HETEROCYCLES, Vol. 53, No. 1, 2000, pp. 93 - 106, Received, 5th August, 1999

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF SOME NEW PYRANO[2,3-*c*]XANTHEN-7-ONE AND PYRANO[3,2-*b*]XANTHEN-6-ONE DERIVATIVES WITH CYTOTOXIC ACTIVITY

Konstantinos Ghirtis, [a] Nicole Pouli, [a] Panagiotis Marakos, [a*] Alexios-Leandros Skaltsounis, [b] Stephane Leonce, [c] Daniel H. Caignard, [c] and Ghanem Atassi [c]

University of Athens, Department of Pharmacy, Division of Pharmaceutical Chemistry [a] and Division of Pharmacognosy, [b] Panepistimiopolis-Zografou, Athens 17345, Greece. Institut de Recherches SERVIER, [c] 11, Rue des Moulineaux, 92150 Suresnes, France

Abstract - The synthesis, conformational analysis and preliminary biological evaluation of some new pyrano[2,3-*c*]xanthen-7-ones and pyrano[3,2-*b*]xanthen-6-ones is described. Certain compounds possess interesting cytotoxic activity against murine leukemia L1210 cells.

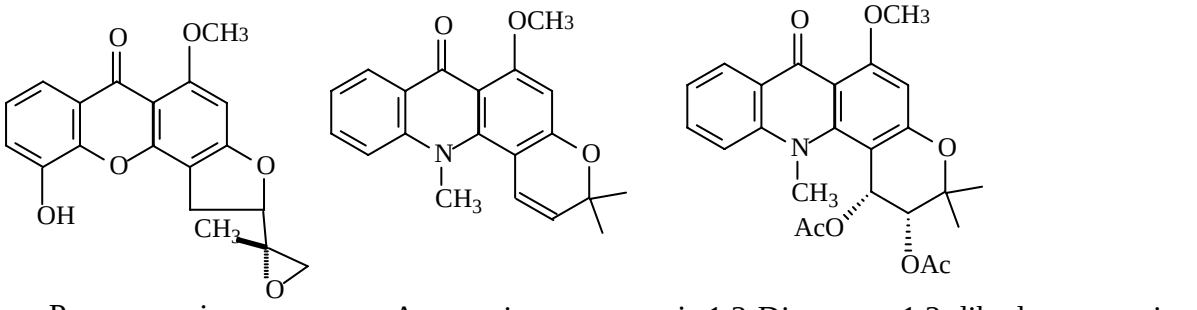
Xanthones possess a number of interesting pharmacological activities, [1-3] and among them, certain members, exhibit inhibition of DNA topoisomerases [4] and cytotoxic activities. [5] On the other hand, significant cytotoxic and antitumor properties have been reported for the furanoxanthone psorospermin, [6] as well as for the acridone alkaloid acronycine. [7,8] Both compounds contain a linear tricyclic ring system and a fourth, oxygen containing ring, fused to it (Figure 1). Promising results have also been reported for 1,2-dihydroxy-1,2-dihydroacronycine diesters.[9] These esters, especially *cis*-1,2-diacetoxy-1,2-dihydroacronycine (Figure 1), proved to have a broad spectrum of activity and increased potency, when compared with acronycine on several tumor cells *in vivo* and *in vitro*.

It appeared therefore of interest to synthesize a number of pyrano[2,3-*c*]xanthen-7-ones, structurally related to the acronycine esters, bearing the isosteric replacement of the oxygen instead of the nitrogen atom. In addition, we have also prepared the isomeric pyrano[3,2-*b*]xanthen-6-ones in order to investigate the influence of this, differently fused ring system to the potential cytotoxic activity of the compounds.

The synthesis of the derivatives is outlined in Scheme 1. We used the 1,3-dihydroxy-9*H*-xanthen-9-ones (**3a**) or (**3b**) as starting material, [10] which were obtained by the reaction of the corresponding benzoic acids (**1a**) or (**1b**) with 1,3,5-trihydroxybenzene (**2**). The deactivation of the 1-hydroxyl group of the derivatives

(**3**), due to its chelation by the carbonyl at the 9-position, made feasible the selective etherification of the 3-hydroxyl group with 3-chloro-3-methyl-1-butyne, in the presence of cuprous salt as catalyst, [11] to give the corresponding ethers, which were not isolated. Methylation of the 1-hydroxyl group of these ethers was then carried out, with dimethyl sulfate in the presence of sodium hydride in THF. Subsequent thermal cyclization of the resulting methylethers in boiling *N*,*N*-diethylaniline, afforded a mixture of two four-ring isomers, namely 6-methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (**4a**, angular isomer) and 5-methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**5a**, linear isomer) as well as the corresponding 11- and 10-methoxy derivatives (**4b** and **5b** respectively). Each isomer was isolated from the mixture by column chromatography. Compounds (**4a**) and (**5a**) have already been prepared by a slightly different procedure, [12] according which, compound (**3a**) was first reacted with 3-chloro-3-methyl-1-butyne and then subjected to Claisen rearrangement to give a mixture of the 6-hydroxy (angular) and 5-hydroxy (linear) isomers. The isomers were separated and the free hydroxyl was then methylated. In our hands, the preceding methylation of 1-OH group and subsequent cyclization facilitated the manipulation of the resulting mixture of isomers and gave better yields of the products, improving the percentage of the angular isomer, which was obtained in a poor yield according to the above mentioned literature procedure.

Figure 1

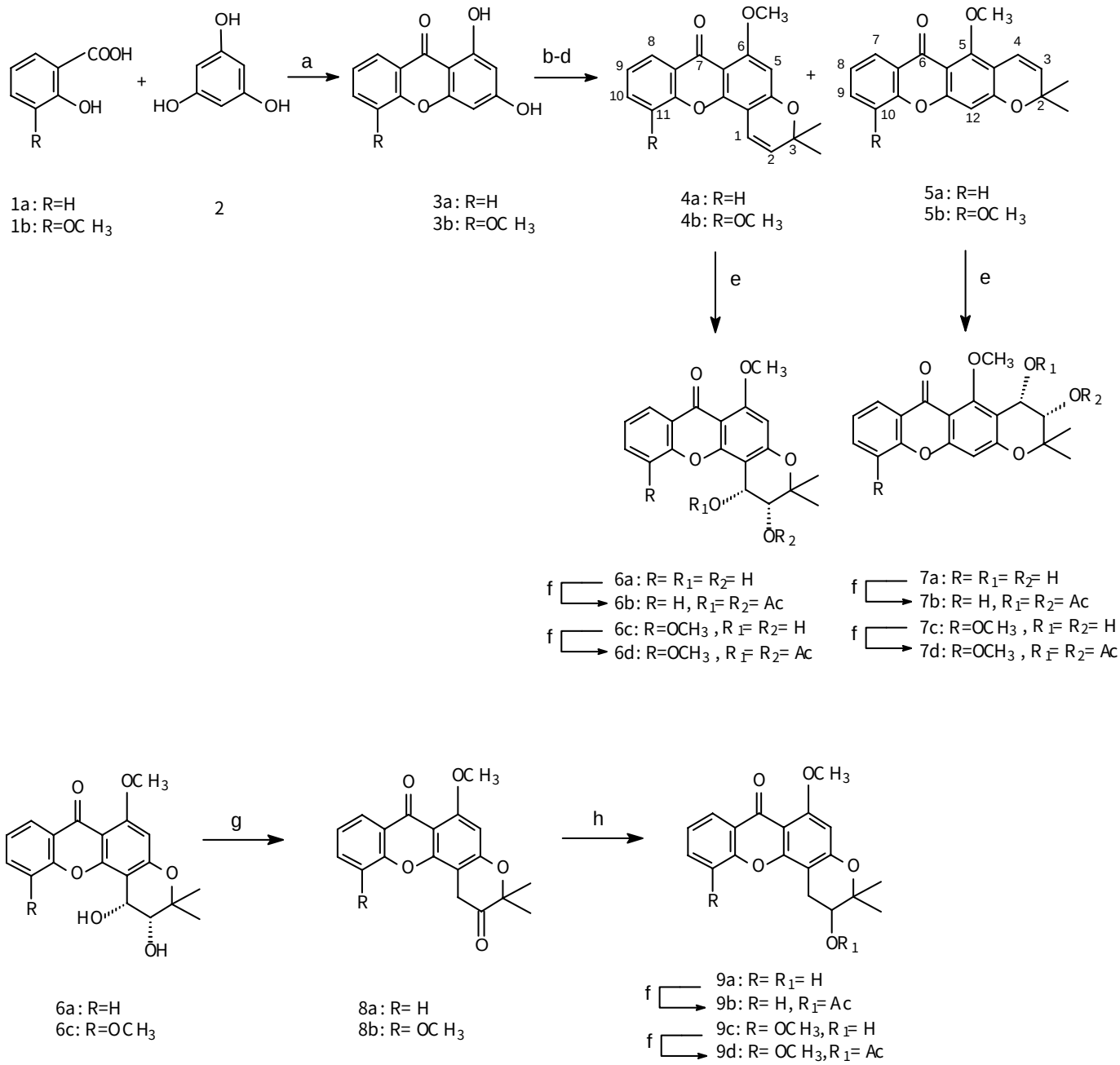


Psorospermine Acronycine *cis*-1,2-Diacetoxy-1,2-dihydroacronycine

In order to identify the angular and linear isomers (**4**) and (**5**), we used 2D-NMR experiments (HMBC, HMQC). From the C-H long-range correlations in the HMBC spectrum we observed for both isomers the appearence of a three bond correlation between the methoxyl hydrogen atom and the 5-carbon atom of the linear isomer (**5**) or the 6-carbon atom of the angular isomer (**4**), but it is only in the case of the linear isomer (**5**) that a three bond correlation between the 5-carbon atom and the 4-hydrogen atom appears. In the case of the angular isomer (**4**), the 6-carbon atom correlates with the aromatic 5-hydrogen atom, while, as expected, this correlation is not present in the case of the linear isomer (**5**). The ^{13}C-NMR spectral data proved to be helpful in afterwards identification of the isomers, since we observed that the chemical shift

of the angular C-5 was always within the range of 95-96 ppm, whereas concerning the linear isomer, C-12 is shifted downfield, at 100-101 ppm.

Scheme 1



a: $ZnCl_2$, $POCl_3$, heat, b:3-chloro-3-methyl-1-butyne, CuI, K_2CO_3, NaI, DMF, c: 1) NaH, THF 2) $(CH_3)_2SO_4$, heat, d: *N*,*N*-DEA, heat, e: 1) OsO_4, *N*-methylmorpholine *N*-oxide 2) $NaHSO_3$, f: Ac_2O, Py, g: $CuSO_4$, toluene, heat, h: $NaBH_4$, CH_3OH.

Addition reactions in the double bond of either 1-position of the angular isomer (**4**), or 3-position of linear isomer (**5**), were then performed in order to obtain the molecules under investigation. Thus, catalytic dihydroxylation with osmium tetroxide and 4-methylmorpholine *N*-oxide as oxidizing agent, yielded the corresponding *cis*-diols (**6a**, **6c**, **7a** and **7c**). The acetates of the above mentioned hydroxy derivatives were prepared by reaction with acetic anhydride in the presence of pyridine.

Furthermore, the 2-keto derivatives (**8a**) and (**8b**) were obtained from the corresponding diols (**6a**) and (**6c**) respectively, by reaction with copper(II) sulfate. Subsequent reduction of the keto compounds provided the 2-hydroxy derivatives (**9a**) and (**9c**) which upon treatment with acetic anhydride in the presence of pyridine gave rise to the esters (**9b**) and (**9d**).

The cytotoxic activity and the cell cycle selectivity studies of the derivatives, were carried out *in vitro* on the L1210 leukemic cell line, with acronycine as the reference compound. Among the pyrano[3,2-*b*]xanthen-6-one series, compound (**7a**) (IC_{50} = 28.2 μM) was equally potent to acronycine (IC_{50} = 27 μM), while compound (**7b**) (IC_{50} = 8.4 μM) was 3 times more potent than acronycine, but showed a partial accumulation of L1210 cells in the G_1 phase of the cell cycle (57%).

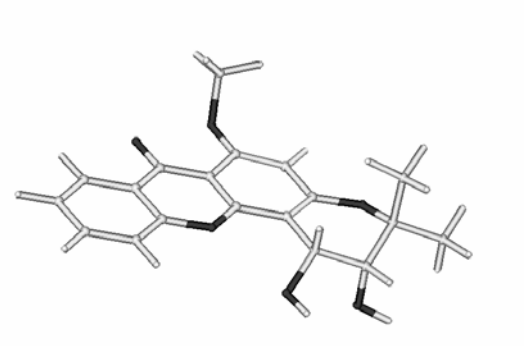
From the pyrano[2,3-*c*]xanthen-7-one series, compounds (**8a**) and (**9a**) were found to be slightly less potent than acronycine (IC_{50} = 50.7 μM and IC_{50} = 33.4 μM respectively). Surprisingly, compounds (**6b**) and (**6d**), while being structurally similar to *cis*-1,2-diacetoxy-1,2 dihydroacronycine (IC_{50} = 5.8 μM) were found to be inactive (IC_{50} >100 μM for both compounds). The cytotoxic activity of the unsaturated isomers (**4a**, **4b**, **5a** and **5b**) was also examined. Among them, compounds (**4a**) and (**5a**) were found to be of interest (IC_{50} = 17.2 μM and IC_{50} = 14 μM respectively).

Taking into account the crucial role of D-ring conformation to the biological activity of acronycine derivatives, [13] it seemed interesting to investigate the D-ring conformation of the 1,2-dihydro-1,2-dihydroxyxanthenone compounds, performing NOESY experiments and computational conformation analysis.

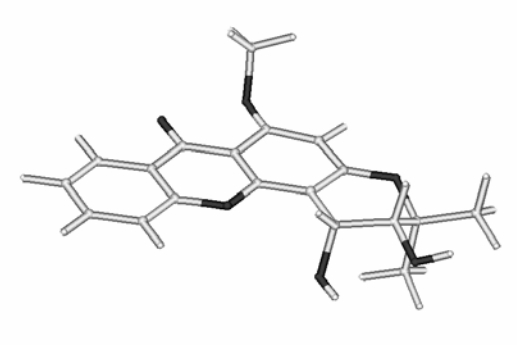
The conformation analysis was performed using molecular mechanics calculations and two lowest energy conformations (structures I and II for compound (**6a**), Figure 2) were predicted. Both conformations are half-chair and their calculated energy difference is small (0.4 $Kcal.mole^{-1}$), suggesting that they could be possibly both present in solution. The NOESY experiments, (mixing time 750 msec) showed a strong cross peak correlation between the H-2 and the β-pyran methyl group, while no correlation between H-1 and the pyran methyl groups is present. These data suggest that 1,2-dihydro-1,2-dihydroxyxanthenone derivatives exist predominantly under the conformation II in solution. In this conformation, the substituents on C-1 and C-2 are relatively hindered by the pyran methyl groups, since the substituent on C-2 is *gauche* with both methyl groups and the substituent on C-1 is homoaxial with the α-CH_3. It is

noteworthy that the pyran ring of the highly active 1,2-dihydro-1,2-dihydroxyacronycine derivatives adopts predominantly the conformation I,[13] where the substituent on C-2 has a pseudoaxial orientation and both C-1 and C-2 substituents are less hindered by the pyran methyl groups. This could possibly explain the fact that the synthesized xanthenone derivatives are less potent than the corresponding acridone derivatives.

Figure 2



Conformer I



Conformer II

EXPERIMENTAL

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H-NMR spectra and 2-D spectra were recorded on a Bruker Avance 400 instrument, whereas ^{13}C-NMR specra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). Flash chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

6-Methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (4a) and 5-Methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (5a).

3-Chloro-3-methyl-1-butyne (1.72 g, 16.8 mmol), was added to a mixture of the xanthone (**3a**) (1.91 g, 8.41 mmol), anhydrous K_2CO_3 (2.35 g, 16.82 mmol), anhydrous KI (2.37 g, 14.23 mmol) and CuI (33 mg, 0.17 mmol), in dry DMF (10 mL) under argon and the reaction was stirred at 70^{o} C for 4 h. Most of the solvent was then vacuum-evaporated, water was added to the residue and it was extracted with dichloromethane. The organic phase was washed with water, a saturated NaCl solution, dried (Na_2SO_4) and evaporated to dryness to give almost pure 1-hydroxy-3-(1,1-dimethyl-2-propynoxy)-9*H*-xanthen-9-one (2.07 g). A small amount of this compound was purified by preparative TLC (silica gel), using a mixture of

dichloromethane : cyclohexane (1 : 1) as mobile phase. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.73 (s, 6H, 2 x gemCH_3), 2.69 (s, 1H, C≡C-H), 6.68 (d, J= ~1 Hz, 1H, H-4), 6.79 (d, J= ~ 1 Hz, 1H, H-2), 7.37 (td, J= 8 Hz, ~1 Hz, 1H, H- 7), 7.43 (dd, J= 8 Hz, ~ 1 Hz, 1H, H-5), 7.70 (dt, J= 8 Hz, ~ 1 Hz, 1H, H-6), 8.23 (dd, J = 8 Hz, ~ 1 Hz, 1H, H-8), 12.82 (s, 1H, 1-OH).

NaH (62 mg, 2.1 mmol, 80% in parafin oil) was added at 0^{o}C to a solution of the above mentioned ether (270 mg, 0.92 mmol) in dry THF (8 mL) and the mixture was refluxed under argon for 1 h. Dimethyl sulfate (260 mg, 2.82 mmol) was then added dropwise and heating was continued for 3.5 h. On cooling at rt, ethanol was added (2 mL), the insoluble material was filtered off and the solvent was vacuum-evaporated to give 1-methoxy-3-(1,1-dimethyl-2-propynoxy)-9*H*-xanthen-9-one (211 mg), which was no further purified. A small amount of this compound was purified by preparative TLC (silica gel), using a mixture of dichloromethane : cyclohexane (1 : 1) as mobile phase. ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.75 (s, 6H, 2 x gemCH_3), 2.72 (s, 1H, C≡C-H), 3.96 (s, 3H, 1-OCH_3), 6.52 (d, J= ~ 1 Hz, 1H, H-4), 7.01 (d, J= ~ 1 Hz, 1H, H-2), 7.30 (dt, J= 8 Hz, ~1 Hz, 1H, H-5), 7.37 (td, J= 8 Hz, ~ 1 Hz, 1H, H-7), 7.51 (dt, J = 8 Hz, ~ 1 Hz, 1H, H-6), 8.23 (dd, J = 8 Hz, ~ 1 Hz, 1H, H-8). A solution of this compound in *N*,*N*-diethylaniline (4 mL) was heated at 210 ^{o}C under argon for 2 h. After cooling, ethyl acetate was added and the mixture was washed successively with a 10% HCl solution, a 10% NaOH solution, water, a saturated NaCl solution and dried (K_2CO_3). The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel 25x2 cm) with cyclohexane : dichloromethane (1:1) as the eluent, to yield **4a** (157 mg, 70 %) and **5a** (28 mg, 13 %).

6-Methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (**4a**).

mp: 198 ^{o}C (lit., [12] 197-198 ^{o}C) (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.50 (s, 6H, 2 x gemCH_3), 3.99 (s, 3H, 6-OCH_3), 5.64 (d, J= 10.5 Hz, 1H, H-2), 6.34 (s, 1H, H-5), 6.92 (d, J= 10.5 Hz, 1H, H-1), 7.33 (dt, J= 8 Hz, ~ 0.5 Hz, 1H, H-9), 7.42 (dd, J= 8 Hz, ~ 0.5 Hz, 1H, H-11), 7.65 (dt, J= 8 Hz, 1.5 Hz, 1H, H-10), 8.31 (dd, J= 8 Hz, 1.5 Hz, 1H, H-8). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 27.80 (2xCH_3), 55.89 (6-OCH_3), 77.64 (C-3), 95.08 (C-5), 101.78 (C-12b), 106.38 (C-6a), 114.09 (C-1), 116.36 (C-11), 122.35 (C-7a), 123.29 (C-9), 126.16 (C-8), 126.53 (C-2), 134.23 (C-10), 153.19 (C-11a), 154.14 (C-12a), 158.37 (C-4a), 161.21 (C-6), 175.14 (C-7).

5-Methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**5a**).

mp: 127-128 ^{o}C (lit., [12] 126.5-127.5 ^{o}C) (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.52 (s, 6H, 2 x gemCH_3), 3.99 (s, 3H, 5-OCH_3), 5.73 (d, J= 10.5 Hz, 1H, H-3), 6.67 (s, 1H, H-12), 6.78 (d, J= 10.5 Hz, 1H, H-4), 7.35 (dt, J= 8 Hz, ~ 0.5 Hz, 1H, H-8), 7.41 (dd, J= 8 Hz, ~ 0.5 Hz, 1H, H-10), 7.67 (dt, J= 8 Hz, 1.5 Hz, 1H, H-9), 8.31 (dd, J= 8 Hz, 1.5 Hz, 1H, H-7). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 28.41 (2xCH_3), 62.71 (5-OCH_3), 78.45 (C-2), 100.68 (C-12), 112.27 (C-4a), 116.05 (C-4), 117.19 (C-10),

122.71 (C-5a), 123.77 (C-8), 126.59 (C-7), 130.17 (C-3), 133.8 (C-6a), 133.92 (C-9), 155.11 (C-10a), 158.02 (C-11a), 158.60 (C-12a), 159.23 (C-5), 175.14 (C-6).

Compounds (**4b**) and (**5b**) were prepared by an analogous procedure.

6,11-Dimethoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (4b).

Yield: 47%. mp: 273-275 ^{o}C (lit., [14] 274-275 ^{o}C) (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.48 (s, 6H, 2 x gemCH_3), 3.95 (s, 3H, OC$\underline{H}_3$), 3.97 (s, 3H, OC$\underline{H}_3$), 5.58 (d, J= 10.5 Hz, 1H, H-2), 6.28 (s, 1H, H-5), 6.93 (d, J= 10.5 Hz, 1H, H-1), 7.11 (dd, J= 7 Hz, ~1 Hz, 1H, H-6), 7.21 (t, J= 7 Hz, 1H, H-9), 7.82 (dd, J= 7 Hz, 1 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 28.25 (2x$\underline{C}H_3$), 56.38 (6-O$\underline{C}H_3$, 11-O$\underline{C}H_3$), 77.65 (C-3), 95.65 (C-5), 102.08 (C-12b), 106.10 (C-6a), 114.61 (C-1), 115.56 (C-9), 117.66 (C-8), 123.23 (C-10), 123.32 (C-7a), 126.97 (C-2), 145.80 (C-11), 147.24 (C-11a), 157.97 (C-4a), 158.05 (C-12a), 161.52 (C-6), 176.22 (C-7).

5,10-Dimethoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (5b).

Yield: 23%. mp: 197 ^{o}C (lit., [15] 196-199 ^{o}C) (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 6H, 2 x gemCH_3), 3.94 (s, 3H, OC$\underline{H}_3$), 3.99 (s, 3H, OC$\underline{H}_3$), 5.69 (d, J= 10.5 Hz, 1H, H-3), 6.70 (d, J= 10.5 Hz, 1H, H-4), 6.73 (s, 1H, H-12), 7.14 (dd, J= 7 Hz, ~1 Hz, 1H, H-9), 7.24 (t, J= 7 Hz, 1H, H-8), 7.84 (dd, J= 7 Hz, ~1 Hz, 1H, H-7). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 28.33 (2x$\underline{C}H_3$), 56.38 (10-OCH_3), 62.65 (5-OCH_3), 78.52 (C-2), 100.95 (C-12), 110.11 (C-4a), 114.73 (C-4), 116.00 (C-8), 117.53 (C-7), 121.60 (C-5a), 123.19 (C-9), 130.26 (C-3), 134.75 (C-6a), 143.10 (C-10), 145.95 (C-10a), 156.45 (C-11a), 159.20 (C-12a), 162.50 (C-5), 174.80 (C-6).

***cis*-1,2-Dihydro-1,2-dihydroxy-6-methoxy-3,3-dimethyl-3*H* ,7*H*-pyrano[2,3-*c*]xanthen-7-one (6a).**

To a solution of osmium tetroxide (2.5% in isopropanol) (0.05 mL, 0.0049 mmol) and *N*-methylmorpholine *N*-oxide (80 mg, 0.68 mmol) in *tert*-BuOH: THF: H_2O (10: 3 : 1, 6 mL), was added compound (**4a**) (150 mg, 0.49 mmol). The reaction mixture was stirred at rt for 3 days. A saturated $NaHSO_3$ solution (0.5 mL) was then added and the mixture was stirred at rt for 2 h. The solvents were vacuum-evaporated and the residue was dry-packed to a silica gel column (20x2 cm). Elution with a mixture of dichloromethane : methanol (98.5 : 1.5) provided **6a** (120 mg, 72 %). mp: 248 ^{o}C (EtOH). ^{1}H-NMR, (400 MHz, DMSO-d_6) δ (ppm): 1.40 (s, 3H, 1 x gemCH_3), 1.41 (s, 3H, 1 x gemCH_3), 3.67 (dd, J= 7.5 Hz, 5 Hz, 1H, H-2), 3.84 (s, 3H, 5-OC$\underline{H}_3$), 5.00 (t, J= 5 Hz, 1H, H-1), 5.05 (d, J= 7.5 Hz, D_2O exch., 1H, 2-O$\underline{H}$), 5.45 (d, J= 5 Hz, D_2O exch., 1H, 1-O$\underline{H}$), 6.36 (s, 1H, H-5), 7.41 (dt, J= 8 Hz, ~ 0.5 Hz, 1H,

H-9), 7.63 (dd, J= 8 Hz, ~ 0.5 Hz, 1H, H-11), 7.78 (dt, J= 8 Hz, 1.5 Hz, 1H, H-10), 8.07 (dt, J= 8 Hz, 1.5 Hz, 1H, H-8). ^{13}C-NMR, (50 MHz, DMSO-d_6) δ (ppm): 21.05 (1x$\underline{C}H_3$), 27.00 (1x$\underline{C}H_3$), 56.12 (6-O$\underline{C}H_3$), 60.13 (C-1), 71.49 (C-2), 79.52 (C-3), 95.63 (C-5), 104.85 (C-12b), 106.27 (C-6a), 117.81 (C-11), 122.29 (C-7a), 124.18 (C-9), 125.70 (C-8), 134.18 (C-10), 154.37 (C-11a), 157.48 (C-4a), 158.25 (C-12a), 160.90 (C-6), 173.71 (C-7). Anal. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.3. Found: C, 66.48; H, 5.14.

The following derivatives were prepared by an analogous procedure.

cis-1,2-Dihydro-1,2-dihydroxy-6,11-dimethoxy-3,3-dimethyl-3_H_,7_H_-pyrano[2,3-_c_]xanthen-7-one (6c).

Yield: 87 %. mp: >250 °C (EtOH). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.39 (s, 3H, 1 x gemCH_3), 1.54 (s, 3H, 1 x gemCH_3), 3.25 (d, J= 4 Hz, D_2O exch., 1H, 2-O$\underline{H}$), 3.91-3.95 (m, 4H, H-2, O$\underline{CH_3}$), 4.01 (s, 3H, O$\underline{CH_3}$), 4.22 (d, J= 4 Hz, D_2O exch., 1H, 1-O$\underline{H}$), 5.29 (t, J= 4 Hz, 1H, H-1), 6.30 (s, 1H, H-5), 7.14 (dd, J= 8 Hz, 1.5 Hz, 1H, H-10), 7.24 (t, J= 8 Hz, 1H, H-9), 7.82 (dd, J= 8 Hz, 1.5 Hz, 1H, H-8). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 22.75 (1x$\underline{C}H_3$), 24.26 (1x$\underline{C}H_3$), 56.33 (6-O$\underline{C}H_3$, 11-O$\underline{C}H_3$), 62.27 (C-1), 70.64 (C-2), 79.23 (C-3), 96.02 (C-5), 102.28 (C-12b), 106.78 (C-6a), 114.12 (C-9), 117.66 (C-8), 123.69 (C-7a), 123.73 (C-10), 144.37 (C-11), 147.72 (C-11a), 158.40 (C-4a), 158.44 (C-12a), 161.55 (C-6), 177.68 (C-7). Anal. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.38; H, 5.42.

cis-3,4-Dihydro-3,4-dihydroxy-5-methoxy-2,2-dimethyl-2_H_,6_H_ -pyrano[3,2-_b_]xanthen-6-one (7a).

Yield: 73 %. mp: 222 °C (EtOH). ^{1}H-NMR, (400 MHz, DMSO-d_6) δ (ppm): 1.36 (s, 6H, 2 x gemCH_3), 3.48 (dd, J= 7.5 Hz, 5 Hz, 1H, H-3), 3.88 (s, 3H, 5-O$\underline{CH_3}$), 4.83 (t, J= 5 Hz, 1H, H-4), 5.17 (d, J= 7.5 Hz, D_2O exch., 1H, 3-O$\underline{H}$), 5.21 (d, J= 5 Hz, D_2O exch., 1H, 4-O$\underline{H}$), 6.56 (s, 1H, H-12), 7.32 (dt, J= 8 Hz, ~ 0.5 Hz, 1H, H-8), 7.42 (dd, J= 8 Hz, ~ 0.5 Hz, 1H, H-10), 7.69 (dt, J= 8 Hz, 1.5 Hz, 1H, H-9), 8.04 (dt, J= 8 Hz, 1.5 Hz, 1H, H-7). ^{13}C-NMR, (50 MHz, DMSO-d_6) δ (ppm): 21.90 (1x$\underline{C}H_3$), 27.58 (1x$\underline{C}H_3$), 61.32 (C-4), 63.33 (5-OCH_3), 71.90 (C-3), 77.92 (C-2), 100.27 (C-12), 110.05 (C-4a), 117.78 (C-10), 122.37 (C-5a), 124.51 (C-8), 126.49 (C-7), 135.13 (C-9), 135.58 (C-6a), 154.99 (C-10a), 158.27 (C-11a), 159.22 (C-12a), 161.87 (C-5), 174.15 (C-6). Anal. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.95; H, 5.15.

***cis*-3,4-Dihydro-3,4-dihydroxy-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (7c).**

Yield: 91 %. mp: 176 °C (EtOH). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.37 (s, 3H, 1 x gemCH_3), 1.48 (s, 3H, 1 x gemCH_3), 3.25 (d, J= 4 Hz, D_2O exch., 1H, 3-O<u>H</u>), 3.87 (t, J= 4 Hz, 1H, H-3), 4.01 (s, 3H, 10-OC<u>H</u>$_3$), 4.07 (s, 3H, 5-OC<u>H</u>$_3$), 4.18 (~s, D_2O exch., 1H, 4-O<u>H</u>), 5.12 (d, J= 4 Hz, 1H, H-4), 6.83 (s, 1H, H-12), 7.17 (dd, J= 8 Hz, 1 Hz, 1H, H-9), 7.25 (t, J= 8 Hz, 1H, H-8), 7.84 (dd, J= 8 Hz, 1 Hz, 1H, H-7). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 22.72 (1x<u>C</u>H_3), 24.52 (1x<u>C</u>H_3), 56.40 (10-O<u>C</u>H_3), 62.30 (5-O<u>C</u>H_3), 62.60 (C-4), 70.60 (C-3), 78.91 (C-2), 101.62 (C-12), 109.89 (C-4a), 113.86 (C-5a), 115.11 (C-8), 117.46 (C-7), 123.27 (C-6a), 123.31 (C-9), 145.51 (C-10), 148.12 (C-10a), 158.29 (C-11a), 158.66 (C-12a), 160.87 (C-5), 174.97 (C-6). Anal. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.42; H, 5.29.

***cis*-1,2-Diacetoxy-1,2-dihydro-6-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (6b).**

To a solution of **6a** (48 mg, 0.14 mmol) in dry pyridine (3 mL) was added acetic anhydride (0.5 mL, 5 mmol) and the reaction mixture was stirred at rt for 12 h. The solvent was then vacuum-evaporated, the last traces being azeotropicaly removed with benzene and the residue was purified by column chromatography (silica gel, 20x1.5 cm) using a mixture of dichloromethane : ethyl acetate (9 : 1) as the eluent to give **6b** (45 mg, 77 %). mp: 249 -250 °C (EtOAc). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.46 (s, 3H, 1 x gemCH_3), 1.51 (s, 3H, 1 x gemCH_3), 2.11 (s, 3H, 1 x C<u>H</u>$_3$COO), 2.14 (s, 3H, 1 x C<u>H</u>$_3$COO), 3.98 (s, 3H, 6-OC<u>H</u>$_3$), 5.28 (d, J= 4.5 Hz, 1H, H-1), 6.31 (s, 1H, H-5), 6.63 (d, J= 4.5 Hz, 1H, H-1), 7.27 (dd, J= 8 Hz, ~ 0 Hz, 1H, H-11), 7.33 (dd, J= 8 Hz, ~ 0 Hz, 1H, H-9), 7.68 (dt, J= 8 Hz, 1 Hz, 1H, H-10), 8.27 (dd, J= 8 Hz, 1 Hz, 1H, H-8). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 20.60 (1x<u>C</u>H_3CO), 20.74 (1x<u>C</u>H_3CO), 21.62 (1x<u>C</u>H_3), 25.89 (1x<u>C</u>H_3), 56.35 (6-O<u>C</u>H_3), 60.69 (C-1), 71.00 (C-2), 77.64 (C-3), 95.53 (C-5), 99.14 (C-12b), 107.34 (C-6a), 116.84 (C-11), 122.74 (C-7a), 124.12 (C-9), 126.54 (C-8), 133.84 (C-10), 154.37 (C-11a), 157.37 (C-4a), 158.37 (C-12a), 162.42 (C-6), 169.75 (1xCH_3<u>C</u>O), 170.08 (1xCH_3<u>C</u>O), 175.01 (C-7). Anal. Calcd for $C_{23}H_{22}O_8$: C, 64.78; H, 5.20. Found: C, 64.77; H, 5.04.

The following derivatives were prepared by an analogous procedure.

***cis*-1,2-Diacetoxy-1,2-dihydro-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (6d).**

Yield: 60 %. mp: 246 °C (decomp) (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 3H, 1 x gemCH_3), 1.49 (s, 3H, 1 x gemCH_3), 2.09 (s, 3H, 1 x C<u>H</u>$_3$COO), 2.12 (s, 3H, 1 x C<u>H</u>$_3$COO), 3.93 (s, 3H, 5-OC<u>H</u>$_3$), 3.97 (s, 3H, 10-OC<u>H</u>$_3$), 6.31 (s, 1H, H-12), 5.32 (d, J= 5 Hz, 1H, H-3), 6.54 (d, J= 5 Hz,

1H, H-4), 7.11 (dd, J= 8 Hz, 1 Hz, 1H, H-10), 7.23 (dd, J= 8 Hz, 1H, H-9), 7.82 (dd, J= 8 Hz, 1 Hz, 1H, H-8). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 20.65 (2x$\underline{C}H_3CO$), 21.84 (1x$\underline{C}H_3$), 25.36 (1x$\underline{C}H_3$), 55.85 (10-O$\underline{C}H_3$), 56.38 (6-O$\underline{C}H_3$), 61.24 (C-1), 70.69 (C-2), 77.62 (C-3), 95.68 (C-5), 99.28 (C-12b), 107.48 (C-6a), 114.33 (C-9), 117.29 (C-8), 123.57 (C-10), 123.77 (C-7a), 144.94 (C-11), 148.22 (C-11a), 157.37 (C-4a), 158.85 (C-12a), 162.35 (C-6), 169.93 (1x$CH_3\underline{C}O$), 170.19 (1x$CH_3\underline{C}O$), 175.14 (C-7). Anal. Calcd for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 63.10; H, 5.57.

***cis*-3,4-Diacetoxy-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (7b).**

Yield: 83 %. mp:163-163 ^{o}C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 3H, 1 x gemCH_3), 1.49 (s, 3H, 1 x gemCH_3), 2.12 (s, 3H, 1 x C$\underline{H}_3$COO), 2.13 (s, 3H, 1 x C$\underline{H}_3$COO), 3.97 (s, 3H, 5-OC$\underline{H}_3$), 5.26 (d, J= 4.5 Hz, 1H, H-3), 6.45 (d, J= 4.5 Hz, 1H, H-4), 6.73 (s, 1H, H-12), 7.35 (dt, J= 8 Hz, ~ 0.5 Hz, 1H, H-8), 7.41 (dd, J= 8 Hz, ~ 0.5 Hz, 1H, H-10), 7.68 (dt, J= 8 Hz, 1.5 Hz, 1H, H-9), 8.28 (dd, J= 8 Hz, 1.5 Hz, 1H, H-7). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 20.64 (1xCH_3CO), 20.83 (1xCH_3CO), 21.72 (1x$\underline{C}H_3$), 26.15 (1x$\underline{C}H_3$), 60.69 (C-4), 62.61 (5-OCH_3), 71.17 (C-3), 77.63 (C-2), 101.04 (C-12), 110.41 (C-4a), 117.13 (C-10), 122.32 (C-5a), 123.85 (C-8), 126.59 (C-7), 133.67 (C-6a), 134.25 (C-9), 154.99 (C-10a), 158.76 (C-11a), 159.11 (C-12a), 161.79 (C-5), 169.79 (1x$CH_3\underline{C}O$), 169.94 (1x$CH_3\underline{C}O$), 174.87 (C-6). Anal. Calcd for $C_{23}H_{22}O_8$: C, 64.78; H, 5.20. Found: C, 64.45; H, 5.42.

***cis*-3,4-Diacetoxy-3,4-dihydro-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (7d).**

Yield: 86 %. mp: 229 ^{o}C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm) 1.42 (s, 3H, 1 x gemCH_3), 1.46 (s, 3H, 1 x gemCH_3), 2.08 (s, 6H, 2 x C$\underline{H}_3$COO), 3.95 (s, 3H, 10-OC$\underline{H}_3$), 4.01 (s, 3H, 5-OC$\underline{H}_3$), 5.24 (d, J= 4 Hz, 1H, H-3), 6.43 (d, J= 4 Hz, 1H, H-4), 6.82 (s, 1H, H-12), 7.20 (m, 2H, H-8, H-9), 7.84 (dd, J = 8 Hz, 1 Hz, 1H, H-7). ^{13}C-NMR, (50 MHz, $CDCl_3$) δ (ppm): 20.66 (1xCH_3CO), 20.85 (1xCH_3CO), 21.73 (1x$\underline{C}H_3$), 26.20 (1x$\underline{C}H_3$), 56.41 (10-OCH_3), 60.78 (5-OCH_3), 62.66 (C-4), 71.26 (C-3), 77.63 (C-2), 101.38 (C-12), 110.45 (C-4a), 110.63 (C-5a), 115.13 (C-8), 117.57 (C-7), 123.33 (C-6a), 123.38 (C-9), 145.40 (C-10), 148.07 (C-10a), 158.83 (C-11a), 158.95 (C-12a), 161.72 (C-5), 169.79 (1x$CH_3\underline{C}O$), 169.96 (1x$CH_3\underline{C}O$), 174.85 (C-6). Anal. Calcd for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.93; H, 5.41.

6-Methoxy-3,3-dimethyl-1*H*,2*H*,3*H*,7*H*-pyrano[2,3-*c*]xanthene-2,7-dione (8a).

A mixture of **6a** (103 mg, 0.30 mmol) and cupric sulfate (40 mg, 0.25 mmol) in dry toluene (20 mL), was heated under Ar at 150 ^{o}C for 20 h. The solvent was vacuum-evaporated and the residue was purified by

column chromatography (dry-pack, 15x2 cm silica gel flash), with cyclohexane : ethyl acetate (80 : 20) as the eluent, yielding **8a** (58 mg, 59 %). mp: 186-188 °C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.48 (s, 6H, 2 x gemCH_3), 3.75 (s, 2H, H-1), 3.95 (s, 3H, 6-O$\underline{C}H_3$), 6.43 (s, 1H, H-5), 7.33 (dt, J= 8 Hz, 0.5 Hz, 1H, H-9), 7.38 (dd, J= 8 Hz, 0.5 Hz, 1H, H-11), 7.63 (dt, J= 8 Hz, 2 Hz, 1H, H-10), 8.28 (dd, J= 8 Hz, 2 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 25.99 (2x$\underline{C}H_3$), 35.87 (C-1), 59.32 (6-O$\underline{C}H_3$), 80.64 (C-3), 98.37 (C-5), 101.22 (C-12b), 107.95 (C-6a), 120.04 (C-11), 125.12 (C-9), 131.73 (C-8), 132.12 (C-7a), 136.02 (C-10), 155.01 (C-11a), 156.07 (C-2), 157.73 (C-4a), 157.44 (C-12a), 161.04 (C-6), 174.71 (C-7). Anal. Calcd for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97. Found: C, 70.68; H, 5.16.

6,11-Dimethoxy-3,3-dimethyl-1*H*,2*H*,3*H*,7*H*-pyrano[2,3-*c*]xanthene-2,7-dione (8b).

The compound was prepared in the same way as **8a**. Yield: 63 %. mp: 238-240 °C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 3H, 1 x gemCH_3), 1.79 (s, 3H, 1 x gemCH_3), 3.47 (s, 2H, H-1), 3.96 (s, 3H, O$\underline{C}H_3$), 4.02 (s, 3H, O$\underline{C}H_3$), 6.44 (s, 1H, H-5), 7.14 (dd, J= 8 Hz, 2 Hz, 1H, H-8), 7.28 (t, J= 8 Hz, 1H, H-9), 7.83 (dd, J= 8 Hz, 2 Hz, 1H, H-10). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 27.63 (1x$\underline{C}H_3$), 27.95 (1x$\underline{C}H_3$), 34.11 (C-1), 57.96 (OCH_3), 58.05 (OCH_3), 80.04 (C-3), 99.50 (C-5), 102.2 (C-12b), 107.58 (C-6a), 116.97 (C-9), 119.38 (C-8), 126.42 (C-10), 146.99 (C-11a), 150.50 (C-11), 157.63 (C-2), 158.37 (C-12a), 162.20 (C-4a), 165.87 (C-6), 179.85 (C-7). Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.53; H, 5.01.

1,2-Dihydro-2-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (9a).

To a solution of **8a** (51 mg, 0.14 mmol) in methanol (20 mL), was added sodium borohydride (50 mg, 1.3 mmol) and the mixture was stirred at 0 °C for 40 min. The reaction mixture was then neutralized (pH= 6) with Amberlite IR-120, filtered, the solvent was evaporated and the residue was purified by column chromatography (silica gel, 15x1.5 cm) with cyclohexane : ethyl acetate (80 : 20) as the eluent, to give **9a** (23 mg, 60 %). mp: 223-224 °C (EtOH). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.33 (s, 3H, 1 x gemCH_3), 1.46 (s, 3H, 1 x gemCH_3), 2.55 (s, D_2O exch., 1H, 2-O$\underline{H}$), 2.97 (dd, J= 14 Hz, 5.6 Hz, 1H, H-1a), 3.13 (dd, J= 14 Hz, 5.6 Hz, 1H, H-1b), 3.91 (s, 3H, 6-O$\underline{C}H_3$), 4.08 (t, J= 5.6 Hz, 1H, H-2), 6.23 (s, 1H, H-5), 7.28 (dt, J= 8 Hz, 0.5 Hz, 1H, H-9), 7.30 (dd, J= 8 Hz, 0.5 Hz, 1H, H-11), 7.55 (dt, J= 8 Hz, 2 Hz, 1H, H-10), 8.27 (dt, J= 8 Hz, 2 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 24.10 (1x$\underline{C}H_3$), 26.81 (1x$\underline{C}H_3$), 28.05 (C-1), 58.80 (OCH_3), 72.01 (C-2), 79.40 (C-3), 97.60 (C-5), 99.85 (C-12b), 107.75 (C-6a), 118.70 (C-11), 124.07 (C-7a), 126.21 (C-9), 129.75 (C-8), 135.70 (C-10), 154.86 (C-11a), 157.95 (C-12a), 158.10 (C-4a), 160.31 (C-6), 174.90 (C-7). Anal. Calcd for $C_{19}H_{18}O_5$: C, 69.92; H, 5.57. Found: C, 69.81; H, 5.28.

1,2-Dihydro-2-hydroxy-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (9c).

The compound was prepared in the same way as **9a**. Yield: 59 %. mp: 264-265 ^{o}C (EtOH). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 3H, 1 x gemCH_3), 1.45 (s, 3H, 1 x gemCH_3), 2.32 (d, J= 6 Hz, D_2O exch., 1H, 2-O$\underline{H}$), 3.01 (dd, J= 17 Hz, 5 Hz, 1H, H-1a), 3.17 (dd, J= 17 Hz, 5 Hz, 1H, H-1b), 3.91 (s, 3H, OC$\underline{H}_3$), 3.93 (s, 3H, OC$\underline{H}_3$), 3.97 (t, J= 5 Hz, 1H, H-2), 6.25 (s, 1H, H-5), 7.07 (dd, J= 8 Hz, 1.5 Hz, 1H, H-10), 7.19 (t, J= 8 Hz, 1H, H-9), 8.27 (dd, J= 8 Hz, 1.5 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 25.21 (1x$\underline{C}H_3$), 29.40 (1x$\underline{C}H_3$), 29.12 (C-1), 56.20 (O$\underline{C}H_3$), 56.59 (O$\underline{C}H_3$), 68.85 (C-2), 77.62 (C-3), 95.88 (C-5), 99.21 (C-12b), 107.11 (C-6a), 113.90 (C-9), 121.21 (C-8), 123.85 (C-7a), 124.13 (C-10), 145.21 (C-11), 147.93 (C-11a), 157.88 (C-4a), 158.23 (C-12a), 160.91 (C-6), 175.38 (C-7). Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.54; H, 5.49.

The following compounds were prepared by a procedure essentially similar to the one described for the preparation of **6b**.

2-Acetoxy-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (9b).

Yield: 60 %. mp: 191-193 ^{o}C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.37 (s, 3H, 1 x gemCH_3), 1.40 (s, 3H, 1 x gemCH_3), 2.08 (s, 3H, 2-C$\underline{H}_3$CO), 2.96 (dd, J= 17.5 Hz, 5 Hz, 1H, H-1a), 3.13 (dd, J= 17.5 Hz, 4.4 Hz, 1H, H-1b), 3.94 (s, 3H, 6-OC$\underline{H}_3$), 5.12 (~t, J= 4.8 Hz, 1H, H-2), 6.31 (s, 1H, H-5), 7.31 (dt, J= 8 Hz, 1.5 Hz, 1H, H-9), 7.37 (dd, J= 8 Hz, 1.5 Hz, 1H, H-11), 7.61 (dt, J= 8 Hz, 1.5 Hz, 1H, H-10), 8.27 (dd, J= 8 Hz, 1.5 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 21.09 ($\underline{C}H_3$CO), 23.01 (1x$\underline{C}H_3$), 23.16 (1x$\underline{C}H_3$), 24.54 (C-1), 56.23 (OCH_3), 69.91 (C-2), 77.62 (C-3), 95.75 (C-5), 98.09 (C-12b), 108.22 (C-6a), 116.90 (C-11), 122.94 (C-7a), 123.90 (C-9), 126.74 (C-8), 133.60 (C-10), 154.66 (C-11a), 158.14 (C-4a), 158.21 (C-12a), 160.72 (C-6), 170.50 ($CH_3\underline{C}O$), 175.22 (C-7). Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.36; H, 5.53.

2-Acetoxy-1,2-dihydro-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (9d).

Yield: 86 %. mp: 179-181 ^{o}C (Et_2O, n-hexane). ^{1}H-NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 3H, 1 x gemCH_3), 1.41 (s, 3H, 1 x gemCH_3), 2.07 (s, 3H, 2-C$\underline{H}_3$CO), 3.04 (dd, J= 17 Hz, 4 Hz, 1H, H-1a), 3.19 (dd, J= 17 Hz, 4 Hz, 1H, H-1b), 3.94 (s, 3H, OC$\underline{H}_3$), 3.97 (s, 3H, OC$\underline{H}_3$), 5.18 (t, J= 4 Hz, 1H, H-2), 6.33 (s, 1H, H-5), 7.14 (dd, J= 8 Hz, 2 Hz, 1H, H-10), 7.23 (t, J= 8 Hz, 1H, H-9), 7.85 (dd, J= 8 Hz, 2 Hz, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 22.90 ($\underline{C}H_3$CO), 24.89 (C-1), 25.13 (1x$\underline{C}H_3$), 26.20 (1x$\underline{C}H_3$), 58.21 (O$\underline{C}H_3$), 58.75 (O$\underline{C}H_3$), 72.01 (C-2), 77.32 (C-3), 97.85 (C-5), 98.44 (C-12b), 107.43 (C-6a), 117.32 (C-9), 120.03 (C-8), 123.17 (C-7a), 125.99 (C-11), 145.90 (C-11), 148.23 (C-11a), 157.13 (C-4a),

158.16 (C-12a), 161.31 (C-6), 171.01 ($CH_3\underline{C}O$), 176.11 (C-7). Anal. Calcd for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.11; H, 5.48.

Biological testing

Cytotoxicity: Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by the microculture tetrazolium assay essentially as described. [16] Cells were exposed for 48 h to nine graded concentrations of the test compound. Results are expressed as IC_{50} (mean, n=3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells. Cell cycle analysis: L1210 cells (2.5 10^5/mL) were incubated for 21 h (approximately two doubling times) with various concentrations of cytotoxic drugs. Cells were then fixed by 70% ethanol, washed twice with phosphate-buffered saline (PBS) and incubated in PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide (PI) for 30 min. For each sample, 10,000 cells were analyzed on an Epics XL Coulter flow cytometer.

Calculations

Molecular calculations were performed using the MM+ force field of the HyperChem program (HyperChem is developed and licensed from Hypercube; the MM+ force field used in this software for molecular mechanics calculations is an extension of MM2 using the MM2 (1991) parameters and atom types with the 1997 functional form). The Polak - Ribiere (conjugate gradient) minimization method with an energy convergence criterion of 0.01 kcal mol^{-1} was used for geometry optimization.

ACKNOWLEDGEMENTS

The present study was supported by a Ministry of Industry, Energy and Technology of Greece Research Grant (Program for Promotion of Research Scientists). The authors would like to thank Dr C. Chasapis (Department of Inorganic Chemistry, University of Athens, Greece) for performing the elemental analyses.

REFERENCES

1 G. M. Kitanov and K. F. Blinova, *Chem. Nat. Compds*, 1987, **2**, 151.

2 K. Hostettman and H. Wagner, *Phytochemistry*, 1977, **16**, 821.

3 A. Banerji, A. D. Deshpande, B. R. Prabhu, and P. Pradhan, *J. Nat. Prod.*, 1994, **57**, 396.

4 H. Tosa, M. Iinuma, T. Tanaka, H. Nosaki, S. Ikeda, K. Tsutsui, M. Yamada, and S. Fujimori, *Chem. Pharm. Bull.*, 1997, **45**, 418.

5 a) G. W. Rewcastle, G. J. Atwell, B. C. Baguley, S. B. Calveley, and W. A. Denny, *J. Med. Chem.*, 1989, **32**, 793. b) G. J. Atwell, G. W. Rewcastle, B. C. Baguley, and W. A. Denny, *J. Med. Chem.*, 1990, **33**, 1345.

6 a) A. M. Habib, D. K. Ho, S. Masuda, T. McCloud, K. S. Reddy, M. Abu-Shoer, A. McKenzie, S. R. Byrn, C. J. Chang, and J. M. Cassady, *J. Org. Chem.*, 1987, **52**, 412. b) D. K. Ho, A. T. McKenzie, S. R. Byrn, and J. M. Cassady, *J. Org. Chem.*, 1987, **52**, 342.

7 G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *J. Pharm. Sci.*, 1966, **55**, 758.

8 R. T. Dorr, J. D. Liddil, D. D. Von Hoff, M. Soble, and C. K. Osborne, *Cancer Res.*, 1989, **49**, 340.

9 A. Elomri, S. Mitaku, S. Michel, A. L. Skaltsounis, F. Tillequin, M. Koch, A. Pierre, N. Guilbaud, S. Leonce, L. Kraus-Berthier, Y. Rolland, and G. Atassi, *J. Med. Chem.*, 1996, **39**, 4762.

10 R. K. M. Pillai, P. Naiksatam, F. Johnson, R. Rajagopalan, P. C. Watts, R. Cricchio, and S. Borras, *J. Org. Chem.*, 1986, **51**, 717.

11 a) D. Bell, M. R. Davies, G. R. Geen, and I. S. Mann, *Synthesis*, 1995, 707. b) J. D. Godfrey Jr, R. H. Mueller, T. C. Sedegran, N. Soundarajan, and V. J. Colandrea, *Tetrahedron Lett.*, 1994, **35**, 6405.

12 J. Schneider, E. L. Evans, E. Grunberg, and R. Ian Fryer, *J. Med. Chem.*, 1972, **15**, 266.

13 E. Mikros, Mitaku, A. L Skaltsounis, F. Libot, F. Tillequin, and M. Koch, *Magn. Reson. Chem.*, 1999, **37**, 498.

14 V. K. Ahluwalia, R. S. Jolly, and A. K. Tehim, *J. Chem. Soc., Perkin Trans. I*, 1983, 1229.

15 O. R. Gottlieb, M. T. Magalhaes, M. O. da Silva Pereira, A. A. L. Mesquita, D. de Barros Correa, and G. G. de Oliveira, *Tetrahedron*, 1968, **24**, 1601.

16 a) S. Leonce, A. Pierre, M. Anstett, V. Perez, A. Genton, J.-P. Bizzari, and G. Atassi, *Biochem. Pharmacol*, 1992, **44**, 1707. b) A. Pierre, T. A. Dunn, L. Kraus-Berthier, S. Leonce, D. Saint-Dizier, G. Regnier, A. Dhainaut, M. Berlion, J.-P. Bizzari, and G. Atassi, *Investigational New Drugs*, 1992, **10**, 137.