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A Study of the Interconversion Between 3,4-Dihydro-4-formyl-2-hydroxy-2*H*-benzopyran and 2,3,3a,8a-Tetrahydro-2-hydroxyfuro[2,3-b]benzofuran Moieties, and its Application to a Formal Synthesis of (±)-Aflatoxin B_1 [1]

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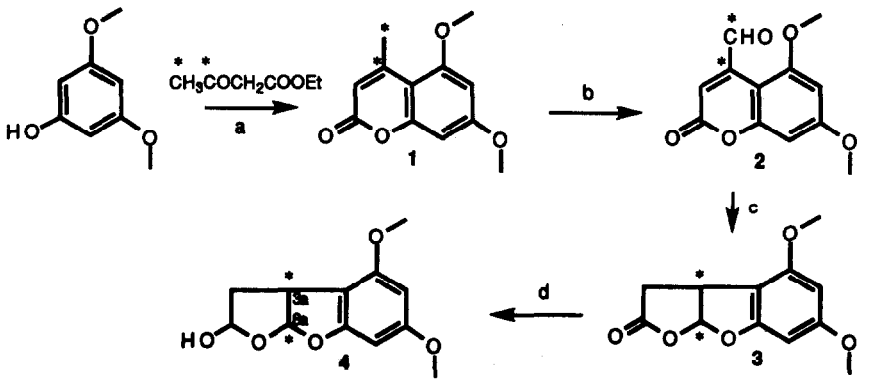
Abstract: The presence of six compounds, i.e., the epimeric hydroxyfuro[2,3-b]benzofurans **4**, a pair of diastereomers of the hydroxylated tetrahydrofuran **6** and the diastereomeric pair of benzopyran aldehydes **8**, was identified in the equilibrium mixture established when hemiacetals **4** were in solution. This identification was carried out by ^1H and ^{13}C NMR techniques using ^{13}C double labeled isotopomers of compound **4**. Theoretical calculations performed at the semiempirical level (PM3) for all of the possible diastereomers involved in the equilibrium mixture afforded a good correlation between the respective heats of formation and the relative abundance determined by NMR. On the other hand, the intervention of aldehydes **8** in the equilibrium mixture was confirmed by their independent synthesis and the observation that they show identical NMR spectra to that exhibited by hemiacetals **4**. Finally, the synthetic value of this equilibrium as an alternative entry to the furo[2,3-b]benzofuran fragment present in aflatoxins has become evident by its application to the convenient preparation of diacetate **17**, an intermediate in the total synthesis of racemic aflatoxin B_1 reported by Büchi and Weinreb. (*J. Am. Chem. Soc.* **1971**, *93*, 746-752).

In the course of our studies on aflatoxin and aflatoxin epoxides, [2] the synthesis of the hydroxyfuro[2,3-b]benzofuran derivative **4**, an intermediate in the preparation of an aflatoxin B_1 (AFB_1) model, was carried out following the procedure shown in Scheme I. However, the chromatographic purification of the corresponding crude reaction mixture afforded samples which showed additional peaks in the ^1H and ^{13}C NMR spectra to those expected for the epimeric mixture of hemiacetals **4**. Although no precedents on the stability of hemiacetal derivatives like **4** were found in the literature, studies carried out on aqueous solutions of AFB_1-diol derivatives containing a similar hemiacetal structure suggested the formation of hydroxyaldehydes and dialdehydes originated from the opening of these hemiacetal moieties. [3] However, this hypothesis could not be confirmed by the identification of the structures involved in the process. On the other hand, the existence of hydroxyfuro[2,3-b]benzofuran moieties in equilibrium with partly cyclized benzofuran or phenol derivatives had been used in synthetic approaches related to an analog of AFB_1 [4] and for the preparation of the toxicophoric fragment of aflatoxin M_1 in the total synthesis of this mycotoxin. [5]

In the same context, different studies had been carried out with aflatoxin B_{2a}, the mycotoxin presenting a hydroxyfuro[2,3-b]benzofuran moiety identical to that exhibited by hemiacetals **4**. Thus, racemization and a pronounced bathochromic shift in the UV spectrum was observed for the natural mycotoxin after treatment with base. [6] This shift was attributed to the formation of a phenoldialdehyde derivative, which could be the species

responsible for the reaction of aflatoxin B_{2a} with proteins, thus accounting for the toxic effects exhibited by this mycotoxin. [7] In this sense, two recent papers on analytical and structural features of aflatoxin B_{2a} reported the identification of the expected epimeric hemiacetals; however, no mention concerning the structure of other species already present in the sample solution was given. [8,9]

Scheme I



a) CH_3SO_3H, 80 °C; b) SeO_2, xylene, reflux; c) Zn / AcOH; d) DIBAH/toluene/ -20 °C.
Asterisks denote positions eventually labeled with ^{13}C.

The above antecedents suggested that the epimeric mixture of hemiacetals **4** could be in equilibrium with other species where the furobenzofuran system would be partly or totally opened. In the present paper, a study on the identification of the compounds present in the equilibrium mixture formed in solutions containing hemiacetal **4** is reported. The study was carried out by ^1H and ^{13}C NMR means, using ^{13}C double labeled isotopomers of compound **4**. In addition, theoretical calculations were performed to determine the relative energy stabilities of the different components of the equilibrium mixture, and an independent synthesis of specific components of the mixture, i.e., the aldehydes **8** (Scheme II) was carried out to confirm their presence in the above mixture and their participation in the equilibrium. Finally, a formal synthesis of (±)-AFB_1 is also presented, by using the former equilibrium as key step for the obtention of the furo[2,3-b]benzofuran intermediate reported in the total synthesis of this aflatoxin published by Büchi et al. (cf. diacetate **17** in Scheme III). [5]

RESULTS AND DISCUSSION

NMR Studies. The pattern of the ^1H NMR spectrum obtained from the purified sample of hemiacetals **4** (Figure 1a) showed a higher complexity than that anticipated for the expected mixture of epimers. Thus, the presence of two doublets at approx. 6.3 ppm (J = 6 Hz) could be assigned to two protons located at the position 8a of two furobenzofuran moieties. However, only two of the complex absorption systems appearing within the 5.4-5.9 ppm region (*i.e.*, the two doublets at 5.83 and 5.62, the doublet of doublets at 5.56 or the singlet at 5.49 ppm) could correspond to the hemiacetal hydrogen atoms of the epimers. Furthermore, two overlapped multiplets were present at approx. 4 ppm, followed by six singlets in the 3.7-3.8 ppm region that could correspond to methoxy groups, and a doublet of doublets at 3.5 ppm. Finally, a complex absorption pattern was

also present within 1.9 and 2.6 ppm. The presence of minor components in the mixture could be also inferred from the detection of several peaks, *inter alia*, the signals at approx. 9.6 ppm. From these observations it could be concluded that at least three major compounds were present in the mixture of hemiacetals **4**.

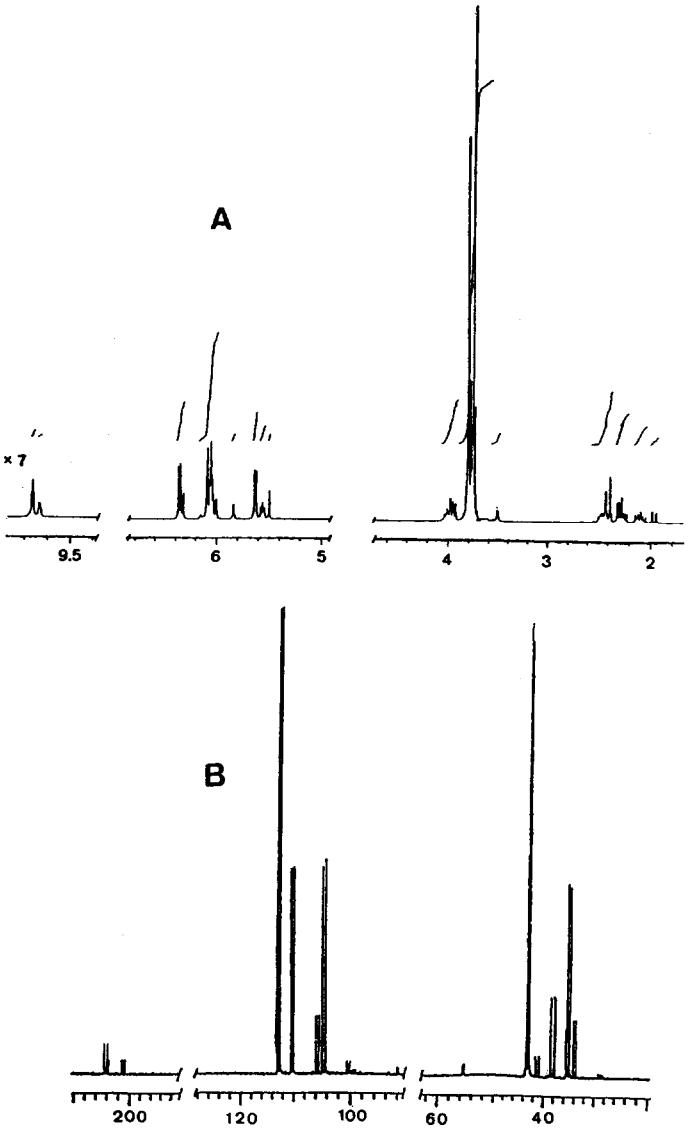


Figure 1. a) ^1H NMR Spectrum (300 MHz, $CDCl_3$-D_2O) of the mixture of compounds in equilibrium with hemiacetal **4**. b) ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of this mixture labeled with ^{13}C.

Experiments carried out under different solvents, presence of base or variable temperature showed that the above sample was in fact an equilibrium mixture in solution. The addition of acid to this mixture led to the small widening of the peaks, together with the appearance of new ones and the disappearance of the singlets at 9.6 ppm. These changes could be the consequence of a process involving the opening and further cyclization of acetal and hemiacetal groups present in the compounds in equilibrium.

In order to shed some light on the structure of the components of this equilibrium mixture, the synthesis of hemiacetals **4** labeled with ^{13}C was carried out. For this purpose, the synthetic sequence shown in Scheme I was used, and ethyl [3,4-$^{13}C_2$]acetoacetate was employed as starting labeled reagent. The ^{13}C NMR spectrum of the purified mixture of labeled hemiacetals **4** (Figure 1b) showed a pattern with six pairs of doublets. These doublets were interrelated by their respective coupling constants, which indicated the existence of six different compounds in a 44:24:20:6:5:1 molecular ratio.

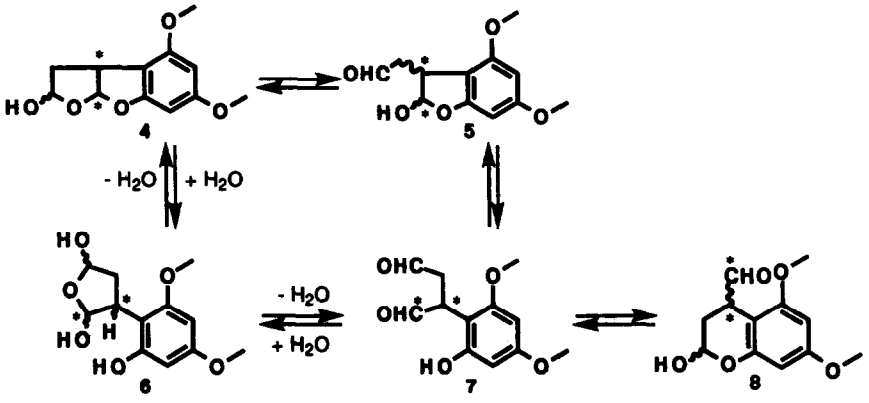
The ^1H NMR spectrum of this labeled sample (not shown), although being far more complex, afforded some interesting data. Thus, the two doublets appearing at the 6.3 ppm region in the spectrum of the non labeled sample (Figure 1a) showed an additional multiplicity (J = 182 Hz), thus indicating that these signals would correspond to acetal protons linked to labeled carbon atoms, *i.e.*, those from position 8a in each furobenzofuran system of epimers **4**. On the other hand, the singlet appearing at 5.49 in Figure 1a was also coupled to ^{13}C (J = 177 Hz), which suggested that it should be a proton, probably from hemiacetal type, directly linked to a labeled carbon atom. In this respect, the doublets present at 112.5, 109.5 and 105.5 ppm in the ^{13}C NMR spectrum would support the former assumptions. Finally, the two small aldehyde signals present in the spectrum of the non labeled sample (approx. at 9.6 ppm) should correspond to protons directly linked to ^{13}C atoms (absorptions at 204.9 and 201.5 ppm, in the spectra of the labeled sample), since J values characteristic for C-H couplings through one bond were again measured (J = 181 and 179 Hz, respectively). This observation led to exclude the possibility that these absorptions could be due to compounds **5** or **7** (see Scheme II), which would bear aldehyde protons in non labeled carbon atoms.

From these data it could be concluded that the three major components of the mixture did not have aldehyde groups, and two of them should contain an acetal and a hemiacetal. The third component should contain two hemiacetal groups in order to justify the two additional absorptions of hemiacetal type (see above). Likewise, the homocorrelation (^1H-^1H DQCOSY) and the heterocorrelation (^1H-^{13}C HETCOR) spectra of the sample containing hemiacetal **4** permitted the assignation of all the peaks corresponding to the three major components present in the equilibrium mixture (see Experimental Section).

The spectroscopic data of the above three major components were compatible with the presence in the mixture of both epimers of hemiacetal **4** and a diastereomer of the hydroxylated tetrahydrofuran derivative **6** (Scheme II). In this respect, the detailed analysis of the coupling constants of both epimers and complementary NOE experiments carried out on the sample permitted the differentiation of the epimer with the hydroxyl group in the *exo* configuration from that with the opposite *endo* configuration, being the latter which was present in higher concentration. Likewise, it was possible to distinguish among the four possible diastereomers of **6** and assign to **6b** (cf. Figure 2) as the third major component present in the equilibrium mixture. Concerning the minor components, the exclusion of aldehyde compounds **5** and **7** led to postulate the presence of the diastereomeric mixture of aldehydes **8** as the responsible for the observed signals at 9.63 and 9.66 ppm in the ^1H NMR spectrum, and the two pairs of doublets at 204.9 and 38.5, and 201.5 and 41.0 ppm in the ^{13}C NMR spectrum of the labeled sample. Finally, the similarity in chemical shifts among the pair of doublets at 105.5 and 33.5 ppm

in the ^{13}C NMR spectrum (Fig. 1b) with those corresponding to the hydrate **6b** led us to assign the sixth component to a second diastereomer of this hydrate, although its relative stereochemistry could not be established.

Scheme II



Asterisks denote positions eventually labeled with ^{13}C.

The need to involve the above compounds, *i.e.*, both epimers of **4**, a pair of diastereomers of **6** and the two diastereomers of aldehyde **8**, in the equilibrium mixture, led to postulate a system with the putative intervention of the five different structures depicted in Scheme II. Thus, although not detected under the NMR conditions used, it appears that aldehydes **5** or **7** should be present in very minor proportion in the equilibrium mixture.

Theoretical calculations. In order to find whether a possible correlation could be established between the relative concentrations of the different components in the equilibrium mixture involving hemiacetals **4** determined experimentally (NMR) and their relative stabilities, the estimation of the heats of formation (ΔH^o_f) for all diastereomers of the structures depicted in Figure 2 was carried out. The calculations were performed at the semiempirical level (MNDO-PM3) [10] and values obtained for ΔH^o_f are given also in Figure 2.

Concerning the hemiacetals **4**, the *endo* isomer appeared to be more stable than the corresponding epimer with the *exo* configuration. Furthermore, if it is assumed that the entropic and solvation contributions are not important in the equilibrium between these epimers, the calculated difference in heats of formation (0.4 kcal/mol) means a value of 0.5 for the corresponding equilibrium constant. From this value, a 2:1 *endo:exo* molecular ratio would be predicted for these epimers, which was in agreement with the ratio determined experimentally.

For the case of hydroxyaldehydes **5**, both compounds appear to be less stable than the above hemiacetals. This unfavourable stability in comparison with hemiacetals **4** was found still more pronounced for the dialdehyde **7**. Therefore, the relative lower stabilities of compounds **5** and **7** could give a reasonable explanation to their absence in the equilibrium mixture under the sensitivity scale of the NMR analysis.

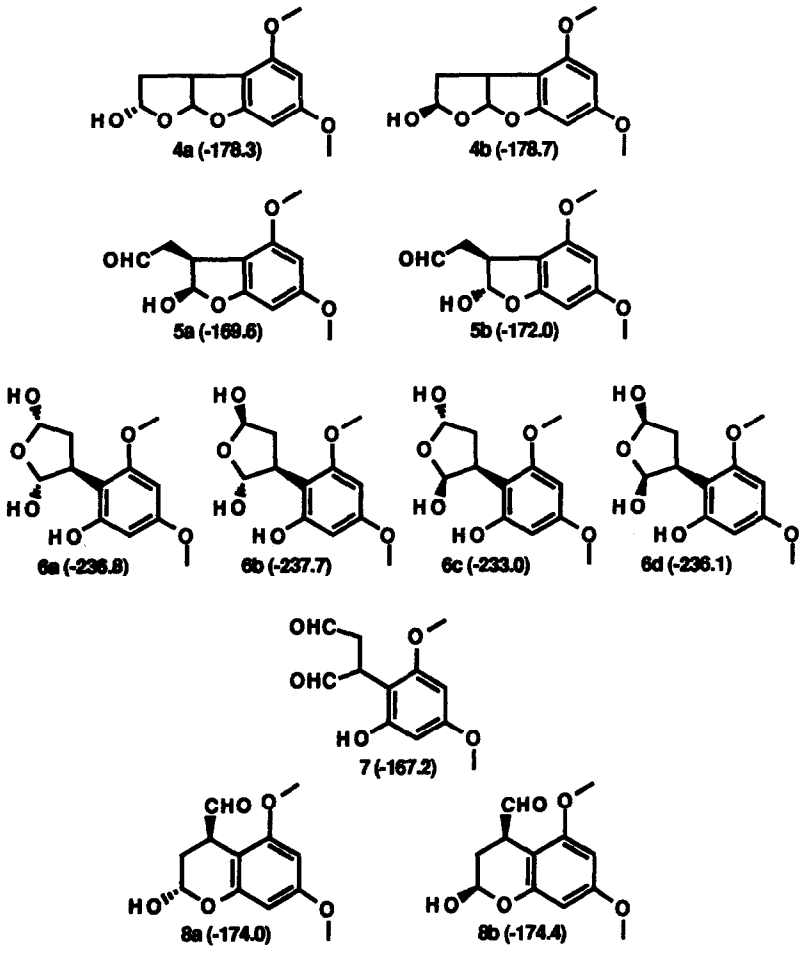


Figure 2. Heats of formation (kcal/mol) calculated at the semiempirical level (MNDO-PM3) for the different diastereomers of compounds **4-8**.

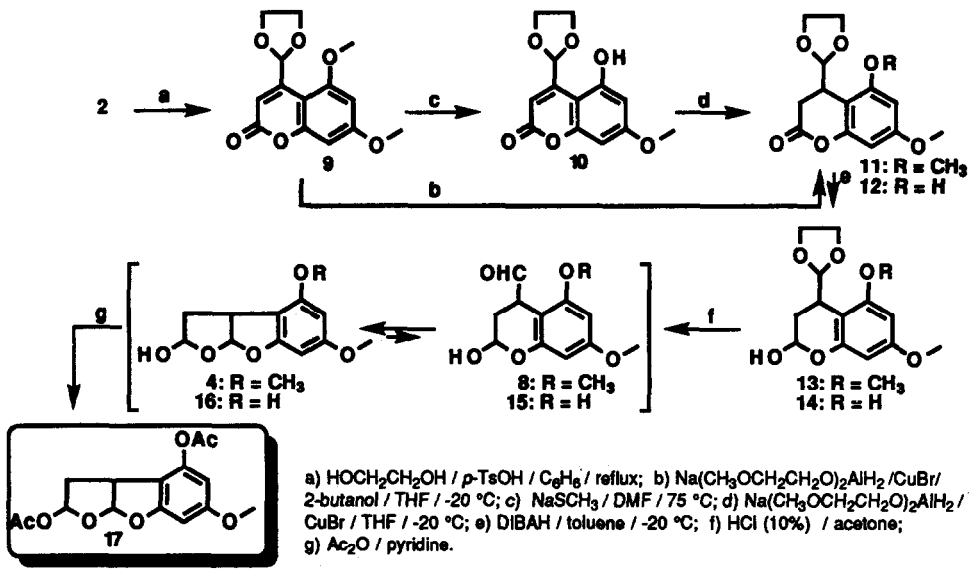
For the calculation of the ΔH^{o}_{f} values for hydrates **6** the possible conformations of all three hydroxyl substituents present in the tetrahydrofuran ring was considered. As shown in Figure 2, the most stable diastereomer, i.e., **6b**, coincides with that identified by NMR as a major component of the equilibrium mixture. According to the ΔH^{o}_{f} values, the next diastereomer in order of stability was **6a**, and the energy difference in respect to **6b** would correspond to a 1:4 **6a:6b** isomeric ratio, which is close to the ratio estimated by NMR between **6b** and the unidentified hydrate diastereomer present as minor component in the equilibrium mixture. This observation could be taken as an indirect evidence for the identification of **6a** as the sixth compound detected in this mixture.

Finally, from the two benzopyran aldehydes **8**, the more stable appeared to be the isomer where both substituents are in a *cis* relative configuration with the formyl group in pseudoaxial and the hydroxyl group in axial orientations. The calculated ΔH^{o}_{f} values for these aldehydes, although higher in comparison with those

obtained for hemiacetals **4**, were lower than those estimated for compounds **5** or **7**. Therefore, they should be favoured in an equilibrium situation, as it was the case since they could be detected by NMR.

Independent synthesis of benzopyran aldehydes 8. The presence of aldehydes **8** as components of the equilibrium mixture was confirmed by the independent synthesis of these compounds (Scheme III). Thus, protection of aldehyde **2** as dioxolane, followed by reduction of the conjugated double bond with Red-Al/CuBr [11] and of the carbonyl group with DIBAH, afforded the protected hemiacetal **13** in 59% overall yield. Final deprotection of **13** in mild acid conditions to render the epimeric mixture of aldehydes **8** led to a crude reaction mixture which showed NMR spectra superimposable to those described above for hemiacetals **4**. This result confirmed the participation of aldehydes **8** in the equilibrium mixture shown in Scheme II and supported the NMR assignations for these compounds.

Scheme III



Formal synthesis of (±)-AFB$_1$. From the identification of aldehydes **8** as constituents of the equilibrium mixture in which hemiacetals **4** are the major components, an important synthetic consequence could be derived: these aldehydes could be envisaged as an alternative entry to the furo[2,3-b]benzofuran toxicophoric fragment present in aflatoxins. Accordingly, as an example of the application of this strategy, the synthesis of diacetate **17**, which is the furo[2,3-b]benzofuran intermediate reported by Büchi et al. in their total synthesis of AFB$_1$, [5] was attempted. To this aim, a key step was the highly regioselective demethylation of dimethoxydioxolane **9** to give phenol **10** (cf. Scheme III). Thus, treatment of **9** with 3 molar equivalents of $NaSCH_3$ in DMF [12] afforded a 15:1 mixture of **10** and its corresponding regioisomer in a 70% conversion yield, which could be separated by chromatographic means. The presence of the free hydroxy group at C-5 in **10** was inferred from the NOE measurements carried out on this phenol and their comparison with those generated in the starting coumarin **9**. The regioselectivity obtained could be due to the influence of the dioxolane ring in the vicinity of the C-5 methoxy group in **9**, by increasing the nucleophilicity of the methanethiolate anion through the interaction of the

dioxolane oxygen atoms with the sodium cation. [13] In fact, when the demethylation reaction was assayed on the 3,4-dihydrocoumarin **11**, both regioselectivity and conversion yields obtained were considerable lower. This result suggested that the perpendicular arrangement of the dioxolane ring in respect to the vicinal aromatic ring, forced by the presence of the α,β-unsaturated double bond in **9**, appeared to be crucial for achieving the optimum activation for the nucleophilic attack of the methanethiolate anion at C-5.

Reduction of the double bond of **10** to give the dihydrocoumarin **12** was assayed with the Red-Al/CuBr reagent used above. In this case, the yields were considerably improved by omitting the recommended addition of 2-butanol to the reaction mixture. [11] Further reduction of the carbonyl group of **12** with DIBAH afforded a diastereomeric mixture of the benzopyran hemiacetals **14** in excellent yields. Different assays carried out under the same conditions led to mixtures of **14** presenting *cis:trans* isomeric ratios varying from 2:1 to 1:2. The fact that these mixtures evolve to give an approximate 1:1 *cis:trans* isomeric ratio indicated that an interconversion between both isomers was taking place. The assignation of the relative stereochemistry of these isomers was deduced from the respective NOE measurements.

Mild hydrolysis of **14** led to a crude reaction mixture which was purified to give a unique chromatographic peak in high yield. As expected, the NMR analysis of this purified sample showed that it was constituted by at least five compounds coexisting in equilibrium. By analogy with the NMR assignments made for the components of the equilibrium mixture involving hemiacetals **4** (see above), the two major components of the mixture originated from dioxolane **14** were identified as the hemiacetal diastereomers **16**. The other three components could be temptatively assigned to a diastereomer of the respective hydrate (cf. compound **6** in Scheme II) and to the diastereomeric aldehydes **15**. In any case, acetylation of this mixture led to the formation of the diasteromeric diacetates **17** as major products. In resume, this diacetate, which according to the total synthesis reported by Büchi et al. is only three steps ahead of racemic aflatoxin B_1, [5] could be synthesized in eight steps from 3,5-dimethoxyphenol (cf. ref. 2) with a 16% overall yield.

In conclusion, results herein reported have shown the advantageous use of the synthetic strategy involving the equilibrium mixture between the title moieties for generating the furo[2,3-b]benzofuran skeleton present in aflatoxin B_1. An extension of this strategy for the synthesis of related mycotoxins, *i.e.*, aflatoxin M_1 is in progress in our laboratory. In addition, the identification of the main components present in the equilibrium mixture of hemiacetal **4** has been used as a basis for the reexamination of the species coexisting in equilibrium in solutions of aflatoxin B_{2a}, [14] thus bringing new potential insights to the toxicity exhibited by this mycotoxin.

Experimental Section

Melting points were performed with a Koffler apparatus and are uncorrected. The IR spectra were recorded with a Bomem model MB120 apparatus. The ^{1}H-(300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Varian Unity 300 spectrometer. All NMR spectra were taken in neutralized $CDCl_3$ solutions and chemical shifts are given in ppm related to tetramethylsilane (TMS) as internal standard. The GC-MS analyses with electron impact (GC-MS-EI) were carried out with a Hewlett-Packard 5995 apparatus provided with a 30 m HP-5 bonded phase capillary column (0.25 mm i.d.). High resolution MS were obtained with a VG Autospec Q apparatus. Elemental analyses were performed with a Carlo Erba 1108 instrument (Microanalysis Service, CID). Compounds **1**, **2** and **3** have been described elsewhere.[2]

Theoretical calculations. The molecular geometries and heats of formation for compounds **4-8** were calculated by using the MOPAC 5.0 (QCPE 455) program package. In all cases PM3 hamiltonians and RHF wave functions were used. [10] The BFGS method [15] was used to optimize the molecular geometries, utilizing the GNORM = .1 and PRECISE MOPAC options. When several local minima with similar ΔH^o_f were obtained (as for compound **6**), the potential hypersurface was explored using the reaction coordinate method. In all cases it was considered that the conformation of the methoxy substituents remained constantly coplanar with the aromatic ring for all points of the evaluated potential hypersurfaces, and their influence on the calculated heat of formation was neglected. In cases where a great conformational mobility was anticipated, i.e., dialdehyde **7**, a previous study by using a molecular mechanics method was carried out and the energy minima derived were refined at the PM3 level.

Safety. Although not containing the structural features of natural aflatoxins, a call for attention should be made for the manipulation of the new compounds reported herein. Thus, use of gloves and safety glasses, well ventilated fume cupboards and careful destruction of residues with NaOCl is strongly recommended.

Labeled compounds. Unless stated otherwise, ^{13}C labeled isotopomers described in this work were synthesized following the same procedure used for the preparation of their corresponding non labeled compounds. This procedure is indicated with the appropriate reference if it has been previously reported. Ethyl [3,4-$^{13}C_2$]-acetoacetate was used as starting labeled material. On the other hand, only the relevant spectroscopic features of the respective ^{13}C isotopomers are given after the description of the respective non labeled derivative.

The NMR studies of the different equilibrium mixtures of compounds **4-8** was carried out in $CDCl_3$ solutions (50 mM concentration) at 20 °C.

4-([^{13}C]Methyl)-5,7-dimethoxy[4-^{13}C]-1-2*H*-benzopyran-2-one (**$^{13}C_2$-1**).[2] ^1H NMR d 6.44 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.29 (d, 1 H, J= 2.5 Hz, H_{Ar}). 5.96 (dq, 1 H, $^2J_{CH}$ = 6.5 Hz, J = 1 Hz, H-3), 3.86 (s, 3 H, CH_3O), 3.85 (s, 3 H, CH_3O), 2.53 (ddd, 3 H, J_{CH} = 130 Hz, $^2J_{CH}$ = 6.5 Hz, J = 1 Hz, CH_3); ^{13}C NMR d 154.6 (d, J = 41 Hz, C-4); 24.2 (d, J = 41 Hz, CH_3); MS m/z (%): 222 (M^+, 100), 194 (86), 179 (58).

4-([^{13}C]Formyl]-5,7-dimethoxy[4-^{13}C]-1-2*H*-benzopyran-2-one (**$^{13}C_2$-2**).[2] 10.49 (dd, 1 H, J_{CH} = 194 Hz, $^2J_{CH}$ = 26 Hz, CHO), 6.51 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.36 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.31 (d, 1 H, $^2J_{CH}$ = 5 Hz, H-3), 3.93 (s, 3 H, CH_3O), 3.89 (s, 3H, CH_3O); ^{13}C NMR d 191.3 (d, J = 48 Hz, CHO), 148.9 (d, J = 48 Hz, C-4); MS m/z (%): 236 (M^+, 100), 208 (14), 179 (45).

2,3,3a,8a-Tetrahydro-4,6-dimethoxy-2-oxo[3a,8a-$^{13}C_2$]furo[2,3-b]-benzofuran (**$^{13}C_2$-3**). [2] ^1H NMR d 6.50 (ddd, 1 H, J_{CH} = 188 Hz, J = 6 Hz, $^2J_{CH}$ = 3.5 Hz, H-8a), 6.14 (d, 1 H, J = 2 Hz, H_{Ar}), 6.09 (dd, 1 H, J = 2 Hz, $^4J_{CH}$ = 1 Hz, H_{Ar}), 4.15 (m, 1 H, J_{CH} = 145 Hz, H-3a), 3.80 (s, 3 H, CH_3O), 3.77 (s, 3 H, CH_3O), 2.95-2.91 (2 H, H-3, H-3'); ^{13}C NMR d 108.4 (d, J = 30 Hz, C-8a), 40.6 (d, J = 30 Hz, C-3a); MS m/z (%): 238 (M^+, 21), 208 (100), 165 (6).

Reduction of lactone 3. A 1.2 M soln of DIBAH in toluene (4 mL, 4.8 mmol) was added to a soln of lactone **3** (0.96 g, 4 mmol) in 100 mL of the same solvent, at - 20 °C under inert atmosphere, and the mixture was stirred for 1.5 h. The crude reaction mixture was acidified with 1N HCl and extracted with AcOEt. The organic extracts were washed with water, brine and dried over $MgSO_4$. The residue obtained after elimination of solvent was purified by flash chromatography on silicagel to render: **a)** a product which was identified as **2,3,3a,8a-Tetrahydro-4,6-dimethoxyfuro[2,3-b]benzofuran** (0.18 g, 20% yield). ^1H NMR d: 6.29 (d, 1 H, J = 6 Hz, H-8a), 6.05 (d, 1 H, J = 2 Hz, H_{Ar}), 6.02 (d, 1 H, J = 2 Hz, H_{Ar}), 4.06 (m, 1 H, H-2), 3.96 (m, 1 H, H-3a), 3.80 (s, 3 H, CH_3O), 3.76 (s, 3 H, CH_3O), 3.64 (m 1 H, H-2'), 2.22-2.02 (2 H, H-3,

H-3'); ^{13}C NMR d : 161.9 ($C_{Ar}O$), 161.2 ($C_{Ar}O$), 156.6 ($C_{Ar}O$), 111.9 (C-8a), 106.2 (C_{Ar}-3b), 91.5 ($C_{Ar}H$), 87.8 ($C_{Ar}H$), 67.4 (C-2), 55.6 (CH_3O), 55.3 (CH_3O), 44.5 (C-3a), 31.5 (C-3); MS m/z (%): 222 (M^+, 100), 207 (20), 193 (22), 178 (14); **b)** a more polar colorless oil (0.70 g, 70% yield) which was identified as a mixture of compounds containing **4a**, **4b** and **6b** in a 2:1:1 molar ratio, respectively (^1H NMR): IR (CCl_4): 3610, 3500-3400, 1625 cm^{-1}; ^1H NMR d : 6.35 (d, 1 H, J = 6 Hz, H-8a, **4a**), 6.32 (d, 1 H, J = 6 Hz, H-8a, **4b**), 6.10-5.98 (6 H, 2 x H_{Ar}, **4a** + **4b** + **6b**), 5.83 (d, 1 H, J = 3.5 Hz, H-5, **6b**), 5.62 (d, 1 H, J = 5 Hz, H-2, **4a**), 5.56 (dd, 1 H, J_1 = 6 Hz, J_2 = 5 Hz, H-2, **4b**), 5.49 (s, 1 H, H-2, **6b**), 4.00 (ddd, 1 H, J_1 = 9 Hz, J_2 = 6 Hz, J_3 = 2.5 Hz, H-3a, **4b**), 3.94 (ddd, 1 H, J_1 = 9 Hz, J_2 = 6 Hz, J_3 = 1 Hz, H-3a, **4a**), 3.81-3.73 (18 H, 2 x CH_3O, **4a** + **4b** + **6b**), 3.50 (dd, 1 H, J_1 = 4 Hz, J_2 = 0.5 Hz, H-3, **4a**), 2.70 (br., OH), 2.49-2.39 (2 H, H-3 **4b** + H-4 **6b**), 2.39 (dd, 1 H, J_1 = 14 Hz, J_2 = 1 Hz, H-3, **4a**), 2.26 (ddd, 1 H, J_1 = 14 Hz, J_2 = 9 Hz, J_3 = 5 Hz, H-3', **4a**), 2.09 (ddd, 1 H, J_1 = 13 Hz, J_2 = 9 Hz, J_3 = 6 Hz, H-3', **4b**), 1.96 (dd, 1 H, J_1 = 11.5 Hz, J_2 = 0.5 Hz, H-4', **6b**); ^{13}C NMR d : 162.2 ($C_{Ar}O$, **4a**), 162.0 ($C_{Ar}O$, **4b**), 160.5 ($C_{Ar}O$, **6b**), 159.9 ($C_{Ar}O$, **4b**), 159.3 ($C_{Ar}O$, **4a**), 156.6 ($C_{Ar}O$, **4b**), 156.5 ($C_{Ar}O$, **6b**), 156.2 ($C_{Ar}O$, **4a**), 152.8 ($C_{Ar}O$, **6b**), 112.6 (C-8a, **4a**), 110.0 (C-8a, **4b**), 108.0 (C-3b, **4a**), 107.2 (C-3b, **4a**), 105.9 (C_{Ar}, **6b**), 104.5 (C-2, **6b**), 100.2 (C-2, **4a**), 99.9 (C-2, **4b**), 99.2 (C-5, **6b**), 93.5 ($C_{Ar}H$, **6b**), 91.8 (2 x $C_{Ar}H$, **4a** + **4b**), 91.6 ($C_{Ar}H$, **6b**), 88.6 ($C_{Ar}H$, **4a**), 88.4 ($C_{Ar}H$, **4b**), 55.6 (CH_3O, **4b**), 55.5 (CH_3O, **4a**), 55.5 (CH_3O, **6b**), 55.3 (CH_3O, **6b**), 55.3 (CH_3O, **4b**), 55.3 (CH_3O, **4a**), 43.2 (C-3a, **4b**), 43.1 (C-3a, **4a**), 38.3 (C-3, **4b**), 38.2 (C-3, **4a**), 35.2 (C-3, **6b**), 28.7 (C-4, **6b**); MS m/z (%): 238 (M^+, 40), 209 (100), 181 (45). Anal. Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.88. Found: C, 60.40; H, 5.87.

When the reduction was carried out on the labeled lactone $^{13}C_2$-**3** (0.120 g, 0.5 mmol), purification of the crude reaction mixture afforded the corresponding isotopomers. a) **2,3,3a,8a-tetrahydro-4,6-dimethoxy[3a,8a-$^{13}C_2$]furo[2,3-b]benzofuran** (0.020 g, 18% yield): ^1H NMR d : 6.29 (ddd, 1 H, J_{CH} = 182.5 Hz, J = 5.5 Hz, $^2J_{CH}$ = 4 Hz, H-8a), 6.05 (d, 1 H, J = 2 Hz, H_{Ar}), 6.02 (d, 1 H, J = 2 Hz, H_{Ar}), 4.06 (m, 1 H, H-2), 3.96 (m, 1 H, H-3a), 3.80 (s, 3 H, CH_3O), 3.76 (s, 3 H, CH_3O), 3.64 (m, 1 H, H-2'), 2.22-2.02 (2 H, H-3, H-3'); ^{13}C NMR d : 111.9 (d, J = 34 Hz, C-8a), 44.5 (d, J = 34 Hz, C-3a); MS m/z (%): 224 (M^+, 100), 209 (21), 194 (23), 179 (28), 166 (31). b) 0.078 g (65% yield) of the mixture of labeled compounds **4a**, **4b**, **6b**, **6a**, **8a** and **8b** in a 44:24:20:6:5:1 molar ratio, respectively (^{13}C NMR). ^1H NMR d : 6.35 (ddd, 1 H, J_{CH} = 182 Hz, J = 6 Hz, $^2J_{CH}$ = 3.5 Hz, H-8a, **4a**), 6.32 (ddd, 1 H, J_{CH} = 182 Hz, J = 6 Hz, $^2J_{CH}$ = 4 Hz, H-8a, **4b**), 6.10-5.98 (6 H, 2 x H_{Ar}, **4a** + **4b** + **6b**), 5.83 (ddd, 1 H, $^3J_{CH}$ = 6.5 Hz, J = 3.5 Hz, $^3J_{CH}$ = 3.5 Hz, H-5, **6b**), 5.62 (m, 1 H, H-2, **4a**), 5.56 (m, 1 H, H-2, **4b**), 5.49 (dd, 1 H, J_{CH} = 177 Hz, $^2J_{CH}$ = 2 Hz, H-2, **6b**), 4.00 (m, 1 H, H-3a, **4b**), 3.94 (m, 1 H, H-3a, **4a**), 3.81-3.73 (18 H, 2 x CH_3O, **4a** + **4b** + **6b**), 3.50 (m, 1 H, H-3, **4a**), 3.00 (br., OH), 2.50-2.35 (3 H, H-3 **4a** + H-3 **4b** + H-4 **6b**), 2.26 (m, 1 H, H-3', **4a**), 2.09 (m, 1 H, H-3', **4b**), 1.96 (dddd, 1 H, J_1 = 11.5 Hz, $^2J_{CH}$ = 8 Hz, $^3J_{CH}$ = 2.5 Hz, J_2 = 0.5 Hz, H-4', **6b**); ^{13}C NMR d : 204.9 (d, J = 38 Hz, CHO, **8a**), 201.5 (d, J = 38.5 Hz, CHO, **8b**), 112.5 (d, J = 33 Hz, C-8a, **4a**), 109.6 (d, J = 32 Hz, C-8a, **4b**), 105.5 (d, J = 36 Hz, C-2, **6a**), 104.4 (d, J = 39.5 Hz, C-2, **6b**), 43.0 (d, J = 32 Hz, C-3a, **4b**), 42.9 (d, J = 33 Hz, C-3a, **4a**), 41.0 (d, J = 39 Hz, C-4, **8b**), 38.5 (d, J = 38 Hz, C-4, **8a**), 34.8 (d, J = 39.5 Hz, C-3, **6b**), 33.5 (d, J = 36 Hz, C-3, **6a**); MS m/z (%): 240 (M^+, 43), 222 (41), 210 (100), 193 (49), 182 (47), 154 (25).

4-(1,3-Dioxolan-2-yl)-5,7-dimethoxy-1-2*H*-benzopyran-2-one (**9**). A mixture of the coumarin **2** (0.150 g, 0.64 mmol), ethyleneglycol 50 mL (0.9 mmol) and *p*-toluenesulphonic acid (0.012 g, 0.064 mmol) in benzene (20 mL) was stirred under reflux for 5 h. The crude reaction mixture was diluted with AcOEt, washed with $NaHCO_3$ satd. soln. and with brine, and dried with $MgSO_4$. The residue obtained after solvent removal was purified by flash chromatography (3:1 hexane:AcOEt) to give compound **9** as a crystalline solid (0.164 g, 92% yield). mp 163-4 °C (hexane-AcOEt). IR (KBr): 1735, 1608, 1112 cm^{-1}; ^1H NMR d 6.56 (d, 1 H, J = 1 Hz, -OCHO-), 6.46 (d, 1 H, J = 1 Hz, H-3), 6.43 (d, 1 H, J = 2.5 Hz, H-8), 6.31 (d, 1 H, J = 2.5 Hz, H-6), 4.03-3.99 (4 H, $-CH_2CH_2-$), 3.89 (s, 3 H, CH_3O-5), 3.82 (s, 3 H, CH_3O-7); ^{13}C NMR d 163.0 ($C_{Ar}O$), 161.3 (C-2), 158.1 ($C_{Ar}O$), 157.2 ($C_{Ar}O$), 151.4 (C-4), 108.4 (C-3), 102.4 (C-4a), 100.0 (-OCHO-), 96.0 ($C_{Ar}H$), 93.7 ($C_{Ar}H$), 65.0 ($-CH_2CH_2-$), 56.2 (CH_3O), 55.7 (CH_3O); MS m/z (%): 278 (M^+, 58), 250 (26), 235 (23), 178 (27), 73 (100). Anal. Calcd. for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found: C, 60.53; H, 5.12. **4-(1,3-[2-^{13}C]Dioxolan-2-yl)-5,7-dimethoxy[4-^{13}C]-1-2*H*-benzopyran-2-one** ($^{13}C_2$-**9**): ^1H NMR d 6.56 (ddd, 1 H, J_{CH} = 179 Hz, $^2J_{CH}$ = 4 Hz, J = 1 Hz, -OCHO-); 6.46 (ddd, 1 H, $^2J_{CH}$ = 5.5 Hz, $^3J_{CH}$ = 1.5 Hz, J = 1 Hz, H-3), 6.44 (d, 1 H, J =2.5 Hz, H-8), 6.31 (dd, 1 H, J = 2.5 Hz, $^4J_{CH}$ = 1 Hz,

H-6), 4.04-3.98 (4H, -CH_2CH_2-), 3.89 (s, 3 H, CH_3O-5), 3.82 (s, 3H, CH_3O-7); ^{13}C NMR d 151.5 (d, J = 50 Hz, C-4), 100.2 (d, J = 50 Hz, -OCHO-); MS m/z (%): 280 (M^+, 100), 252 (40), 237 (34), 224 (20), 179 (41), 74 (99).

4-(1,3-Dioxolan-2-yl)-5-hydroxy-7-methoxy-1-2*H*-benzopyran-2-one (10). A solution of the coumarin **9** (118 mg, 0.4 mmol) in anhydrous DMF (2 mL) was treated with $NaSCH_3$ (89 mg, 1.3 mmol) and the mixture was heated for 6 h at 75 °C. The crude reaction mixture was diluted with AcOEt, washed with 1N HCl, brine and dried with $MgSO_4$. The residue obtained after solvent removal was crystallized from acetone to render pure hydroxycoumarin **10** (70 mg, 63% yield). Chromatographic separation of the mother liquors afforded 14 mg (14% yield) of a 2:1 (^1H NMR analysis) mixture of **10** and its isomeric 7-hydroxycoumarin derivative, and 10 mg of a product which was identified by ^1H NMR and MS as the corresponding dihydroxy derivative. **10**: mp 216-8 °C; IR (KBr): 3400-3100, 1681, 1620, 1598 cm^{-1}; ^1H NMR (CD_3COCD_3), d: 6.62 (s 1 H, OCHO), 6.45 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.39 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.33 (s, 1 H, H-3), 4.12-3.99 (4 H, CH_2-CH_2), 3.86 (s, 3 H, CH_3O); ^1H NMR (CD_3COCD_3), d: 163.9 ($C_{Ar}O$), 161.0 (C-2), 158.2 ($C_{Ar}O$), 157.1 ($C_{Ar}O$), 152.4 (C-4), 108.4 (C-3), 102.1 (C-4a), 100.4 (OCHO), 99.6 ($C_{Ar}H$), 94.2 ($C_{Ar}H$), 65.7 (CH_2-CH_2), 56.0 (CH_3O); MS m/z (%): 264 (M^+, 71), 220 (25), 192 (29), 164 (100). Anal. Calcd. for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.06; H, 4.54.

3,4-Dihydro-4-(1,3-dioxolan-2-yl)-5,7-dimethoxy-1-2*H*-benzopyran-2-one (11). A solution of Red-Al® (2.8 mL, 9.8 mmol) in toluene was added dropwise to a suspension of CuBr (686 mg g, 4.8 mmol) in anhydrous THF (8 mL) maintained at 0 °C and under inert atmosphere, and the mixture was stirred for 45 min. Then the mixture was cooled down to -70 °C and anhydrous 2-butanol (814 mg mL, 11 mmol) was added dropwise to the reaction flask. After 10 min of stirring at that temperature, a solution of coumarin **9** (166 mg, 0.60 mmol) in THF (12 mL) was added to the reaction flask and the mixture was stirred for 3 h at -20 °C. The crude reaction mixture was diluted with Et_2O, poured onto a NH_4Cl saturated solution and stirred until a blue coloration was developed. After separation of layers, the aqueous fraction was extracted with Et_2O. The organic extracts were washed with brine and dried with $MgSO_4$. The residue obtained after solvent removal was purified by flash chromatography (5:1 hexane:AcOEt) to give compound **11** as a crystalline solid (109 mg, 65% yield). **11**: mp 146-146.5 °C (hexane-AcOEt). IR (KBr): 1762, 1627, 1600, 1591, 1145, 1120, 1105 cm^{-1}; ^1H NMR d: 6.23 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.23 (d, 1 H, J = 2.5 Hz, H_{Ar}), 5.01 (d, 1 H, J = 2 Hz, OCHO), 4.10-3.75 (4 H, CH_2-CH_2), 3.80 (s, 3 H, CH_3O), 3.76 (s, 3 H, CH_3O), 3.59 (dt, 1 H, J_1 = 7.5 Hz, J_2 = 1.5 Hz, H-4), 2.96 (dd, 1 H, J_1 = 16.5 Hz, J_2 = 1.5 Hz, H-3), 2.48 (dd, 1 H, J_1 = 16.5 Hz, J_2 = 7.5 Hz, H-3'); ^{13}C NMR d: 167.9 (C-2), 160.8 ($C_{Ar}O$), 157.4 ($C_{Ar}O$), 153.7 ($C_{Ar}O$), 103.8 (OCHO), 101.6 (C-4a), 94.5 ($C_{Ar}H$), 93.9 ($C_{Ar}H$), 65.8 (CH_2), 65.2 (CH_2), 55.6 (CH_3O), 55.5 (CH_3O), 33.4 (C-4), 27.1 (C-3); MS m/z (%): 280 (M^+, 10), 207 (14), 73 (100). Anal. Calcd. for $C_{14}H_{16}O_6$: C, 60.00; H, 5.75. Found: C, 60.11; H, 5.73. **3,4-Dihydro-4-(1,3-[2-^{13}C]dioxolan-2-yl)-5,7-dimethoxy[4-^{13}C]-1-2*H*-benzopyran-2-one ($^{13}C_2$-11)**: ^1H NMR d: 6.23 (d 1 H, J = 2.5 Hz, H_{Ar}), 6.21 (d 1 H, J = 2.5 Hz, H_{Ar}), 5.00 (ddd, 1 H, J_{CH} = 173 Hz, $^2J_{CH}$ = 6.5 Hz, J = 1.5 Hz, OCHO), 4.10-3.75 (4 H, CH_2-CH_2), 3.79 (s, 3 H, CH_3O), 3.76 (s, 3 H, CH_3O), 3.58 (m 1 H, H-4), 2.95 (dtd, 1 H, J_1 = 16 Hz, $^2J_{CH}$ = $^3J_{CH}$ = 3 Hz, J_2 = 1 Hz, H-3), 2.47 (dtd, 1 H, J_1 = 16 Hz, J'_{HH} = $^2J_{CH}$ = 7 Hz, $^3J_{CH}$ = 4.5 Hz, H-3'); ^{13}C NMR d: 103.9 (d, J = 43 Hz, OCHO), 33.5 (d, J = 43 Hz, H-3'); MS m/z (%): 282 (M^+, 8), 208 (14), 74 (100).

3,4-Dihydro-4-(1,3-dioxolan-2-yl)-5-hydroxy-7-methoxy-1-2*H*-benzopyran -2-one (12). Following a similar procedure to that described above, with the exception of the 2-butanol addition, 5-hydroxycoumarin **10** (92 mg, 0.35 mmol) were reduced to give the corresponding dihydro derivative **12** (60 mg, 64 % yield), which was isolated by flash chromatography eluting with a 3:1 hexane:AcOEt mixture. **12**: mp 175-6 °C; IR (film): 3500-3000, 1766, 1631, 1602, 1145 cm^{-1}; ^1H NMR d: 6.82 (s, 1 H, OH), 6.32 (d, 1 H, J = 2.5 Hz, H_{Ar}), 6.27 (d, 1 H, J = 2.5 Hz, H_{Ar}), 5.09 (d, 1 H, J = 3 Hz, OCHO), 3.92-3.80 (4 H, CH_2-CH_2), 3.77 (s, 3 H, CH_3O), 3.49 (ddd, 1 H, J_1 = 8 Hz, J_2 = 3 Hz, J_3 = 1 Hz, H-4), 2.94 (dd, 1 H, J_1 = 16 Hz, J_2 = 1 Hz, H-3), 2.74 (dd, 1 H, J_1 = 16 Hz, J_2 = 8 Hz, H-3'); ^{13}C NMR d: 167.0 (C-2), 161.0 ($C_{Ar}O$), 155.3 ($C_{Ar}O$), 153.9 ($C_{Ar}O$), 105.6 (OCHO), 100.3 (C-4a), 99.5 ($C_{Ar}H$), 95.9 ($C_{Ar}H$), 66.0 (CH_2), 65.5 (CH_2), 55.5 (CH_3O), 34.8 (C-4), 29.6 (C-3); MS m/z (%): 266 (M^+, 3), 193 (3), 73 (100). Anal. Calcd. for $C_{13}H_{14}O_6$: C, 58.65; H, 5.30. Found: C, 58.59; H, 5.37.

3,4-Dihydro-4-(1,3-dioxolan-2-yl)-2-hydroxy-5,7-dimethoxy-1-2*H*-benzopyran (13). A solution of 1 M DIBAH (1.8 mL, 1.8 mmol) in toluene was added dropwise to a solution of the lactone **11**

(420 mg, 1.5 mmol) in toluene (65 mL), at -20 °C under N_2, and the mixture was stirred until reaction was completed (3 h). The crude reaction mixture was quenched with 1N HCl and extracted with AcOEt. The organic extract was washed with brine and dried with $MgSO_4$. The residue obtained after solvent removal was purified by flash chromatography (5:1 hexane:AcOEt) to give compound **13** as a crystalline mixture of diastereomers in a 3:2 *cis*:*trans* ratio (^{1}H NMR), which were in equilibrium in solution and could not be resolved by chromatographic means (415 mg, 98% yield). IR (KBr): 3398, 1618, 1593, 1149, 1134, 1112, 1066 cm^{-1}; ^{1}H NMR d: 6.83 (d, 1 H, J = 11.5 Hz, OH *cis*), 6.11 (d, 1 H, J = 2.5 Hz, H_{Ar} *cis*), 6.08 (d, 1 H, J = 2.5 Hz, H_{Ar} *cis*), 6.08 (d, 1 H, J = 2 Hz, H_{Ar} *trans*), 6.07 (d, 1 H, J = 2 Hz, H_{Ar} *trans*), 5.68 (ddd, 1 H, J_1 = 9.5 Hz, J_2 = 8 Hz, J_3 = 3 Hz, H-2 *trans*), 5.44 (ddd, 1 H, J_1 = 11.5 Hz, J_2 = 4 Hz, J_3 = 1 Hz, H-2 *cis*), 5.44 (d, 1 H, J = 2 Hz, OCHO *cis*), 5.29 (d, 1 H, J = 2 Hz, OCHO *trans*), 4.16-3.85 (8 H, CH_2-CH_2 *cis* + *trans*), 3.78 (s, 3 H, CH_3O, *cis*), 3.77 (s, 3 H, CH_3O, *trans*), 3.75 (s, 3 H, CH_3O, *cis*), 3.74 (s, 3 H, CH_3O, *trans*), 3.51 (ddd, 1 H, J_1 = 8.5 Hz, J_2 = 2 Hz, J_3 = 1 Hz, H-4 *cis*), 3.43 (dt, 1 H, J_1 = 7 Hz, J_2 = 2 Hz, H-4 *trans*), 3.15 (d, 1 H, J = 8 Hz, OH *trans*), 2.45 (ddd, 1 H, J_1 = 14 Hz, J_2 = 3 Hz, J_3 = 2 Hz, H-3 *trans*), 2.41 (dt, 1 H, J_1 = 15 Hz, J_2 = 1 Hz, H-3 *cis*), 2.10 (ddd, 1 H, J_1 = 15 Hz, J_2 = 8.5 Hz, J_3 = 4 Hz, H-3'*cis*), 1.62 (ddd, 1 H, J_1 = 14 Hz, J_2 = 9.5 Hz, J_3 = 7 Hz, H-3' *trans*); ^{13}C NMR d: 160.4 ($C_{Ar}O$ *cis*), 160.3 ($C_{Ar}O$ *trans*), 158.5 ($C_{Ar}O$ *cis*), 158.3 ($C_{Ar}O$ *trans*), 155.2 ($C_{Ar}O$ *trans*), 153.6 ($C_{Ar}O$ *cis*), 103.7 (OCHO *trans*), 102.6 (OCHO *cis*), 100.9 (C-4a *trans*), 99.6 (C-4a *cis*), 94.4 ($C_{Ar}H$ *cis*), 93.6 ($C_{Ar}H$ *trans*), 93.0 (C-2 *trans*), 92.1 ($C_{Ar}H$ *cis*), 91.8 ($C_{Ar}H$ *trans*), 89.5 (C-2 *cis*), 65.2 (CH_2 *trans*), 65.0 (CH_2 *cis*), 65.0 (CH_2 *trans*), 64.9 (CH_2 *cis*), 55.4 (2 x CH_3O *cis* + *trans*), 55.3 (2 x CH_3O *cis* + *trans*), 32.6 (C-4 *trans*), 30.0 (C-4 *cis*), 28.2 (C-3 *trans*), 25.2 (C-3 *cis*); MS m/z (%): *cis*: 282 (M^+, 15), 209 (100), 181 (30), 73 (93); *trans* 282 (M^+, 15), 221 (50), 192 (100), 191 (89), 177 (24), 73 (10). Anal. Calcd. for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43. Found: C, 59.48; H, 6.45. **3,4-Dihydro-4-(1,3-[2-^{13}C]dioxolan-2-yl)-2-hydroxy-5,7-dimethoxy[4-^{13}C]-1-2*H*-benzopyran ($^{13}C_2$-13)**: ^{1}H NMR d: 6.86 (d, 1 H, J = 11.5 Hz, OH *cis*), 6.11 (d, 1 H, J = 2 Hz, H_{Ar} *cis*), 6.08 (d, 1 H, J = 2 Hz, H_{Ar} *cis*), 6.08 (d, 1 H, J = 2 Hz, H_{Ar} *trans*), 6.06 (d, 1 H, J = 2 Hz, H_{Ar} *trans*), 5.68 (m, 1 H, H-2 *trans*), 5.44 (dddd, 1 H, J_1 = 11.5 Hz, $^3J_{CH}$ = 5.5 Hz, J_2 = 4 Hz, J_3 = 1.5 Hz, H-2 *cis*), 5.44 (ddd, 1 H, J_{CH} = 176 Hz, $^2J_{CH}$ = 4.5 Hz, J = 2 Hz, OCHO *cis*), 5.28 (ddd, 1 H, J_{CH} = 173 Hz, $^2J_{CH}$ = 5 Hz, J = 2 Hz, OCHO *trans*), 4.16-3.80 (8 H, CH_2-CH_2 *cis* + *trans*), 3.78 (s, 3 H, CH_3O, *cis*), 3.77 (s, 3 H, CH_3O, *trans*), 3.75 (s, 3 H, CH_3O, *cis*), 3.74 (s, 3 H, CH_3O, *trans*), 3.51 (dddd, 1 H, J_{CH} = 135 Hz, J_1 = 8.5 Hz, $^2J_{CH}$ = 6.5 Hz, J = 2 Hz, H-4 *cis*), 3.41 (m, 1 H, H-4 *trans*), 3.22 (d, 1 H, J = 7.5 Hz, OH *trans*), 2.50-2.35 (2 H, H-3 *cis* +*trans*), 2.11 (m, 1 H, H-3' *cis*), 1.62 (m, 1 H, H-3'*trans*); ^{13}C NMR d: 103.9 (d, J = 43 Hz, OCHO *trans*), 102.8 (d, J = 43 Hz, OCHO *cis*), 32.7 (d, J = 43 Hz, C-4 *trans*), 30.1 (d, J = 43 Hz, C-4 *cis*); MS m/z (%): *cis*: 284 (M^+, 17), 210 (100), 193 (33), 192 (38), 182 (34), 74 (87); *trans* 284 (M^+, 4), 223 (43), 193 (100), 192 (75), 178 (18), 154 (20), 74 (9).

3,4-Dihydro-4-(1,3-dioxolan-2-yl)-2,5-dihydroxy-7-methoxy-1-2*H*-benzopyran (14). By using a similar procedure to that described above, reduction of the lactone **12** (170 mg, 0.64 mmol) with DIBAH afforded a crude reaction mixture which was purified by flash chromatography (2:1 hexane:AcOEt) to render compoud **14** as a mixture of diastereomers in a 2:1 *cis*:*trans* ratio (^{1}H NMR) (160 mg, 93% yield). IR (film): 3600-3000, 1629, 1585, 1145 cm^{-1}; ^{1}H NMR d: 8.00 (s, 1 H, OH *trans*), 7.96 (s, 1 H, OH *cis*), 6.17 (d, 1 H, J = 2.5 Hz, H_{Ar} *cis*), 6.15 (d, 1 H, J = 2.5 Hz, H_{Ar} *trans*), 6.08 (d, 1 H, J = 2 Hz, H_{Ar} *cis*), 6.07 (d, 1 H, J = 2.5 Hz, H_{Ar} *trans*), 5.63-5.53 (2 H, 2 x H-2 *trans* + *cis*), 5.31 (d, 1 H, J = 3.5 Hz, OCHO *cis*), 5.03 (d, 1 H, J = 4 Hz, OCHO *trans*), 4.94 (d, 1 H, J = 9 Hz, OH *cis*), 4.12-3.85 (8 H, CH_2-CH_2 *cis* + *trans*), 3.74 (s, 3 H, CH_3O, *cis*), 3.74 (s, 3 H, CH_3O, *trans*), 3.30-3.24 (2 H, 2 x H-4 *cis* + *trans*), 3.10 (d, 1 H, J = 7 Hz, OH *trans*), 2.35 (dt, 1 H, J_1 = 14.5 Hz, J_2 = 1.5 Hz, H-3 *cis*), 2.33 (dt, 1 H, J_1 = 14 Hz, J_2 = 2 Hz, H-3 *trans*), 2.14 (ddd, 1 H, J_1 = 14.5 Hz, J_2 = 8 Hz, J_3 = 3.5 Hz, H-3'*cis*), 1.86 (ddd, 1 H, J_1 = 14 Hz, J_2 = 10 Hz, J_3 = 7 Hz, H-3' *trans*); ^{13}C NMR d: 160.8 ($C_{Ar}O$ *trans*), 160.7 ($C_{Ar}O$ *cis*), 156.5 (2 x $C_{Ar}O$ *cis* +*trans*), 155.6 ($C_{Ar}O$ *trans*), 153.6 ($C_{Ar}O$ *cis*), 107.5 (OCHO *trans*), 107.2 (OCHO *cis*), 99.4 (C-4a *trans*), 98.7 (C-4a *cis*), 96.6 (C-2 *cis*), 96.5 (C-2 *trans*), 95.2 ($C_{Ar}H$ *cis*), 94.6 ($C_{Ar}H$ *trans*), 91.5 ($C_{Ar}H$ *trans*), 90.5 ($C_{Ar}H$ *cis*), 65.7 (CH_2 *cis*), 65.7 (CH_2 *trans*), 65.2 (CH_2 *cis*), 64.8 (CH_2 *trans*), 55.2 (2 x CH_3O *cis* + *trans*), 34.6 (C-4 *trans*), 31.5 (C-4 *cis*), 31.0 (C-3 *trans*), 28.3 (C-3 *cis*); MS m/z (%): *cis*: 268 (M^+, 4), 177 (96), 73 (100). MS (high resolution): calcd for $C_{13}H_{16}O_6$: 268.09469. Found: 268.09355.

Hydrolysis of benzopyrans 13-14: A soln. of the diastereomeric mixture of hemiacetals **13** (18 mg, 0.06 mmol) in acetone (1.8 mL), was treated with 10% HCl (0.2 mL) for 2 h at 25 °C. The crude reaction mixture was diluted with AcOEt, washed with brine and dried with $MgSO_4$.The residue obtained after solvent removal (14 mg, 92 % yield), afforded superimposable IR, NMR and MS spectra with those obtained for the

equilibrium mixture containing hemiacetals **4**. When the reaction was carried out with a sample of $^{13}C_2$-**13**, the residue obtained was identical to the equilibrium mixture constituted by the ^{13}C isotopomers of compounds **4a**, **4b**, **6a**, **6b**, **8a** and **8b**. Similarly, 20 mg (89% yield) of the corresponding equilibrium mixture containing the two diastereomeric hemiacetals **16** as major components were obtained from the hydrolysis of 27 mg (0.1 mmol) of the mixture of hemiacetals **14**. ^1H NMR (CD_3COCD_3) d: 8.55 (s, 1 H, OH, **16a**), 8.53 (s, 1 H, OH, hydrate), 8.41 (s, 1 H, OH, **16b**), 6.28 (d, 1 H, J = 6.5 Hz, H-8a, **16b**), 6.25 (d, 1 H, J = 6 Hz, H-8a, **16a**), 6.04 (d, 1 H, J = 2.5 Hz, H_{Ar}, hydrate), 5.98 (d, 1 H, J = 2 Hz, H_{Ar}, **16b**), 5.95 (d, 1 H, J = 2 Hz, H_{Ar}, **16a**), 5.90 (d, 1 H, J = 2 Hz, H_{Ar}, **16a**), 5.86 (d, 1 H, J = 2 Hz, H_{Ar}, **16b**), 5.85 (d, 1 H, J = 2.5 Hz, H_{Ar}, hydrate), 5.80-5.74 (2 x OH), 5.63 (dd, 1 H, J_1 = 6 Hz, J_2 = 4 Hz, H-2, **16b**), 5.45 (td, 1 H, J_1 = 6.5 Hz, J_2 = 4.5 Hz, H-2, **16a**), 5.38 (d, 1 H, J = 5 Hz, H-2, hydrate), 4.96 (d, 1 H, J = 4 Hz, H-5, hydrate), 3.98 (ddd, 1 H, J_1 = 8.5 Hz, J_2 = 6 Hz, J_3 = 2 Hz, H-3a, **16a**), 3.93 (m, 1 H, H-3a, **16b**), 3.69 (s, 3 H, CH_3O, **16a**), 3.67 (s, 3 H, CH_3O, **16b**), 3.66 (s, 3 H, CH_3O, hydrate), 3.40 (d, 1 H, J = 4 Hz, H-3, hydrate), 2.44 (dt, 1 H, J_1 = 11.5 Hz, J_2 = 4 Hz, H-4, hydrate), 2.34 (ddd, 1 H, J_1 = 13 Hz, J_2 = 4.5 Hz, J_3 = 2 Hz, H-3, **16a**), 2.25 (m, 1 H, H-3, **16b**), 2.08 (m, 1 H, H-3', **16b**), 2.02 (ddd, 1 H, J_1 = 13 Hz, J_2 = 8.5 Hz, J_3 = 6.5 Hz, H-3', **16a**), 1.85 (dd, 1 H, J_1 = 11.5 Hz, J_2 = 0.5 Hz, H-4', hydrate); ^{13}C NMR (CD_3COCD_3) d: 162.6 (2 x $C_{Ar}O$, **16b** + hydrate), 162.4 ($C_{Ar}O$, **16b**), 161.6 ($C_{Ar}O$, **16a**), 161.4 ($C_{Ar}O$, **16a**), 155.1 (2 x $C_{Ar}O$, hydrate), 154.9 ($C_{Ar}O$, **16b**), 154.6 ($C_{Ar}O$, **16a**), 113.4 (C-8a, **16b**), 110.6 (C-8a, **16a**), 108.9 (C-3b, **16b**), 107.5 (C-3b, **16a**), 106.4 (C_{Ar}, hydrate), 105.5 (C-2, hydrate), 100.8 (C-2, **16b**), 100.5 (C-2, **16a**), 99.6 (C-5, hydrate), 95.3 ($C_{Ar}H$, **16a**), 95.0 (C_{Ar}, hydrate), 94.9 ($C_{Ar}H$, **16b**), 94.0 ($C_{Ar}H$, hydrate), 88.7 ($C_{Ar}H$, **16b**), 88.5 ($C_{Ar}H$, **16a**), 55.5 (CH_3O, **16a**), 55.4 (CH_3, **16b**), 55.3 (CH_3O, hydrate), 43.8 (C-3a, **16b**), 43.5 (C-3a, **16a**), 39.1 (C-3, **16a**), 38.9 (C-3, **16b**), 36.5 (C-3, hydrate), (C-4, hydrate, masked by the solvent absorption); MS m/z (%): 224 (M^+, 33), 206 (12), 195 (100), 178 (26), 177 (45), 167 (72).

2,3,3a,8a-Tetrahydro-2,4-diacetoxy-6-methoxyfuro-[2,3-b]-benzofuran (17). A soln of the equilibrium mixture constituted by compounds **15-16** (20 mg, 0.09 mmol) in CH_2Cl_2 (2 mL) was treated with Ac_2O (180 mg, 1.8 mmol) and pyridine (710 mg, 9 mmol) and the mixture was stirred for 5 h at 25 °C. The usual work-up of the crude reaction mixture led to a residue which was purified by preparative TLC on silica gel eluting with 2:1 hexane:AcOEt to give pure diacetates as a mixture of diastereomers, namely **17a** and **17b**, in a 2:1 ratio (^1H NMR), respectively (20 mg, 73% yield). **17** [5]: ^1H NMR d: 6.42-6.32 (4 H, 2 x H-2, 2 x H-8a, **17a** + **17b**), 6.30 (d, 1 H, J = 2.5 Hz, H_{Ar}, **17b**), 6.29 (d, 1 H, J = 2 Hz, H_{Ar}, **17a**), 6.26 (d, 1 H, J = 2.5 Hz, H_{Ar}, **17b**), 6.24 (d, 1 H, J = 2 Hz, H_{Ar}, **17a**), 4.14-3.92 (2 H, 2 x H-3a, **17a** + **17b**), 3.76 (s, 3 H, CH_3O, **17a**), 3.74 (s, 3 H, CH_3O, **17b**), 2.42-2.28 (4 H, 4 x H-3, **17a** + **17b**), 2.35 (s, 3 H, CH_3, **17b**), 2.30 (s, 3 H, CH_3, **17a**), 2.08 (s, 3 H, CH_3, **17a**), 2.07 (s, 3 H, CH_3, **17b**); ^{13}C NMR d, **17a**: 171.3 (CO), 169.4 (CO), 164.5 ($C_{Ar}O$), 160.4 ($C_{Ar}O$), 156.7 ($C_{Ar}O$), 115.0 (C-8a), 108.3 (C-3b), 99.7 (C-2), 93.1 ($C_{Ar}H$), 90.0 ($C_{Ar}H$), 55.5 (CH_3O), 43.2 (C-3a), 37.2 (C-3), 22.1 (CH_3), 21.3 (CH_3); MS (GC/MS, 3 peaks), m/z (%): (i) 248 (22), 206 (38), 178 (27), 177 (100), 43 (35); (ii) 308 (M^+, 16), 207 (31), 206 (58), 177 (80), 43 (100); (iii) 308 (M^+, 13), 249 (23), 207 (34), 206 (78), 178 (43), 177 (89), 43 (100).

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