 22.63 MHz), *Varian-VXR-400* spectrometer with *Fourier* transform (^{1}H, 400 MHz; ^{13}C, 101 MHz); in $CDCl_3$; chemical shifts in ppm downfield from internal TMS. MS: *VG-70-250* spectrometer, CI (chemical ionization) by NH_3. DCC = dicyclohexylcarbodiimide, PPTS = pyridinium *p*-toluenesulfonate.

*12,13-Epoxy-3α-[(tetrahydro-2*H*-pyranyl)oxy]trichothec-9-ene-4β,15-diyl Diacetate* (= *5aα-(Acetoxymethyl)-2β,3,4,5,5a,6,7,9aα-octahydro-5β,8-dimethyl-3α-[(tetrahydro-2*H*-pyranyl)oxy]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-4β-yl Acetate;* **8**). To a soln. of 2.077 g (5.67 mmol) of **5** in 30 ml of CH_2Cl_2, 1.03 ml (11.34 mmol) of 3,4-dihydro-2*H*-pyran and 144 mg (0.57 mmol) of PPTS were added. The mixture was stirred at r.t. for 15 h. After removal of the solvent, the residue was filtered with Et_2O on SiO_2. Removal of the solvent afforded 2.45 g (96%) of **8** as a white foam. IR ($CHCl_3$): 2950, 1735 (ester), 1235. ^{1}H-NMR (90 MHz): 0.77 (*s*, CH_3(14)); 1.75 (*s*, CH_3(16)); 2.07 (*s*, Ac); 2.12 (*s*, Ac); 2.80, 3.05 (*AB*, *J* = 4, CH_2(13)); 3.77 (*d*, *J* = 5, H–C(2)); 4.77 (br., 1 H, acetal); 5.52 (br. *m*, H–C(10)); 5.68 (*d*, H–C(4)).

*12,13-Epoxy-3α-[(tetrahydro-2*H*-pyranyl)oxy]trichothec-9-ene-4β,15-diol* (= *2β,3,4,5,5a,6,7,9aα-Octahydro-5aα-(hydroxymethyl)-5β,8-dimethyl-3α-[(tetrahydro-2*H*-pyranyl)oxy]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-4β-ol;* **9**). To a soln. of 2.45 g (5.44 mmol) of **8** in 60 ml of MeOH, 120 ml (36 mmol) of 0.3N NaOH were added. The mixture was stirred for 1 h, MeOH evaporated, and the aq. soln. saturated with NaCl and extracted with Et_2O. The Et_2O soln. was washed with brine and the solvent evaporated: 1.93 g (97%) of **9** as a white foam. IR ($CHCl_3$): 3460 (OH). ^{1}H-NMR (60 MHz): 0.90 (*s*, CH_3(14)); 1.72 (*s*, CH_3(16)); 2.72, 2.98 (*AB*, *J* = 4, CH_2(13)); 4.46 (*m*, H–C(4)); 4.70, 4.93 (br., 1 H, acetal); 5.50 (*m*, H–C(10)). CI-MS: 367 ($[M + H]^+$), 281, 265, 247, 85 (100).

*12,13-Epoxy-4β-hydroxy-3α-[(tetrahydro-2*H*-pyranyl)oxy]trichothec-9-en-15-yl Levulinate* (= *2β,3,4,5,5a,6,7,9aα-Octahydro-4β-hydroxy-5β,8-dimethyl-3α-[(tetrahydro-2*H*-pyranyl)oxy]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-5aα-methyl 4-Oxopentanoate;* **10**). To a soln. of 1.582 g (13.62 mmol) of levulinic acid (= 4-oxopentanoic acid) and 4.994 g (13.63 mmol) of **9** in 80 ml of CH_2Cl_2, 145.7 mg (1.19 mmol) of (Me_2N)Py were added, and the mixture was stirred at 0°. After 10 min, 3.63 g (17.59 mmol) of DCC were added and stirring was continued for 4 h at 0°. Then the mixture was filtered, the filtrate diluted with CH_2Cl_2 and washed with ice-cold 1N HCl, sat. $NaHCO_3$ soln., and brine. After removal of the solvent, CC (Al_2O_3, AcOEt/petroleum ether 1:1) yielded 3.29 g (52%) of the THP diastereoisomers as a pale yellow oil. IR (film): 3460 (OH); 2950, 1740, 1720 (C=O, ester, ketone). ^{1}H-NMR (90 MHz): 0.84 (*s*, CH_3(14)); 1.72 (br. *s*, CH_3(16)); 2.21 (*s*, $COCH_3$); 2.4–3.2 (*m*, $OCCH_2CH_2CO$); 2.77, 3.04 (*AB*, *J* = 4, CH_2(13)); 3.78 (*d*, *J* = 5, H–C(2)); 4.05 (CH_2(15)); 4.97 (br., 1 H, acetal); 5.56 (br. *d*, *J* = 5, H–C(10)). CI-MS: 482 ($[M + NH_4]^+$), 465 ($[M + H]^+$), 363, 365, 102, 85 (100).

S-{*12,13-Epoxy-15-[(levulinoyl)oxy]-3α-[(tetrahydropyranyl)oxy]trichothec-9-en-4β-yl*} *1*H*-Imidazol-1-carbothioate* (= S-{*2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethyl-5aα-[(4-oxopentanoyl)oxy)methyl]-3α-[(tetrahydro-2*H*-pyranyl)oxy]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-4β-yl*} *1*H*-Imidazol-1-carbothioate;* **11**). To a soln. of 2.456 g (5.286 mmol) of **10** in 65 ml of 1,2-dichloroethane, 2.263 g (12.697 mmol) of 1,1-thiocarbonyldiimidazole were added. The soln. was refluxed for 6 h, then the solvent evaporated, and the residue dissolved in CH_2Cl_2 and washed with ice-cold 1N HCl, sat. $NaHCO_3$ soln. and brine. Removal of the solvent yielded 2.977 g (98%) of **12** as a yellow foam which showed to be homogeneous on TLC. IR: 3450, 3130, 2950, 1740, 1720 (ester, ketone). ^{1}H-NMR (90 MHz): 0.82, 0.86 (*s*, CH_3(14)); 1.74 (br. *s*, CH_3(16)); 2.16 (*s*, $COCH_3$); 2.20–2.90 (*m*, $OCCH_2CH_2CO$); 2.82, 3.12 (*AB*, *J* = 4, CH_2(13)); 3.87 (*d*, *J* = 5, H–C(2)); 4.78 (*m*, 1 H, acetal); 5.52 (br., H–C(10)); 6.39 (*d*, *J* = 3, H–C(4)); 7.04 (*s*, 1 H, imidazole); 7.65 (*s*, 1 H, imidazole); 8.38 (*s*, 1 H, imidazole). CI-MS: 575 ($[M + H]^+$), 366, 282, 85, 69 (100).

*12,13-Epoxy-3α-[(tetrahydro-2*H*-pyranyl)oxy]trichothec-9-en-15-yl Levulinate* (= *2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethyl-3α-[(tetrahydro-2*H*-pyranyl)oxy]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-5aα-methyl 4-Oxopentanoate;* **12**). A soln. of 1.34 ml (5.06 mmol) of Bu_3SnH in 65 ml of toluene was heated at 110°. A soln. of 1.466 g (2.551 mmol) of **11** in 65 ml of toluene was added dropwise to the stirred, refluxing soln. within 45 min. After 3 h, the solvent was evaporated, and the residue purified by CC (Et_2O): 1.144 g (98%) of a pale-yellow

oil. IR (KBr): 2950, 1740, 1735, 1725 (ester, ketone). ^{1}H-NMR (90 MHz): 0.81 (*s*, CH_3(14)); 1.72 (br. *s*, CH_3(16)); 2.16 (*s*, $COCH_3$); 2.44–2.78 (*m*, $OCCH_2CH_2CO$); 2.83, 3.06 (*AB*, *J* = 4, CH_2(13)); 4.73 (*m*, 1 H; acetal); 5.50 (*m*, H–C(10)). CI-MS: 466 ($[M + NH_4]^+$), 449 ($[M + H]^+$), 382, 102 (100), 85.

12,13-Epoxy-3α-hydroxytrichothec-9-en-15-yl Levulinate (= *2β,3,4,5,5a,6,7,9aα-Octahydro-3α-hydroxy-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-5aα-methyl 4-Oxopentanoate;* **13**). A soln. of 2.174 g (4.846 mmol) of **12** and 120 mg (0.478 mmol) of PPTS in 35 ml of MeOH was heated at 50°. After 4 h, the MeOH was evaporated, the residue dissolved in Et_2O and washed with brine, and the Et_2O evaporated: 1.289 g (73%) of **13** which crystallized spontaneously. An anal. sample was recrystallized from CH_2Cl_2/Et_2O. M.p. 133–135°. $[\alpha]_D^{20} = -19.5$ (*c* = 0.945, $CHCl_3$). IR (KBr): 3410 (OH), 2980, 2965, 2910, 2890, 1745, 1730, 1715 (ester, ketone). ^{1}H-NMR (90 MHz): 0.85 (*s*, CH_3(14)); 1.76 (br. *s*, CH_3(16)); 2.21 (*s*, $COCH_3$); 2.47–2.83 (*m*, $OCCH_2CH_2CO$); 2.87, 3.10 (*AB*, *J* = 4, CH_2(13)); 3.50 (*d*, *J* = 5, H–C(2)); 3.88, 4.13 (*AB*, *J* = 12, CH_2(15)); 4.21 (*d*, *J* = 5, H–C(11)); 4.42 (*m*, H–C(3)); 5.52 (br. *d*, *J* = 5, H–C(10)). CI-MS: 382 ($[M + NH_4]^+$), 365 ($[M + H]^+$), 249 (100). Anal. calc. for $C_{20}H_{28}O_6$ (364.44): C 65.91, H 7.74; found: C 65.83, H 8.05.

1-(12,13-Epoxy-15-hydroxytrichothec-9-en-3α-yl) 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate (= *1-{2β,3,4,5,5a,6,7,9aα-Octahydro-5aα-(hydroxymethyl)-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-3α-yl} 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate;* **16**). To a soln. of 500 mg (1.372 mmol) of **13** and 366 mg (1.511 mmol) of 6-[2-(trimethylsilyl)ethyl] hydrogen (2*Z*,2*E*)-2,4-hexadienedioate (**7**) in 6.5 ml of CH_2Cl_2, 34 mg (0.278 mmol) of (Me_2N)Py were added. The soln. was cooled to −50°, and after the addition of 367 mg (1.779 mmol) of DCC, the mixture was kept at −20° for 18 h. Then, the precipitated urea was filtered off, the solvent evaporated, and the residue immediately redissolved in Et_2O. Column chromatography (Et_2O) afforded **14**, which was immediately redissolved in a mixture of 14 ml of pyridine and 3.6 ml of AcOH. This soln. was cooled for 5 min at 0°. Then, 0.5 ml (9.6 mmol) of $NH_2NH_2 \cdot H_2O$ were added. Stirring was continued for 100 min. Then, 50 ml of ice/H_2O were added, the pH was adjusted to 2–3 by careful addition of conc. HCl soln., and the aq. layer extracted with CH_2Cl_2 (3 × 150 ml). The org. layers were washed with 1N HCl, sat. $NaHCO_3$ soln., and brine and evaporated: 550 mg (82%) of pure **16**, after CC (Et_2O). IR ($CHCl_3$): 3480 (OH); 2960, 1725 (ester); 1640, 1605. ^{1}H-NMR (90 MHz; for numbering, see **1**): 0.07 (*s*, $(CH_3)_3Si$); 1.03 (*m*, CH_2Si); 0.93 (*s*, CH_3(14)); 1.60 (br. *s*, OH); 1.71 (br. *s*, CH_3(16)); 2.88, 3.12 (*AB*, *J* = 4, CH_2(13)); 3.56, 3.78 (*AB*, *J* = 12, CH_2(15)); 3.81 (*d*, *J* = 5, H–C(2)); 4.28 (*m*, $OCOCH_2$, H–C(11)); 5.32 (*m*, H–C(3)); 5.48 (br. *d*, *J* = 5, H–C(10)); 6.03 (*d*, *J* = 12, H–C(2′)); 6.13 (*d*, *J* = 16, H–C(5′)); 6.71 (*dd*, *J* = 12, 12, H–C(3′)); 8.42 (*dd*, *J* = 12, 16, H–C(4′)). CI-MS: 510, 508 ($[M + NH_4]^+$), 493, 491 ($[M + H]^+$), 465, 463, 249, 90 (100).

1-{2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethyl-5aα-[((4-oxopentanoyl)oxy)methyl]spiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-3α-yl} 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate (**14**). ^{1}H-NMR (90 MHz, int. standard benzene; for numbering, see **1**): 0.07 (*s*, $(CH_3)_3Si$); 0.87 (*s*, CH_3(14)); 1.07 (*m*, CH_2Si); 1.73 (br. *s*, CH_3(16)); 2.20 (*s*, $COCH_3$); 2.67 (*m*, CH_2COO); 2.89, 3.13 (*AB*, *J* = 4, CH_2(13)); 3.83 (*d*, *J* = 5, H–C(2)); 4.04 (H–C(11), CH_2(15)); 4.31 (*m*, CH_2O); 5.29 (*m*, H–C(3)); 5.49 (br. *d*, *J* = 5, H–C(10)); 6.03 (*d*, *J* = 11, H–C(2″)); 6.12 (*d*, *J* = 16, H–C(5″)); 6.70 (*dd*, *J* = 11, 11, H–C(3″)); 8.42 (*dd*, *J* = 11, 16, H–C(4″)).

1-{2β,3,4,5,5a,6,7,9aα-Octahydro-5aα-[((4-hydroxypentanoyl)oxy)methyl]-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-3α-yl} 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate (**15**). ^{1}H-NMR (90 MHz; for numbering, see **1**): 0.05 (*s*, $(CH_3)_3Si$); 0.85 (*s*, CH_3(14)); 1.04 (*m*, CH_2Si); 1.22 (*d*, *J* = 6, $CH_3CH(OH)$); 1.71 (br. *s*, CH_3(16)); 2.88, 3.12 (*AB*, *J* = 4, CH_2(13)); 3.70–4.18 (*m*, H–C(2), H–C(11), CH_2(15)); 4.30 (*m*, $OCOCH_2$); 5.29 (*m*, H–C(3)); 5.49 (br. *d*, *J* = 5, H–C(10)); 6.01 (*d*, *J* = 11, 1 H, H–C(2″)); 6.09 (*d*, *J* = 16, H–C(5″)); 6.67 (*dd*, *J* = 11, 11, H–C(3″)); 8.39 (*dd*, *J* = 11, 16, H–C(4″)).

1-{15-[(2S,3R)-5-[((tert-Butyl)dimethylsilyl)oxy]-3-methyl-2-[(tetrahydro-2H-pyranyl)oxy]pentanoyloxy]-12,13-epoxytrichothec-9-en-3α-yl} 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate (= *1-{5aα-{[(2S,3R)-5-[((tert-Butyl)dimethylsilyl)oxy]-3-methyl-2-[(tetrahydro-2H-pyranyl)oxy]pentanoyloxy]methyl}-2β,3,4,5,5a,6,7,9aα-octahydro-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-3α-yl} 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-Hexadienedioate;* **17**). A soln. of 224 mg (0.457 mmol) of **16**, 311 mg (0.898 mmol) of (2*S*,3*R*)-5-[((*tert*-butyl)dimethylsilyl)oxy]-3-methyl-2-[(tetrahydro-2*H*-pyranyl)oxy]pentanoic acid (**6**), and 15 mg (0.123 mmol) of (Me_2N)Py in 13 ml of CH_2Cl_2 was cooled to −70° for 5 min. Then, 190 mg (0.921 mmol) of DCC were added. The mixture was warmed up to −20° and kept at −20° for 18 h. Then, the precipitated urea was filtered off, the filtrate diluted with Et_2O, and the Et_2O soln. washed with 0.5N HCl, sat. $NaHCO_3$ soln., and brine. Removal of the solvent and subsequent CC (Et_2O/petroleum ether 3:7) afforded 273 mg (72%) of pure **17** according to TLC. ^{1}H-NMR (90 MHz): 0.08 (*s*, $(CH_3)_2Si$, $(CH_3)_3Si$); 0.90 (*s*, CH_3(14), $(CH_3)_3CSi$); 1.73 (br. *s*, CH_3(16)); 2.88, 3.14 (*AB*, *J* = 4, CH_2(13)); 5.30 (*m*, H–C(3)); 5.49 (br. *d*, *J* = 5, H–C(10)); 6.03 (*d*, *J* = 12, H–C(2″)); 6.13 (*d*, *J* = 16, H–C(5″)); 6.71 (*dd*, *J* = 12,12, H–C(3″)); 8.43 (*dd*, *J* = 12, 16, H–C(4″)). CI-MS: 838, 836 ($[M + NH_4]^+$), 819 ($[M + H]^+$), 703, 90 (100).

Cleavage of the Silyl Protecting Groups of **17**. To a soln. of 117 mg (0.143 mmol) of **17** in 2 ml of THF, 223 mg (0.707 mmol) of Bu_4NF were added. The soln. was stirred for 2 h, then diluted with Et_2O, and washed with H_2O. The Et_2O was evaporated and the residue dried over P_2O_5 (20°/< 0.05 Torr, 2 h): 86 mg of crude *1-{2β,3,4,5,5a,6,7,9aα-Octahydro-5aα-{[(2S,3R)-5-hydroxy-3-methyl-2-[(tetrahydro-2H-pyranyl)oxy]pentanoyloxy]methyl}-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2'-oxirane]-3α-yl} Hydrogen (2Z,4E)-2,4-Hexadienedioate* (**18**), which were submitted to the lactonization according to *Method A*, *B*, or *C*.

Macrolactonization of **18**. *Method A*. A soln. of 60 mg (0.114 mmol) of **18**, 0.017 ml (0.122 mmol) of Et_3N, and 30 mg (0.123 mmol) of 2,4,6-trichlorobenzoyl chloride in 1.5 ml of THF was stirred for 2 h. The precipitate was filtered off and the filtrate dissolved in 55 ml of toluene, which were added within 105 min to a stirred, refluxing soln. of 84 mg (0.688 mmol) of $(Me_2N)Py$ in 11 ml of toluene. After additional 15 min, the toluene was evaporated, the residue dissolved in Et_2O and washed with 1N HCl, sat. $NaHCO_3$ soln., and H_2O. The solvent was removed and the residue purified by CC (Et_2O), which afforded 63 mg of a mixture which was dissolved in 4 ml of EtOH. After the addition of 22 mg (0.088 mmol) of PPTS, the soln. was stirred for 16 h at 50°. The solvent was evaporated and the products separated by CC (Et_2O/petroleum ether 1:1) to yield 29 mg (50%) of **3** and 7 mg (16%) of **20**. (Verrucinol (**19**) was not formed in this experiment.)

Method B. After 10 min, a soln. was obtained from 90 mg (0.148 mmol) of **18** and 0.103 ml (0.739 mmol) of Et_3N in 78 ml of CH_2Cl_2. Then, 0.072 ml (0.585 mmol) of pivaloyl chloride were added, and stirring was continued for 20 min. Finally, 18.2 mg (0.149 mmol) of $(Me_2N)Py$ were added, and the soln. was stirred for an additional 90 min (colourless→yellow soln.). The CH_2Cl_2 was evaporated and the residue purified by CC (Et_2O/petroleum ether 1:1) to yield *ca.* 50 mg of the crude product. The THP group was cleaved as described in *Method A*. CC afforded 32 mg (43%) of **3**. The products **19** and **20** were detected in small amounts by TLC but could not be isolated.

Method C. To a soln. of 47 mg (0.078 mmol) of **18** in 38 ml of benzene, 31 mg (0.118 mmol) of Ph_3P were added under stirring. After 5 min, 0.0183 ml (0.117 mmol) of diethyl azodicarboxylate were dissolved in 1 ml of benzene and added within 4 min to the soln. The mixture was stirred for 66 h. The solvent was evaporated and the residue purified by CC (Et_2O/petroleum ether 1:1). A second CC (CH_2Cl_2/acetone 95:5) afforded 15 mg of the product. The THP group was cleaved as described in *Method A*. Finally, 11 mg (28%) of pure **3** were obtained by prep. TLC (Et_2O).

3-Isoverrucarin A (*(=1''-O)(3→4)*abeo-*Verrucarin A*, *=12,1-(2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2'-oxirane]-5aα-methyl-3α-yl) (10R,11S,2Z,4E)-11-Hydroxy-10-methyl-6-oxo-7-oxa-2,4-dodecadienedioate;* **3**). An anal. sample was crystallized from CH_2Cl_2, Et_2O, and petroleum ether. M.p. 234–237°. $[\alpha]_D^{20} = -5.5$, $[\alpha]_{436}^{20} = -18.9$ ($c = 0.355$, $CHCl_3$). ^{1}H-NMR (400 MHz): 0.78 (*d*, $J = 7$, $CH_3(6')$); 0.83 (*s*, $CH_3(14)$); 1.74 (*s*, $CH_3(16)$); 2.20 (*m*, 1 H–C(4)); 2.77 (*d*, $J = 6$, OH); 2.88, 3.13 (*AB*, $J = 4$, $CH_2(13)$); 2.91 (*m*, 1 H–C(4)); 3.85, 4.73 (*AB*, $J = 12$, $CH_2(15)$); 4.07 (*d*, $J = 4.5$, H–C(2)); 4.10 (*m*, 1 H–C(5')); 4.35 (*dd*, $J = 2$, 6, H–C(2')); 4.54 (br. *d*, $J = 5$, H–C(11)); 4.68 (*m*, H–C(5')); 5.18 (*ddd*, $J = 4.5$, 4.5, 11, H–C(3)); 5.55 (br. *d*, $J = 5$, H–C(10)); 5.98 (*d*, $J = 12$, H–C(2'')); 6.13 (*d*, $J = 16$, H–C(5'')); 6.65 (*dd*, $J = 12$, 12, H–C(3'')); 7.98 (*dd*, $J = 12$, 16, H–C(4'')). ^{13}C-NMR (101 MHz): 11.2 (C(6')); 12.7 (C(14)); 23.1 (C(7)); 23.6 (C(16)); 27.7 (C(8)); 32.0 (C(3')); 32.0 (C(4')); 38.8 (C(4)); 42.2 (C(5)); 43.9 (C(6)); 49.0 (C(13)); 61.7 (C(5')); 64.9 (C(12)); 67.5 (C(15)); 68.3 (C(11)); 71.9 (C(3)); 73.5 (C(2')); 77.5 (C(2)); 119.5 (C(10)); 125.7, 129.5 (C(2''), C(5'')); 137.7 (C(4'')); 138.3 (C(3'')); 139.5 (C(9)); 165.3, 165.3 (C(1''), C(6'')); 175.0 (C(1')). CI-MS: 520 ($[M + NH_4]^+$), 503 ($[M + H]^+$), 249, 148 (100).

Verrucinol (*=9,1-(2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2'-oxirane]-5aα-methyl-3α-yl) (8S,2Z,4E)-8-[(1R)-3-Hydroxy-1-methylpropyl]-6-oxo-7-oxa-2,4-nonadienedioate;* **19**). ^{1}H-NMR (400 MHz): 0.88 (*s*, $CH_3(14)$); 1.06 (*d*, $J = 6$, $CH_3(6')$); 1.45 (*m*, 1 H–C(4')); 1.69 (*s*, $CH_3(16)$); 1.74 (*m*, 1 H–C(4')); 2.17 (*m*, 1 H–C(4)); 2.43 (*m*, H–C(3')); 2.86, 3.13 (*AB*, $J = 4$, $CH_2(13)$); 3.16 (*m*, 1 H–C(4)); 3.73 (*m*, 2 H–C(5')); 3.84, 4.40 (*AB*, $J = 12$, $CH_2(15)$); 3.91 (*d*, $J = 4.5$, H–C(2)); 4.72 (br. *d*, $J = 5.5$, H–C(11)); 5.34 (*ddd*, $J = 4$, 4.5, 11.5, H–C(3)); 5.42 (br. *d*, $J = 5.5$, H–C(10)); 5.61 (*d*, $J = 3.5$, H–C(2')); 6.02 (*d*, $J = 11.5$, H–C(2'')); 6.12 (*d*, $J = 16$, H–C(5'')); 6.81 (*dd*, $J = 11.5$, 11.5, H–C(3'')); 8.74 (*dd*, $J = 11.5$, 16, H–C(4'')). ^{13}C-NMR (101 MHz): 11.4 (C(6')); 15.2 (C(14)); 21.9 (C(7)); 23.1 (C(16)); 27.8 (C(8)); 33.4 (C(4)); 35.3 (C(3')); 39.1 (C(4)); 42.0 (C(5)); 44.5 (C(6)); 49.1 (C(13)); 60.2 (C(5')); 65.3 (C(12)); 66.3 (C(11)); 67.1 (C(15)); 71.9 (C(3)); 74.8 (C(2')); 77.7 (C(2)); 119.0 (C(10)); 126.2, 126.4 (C(2''), C(5'')); 138.5, 139.4 (C(3''), C(4'')); 140.6 (C(9)); 164.4, 164.5 (C(1''), C(6'')); 169.9 (C(1')). CI-MS: 520 ($[M + NH_4]^+$), 503 ($[M + H]^+$), 396, 266, 148 (100).

Verrucene (*=6,1-(2β,3,4,5,5a,6,7,9aα-Octahydro-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2'-oxirane]-5aα-methyl-3α-yl) (2Z,4E)-2,4-Hexadienedioate;* **20**). M.p. > 300°. $[\alpha]_D^{21} = +13.2$; $[\alpha]_{436}^{21} = +31.2$ ($c = 0.175$, $CHCl_3$). IR (KBr): 3440 (H_2O); 2970, 2940, 1725, 1715 (ester). ^{1}H-NMR (90 MHz): 0.93 (*s*, $CH_3(14)$); 1.72 (br. *s*, $CH_3(16)$); 2.37 (*m*, 1 H–C(4)); 2.96–3.15 (*m*, 1 H–C(4)); 2.92, 3.18 (*AB*, $J = 4$, $CH_2(13)$); 3.78 (*d*, $J = 5$, H–C(2)); 3.97, 4.81 (*AB*, $J = 12$, $CH_2(15)$); 4.72 (br. *d*, $J = 5$, H–C(11)); 5.40 (br. *d*, $J = 5$, H–C(10)); 5.74 (*m*,

H–C(3)); 5.93 (*d*, J = 16, H–C(5′)); 6.20 (*d*, J = 11, H–C(2′)); 6.80 (*dd*, J = 11, 11, H–C(3′)); 8.40 (*dd*, J = 11, 16, H–C(4′)). CI-MS: 390 ($[M + NH_4]^+$), 373 ($[M + H]^+$), 355, 342, 249. Anal. calc. for $C_{21}H_{24}O_6$ (372.41): C 67.73, H 6.50; found: C 67.55, H 6.73.

Acetate **21**. ^{1}H-NMR (90 MHz): 0.87 (*s*, CH_3(14)); 1.08 (*d*, J = 7, CH_3(6′)); 1.69 (br. *s*, CH_3(16)); 2.05 (*s*, Ac); 2.86, 3.14 (*AB*, J = 4, CH_2(13)); 3.14 (*m*, 1 H–C(4)); 3.85, 4.41 (*AB*, J = 12, CH_2(15)); 3.91 (*d*, J = 4.5, H–C(2)); 4.15 (br. *t*, J = 6, CH_2(5′)); 4.70 (br. *d*, J = 5, H–C(11)); 5.35 (*m*, H–C(3)); 5.43 (br. *d*, J = 5, H–C(10)); 5.59 (*d*, J = 3, H–C(2′)); 6.04 (*d*, J = 11.5, H–C(2″)); 6.11 (*d*, J = 16, H–C(5″)); 6.83 (*dd*, J = 11.5, 11.5, H–C(3″)); 8.73 (*dd*, J = 11.5, 16, H–C(4″)).

12,13-Epoxy-4β-hydroxytrichothec-9-en-15-yl Levulinate (= *2β,3,4,5,5a,6,7,9aα-Octahydro-4β-hydroxy-5β,8-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxirane]-5aα-methyl 4-Oxopentanoate;* **23**). As described for **10**, with 300 mg (1.13 mmol) of verrucarol (**22**), 132 mg (1.13 mmol) of levulinic acid, 29 mg (0.237 mmol) of (Me_2N)Py, 302 mg (1.46 mmol) of DCC, and 7 ml of CH_2Cl_2. CC (CH_2Cl_2/acetone 8:2) yielded 282 mg (68%) of **23**. ^{1}H-NMR (60 MHz): 0.83 (*s*, CH_3(14)); 1.72 (br. *s*, CH_3(16)); 2.17 (*s*, $COCH_3$); 2.25–2.97 (*m*, CH_2(3), CH_2(13), OCH_2CH_2CO); 3.08 (*d*, J = 4, H–C(13)); 3.60 (br. *d*, H–C(11)); 3.78 (*d*, J = 5, H–C(2)); 3.92, 4.81 (*AB*, J = 12, CH_2(15)); 4.52 (*m*, H–C(4)); 5.40 (br. *d*, J = 5, H–C(10)).

1-(12,13-Epoxy-15-hydroxytrichothec-9-en-4β-yl) 6-[2-(Trimethylsilyl)ethyl] (2Z,4E)-2,4-hexadienedioate (**24**). As described for **16**, with 282 mg (0.774 mmol) of **23**, 206 mg (0.851 mmol) of **7**, 20 mg (0.163 mmol) of (Me_2N)Py, 207 mg (1.01 mmol) of DCC, and 7 ml of CH_2Cl_2. The product was separated by CC (Et_2O) and treated with 0.28 ml (5.418 mmol) of $NH_2NH_2 \cdot H_2O$ in 10 ml of pyridine/AcOH 8:2. Purification by CC (Et_2O) yielded 191 mg (50%) of **24** and its (*E*/*E*)-isomer (ratio *ca.* 7:3). ^{1}H-NMR (400 MHz): 0.09 (*s*, $(CH_3)_3Si$); 0.84 (*s*, CH_3(14)); 1.05 (*m*, CH_2Si); 1.73 (*s*, CH_3(16)); 2.13 (*ddd*, J = 4, 5, 15, 1 H–C(3)); 2.51 (*dd*, J = 8, 15, 1 H–C(3)); 2.55 (br., OH, exchangeable with D_2O); 2.83, 3.14 (*AB*, J = 4, CH_2(13)); 3.69, 3.84 (*AB*, J = 12, CH_2(15)); 3.85 (*d*, J = 5, H–C(2)); 3.95 (br. *d*, J = 5.5, H–C(11)); 4.28 (*m*, 2 H, OCH_2CH_2Si); 5.51 (br. *d*, J = 5.5, H–C(10)); 6.02 (*d*, J = 11.5, H–C(2′)); 6.11 (*d*, J = 15.5, H–C(5′)); 6.17 (*dd*, J = 4, 8, H–C(4)); 6.68 (*dd*, J = 11.5, 11.5, H–C(3′)); 8.41 (*dd*, J = 11.5, 15.5, H–C(4′)). CI-MS: 510, 508 ($[M + NH_4]^+$), 491 ($[M + H]^+$), 321, 249 (100).

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