74. A Novel Rearrangement Product of Podophyllotoxone – Ester Derivatives and *in vitro* Cytotoxicity Studies

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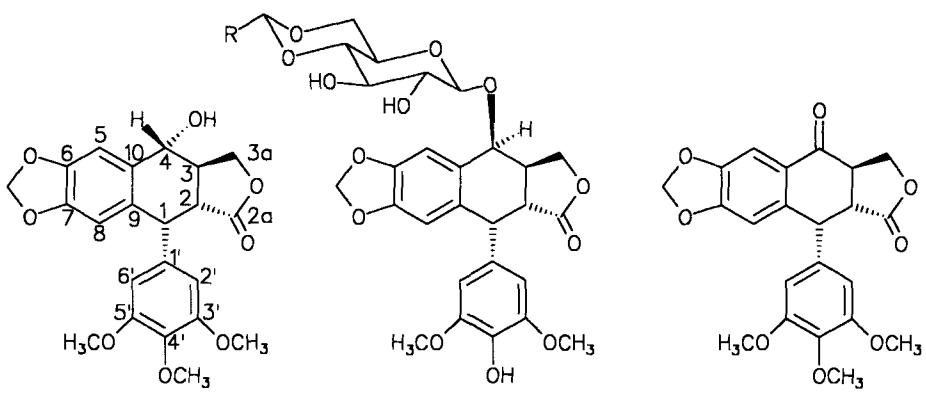
(31.I.94)

A new rearrangement product of podophyllotoxone was obtained by reaction with strong bases. *In vitro* cytotoxities of this substance and some derivatives were determined using L-1210 and KB cell lines.

Introduction. – Podophyllotoxin (**1**), the most important compound of *Podophyllum peltatum* (Berberidaceae), is a strong antimitotic substance which blocks cell division in metaphase II similar to colchicine [1]. It is used in the therapy of condyloma acuminata (genital warts) [2]. Etoposide (**2**) and teniposide (**3**), which are derivatives of podophyllotoxin, do not inhibit micro tubule assembly *in vivo*, but they are non-DNA-intercalating inhibitors of eukaryotic topoisomerase II [3].



1 Podophyllotoxin **2** R = CH_3, Etoposide **4** Podophyllotoxone

3 R =  Teniposide

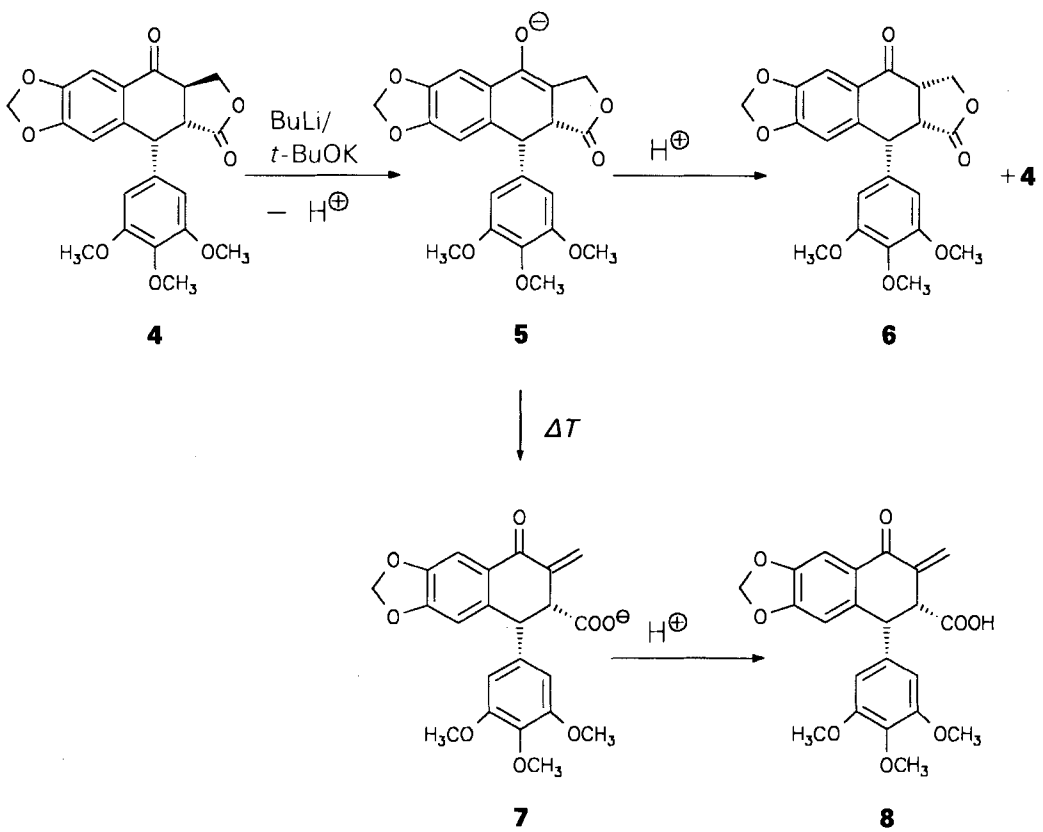
Podophyllotoxone (**4**), an oxidation product of podophyllotoxin, is a naturally occurring compound of *Podophyllum*, too [4]. To understand more about the structure-activity relationships of podophyllotoxin congeners, we were interested in new derivatives of podophyllotoxone.

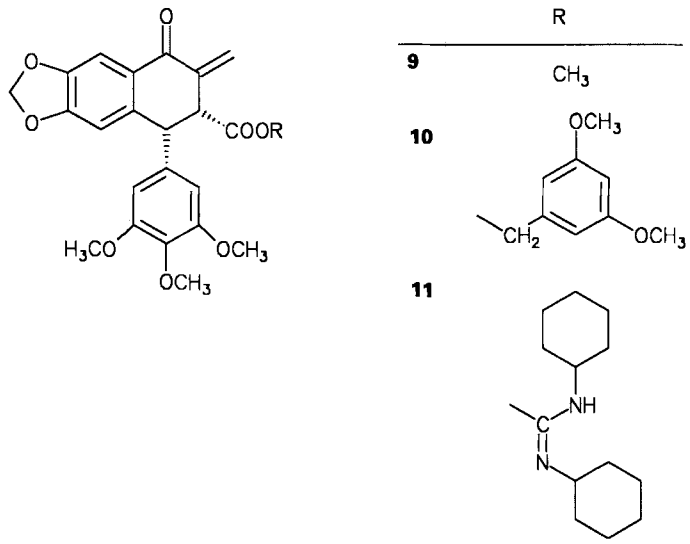
Results and Discussion. – Podophyllotoxone can be synthesized by oxidation of podophyllotoxin with freshly prepared MnO_2 [5] or pyridinium chlorochromate. Podophyllotoxone does not readily react with nucleophiles because of the electron-rich aromatic system next to the ketone group. Thus, podophyllotoxone does not undergo a *Knoevenagel* reaction with malononitrile and ammonium acetate or β-alanine as catalysts (in benzene). Furthermore, it is inert to *Grignard* reagents, and does not condense with *p*-hydroxyaniline in refluxing toluene (in the presence of TsOH as catalyst).

With stronger bases like BuLi (1 equiv. in refluxing Et_2O), the most acidic proton of **4**, H–C(3), is abstracted and podophyllotoxone is transformed into its enolate **5**. After addition of HCl, a mixture of podophyllotoxone (90%) and isopodophyllotoxone (**6**, 10%), the C(3) epimer, is obtained. When reacting podophyllotoxone with *t*-BuOK in refluxing *t*-BuOH (under N_2), a rearrangement reaction of the enolate **5** to the β-methylidene-carboxylate anion **7** occurs (*Scheme 1*). The corresponding carboxylic acid **8** can be isolated after addition of HCl.

Compound **8** contains a methylidene group in α-position to C=O and, therefore, may show alkylating properties towards nucleic acids or, as it has merely soft electrophilic properties, especially to sulfhydryl groups of proteins [6] [7]. An alkylation of sulfhydryl

Scheme 1

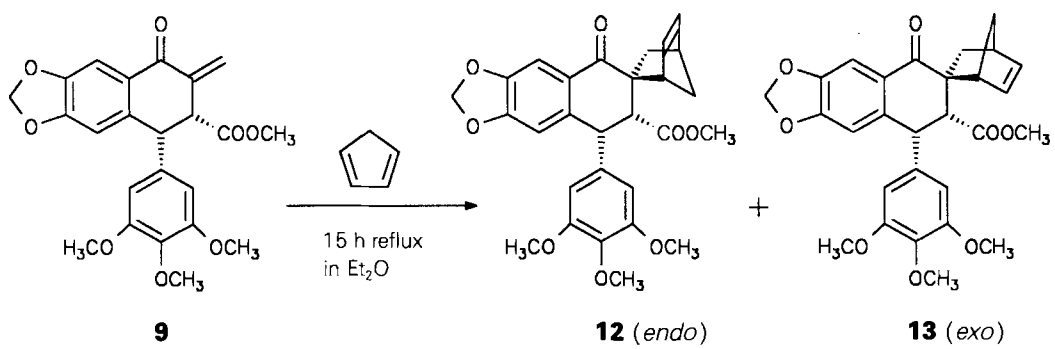




groups of the micro tubule apparatus might be in competition with the micro tubule disassembly effect of the trimethoxy-phenyl substructure or even evoke a synergistic effect.

To modify the lipophilic quality of **8**, we synthesized the methyl ester **9**, the 3,5-dimethoxybenzyl ester **10**, and the intermediate 1,3-dicyclohexylisourea derivative **11**. Compounds **8** to **11**, two-π-electron systems, are potential dienophiles which may undergo [4 + 2] cycloaddition reactions. Therefore, we performed *Diels-Alder* reaction of cyclopentadiene with the methyl ester **9**. This afforded a mixture of the *endo*- and *exo*-product, **12** and **13**, respectively, generated by front attack of the diene to the

Scheme 2



dienophil (*Scheme 2*). The mixture **12**/**13** was separated by HPLC and the structures elucidated by ^{1}H- and ^{13}C-NMR, and the H,H- and C,H-COSY-spectra taking into account the anisotropic effects of the C=C and the C=O bonds (*Fig.*).

The different congeners **1**, **4**, **6**, **8**–**11**, and the mixture **12**/**13** were tested *in vitro* for cytotoxic activity (*Table*). None of the synthesized congeners was as cytotoxic as podo-

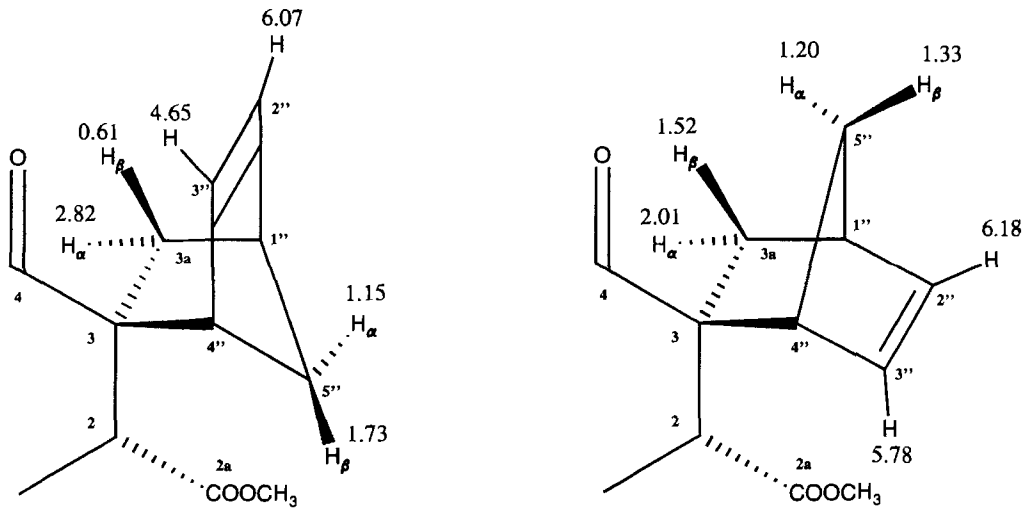


Figure. *Chemical shifts of bicycloheptene protons of* **12** *and* **13** *influenced by their neighborship to double bonds* (anisotropic effects)

Table. *Cytotoxicity of Podophyllotoxin* (**1**), *Etoposide* (**2**), *Podophyllotoxone* (**4**), *and Congeners* (L-1210 = murine leucaemia, KB = human squamous carcinoma)

Cytotoxicity EC_{90}	Congener								
	1	2	4	6	8	9	10	11	12 and 13
L-1210	0.003	0.1	0.3	10	> 10	0.03	0.03	3	3
KB	0.002	0.3	0.3	10	3	0.3	0.2	10	> 10

phyllotoxin, which cannot be used in cancer therapy because of its severe toxic side effects, but the EC_{90} values of the esters **9** and **10** should still be high enough for a possible therapeutic effect.

Conclusion. – Podophyllotoxone can be transformed into an α-methylidene ketone when heating with strong bases. The product **8** might be considered as an interesting soft alkylating agent. Its methyl ester **9** and its 3,5-dimethoxybenzyl ester **10** show good cytotoxic activity in the *in vitro* assay using L-1210 and KB cell lines.

Experimental Part

General. Chemicals and solvents: Phodophyllin *ex Podophyllum hexandrum* from *Roth*, 4-(pyrrolidin-1-yl)pyridin, '3,5-dimethoxybenzyl alcohol', and *t*-BuOK (1.0M soln. in *t*-BuOH) from *Aldrich*, *t*-BuOH and HCl from *Riedel de Haën*, pyridinium chlorochromate, BuLi (15% in hexane), cyclopentadiene, and all other solvents from *Merck*, all solvents *p.a.* or prepsolv.-grade except for Et_2O which was distilled and filtered over basic Al_2O_3 (*Macherey-Nagel*). HPLC: For anal. separations, we used a liquid chromatograph consisting of the following components supplied by *Merck Hitachi*: HPLC pump *665A-11*, UV detector *655-A*, integrator *655-61*. Prep. separations were carried out on the *Prep LC4000* system supplied by *Waters Association* consisting of pump, photodiode array detector 990 (changed for prep. purposes, 0.5-mm path length), *NEC APC IV*, Plotter *990*. Both

anal. and prep. chromatographs were equipped with a *Rheodyne* injection valve. M.p.: Microscope *HM-LUX* from *Leitz*, uncorrected, IR: *Hitachi 270-30* or *Nicolet 510P*, UV [nm]: *Waters* photodiode array detector 990 (resol. 3 nm, same solvent as *k'*). ^{1}H and ^{13}C-NMR: *Jeol FX 90* or *Jeol GX 400* spectrometers, $CDCl_3$ solns., δ in ppm downfield from internal Me_4Si, *J* in Hz. MS: *VG 7070H* from *Vacuum Generators*, 70 eV, fragment ions in *m/z* with relative intensities in parantheses.

Bioassays[1]): *Material*: Tissued culture dishes *d* = 35 mm, *Nunc*; *RPMI-1640*, *Gibco*, L-glutamine (200 mmol/l), *Gibco*; nonessential amino acids (100×), *Seromed*; sodium pyruvate (100 mmol/l), *Seromed*; Hepes-soln. 1 mol/l, *Merck*; mercaptoethanol, *Merck*, 3-mercaptopropane-1,2-diol, pure, *Serva*; penicilline (10,000 U/ml)/streptomycin (10,000 µg/ml), *Gibco*; FCS, *Seromed*; distilled water (Ampuwa), *Fresenius*; PBS, *Gibco*; trypanblue-soln. 0.5% in PBS, *Bacto-Agar* (*Difco*), 3% in Ampuwa. *Cells*: transplantable murine leukemia L-1210, ATCC CCL 219, human squamous carcinoma KB, ATCC CCL 17. *Medium 1* for L-1210: RPMI-1640 (90 ml), FCS active (10 ml), Hepes-soln. (2.25 ml), mercaptoethanol (1:1000, 0.37 ml), penicillin/streptomycin (1 ml). *Medium 2* for L-1210: RPMI-1640 (80 ml), FCS active (20 ml), Hepes-sol. (2 ml), mercaptoethanol (1:1000, 0.37 ml), penicillin/streptomycin (1 ml). *Medium 1* = *Medium 2* for KB: RPMI-1640 (80 ml), FCS inactivated (20 ml), Hepes-soln. (1 ml), nonessential amino acids (1 ml), glutamine (1 ml), sodium pyruvate (1 ml), 3-mercaptopropane-1,2-diol (1:5000, 1 ml), penicillin/streptomycin (1 ml). *Procedure*: 5 parts of 37° warm *Medium 1* are mixed with 1 part of hot agar soln. 1 ml of this mixture is pipetted to each tissue culture dish and left to gel. Tumor cells are grown in fluid culture, tested for vitality by incubation with trypanblue and counted. 9 parts of 37° warm *Medium 2* with the cells (for cell concentration, see below) are mixed with 1 part of hot agar soln. 1 ml of this mixture is pipetted into each tissue culture dish onto the first agar layer and is also left to gel. The test substance is dissolved in PBS in a concentration three times the desired test concentration, 1 ml here of is layered on top of the agar. The tissue culture dishes are placed in an incubator (for conditions, see below). After the incubation, cell colonies are counted using either a binocular microscope or an automatic image analyzer. All aggregates of more than 50 cells are counted as colonies. Results are expressed as %-inhibition of colony formation relative to the control without test substance. The concentration inhibiting colony formation by 90% (EC_{90}) is determined graphically. *Experimental conditions for L-1210*: *in vitro* application, number of cells/dish = 100, concentrations tested 0.464–464 µg/ml, incubation conditions 5% CO_2, 95% rel. humidity, 37°, incubation time 6 d. *Experimental conditions for KB*: *in vitro* application, number of cells/dish = 1000, concentrations tested 0.464–464 µg/ml, incubation conditions 5% CO_2, 95% rel. humidity, 37°, incubation time 8 d.

Podophyllotoxin (**1**) was isolated from Podophyllin *ex Podophyllum hexandrum* (80 g) *via* column chromatography (*Si 60*, *Merck*, 63–200 µm, CH_2Cl_2/MeOH 95:5) followed by HPLC (*Diol*, *Merck*, Lichrosorb, 250 mm × 50 mm, 5 µm, CH_2Cl_2). The resulting material (20 g) was of > 95% purity. M.p. 89°, *k'* = 3.63 (*RP18*, MeOH/H_2O 60:40). UV: 206, 290. IR (KBr): 3454, 2896, 2830, 2248, 1773, 1587, 1606, 1482, 1419, 1374, 1329, 1290, 1236, 1185, 1155, 1125, 1080, 1038, 996, 930, 876, 789, 765, 729, 696, 672, 648, 624, 594, 573, 528. ^{1}H-NMR (400 MHz): 7.11 (*s*, H–C(5)); 6.48 (*s*, H–C(8)); 6.37 (*s*, H–C(2′ and 6′)); 5.96, 5.94 (*d*, ^2J = 1.3, OCH_2O); 4.72 (*d*, $^3J(3,4)$ = 8.5, H–C(4)); 4.56 (*d*, $^3J(1,2)$ = 3.8, H–C(1)); 4.53 (*m*, H_α–C(3a)); 3.99 (*m*, H_β–C(3a)); 3.79 (*s*, MeO–C(4′)); 3.72 (*s*, MeO–C(3′ and 5′)); *ca.* 2.7–2.9 (*m*, H–C(2), H–C(3)). ^{13}C-NMR (100 MHz): 175.0 (C(2a)); 152.5 (C(3′), C(5′)); 147.6 (C(6), C(7)); 137.0 (C(4′)); 135.8 (C(1′)); 133.5 (C(10)); 131.0 (C(9)); 109.6 (C(8)); 108.5 (C(2′), C(6′)); 106.5 (C(5)); 101.4 (OCH_2O); 72.4 (C(4)); 71.6 (C(3a)); 60.8 (CH_3O–C(4)); 56.2 (CH_3O–C(3′ and 5′)); 45.3 (C(2)); 44.1 (C(1)); 40.6 (C(3)). MS (350°): 414 (100, M^+), 415 (36), 168 (15), 169 (11), 400 (8), 181 (8), 189 (7), 153 (7), 417 (7), 201 (7); ($C_{22}H_{22}O_8$).

Podophyllotoxone (**4**): 414 mg of **1** (1 mmol) were dissolved in CH_2Cl_2 (10 ml), 215 mg of pyridinium chlorochromate (1 mmol) were added, and the suspension was stirred overnight. FC with CH_2Cl_2 gave 320 mg of pure **4** (77%). M.p. 174°, *k'* = 5.63 (*RP18*, MeOH/H_2O 60:40). UV: 205, 236, 280, 320. IR (KBr): 3448, 2926, 2830, 1779, 1665, 1614, 1584, 1503, 1479, 1458, 1419, 1377, 1320, 1284, 1245, 1179, 1158, 1128, 1068, 1032, 990, 924, 879, 834, 795, 762, 720, 678, 636, 615, 531. ^{1}H-NMR (400 MHz): 7.55 (*s*, H–C(5)); 6.70 (*s*, H–C(8)); 6.39 (*s*, H–C(2′ and 6′)); 6.10, 6.08 (*d*, ^2J = 1.3, OCH_2O); 4.85 (*d*, $^3J(1,2)$ = 4.4, H–C(1)); 4.56 (*dd*, ^2J = 9.2, $^3J(3,3a\alpha)$ = 7.6, H_α–C(3a)); 4.36 (*dd*, $^3J(3,3a\beta)$ = 10.4, H_β–C(3a)); 3.82 (*s*, MeO–C(4′)); 3.75 (*s*, MeO–C(3′ and 5′)); 3.52 (*ddd*, $^3J(2,3)$ = 15.5, H–C(3)); 3.28 (*dd*, H–C(2)). ^{13}C-NMR (100 MHz): 192.4 (C(4)); 173.1 (C(2a)); 153.2 (C(7)); 153.1 (C(3′), C(5′)); 148.1 (C(6)); 141.5 (C(9)); 137.8 (C(4′)); 132.1 (C(1′)); 128.2 (C(10)); 109.7 (C(8)); 107.7 (C(2′), C(6′)); 106.1 (C(5)); 102.4 (OCH_2O); 67.0 (C(3a)); 60.8 (CH_3O–C(4′)); 56.3 (CH_3O–C(3′, and 5′)); 46.7 (C(2)); 44.7 (C(1)); 43.5 (C(3)). MS (190°): 412 (100, M^+), 413 (27), 168 (19), 367 (17), 153 (7), 337 (7), 397 (6), 353 (6), 336 (5), 368 (5); ($C_{22}H_{20}O_8$).

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[1]) We thank *J. Engel* and *R. Vögeli*, *ASTA Medica AG*, for performing the biological tests.

Isopodophyllotoxone (**6**): 41 mg of **4** (0.1 mmol) were solved in dry Et_2O (10 ml). After addition of 1.5 equiv. of BuLi, the mixture was heated for 1 h under reflux. FC (CH_2Cl_2), followed by HPLC (*Diol*, hexane/i-PrOH 85:15) gave 8 mg (19%) of pure **6**. M.p. 88°, $k' = 3.75$ (*RP18*, MeOH/H_2O 60:40). UV: 203, 239, 284, 329. IR (KBr): 3526, 3052, 2902, 2830, 2644, 2296, 1953, 1776, 1668, 1614, 1587, 1503, 1479, 1419, 1338, 1254, 1191, 1125, 1032, 1020, 930, 891, 852, 816, 786, 765, 732, 714, 687, 522. ^{1}H-NMR (400 MHz): 7.50 (*s*, H–C(5)); 6.69 (*s*, H–C(8)); 6.24 (*s*, H–C(2′ and 6′)); 6.05, 6.04 (*d*, $^2J = 1.3$, OCH_2O); 4.76 (*d*, $^2J = 9.4$, H_α–C(3a)); 4.69 (*s*, H–C(1)); 4.35 (*ddd*, $^3J(3,3a\beta) = 4.5$, $^4J(2,3a\beta) = 1.6$, H_β–C(3a)); 3.80 (*s*, C(4′)); 3.76 (*s*, MeO–C(3′ and 5′)); 3.31 (*m*, H–C(2, H–C(3)). ^{13}C-NMR (100 MHz): 193.5 (C(4)); 175.6 (C(2a)); 153.8 (C(7)); 153.7 (C(3′, C(5′)); 148.4 (C(6)); 139.5 (C(9)); 138.0 (C(1′)); 137.2 (C(4′)); 127.2 (C(10)); 109.4 (C(8)); 106.0 (C(5)); 104.6 (C(2′), C(6′)); 102.2 (OCH_2O); 70.5 (C(3a)); 60.8 (CH_3O–C(4′)); 56.2 (CH_3O–C(3′, and 5′)); 46.7 (C(2)); 43.4 (C(1)); 43.3 (C(3)). MS: (180°): 412 (100, M^+), 367 (66), 413 (27), 368 (18), 337 (16), 297 (13), 354 (10), 115 (9), 168 (9), 139 (8); ($C_{22}H_{20}O_8$).

*[5R-(5α,6α)]-5,6,7,8-Tetrahydro-7-methylidene-8-oxo-5-(3,4,5-trimethoxyphenyl)naphtho[2,3-*d*][1,3]-dioxole-6-carboxylic Acid* (**8**): 412 mg of **4** (1 mmol) were dissolved in dry *t*-BuOH (14 ml), 1 equiv. of *t*-BuOK was added under N_2, and the mixture was allowed to react under reflux for 24 h. Then the mixture was dried under vacuum and extracted twice with 5 ml of MeOH. Prep. HPLC (*Diol*, CH_2Cl_2) gave 318 mg (79%) pure **8**. M.p. 99°, $k' = 0.44$ (*RP18*, MeOH/H_2O 60:40). UV: 203, 239, 287, 341. IR (KBr): 3886, 3442, 2926, 2830, 2638, 1731, 1668, 1590, 1503, 1479, 1461, 1419, 1389, 1332, 1293, 1251, 1182, 1125, 1032, 1002, 930, 885, 843, 819, 732, 696, 594, 525. ^{1}H-NMR (400 MHz): 7.58 (*s*, H–C(5)); 6.55 (*s*, H–C(8)); 6.38 (*s*, H–C(3a)); 6.22 (*s*, H–C(2′ and 6′)); 6.02 (*s*, OCH_2O); 5.39 (*s*, H–C(3a)); 4.62 (*d*, $^3J(1,2) = 3.2$, H–C(1)); 3.90 (*d*, H–C(2)); 3.79 (*s*, MeO–C(4′)); 3.73 (*s*, MeO–C(3′ and 5′)). ^{13}C-NMR (100 MHz): 184.0 (C(4)); 176.5 (C(2a)); 153.2 (C(3′), C(5′)); 153.0 (C(7)); 148.0 (C(6)); 139.4 (C(9)); 137.7 (C(1′)); 137.1 (C(4′)); 137.0 (C(3)); 127.3 (C(10)); 126.7 (C(3a)); 108.9 (C(8)); 106.7 (C(5)); 105.3 (C(2′), C(6′)); 102.0 (CH_2O); 60.8 (CH_3O–C(4′)); 56.1 (CH_3O–C(3′ and 5′)); 55.2 (C(2)); 47.9 (C(1)). MS (22°): 412 (92, M^+), 368 (100), 367 (68), 353 (47), 369 (29), 413 (23), 354 (18), 152 (16), 168 (16), 337 (16). Anal. calc. for $C_{22}H_{20}O_8$: C 64.08, H 4.89; found: C 63.62, H 4.58.

*Methyl [5R-(5α,6α)]-5,6,7,8-Tetrahydro-7-methylidene-8-oxo-5-(3,4,5-trimethoxyphenyl)naphtho[2,3-*d*]-[1,3]dioxole-6-carboxylate* (**9**): 41.2 mg of **8** (0.1 mmol) were dissolved in dry CH_2Cl_2 (10 ml). 41.2 mg of dicyclohexylcarbodiimide (DCC, 0.2 mmol), *ca.* 50 µl of MeOH; and a few crystals of 4-(pyrrolidin-1-yl)pyridine were added and the soln. was stirred overnight. Filtration and HPLC (*Diol*, hexane/i-PrOH 90:10) led to 32 mg (75%) of **9**. M.p. 61°, $k' = 1.16$ (*RP18*, MeOH/H_2O 80:20). UV: 205, 238, 289, 340. IR (KBr): 3448, 2930, 2850, 1734, 1671, 1590, 1503, 1479, 1434, 1326, 1293, 1251, 1185, 1125, 1032, 1008, 933, 882, 819, 702. ^{1}H-NMR (400 MHz): 7.60 (*s*, H–C(5)); 6.57 (*s*, H–C(8)); 6.37 (*d*, $^2J = 1.0$, H–C(3a)); 6.25 (*s*, H–C(2′ and 6′)); 6.03 (*s*, OCH_2O); 5.36 (*d*, H–C(3a)); 4.65 (*d*, $^3J(1,2) = 3.6$, H–C(1)); 3.91 (*d*, H–C(2)); 3.81 (*s*, MeO–C(4′)); 3.75 (*s*, MeO–C(3′ and 5′)); 3.62 (*s*, COOMe). ^{13}C-NMR (100 MHz): 184.1 (C(4)); 172.0 (C(2a)); 153.3 (C(3′), C(5′)); 152.9 (C(7)); 148.0 (C(6)); 139.7 (C(9)); 138.3 (C(1′)); 137.1 (C(4′), C(3)); 127.5 (C(10)); 126.1 (C(3a)); 108.9 (C(8)); 106.7 (C(5)); 105.4 (C(2′), C(6′)); 102.0 (OCH_2O); 60.8 (CH_3O–C(4′)); 56.1 (CH_3O–C(3′ and 5′)); 55.5 (C(2)); 52.6 (COOMe); 48.3 (C(1)). MS (160°): 426 (71, M^+), 367 (100), 368 (27), 427 (17), 336 (14), 336 (12), 351 (11), 335 (9); 337 (7), 152 (6). Anal. calc. for $C_{23}H_{22}O_8$: C 64.78, H 5.20; found: C 64.30, H 4.99.

*3,5-Dimethoxybenzyl [5R-(5α,6α)]-5,6,7,8-Tetrahydro-7-methylidene-8-oxo-5-(3,4,5-trimethoxyphenyl)-naphtho[2,3-*d*][1,3]dioxole-6-carboxylate* (**10**): Synthesis as described for **9** with 16.8 mg of 3,5-dimethoxybenzyl alcohol (0.1 mmol) instead of MeOH and HPLC led to 38 mg (69%) of **10**. M.p. 71°, $k' = 2.08$ (*RP18*, MeOH/H_2O 80:20). UV: 202, 232, 283, 343. IR (KBr): 3448, 2920, 2830, 2638, 1842, 1734, 1671, 1596, 1503, 1479, 1461, 1431, 1383, 1329, 1293, 1251, 1203, 1152, 1125, 1068, 1032, 1008, 933, 885, 834, 759, 702, 594, 528. ^{1}H-NMR (400 MHz): 7.54 (*s*, H–C(5)); 6.50 (*s*, H–C(8)); 6.37 (*d*, $^4J(2'',4'') = 2.2$, H–C(4″)); 6.37 (*d*, $^2J = 0.9$, H–C(3a)); 6.29 (*d*, H–C(2″ and 6″)); 6.22 (*s*, H–C(2′ and 6′)); 6.03 (*s*, OCH_2O); 5.35 (*d*, H–C(3a)); 5.02, 4.94 (*d*, $^2J = 12.3$, 2 H–C(7″)); 4.63 (*d*, $^3J(1,2) = 3.8$, H–C(1)); 3.95 (*d*, H–C(2)); 3.80 (*s*, MeO–C(4′)); 3.76 (*s*, MeO–C(3″ and 5″)); 3.73 (*s*, MeO–C(3′ and 5′)). ^{13}C-NMR (100 MHz): 184.1 (C(4)); 171.3 (C(2a)); 160.9 (C(3″), C(5″)); 153.3 (C(3′), C(5′)); 152.9 (C(7)); 148.0 (C(6)); 139.3 (C(9)); 138.3 (C(1′)); 137.5 (C(1″)); 137.1 (C(4′)); 136.9 (C(3′)); 127.6 (C(10)); 126.0 (C(3a)); 108.8 (C(8)); 106.7 (C(5)); 105.7 (C(2″), C(6′)); 105.4 (C(2′), C(6′)); 102.0 (OCH_2O); 100.3 (C(4″)); 66.9 (C(7″)); 60.8 (C(4′)–OMe); 56.1 (CH_3O–(C3′, and 5′)); 55.7 (C(2)); 48.5 (C(1)). MS (280°): 562 (1, M^+), 367 (100), 368 (33), 41 (20), 336 (16), 67 (16), 69 (15), 57 (15), 366 (15), 82 (13). Anal. calc. for $C_{31}H_{30}O_{10}$: C 66.19, H 5.38; found: C 65.85, H 5.01.

*(Cyclohexylamino)(cyclohexylimino)methyl [5R-(5α,6α)]-5,6,7,8-Tetrahydro-7-methylidene-8-oxo-5-(3,4,5-trimethoxyphenyl)naphtho[2,3-*d*][1,3]dioxole-6-carboxylate* (**11**): Synthesis was performed as described for **9** without MeOH and 4-(pyrrolidin-1-yl)pyridine and following HPLC led to 50 mg (81%) of **11**. M.p. 112°, $k' = 4.22$ (*RP18*, MeOH/H_2O 80:20). UV: 202, 240, 292, 343. IR (KBr): 3412, 2920, 2848, 1842, 1701, 1665, 1590, 1503, 1479, 1455, 1431, 1386, 1332, 1290, 1251, 1125, 1035, 927, 888, 819, 729, 591. ^{1}H-NMR (400 MHz): 7.60 (*s*,

H–C(5)); 6.384 (*s*, H–C(8)); 6.376 (*s*, H–C(2′ and 6′)); 6.30 (*s*, H–C(3a)); 6.01, 6.00 (*d*, ^2J = 1.3, OCH_2O); 5.35 (*s*, H–C(3a)); 4.56 (*d*, $^3J(1,2)$ = 7.6, H–C(1)); 4.12 (*d*, H–C(2)); 4.00 (*m*, CH(cyclohexyl)); 3.818 (*s*, MeO–C(4′)); 3.816 (*s*, MeO–C(3′ and 5′)); 3.63 (*m*, CH(cyclohexyl)); 2–1 (*m*, CH_2(cyclohexyl)). ^{13}C-NMR (100 MHz): 184.4 (C(4)); 169.2 (C(2a)); 153.7 (C(1″)); 153.3 (C(3′), C(5′)); 152.8 (C(7)); 147.6 (C(6)); 141.7 (C(9)); 141.5 (C(1′)); 137.2 (C(4′)); 136.8 (C(3)); 127.2 (C(10)); 122.9 (C(3a)); 108.8 (C(8)); 106.6 (C(5)); 106.0 (C(2′), C(6′)); 101.9 (OCH_2O); 60.8 (CH_3O–C(4′)); 56.1 (CH_3O–C(3′ and 5′)); 55.3 (C(2)); 54.5 (CH(cyclohexyl)); 50.3 (CH(cyclohexyl)); 49.2 (C(1)); 32.7, 32.3, 31.1, 30.5, 26.0, 25.9, 25.4, 25.3, 24.6; (CH(cyclohexyl)). MS (240°): 618 (0.2, M^+), 368 (100), 353 (62), 367 (47), 369 (29), 337 (26), 394 (23), 354 (14), 366 (14); ($C_{25}H_{42}N_2O_8$).

*Methyl (6*S*,7*R*,8*R*,1′*S*,4′*S*)-5,6,7,8-Tetrahydro-5-oxo-8-(3,4,5-trimethoxyphenyl)naphtho[2,3-*d*][1,3]dioxole-6-*endo*-spiro-5′-(bicyclo[2.2.1]hept-2-ene)-7-carboxylate* (**12**) *and Methyl (6*S*,7*R*,8*R*,1′*R*,4′*R*)-5,6,7,8-Tetrahydro-5-oxo-8-(3,4,5-trimethoxyphenyl)naphtho[2,3-*d*][1,3]dioxole-6-*exo*-spiro-5′-(bicyclo[2.2.1]hept-2-ene)-7-carboxylate* (**13**): To 42.6 mg of **9** *ca.* 1 ml of cyclopentadiene (freshly distilled) and Et_2O (10 ml) were added. After 15 h reflux, HPLC (*Diol*, hexane/i-PrOH 85:15) led to a mixture of *ca.* 10 mg of **12** (20%) and *ca.* 5 mg of **13** (10%). *k′* (**12**) = 2.29, *k′* (**13**) = 2.01 (*RP18*, MeOH/H_2O 80:20). UV: 205, 235, 277, 316. ^{1}H-NMR of **12** (400 MHz): 7.66 (*s*, H–C(5)); 6.54 (*s*, H–C(8)); 6.43 (*s*, H–C(2′ and 6′)); 6.07 (*dd*, $^3J(1,2)$ = 2.9, $^3J(2,3)$ = 6.0, H–C(2″)); 6.06, 6.00 (*d*, ^2J = 1.3, OCH_2O); 4.65 (*dd*, $^3J(3'',4'')$ = 2.8, H–C(3″)); 4.48 (*d*, $^3J(1,2)$ = 1.6, H–C(1)); 3.87 (*s*, MeO–C(4′)); 3.80 (*s*, MeO–C(3′ and 5′)); 3.62 (*s*, COOMe); 3.11 (*d*, H–C(2)); 2.82 (*dd*, ^2J = 11.9, $^3J(1'',3a\alpha)$ = 3.8, H_α–C(3a)); 2.78 (br. *s*, H–C(1″)); 2.72 (br. *s*, H–C(4″)); 1.73 (*d*, ^2J = 8.5, H_β–C(5″)); 1.15 (*d*, H_α–C(5″)); 0.61 (*dd*, $^3J(1'',3a\beta)$ = 2.8, H_β–C(3a)). ^{13}C-NMR of **12** (100 MHz): 197.1 (C(4)); 174.6 (C(2a)); 153.3 (C(3′), C(5′)); 151.7 (C(7)); 147.7 (C(6)); 139.2 (C(2″)); 139.0 (C(9)); 137.7 (C(4′)); 135.4 (C(1′)); 133.7 (C(3″)); 128.0 (C(10)); 109.4 (C(8)); 107.1 (C(5)); 106.3 (C(2′), C(6′)); 101.8 (OCH_2O); 61.1 (CH_3O–C(4′)); 58.2 (C(2)); 56.5 (CH_3O–C(3′ and 5′)); 53.5 (C(3)); 52.2 (COOMe); 51.0 (C(4″)); 46.3 (C(1)); 46.1 (C(5″)); 41.4 (C(1″)); 35.6 (C(3a)). ^{1}H-NMR of **13** (400 MHz): 7.43 (*s*, H–C(5)); 6.50 (*s*, H–C(8)); 6.43 (*s*, H–C(2′, and 6′)); 6.18 (*dd*, $^3J(1'',2'')$ = 3.2, $^3J(2'',3'')$ = 5.7, H–C(2″)); 6.04, 5.99 (*d*, ^2J = 1.3, OCH_2O); 5.78 (*dd*, $^3J(3'',4'')$ = 3.0, H–C(3″)); 4.57 (*d*, $^3J(1,2)$ = 3.5, H–C(1)); 3.85 (*s*, CH_3O–C(4′)); 3.80 (*s*, CH_3O–C(3″ and 5″)); 3.63 (*s*, COOMe); 3.57 (*d*, H–C(2)); 2.92 (br. *s*, H–C(4″)); 2.81 (br. *s*, H–C(1″)); 2.01 (*dd*, ^2J = 11.0, $^3J(1'',3a\alpha)$ = 2.5, H_α–C(3a)); 1.52 (*dd*, $^3J(1'',3a_\beta)$ = 3.6, H_β–C(3a)); 1.33 (*d*, ^2J = 10.0, H_β–C(5″)); 1.20 (*d*, H_α–C(5″)). ^{13}C-NMR of **13** (100 MHz): 196.6 (C(4)); 174.0 (C(2a)); 153.1 (C(3′), C(5′)); 151.5 (C(7)); 147.5 (C(6)); 139.8 (C(9)); 137.1 (C(4′)); 136.0 (C(1′)); 133.5 (C(3″)); 128.6 (C(10)); 109.4 (C(8)); 106.9 (C(5)); 106.5 (C(2), C(6′)); 101.7 (OCH_2O); 61.0 (CH_3O–C(4′)); 58.2 (C(2)); 56.4 (CH_3O–C(3′ and 5′)); 54.7 (C(3)); 52.2 (COOMe); 48.6 (C(4″)); 47.4 (C(5″)); 46.2 (C(1)); 42.9 (C(1″)); 35.7 (C(3a)). MS of the mixture of 65% **12** and 35% **13** (90°): 492 (7, M^+), 367 (100), 491 (56), 426 (51, *retro*-DA-product), 84 (43), 368 (36), 86 (32), 66 (32, *retro-Diels-Alder* product), 427 (29), 79 (29). Anal. calc. for $C_{28}H_{28}O_8$: C 68.28, H 5.73; found: C 68.00, H 5.65.

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