114. Biphenyltetrols and Dibenzofuranones from Oxidative Coupling of Resorcinols with 4-Alkylpyrocatechols: New Clues to the Mechanism of Insect Cuticle Sclerotization

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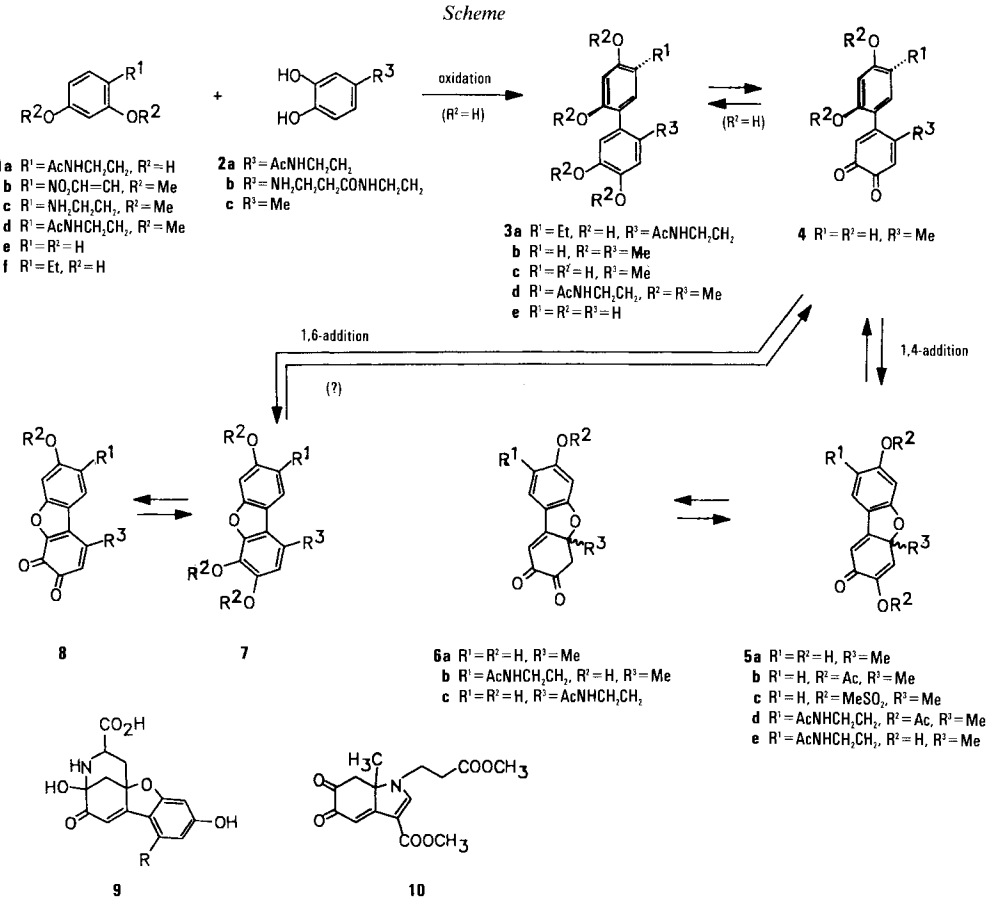
Oxidation of 4-alkylpyrocatechols **2** by means of an insect diphenoloxidase (laccase) or $K_3[Fe(CN)_6]$ yields, in the presence of resorcinols **1** (R^2 = H), complex mixtures of products from which biphenyltetrols **3** (R^3 = H) and dibenzofuranones **5** and **6** were isolated. It is suggested that similar homo-coupling products are formed from pyrocatechols **2b** and **2c** in insects during cuticle sclerotization.

Introduction. – The main components of the exoskeleton of insects are chitin and protein [1]. The soft hydrated matrix of those biopolymers is stabilized in a complex sequence of reactions, initialized by the enzymatic oxidation of 4-alkylbenzene-1,2-diols, resulting ultimately in extrusion of H_2O and hardening of the cuticle. Reactive intermediates in this vital sclerotization process are quinones [2] and quinone methides [3–5] which are thought to modify functional groups of proteins by conjugate addition (for review, see [6]). Also, degradation of the alkyl side chain may occur [7]. Nondestructive analysis by solid-state NMR spectroscopy confirms that the heterogeneity of the phenolic component of sclerotized insect cuticles is comparable to that of lignocelluloses or melanoproteins [8] [9]. At least, isotope labeling in combination with solid-state NMR provides convincing evidence for the addition of imidazole N-atoms of histidyl residues to ring positions of unidentified derivatives of dopamine [10]. In addition, it was argued that benzenediol oxidation should result in formation of polyphenols and that hydrophobic interactions of those polymers with proteins represent another important aspect of insect cuticle sclerotization [2] [3] [6] [8] [11].

Recently, it was observed that *N*-acetyl-2′-hydroxytyramine (**1a**), an analogue of the natural sclerotization agent *N*-acetyldopamine (**2a**), inhibits cuticle sclerotization reactions *in vitro* [12]. In order to obtain some ideas about the mechanism of this inhibition, it was of interest to investigate analogous enzymatic and biomimetic reactions in more detail. We now report on the isolation of cross-coupling products of types **3**, **5**, and **6**. These structures are formed under oxidative conditions from reactive quinonoid intermediates in trapping reactions by resorcinols (= benzene-1,3-diols). It is suggested, that analogous homo-coupling products are generated from 4-alkylpyrocatechols (= 4-alkylbenzene-1,2-diols) in the cuticle of insects during the process of sclerotization. Therefore,

[1]) This contribution contains, in part, results from the Diploma Thesis of *M. M.*, University of Bonn, 1990.

Scheme



the above mentioned inhibition of sclerotization reactions by **1a** results from the competition of a resorcinol with other reactants – such as pyrocatecols and/or nucleophilic functional groups of proteins – in oxidative phenolic homo-coupling and conjugate addition.

Results and Discussion. – The resorcinol derivative **1a** was prepared from nitrostyrene **1b** in an improved procedure (*cf.* [12]) by reduction with $LiBH_4/Me_3SiCl$ in THF [13]. The resulting amine **1c** was then acetylated to give **1d**, and the MeO groups were cleaved by means of BBr_3 (→**1a**).

Reaction mixtures containing equimolar amounts of **1a** and **2a** or **2b** and laccase or tyrosinase or pieces of cuticle from larvae of the giant silk moth (*Hyalophora cecropia*), the tobacco hornworm (*Manduca sexta*), or the migratory locust (*Locusta migratoria*) in phosphate buffer display a stable deep-red color (λ_{max} 490 nm) [12]. The same chromophore is observed when various other resorcinols, such as **1e** or **1f** are mixed with **2a** under the same conditions. HPLC analysis of the reaction mixture obtained after incubation of locust cuticle with **1f** and **2a** reveals formation of **3a** as the major product (λ_{max} 284 nm), besides minor amounts of known benzodioxin-type homodimers of **2a** [14]. The biphenyltetrol **3a** was identified by NMR and mass spectroscopy (see *Exper. Part*).

Attempts to isolate colored compounds from the enzymatic oxidation of **2a** in the presence of **1a** or **1f** resulted in complex mixtures, insufficient for spectroscopic characterization. Therefore, the reaction was repeated under biomimetic conditions using $K_3[Fe(CN)_6]$ as the oxidant. Again, frustratingly complex mixtures of mostly instable components were observed. Consequently, we turned to more simple benzenediols.

When **2c** was oxidized by means of K_3 $[Fe(CN)]_6$ in alkaline solution in the presence of **1e**, a dark reddish brown solid precipitated. Extraction of the reaction mixture with AcOEt followed by TLC, afforded a yellow solid. Spectral data showed it to be the dibenzofurandione **6a**, containing its tautomer **5a** to 11%.

HR-MS of the yellow solid shows an intensive M^+ peak ($C_{13}H_{10}O_4$), in addition to $[M + 2H]^+$. The basis peak at *m/z* 188 arises from loss of ketene. Another intensive fragment ion at *m/z* 160 forms on additional decarbonylation. The ^{1}H-NMR spectrum shows, besides the *s* of an olefinic proton at 6.61 ppm, an 1,2,4-trisubstituted aromatic unit. The aliphatic region displays the diastereotopic protons of a methylene group as a *d* at 3.41 and a *dd* at 3.37 ppm ($^2J = 15$, $^4J = 0.8$ Hz). The long-range coupling is also detected in the *d* of a Me group at 1.59 ppm. From these data, structure **6a** is assigned. Further support is obtained from the ^{13}C-NMR spectrum which shows two carbonyl-C at 192.49 and 179.26 ppm. A quaternary C-atom linked to an O-atom appears at 88.54 ppm, whereas a secondary C-atom shows up at 52.55 ppm. The data compare very well with the NMR spectra of the tetrahydro-methanobenzofuroazocines **9** (R = H, OH) [15] and with the indole derivative **10** [16]. Those contain a similar cyclohexenone moiety with a quaternary C-atom that is linked to a ring-CH_2, a hetero atom, and an exocyclic CH_2 or Me group, respectively. Careful analysis of the ^{1}H-NMR spectrum reveals the presence of **5a** as a minor component (11%). Two olefinic protons appear as *s* at 6.28 and 6.22 ppm.

Treatment of either **6a** or the crude reddish brown product with Ac_2O in pyridine yielded diacetate **5a** (λ_{max} 372 nm). Likewise, treatment of the crude oxidation product with methanesulfonyl chloride afforded the dimesylate **5c**. The tetramethyl ether **3b** was isolated after treating the crude oxidation product with dimethyl sulfate in acetone. Compounds **5b**, **5c**, and **3b** were characterized by their spectral data.

The ^{1}H-NMR spectrum of **5b** reveals, besides the 1,2,4-trisubstituted aromatic moiety, two olefinic protons at 6.94 and 6.34 ppm. The quaternary C-atom linked to the O-atom and Me group is detected in the ^{1}H-coupled ^{13}C-NMR spectrum by a *s* 87.34 ppm. The low-field region of the ^{13}C-NMR spectrum reveals 7 *s* between 180 and 145 ppm which are assigned to 2 AcO groups, one ring C=O group, two phenoxy and two olefinic C-atoms.

Apparently, compound **6a** is formed by oxidative coupling of resorcinol (**1e**) with 4-methylpyrocatechol (**2c**), followed by reoxidation of the resulting biphenyltetrol **3c** to *o*-quinone **4** which then undergoes intramolecular *Michael*-type 1,4-addition at the C-atom bearing the Me group. The cyclohexadienone **5a** is finally stabilized giving the dibenzofurandione **6a** (see *Scheme*). Alternatively, *o*-quinone **4** could cyclize by intramolecular 1,6-*Michael*-type addition to give dibenzofuran **7** as an isomer of **5a**. This could then be oxidized to the dibenzofurandione **8**. However, failure to detect products of type **7** or **8** indicates that this pathway is less likely, though its occurrence cannot be excluded with certainty.

In complete analogy, coupling of **2c** with **1a** gave a dark solid from which the diacetate **5d** and the tetramethyl ether **3d** were prepared. Also in this case, a yellow solid was extracted from the oxidation mixture that consisted of dibenzofurandione **6b** and dibenzofuranone **5e** in a ratio of 1:0.08 (^{1}H-NMR).

Oxidation mixtures from **2a** and **1e** show a more complex composition. TLC analysis indicates mostly polar material, in addition to a minor yellow component. A variety of aromatic and quinonoid signals appears in the ^{1}H-NMR spectrum. Formation of the

heterodimer **6c** is evident from the MS which displays a major $[M + 2]^+$ peak at *m/z* 303 ($C_{16}H_{17}NO_5$) besides a minor M^+ at *m/z* 301. In addition, HPLC reveals a major component (λ_{max} 407 nm). Even more complex mixtures were obtained from oxidation of **2a** in the presence of **1a**. Though HPLC indicates two quinonoid compounds (λ_{max} 458 and 443 nm, resp.), the ^{1}H-NMR spectrum did not allow structural assignments and the EI-MS did not show an M^+ peak. Attempts to isolate acetates or methyl ethers from the oxidation of **2a** in the presence of either **1a** or **1e** were so far unsuccessful.

Oxidative coupling of benzenediols is a well known reaction of fundamental importance in natural-products chemistry [17] [18]. Comprehensive studies have shown that both radical coupling and nucleophilic addition explain the mechanism of dimerization [19] [20]. Usually, formation of homodimers has been observed in the reactions of either hydroquinones, pyrocatechols, or resorcinols.

Though coupling of resorcinols has been extensively studied [19], cross-coupling of resorcinols with pyrocatechols has, apparently, received little attention. Thus, it was assumed that mixtures of the three possible regioisomeric 4-(dihydroxyphenyl)-5-methylcyclohexa-3,5-diene-1,2-diones are formed in the enzymatic cross-coupling of **1e** and **2c** [21], whereas autoxidation of 3-isopropylpyrocatechol in the presence of orcinol (= 5-methylbenzene-1,3-diol) leads to 1-methyl-6-isopropyldibenzofuran-3,7,8-triol [22]. Only recently, the structure of the natural product sappanin [23] has been described as the biphenyltetrol **3e** [24]. Isolation of dibenzofurandiones from cross-coupling reactions of 4-alkylresorcinols and 4-alkylpyrocatechols is preceded so far only by coupling products **9** of dopa with resorcinol or phloroglucinol (= benzene-1,3,5-triol), respectively [15]. These results suggest that analogous compounds may also form by homo-coupling of 4-alkylpyrocatechols. This idea is supported further by recent identifications of biphenyltetrols and dibenzofurans in addition to side chain allylic coupling products in the enzymatic oxidation of urushiol (= mixture containing 3-(pentadecatrienyl)benzene-1,2-diol) [25] and of phenylbenzoquinones from incubations of 4-alkylcatechols with insect cuticle [26]. We believe that the results of this study provide the mechanistic basis for a hitherto much neglected aspect of the various reactions occurring during the hardening of insect cuticles.

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Experimental Part

General. Solvents were redistilled before use. Benzene-1,2-diol **2a** was prepared by literature methods [3] [27]. Otherwise, resorcinols and pyrocatechols, except **1a** (see below), as well as reagents and solvents were from *Aldrich, Fluka*, or *E. Merck*. Evaporation procedures in a rotary evaporator, bath temp. *ca.* 30°. TLC: silica gel *60* F_{254} on alumina sheets (*Merck*). Flash chromatography (FC): silica gel *60* (30–40 μm; *Merck*). HPLC: instrumentation from *Waters* or from *LKB*; *Nucleosil 5* RP_{18} (*Knauer*), with 0.2N AcOH/MeOH at 1.5 ml · min^{-1}; *Spherisorb ODS-2* (*LKB*), with 0.1% aq. CF_3COOH/MeOH at 0.8 ml · min^{-1}; retention times (t_R) in min. M.p.: *Büchi-SMP-20* apparatus, uncorrected. UV/VIS spectra: *Cary-UV-VIS* spectrophotometer (*Varian*), scanning speed 2 nm · s^{-1}; λ_{max} (logε) in nm; ε_{rel} indicates that spectra were recorded from mixtures or during HPLC with a

photodiode array detector (with reference to the most intensive band ($\varepsilon_{rel} = 1$)). IR-spectra: *Perkin-Elmer* model *IR-1420*, in cm^{-1}. ^1H- and ^{13}C-NMR spectra: at r.t.; *Bruker-AC-270* (270 and 67.9 MHz, resp.), *Bruker-WM 200* (200 and 50.3 MHz, resp.) or *Varian EM 390* (90 MHz); δ in ppm rel. to TMS (= 0 ppm, ^1H) or solvent (^{13}C). MS: *AEI MS-50*, direct inlet, ionization energy 70 eV, if not indicated otherwise; in *m/z* (% of basis peak).

1. *N*-[2-(2,4-Dihydroxyphenyl)ethyl]acetamide (1a). – *2-(2,4-Dimethoxyphenyl)ethylamine* (**1c**). Me_3SiCl (8.64 g, 80 mmol) is added at 22° to a stirred suspension of $LiBH_4$ (0.87 g, 40 mmol) in THF (20 ml) [13]. Within 5 min, 2,4-dimethoxy-1-(2-nitroethenyl)benzene (**1b**; *cf.* [28]; 2.09 g, 10 mmol) is added in small portions. Stirring is continued for 24 h at 22°. MeOH (30 ml) is added, followed by evaporation and subsequent addition of 20% KOH/H_2O (30 ml). The resulting soln. is extracted with CH_2Cl_2 (3 × 50 ml). Drying ($MgSO_4$) of the combined extracts, evaporation, and bulb-to-bulb distillation give 1.35 g (72%) of **1c**. Colorless oil. B.p. 150°/1.3 · 10^{-2} Pa (bulb-to-bulb dist.). TLC (PrOH/H_2O/AcOH 3:2:2): R_f 0.72. ^1H-NMR (90 MHz, $CDCl_3$): 7.06 (*d*, ^2J = 9, 1 arom. H); 6.43 (*m*, 2 arom. H); 3.80 (*s*, 2 MeO); 3.03–2.56 (*m*, $2CH_2$); 1.33 (*s*, NH_2).

N-*[2-(2,4-dimethoxyphenyl)ethyl]acetamide* (**1d**; *cf.* [29]). A soln. of **1c** (1.8 g, 10 mmol) and Ac_2O (1.6 g, 15.6 mmol) in MeOH (15 ml) is stirred for 10 h at 20°. After evaporation, $CHCl_3$/MeOH 1:1 is added repeatedly to the residue and evaporated. The product crystallizes at −20° after addition of Et_2O: 1.74 g (78%) of **1d**. M.p. 83–84°. TLC ($CHCl_3$/MeOH 9:1): R_f 0.62. ^1H-NMR (90 MHz, $CDCl_3$): 7.03 (*d*, ^2J = 9, 1 arom. H); 6.40 (*m*, 2 arom. H); 3.76 (*s*, 2 MeO); 3.36 (*m*, CH_2); 2.70 (*t*, ^2J = 6.9, CH_2); 1.90 (*s*, Ac).

Acetamide **1a**. At −70°, BBr_3 (5 g, 20 mmol) is added under Ar to a soln. of **1d** (1.1 g, 5 mmol) in dry CH_2Cl_2 (25 ml). The mixture is stirred 5 min at 22° and subsequently cooled again to −70°. Addition of MeOH (30 ml) is followed by evaporation. The light brown oil crystallizes after addition of H_2O (15 ml) in light pink needles: 0.84 g (87%) of **1a**. M.p. 193–195°. TLC ($CHCl_3$/MeOH 9:1): R_f 0.26. ^1H-NMR (200 MHz, CD_3OD): 6.84 (*d*, ^2J = 8.1, 1 arom. H); 6.25 (*dd*, ^2J = 8.1, ^4J = 1.8, 1 arom. H); 6.18 (*d*, ^4J = 1.8, 1 arom. H); 3.30 (*m*, CH_2); 2.68 (*t*, ^2J = 8.1, CH_2); 1.90 (*s*, Ac). Anal. calc. for $C_{10}H_{13}NO_3$ (192.22): C 61.53, H 6.71, N 7.18; found: C 60.98, H 6.75, N 7.13.

2. General Procedures. – 2.1. *Enzymatic Oxidation* [30]. A mixture of **1a** or **1f** (0.5 mmol), **2a** (0.5 mmol), and cuticle, either 500 mg (wet weight) from 5th instar larvae of the giant silkmoth *Hyalophora cecropia* or 200 mg (wet weight) from adult *Locusta migratoria*, in 0.1M potassium phosphate buffer pH 7.0 (50 ml) is gently shaken for 16 h in a water bath at 40° under air. The buffer soln. is then decanted, acidified with HCOOH (*ca.* pH 3), and subjected to CC on *Bio-Beads SM-16* (1.5 × 15 cm column; equilibration with 0.2M AcOH, elution with linear gradient 0→100% MeOH, flow rate 8 ml · min^{-1}). Fractions showing λ_{max} 280 nm are combined, evaporated, and, if necessary, repurified by repeated CC under the same conditions. The chromatographically homogeneous compound is subjected to spectroscopic analysis without further purification.

2.2. *Oxidation with $K_3[Fe(CN)_6]$ in Alkaline Solution.* A degassed soln. of $K_3[Fe(CN)_6]$ (2.3 g, 6.9 mmol) in 1.6% KOH/H_2O (10 ml) is added under Ar at 22° within 60 min to a stirred degassed soln. of **1a**, **1e**, or **1f** (1.0 mmol) and **2** (1.1 mmol) in 1.6% KOH/H_2O (10 ml). After further stirring for 30 min, 0.1N citric-acid soln. (5 ml) is added, the red precipitate is collected by filtration, washed 3 times with ice-cold H_2O, and dried at 1.3 · 10^{-2} Pa to give crude *Fraction A*. The filtrate is extracted 5 times with AcOEt, the combined extract dried ($MgSO_4$) and evaporated, the residue stirred with MeOH (6 ml), and the solid collected by filtration and dried at 1.3 · 10^{-2} Pa to give *Fraction B*.

2.3. *Oxidation with $K_3[Fe(CN)_6]$ at pH 8.* A soln. of $K_3[Fe(CN)_6]$ (1.32 g, 4 mmol) in H_2O (20 ml) is added rapidly (within *ca.* 5 s) to a stirred soln. of **1a**, **1e**, or **1f** (1 mmol) and **2** (1 mmol) in 0.2M $(NH_4)HCO_3$ pH 8.2 (120 ml). The flask is stoppered and the soln. stirred for 45 min. After acidification with 1M HCl to pH 3, the mixture is extracted with AcOEt (4 times); the combined extracts are washed with H_2O (3 times), dried (Na_2SO_4), and evaporated to give crude *Fraction A*. The reddish brown residue is taken up in MeOH and separated by TLC (silica gel, benzene/AcOEt 6:4) to give *Fraction B*.

2.4. *Preparation of Acetates.* According to standard methods (Ac_2O/pyridine). The mixture is diluted with H_2O and extracted with $CHCl_3$. Washing of the combined extracts with sat. aq. $NaHCO_3$ soln. and H_2O is followed by drying ($MgSO_4$) and evaporation. The residue is separated by FC.

2.5. *Preparation of Mesylates.* According to standard methods ($MsCl/Et_3N$ in dry CH_2Cl_2). Stirring for 1.5 h is followed by addition of sat. aq. $NaHCO_3$ soln. (12 ml) and H_2O (40 ml). The mixture is extracted with CH_2Cl_2, the combined extract washed with H_2O, dried ($MgSO_4$), and evaporated, and the residue separated by FC.

2.6. *Preparation of Methyl Ethers.* According to standard methods (Me_2SO_4/K_2CO_3 in dry acetone). Excess Me_2SO_4 is decomposed by addition of 2M NH_4OH followed by exctraction with AcOEt. The combined extract is evaporated, the residue dissolved in $CHCl_3$ (30 ml), the soln. washed with sat. aq. $NaHCO_3$ soln. and H_2O and evaporated, and the residue separated by FC.

3. Coupling Products and Derivatives. – 3.1. *N-[2-(5'-Ethyl-2',4,4',5-tetrahydroxybiphenyl-2-yl)-ethyl]acetamide* (**3a**). From oxidation (see *2.1*) of **1f** (69.0 mg, 0.50 mmol) and **2a** (97.5 mg, 0.50 mmol): 30 mg (18.1%). Colorless crystals. HPLC (*Spherisorb*, linear gradient 0→70% MeOH): t_R 22.9. UV/VIS (MeOH/H_2O/0.01% CF_3COOH): 284 (ε_{rel} = 1). ^{1}H-NMR (200 MHz, (D_6)DMSO): 9.05 (*s*, OH); 8.71 (*s*, OH); 8.68 (*s*, OH); 8.64 (*s*, OH); 7.70 (br. *t*, ^2J = 5.0, NH); 6.60 (*s*, H–C(6')); 6.59 (*s*, H–C(6)); 6.44 (*s*, H–C(3)); 6.38 (*s*, H–C(3')); 3.0 (br. *m*, CH_2CH_2NH); 2.43 (*t*, ^2J = 7.0, CH_2CH_2NH); 2.37 (*q*, ^2J = 7.0, CH_3CH_2); 1.72 (*s*, Ac); 1.07 (*t*, ^2J = 7.0, CH_3CH_2). ^{13}C-NMR (50.3 MHz, (D_6)DMSO): 168.97 (*s*, CH_3CON); 154.36 (*s*, C(4')); 152.79 (*s*, C(2')); 143.88 (*s*, C(4)); 142.80 (*s*, C(5)); 131.02 (*d*, C(6')); 129.93 (*s*, C(2)); 128.78 (*s*, C(1)); 120.15 (*s*, C(1')); 119.03 (*s*, C(5')); 118.17 (*d*, C(3)); 115.86 (*d*, C(6)); 102.40 (*d*, C(3')); 40.09 (*t*, CH_2CH_2NH); 32.30 (*t*, CH_2CH_2NH); 22.62 (*q*, CH_3CON); 22.09 (*t*, CH_3CH_2); 14.66 (*q*, CH_3CH_2). MS: 331.1417 (81, M^+, $C_{18}H_{21}NO_5$, calc. 331.1419), 273 (10), 272 (78, $[M-MeCH_2NO]^+$), 271 (11), 258 (12), 257 (100, $[M-MeCH_2NO-Me]^+$), 256 (7), 255 (51), 244 (13), 243 (75, $[M-MeCH_2NO-Et]^+$), 242 (7), 241 (22), 231 (8), 230 (56), 229 (14), 227 (8), 226 (7), 213 (12), 184 (10), 138 (14), 123 (15), 43 (19).

3.2. *4,4a-Dihydro-7-hydroxy-4a-methyldibenzofuran-2,3-dione* (**6a**). From oxidation (see *2.2*) of **1e** (110 mg, 1.0 mmol) and **2c** (136 mg, 1.1 mmol): 164 mg (70%) of crude *Fraction A* (TLC ($CHCl_3$/MeOH (9:1)): R_f 0–0.28, 0.43, 0.6) and 12.3 mg (5.3%) of *Fraction B*, containing 11% **5a** (see *3.3*). From oxidation (see *2.3*) of **1e** (154 mg, 1.4 mmol) and **2c** (124 mg, 1.0 mmol): 264 mg of crude *Fraction A* and 6.0 mg (2.6%) of *Fraction B*. *Fraction B:* Yellow solid (**6a**). TLC ($CHCl_3$/MeOH 9:1): R_f 0.6. TLC (benzene/AcOEt 6:4): R_f 0.45. HPLC (*Nucleosil*, linear gradient 20→80% MeOH within 30 min): t_R 21.4, 22.5 (sh). UV/VIS (MeOH): 264 (4.09), 289 (4.23), 381 (4.36). IR (KBr): 3025, 2930, 1725, 1655, 1608*s*, 1475, 1440, 1365*s*, 1325, 1255, 1175*s*, 1130, 1040, 960*s*, 830, 800. ^{1}H-NMR (270 MHz, (D_6)acetone): 7.72 (*d*, ^2J = 8.5, H–C(9)); 6.70 (*dd*, ^2J = 8.5, ^4J = 2.1, H–C(8)); 6.61 (*s*, H–C(1)); 6.52 (*d*, ^4J = 2.1, H–C(6)); 3.41 (*d*, ^2J = 15, H_A–C(4)); 3.37 (*dd*, ^2J = 15, ^4J = 0.8, H_B–C(4)); 1.59 (*d*, ^4J = 0.8, Me). ^{13}C-NMR (67.9 MHz, (D_6)acetone): 192.29 (*s*, C(3)); 179.26 (*s*, C(2)); 169.60 (*s*, C(7)); 167.64, 166.63 (2*s*, C(5a), C(9b)); 126.98 (*d*, C(9)); 114.38 (*s*, C(9a)); 113.64, 112.90 (2*d*, C(6), C(8)); 99.06 (*d*, C(1)); 88.54 (*s*, C(4a)); 52.55 (*t*, C(4)); 30.30 (*q*, Me). MS (40 eV): 232 (19, $[M+2H]^+$), 231 (11), 230.0587 (68, M^+, $C_{13}H_{10}O_4$, calc. 230.0579), 229 (8), 202 (31), 201 (44), 189 (12), 188 (100, $[C_{11}H_8O_3]^+$), 187 (72), 185 (26), 174 (22), 173 (12), 161 (13), 160 (32), 147 (5), 131 (15), 129 (5), 103 (5), 77 (8), 43 (10).

3.3. *3,7-Dihydroxy-4a-methyldibenzofuran-2(4aH)-one* (**5a**). Minor component (11%) in **6a** (see *3.2*, oxidation procedure *2.2*). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.57 (*d*, ^2J = 8.5, H–C(9)); 6.52 (*dd*, ^2J = 8.5, ^4J = 2.1, H–C(8)); 6.40 (*d*, ^4J = 2.1, H–C(6)); 6.28, 6.22 (2*s*, H–C(1), H–C(4)); 1.60 (*s*, Me).

3.4. *2,4a-Dihydro-4a-methyl-2-oxodibenzofuran-3,7-diyl Diacetate* (**5b**). From acetylation (see *2.4*) of 200 mg of crude *Fraction A*, prepared according to *2.2* (see *3.2*). Purified by FC (cyclohexane/AcOEt 5:3): 44.3 mg (14%) of **5b**. From 36 mg (0.16 mmol) of *Fraction B*, prepared according to *2.2*: 14.9 mg (30.1%) of **5b**. Yellow needles. M.p. 118–119° (CH_2Cl_2/hexane). TLC (cyclohexane/AcOEt 5:3): R_f 0.38. UV/VIS (MeOH): 206 (4.36), 258 (4.08), 304 (3.69), 372 (4.05). ^{1}H-NMR (200 MHz, $CDCl_3$): 7.52 (*dd*, ^2J = 8.0, ^5J = 0.8, H–C(9)); 6.94 (*s*, H–C(1)); 6.80 (*dd*, ^2J = 8.0, ^4J = 2.0, H–C(8)); 6.75 (*d*, ^4J = 2.0, ^5J = 0.8, H–C(6)); 6.34 (*s*, H–C(4)); 2.30 (*s*, Ac); 2.28 (*s*, Ac); 1.75 (*s*, Me). ^{13}C-NMR (50.3 MHz, $CDCl_3$): 178.26 (*s*, C(2)); 168.85 (*s*); 168.67 (*s*); 165.26 (*s*, C(7)); 164.39 (*s*, C(5a)); 155.35 (*s*, C(9b)); 145.45 (*s*, C(3)); 131.24 (*d*, C(4)); 124.29 (*d*, C(9)); 120.30 (*s*, C(9a)); 116.42 (*d*, C(8)); 114.64 (*d*, C(6)); 106.51 (*d*, C(1)); 87.34 (*s*, C(4a)); 32.26 (*q*); 21.24 (*q*); 20.55 (*q*). MS (60 eV): 314.0783 (4, M^+, $C_{17}H_{14}O_6$, calc. 314.0790), 230 (4), 202 (30), 201 (43), 152 (22), 124 (27), 110 (100, $[C_6H_6O_2]^+$), 43 (48).

3.5. *2,4a-Dihydro-4a-methyl-2-oxodibenzofuran-3,7-diyl Bis(methanesulfonate)* (**5c**). From mesylation (see *2.5*) of 370 mg of crude *Fraction A*, prepared according to *2.2* (see *3.2*). Purified by FC (cyclohexane/AcOEt 1:1): 80.1 mg (13%). Light green platelets. M.p. 193–195° (Et_2O). TLC (cyclohexane/AcOEt 1:1): R_f 0.46. UV/VIS (MeOH): 256 (3.56), 304 (3.15), 370 (3.47). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.98 (*d*, ^2J = 8.4, H–C(9)); 7.66 (*s*, H–C(1)); 7.21 (*d*, ^4J = 2.2, H–C(6)); 7.14 (*dd*, ^2J = 8.4, ^4J = 2.2, H–C(8)); 6.74 (*s*, H–C(4)); 3.34 (*s*, 2 MeS); 1.74 (*s*, Me). MS: 388 (13, $[M+2H]^+$), 387 (5), 386.0139 (80, M^+, $C_{15}H_{14}O_8S_2$, calc. 386.0130), 310 (8), 309 (16), 308 (15), 301 (70), 280 (26), 231 (12), 230 (9), 229 (44), 228 (30), 215 (24), 214 (10), 213 (10), 212 (10), 202 (11), 201 (88), 200 (100, $[C_{12}H_8O_3^+]$), 199 (66), 173 (16), 172 (23), 171 (10), 145 (5), 144 (15), 116 (12), 115 (34), 80 (11), 79 (15), 65 (20), 64 (10), 63 (8).

3.6. *2',4,4',5-Tetramethoxy-2-methylbiphenyl* (**3b**). From methylation (see *2.6*) of 400 mg crude of *Fraction A*, prepared according to *2.2* (see *3.2*). Purified by FC (cyclohexane/AcOEt 5:1.5): 117.6 mg (23.5%) of **3b**. Colorless needles. M.p. 90–92° (CH_2Cl_2/hexane). TLC (cyclohexane/AcOEt 5:1.5): R_f 0.36. UV/VIS (MeOH): 283 (3.98). ^{1}H-NMR (200 MHz, $CDCl_3$): 7.06 (*d*, ^2J = 8.8, H–C(6')); 6.76, 6.71 (2*s*, H–C(3), H–C(6)); 6.55 (*d*, ^4J = 2.1, *dd*, ^2J = 8.8, ^4J = 2.1, H–C(3'), H–C(5')); 3.90, 3.86, 3.84, 3.77 (4*s*, 4 MeO); 2.08 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 160.16,

157.70, 147.78, 146.47, 131.61, 130.09, 129.17, 123.39, 113.76, 112.76, 104.03, 98.55, 55.96, 55.84, 55.48, 55.43, 19.55. MS (40 eV): 289 (18), 288.1367 (100, M^+, $C_{17}H_{20}O_4$, calc. 288.1361), 274 (4), 273 (23), 257 (3), 245 (8), 242 (8), 230 (4), 227 (3), 199 (5).

3.7. N-*[2-(5a,6,7,8-Tetrahydro-3-hydroxy-5a-methyl-7,8-dioxodibenzofuran-2-yl)ethyl]acetamide* (**6b**). From oxidation (see *2.2*) of **1a** (270 mg, 1.2 mmol) and **2c** (175 mg, 1.4 mmol): 317 mg (70.7%) of crude *Fraction A* (TLC ($CHCl_3$/MeOH 9:1): R_f 0–0.14, 0.41) and 12.3 mg (5.3%) of *Fraction B*, containing 7% of **5e**, see *3.8*. **6b**: Yellow solid. TLC ($CHCl_3$/MeOH 9:1): R_f 0.41. HPLC (*Nucleosil;* linear gradient 20→80% MeOH within 30 min): t_R 20.59. UV/VIS (H_2O/MeOH/0.2N AcOH; ε_{rel}): 255 (0.31), 306 (0.30), 438 (1). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.95 (*t*, ^2J = 2.6, NH); 7.64 (*s*, H–C(1)); 6.67 (*s*, H–C(9)); 6.50 (*s*, H–C(4)); 3.21 (*m*, H–C(6), CH_2CH_2NH); 2.64 (*t*, ^2J = 7.6, CH_2CH_2N); 1.88 (*s*, Ac); 1.50 (*s*, Me). ^{13}C-NMR (50.3 MHz, (D_6)DMSO): 197.34 (*s*, C(7)); 184.84 (*s*, C(8)); 175.71 (*s*, C(3)); 173.10 (*s*, C(4a)); 172.81 (*s*, C(9a)); 172.43 (*s*, CH_3CON); 132.45 (*s*, C(2)); 125.58 (*d*, C(1)); 109.86 (*s*, C(9b)); 105.72 (*d*, C(4)); 99.81 (*d*, C(9)); 90.07 (*s*, C(5a)); 51.63 (*d*, C(6)); 41.25 (*t*, CH_2CH_2N); 31.33 (*t*, CH_2CH_2N); 30.26 (*q*, Me); 22.63 (*q*, CH_3CON). MS: 317.1266 (19, $[M + 2H]^+$, $C_{17}H_{19}NO_5$, calc. 317.1263), 259 (25), 258 (100, $[C_{15}H_{14}O_4]^+$), 257 (5), 246 (8), 245 (36), 243 (3), 199 (4), 91 (6), 44 (5), 43 (6), 32 (43). FAB-MS (thioglycerol): 632 (3), 631 (4, $[2M + H]^+$), 630 (1), 450 (5), 448 (5), 446 (7), 433 (8), 431 (8), 429 (12), 424 (11), 348 (22), 342 (22), 340 (18), 327 (11), 325 (74), 324 (15), 323 (55), 318 (11), 317 (15), 316 (37, $[M + H]^+$), 307 (22), 292 (9), 290 (7), 288 (5), 283 (9), 280 (9), 258 (5).

3.8. N-*[2-(5a,8-Dihydro-3,7-dihydroxy-5a-methyl-8-oxodibenzofuran-2-yl)ethyl]acetamide* (**5e**). Minor component (7%) in **6b** (see *3.7*). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.47 (*s*, H–C(1)); 6.45 (*s*, H–C(4)); 6.30 (*s*, H–C(9)); 6.20 (*s*, H–C(6)); 3.21 (*m*, CH_2CH_2NH); 2.64 (*t*, ^2J = 7.6, CH_2CH_2N); 1.88 (*s*, Ac); 1.61 (*s*, Me).

3.9. *8-[2-(Acetamido)ethyl]-2,4a-dihydro-4a-methyl-2-oxodibenzofuran-3,7-diyl Diacetate* (**5d**). From acetylation (see *2.4*) of 120 mg of crude *Fraction A* (see *3.8*). Purified by repeated FC ($CHCl_3$/MeOH 9:1): 0.5 mg of **5d**. TLC ($CHCl_3$/MeOH 9:1): R_f 0.48. MS (60 eV): 401 (2, $[M + 2H]^+$), 400 (9), 399.1314 (5, M^+, $C_{21}H_{21}NO_7$, calc. 399.1318), 359 (3), 358 (3), 357 (22), 315 (22), 286 (22), 258 (15), 257 (5), 256 (30), 244 (9), 243 (27), 237 (28), 228 (11), 227 (15), 178 (31), 137 (12), 136 (100), 123 (38), 43 (41).

3.10. N-*[2-(4,4',5,6-Tetramethoxy-2'-methylbiphenyl-3-yl)ethyl]acetamide* (**3d**). From methylation (see *2.6*) of 100 mg of crude *Fraction A* (see *3.8*). Purified by repeated FC ($CHCl_3$/MeOH 95:5): 9.6 mg (6.4%) of **3d**. Light yellow oil. TLC ($CHCl_3$/MeOH 95:5): R_f 0.45. ^{1}H-NMR (200 MHz, $CDCl_3$): 6.80, 6.74, 6.67, 6.52 (4*s*, 4 arom. H); 3.90, 3.88, 3.85, 3.76 (4*s*, 4 MeO); 3.44 (*m*, CH_2CH_2NH); 2.78 (*t*, ^2J = 6.9, CH_2CH_2N); 2.08 (*s*, Me), 1.92 (*s*, Ac). MS: 373.1891 (52, M^+, $C_{21}H_{27}NO_5$, calc. 373.1889), 315 (30), 314 (100, $[C_{19}H_{22}O_4]^+$), 303 (10), 301 (52), 299 (6), 285 (5), 157 (6), 43 (6).

3.11. *Oxidation of* N-*Acetyldopamine* (**2a**) *in the Presence of Resorcinol* (**1e**). Oxidation (see *2.2*) of **1e** (125 mg, 1.1 mmol) and **2a** (257 mg, 1.15 mmol): 219 mg (53%) of *Fraction A* (TLC ($CHCl_3$/MeOH 9:1): R_f 0, 0.31) and 6.9 mg (1.7%) of *Fraction B*. *Fraction B* (presumably **6c**): yellow solid. TLC ($CHCl_3$/MeOH 9:1): R_f 0.31. HPLC (*Nucleosil*, linear gradient 20→80% MeOH within 60 min): t_R 23.09. UV/VIS (H_2O/MeOH/0.2N AcOH; ε_{rel}): 255 (0.32), 302 (0.32), 407 (1). IR (KBr): 3320*s*, 1729, 1650, 1610*s* (amide I), 1570*s* (amide II), 1550*s*, 1455, 1400, 1329, 1285, 1249, 1170, 1120, 975. MS: 303.1123 (39, $[M + 2]^+$, $C_{16}H_{17}NO_5$, calc. 303.1106), 245 (12), 244 (100), 243 (49), 242 (15), 232 (5), 231 (9), 230 (21), 229 (94), 228 (8), 227 (50), 226 (10), 214 (11), 213 (47), 201 (7), 200 (6), 197 (6), 185 (16), 65 (8), 43 (12), 32 (47).

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