New Anti-tumor Agents. 3. Phenolic Benzopyran Lactone and Amine Derivatives

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2-Aminopyridine, sesamol and 4-hydroxy-3,5-dimethoxybenzaldehyde condense to form a Mannich base **3b** which reacts with tetronic acid to yield lactone **4c**. Cyclization of **4c** yields the benzopyran lactone **1b**; this inhibits growth of tumors in NCI *in vitro* tests. Alkaline hydrolysis of acetylated and methylated intermediate lactones, *e.g.* **4e**, in the presence of acetone leads to the formation of novel lactones, *e.g.*, of type **6**, which incorporate the acetone nucleus. Compound **6** is of interest since it inhibits tumor growth *in vitro* and has been selected by NCI for ongoing *in vivo* testing with human cancers. Heating **3b** with mixtures of propionaldehyde and secondary amines such as morpholine leads to 3-methylbenzopyrans containing the amine nucleus, *e.g.* **7b**. Unlike 3,4,5-trimethoxyphenyl compounds, *e.g.* **7a**, phenolic analogs of type **7b** do not inhibit growth of tumors *in vitro*.

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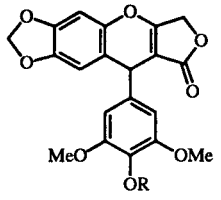
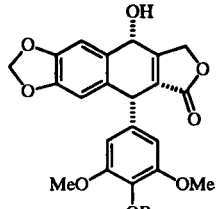
Earlier work in this laboratory on the preparation of potential insect growth regulators led to the synthesis of new types of benzopyran lactones [1] and benzopyranylamine derivatives [2]. Many of these benzopyrans proved to be active in the National Cancer Institute *in vitro* screening program [3,4] designed to detect possible anti-tumor agents. The most effective lactone in these tests proved to be the 3,4,5-trimethoxyphenyl compound **1a**, which inhibited growth of diverse tumor cells *in vitro* at concentrations as low as 10^{-6}-10^{-7} moles/L. As earlier noted, lactone **1a** is structurally similiar to podophyllotoxin **2a**, a naturally occurring anti-tumor agent which has long been considered to be too toxic to be useful. However, a related compound, etoposide, which is a glycosidic derivative of the phenolic 4-hydroxy-3,5-dimethoxyphenyl analog **2b** of podophyllotoxin, is a highly effective chemotherapeutic agent in current clinical practice. The anti-tumor action of podophyllotoxin and etoposide apparently involve distinctly different enzyme systems [4]. In view of these differences it was of some interest to synthesize and test 4-hydroxy-3,5-dimethoxyphenylbenzopyran analogs of active 3,4,5-trimethoxyphenyl compounds.

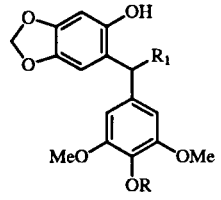
As previously observed [5], the first step in the novel synthetic route to benzopyrans involves the ability of aromatic aldehydes to consense easily with sesamol and morpholine, piperidine or pyrrolidine, to form an easily purified crystalline Mannich base, *e.g.* 3,4,5-trimethoxybenzaldehyde, morpholine and sesamol form the base **3a**. Treated with tetronic acid these bases readily yield intermediate lactones of type **4** which can then be cyclized to unsaturated lactones of type **1**, *e.g.* **3a** → **4a** → **1a**. However, early attempts to synthesize **1b** and other phenolic benzopyran analogs were abandoned when it was found that the reaction of 4-hydroxy-3,5-dimethoxybenzaldehyde with sesamol and morpholine, piperidine or pyrrolidine gave only intractable, highly colored tars which could not be purified. It was later found that, unlike these secondary amines, 2-aminopyridine reacts readily with sesamol and 4-hydroxy-3,5-dimethoxybenzaldehyde to give high yields of the colorless, crystalline Mannich base **3b**. 2-Aminopyrimidine similarly forms the crystalline base **3c**. Warmed with tetronic acid in methanol **3b** gives the lactone **4c**. Acetylation of **4c** yields the crystalline diacetate **4d** which on methylation gives the monomethyl derivative **4e**. Warmed with methanolic sodium hydroxide **4e** undergoes hydrolysis and cyclization to yield the desired unsaturated lactone **1b**. The intermediate cyclized product **5** can also be isolated in small amounts as a



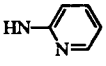
1a, R = Me
1b, R = H
1c, R = $COCH_3$
1d, R = $CH_2CO_2C_2H_5$

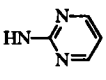


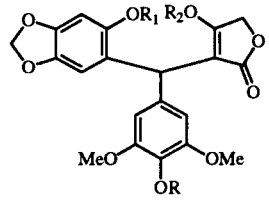
2a, R = Me
2b, R = H



3a, R = Me, R_1 = 

3b, R = H, R_1 = 

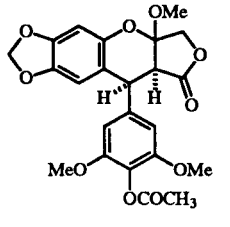
3c, R = H, R_1 = 



4a, R = Me, R_1 = R_2 = H
4b, R = R_2 = Me, R_1 = $COCH_3$
4c, R = R_1 = R_2 = H
4d, R = R_1 = $COCH_3$, R_2 = H
4e, R = R_1 = $COCH_3$, R_2 = Me

minor product in these hydrolysis experiments. NCI tests showed that *in vitro* the lactone **1b** and its acetate **1c** were active inhibitors of a number of cancer cell types at concentrations of approximately 10^{-5} moles/L.

As already noted [1] hydrolysis of methylated acetates such as **4b** and **4e** in methanolic sodium hydroxide yields the unsaturated lactones **1a** and **1b** respectively. Addition of acetone to the methanolic sodium hydroxide reaction mixture, however, results in the formation of new products which incorporate elements of the acetone nucleus. Thus, hydrolysis of **4b** in the presence of acetone yields a colorless, crystalline product, $C_{24}H_{24}O_9$, which on the basis of its nmr spectra (see experimental) is considered to be the cyclic product **6**. In accord with this structure the *gem*-dimethyl group derived from acetone appears in the ^1H nmr spectrum as singlets at δ 1.32 (CH_3) and δ 1.39 (CH_3) and the benzylic methine as a singlet at δ 4.78 (CH). The ^{13}C nmr spectrum shows signals *inter al* of the *gem*-dimethyl group at δ 26.7 (CH_3) and 25.6 (CH_3), the benzylic CH at δ 37.2, two methylene (CH_2) groups and a total of twelve quaternary C's. This acetone product proved to be active in the NCI *in vitro* screening program. Based on their *in vitro* results NC1 has extended their investigation of **6** to on-going *in vivo* experiments with sensitive cancers [6].



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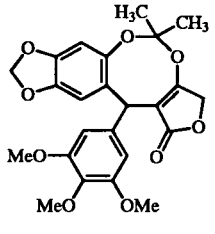

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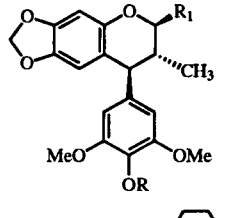
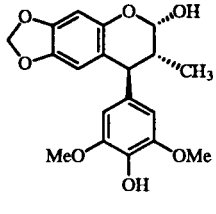


7a, R = Me, R_1 = N O

7b, R = H, R_1 = N O

7c, R = H, R_1 = N

7d, R = H, R_1 = OH



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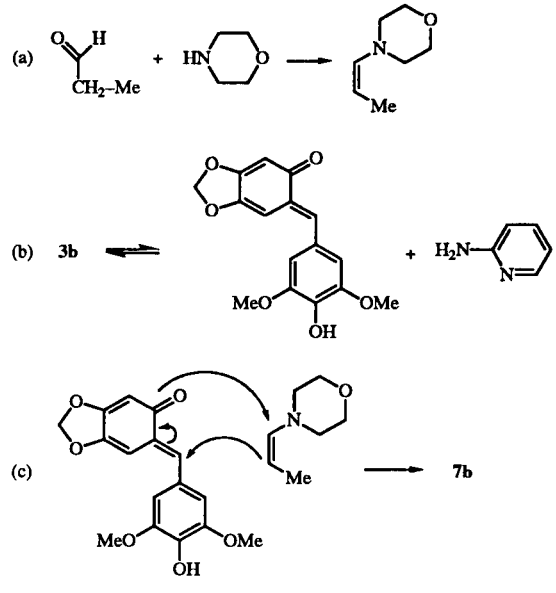
In Part 2 it was reported that pyrrolidine, piperidine and morpholine derived Mannich bases react rapidly with propionaldehyde to form 3-methylbenzopyrans, *e.g.* **3a** reacts with propionaldehyde to form **7a** [2]. The aminopyridine base **3b** does not react with propionaldehyde under similiar conditions and, as indicated above, Mannich bases have not been successfully isolated from reactions of secondary amines such as morpholine with sesamol and 4-hydroxy-3,5-dimethoxybenzaldehyde. However, a slight modification of the synthetic sequence allows easy access to benzopyranylamines containing this phenolic nucleus. For example, when the 2-aminopyridine base **3b** is warmed in methanol with excess of propionaldehyde and morpholine or pyrrolidine, it rapidly gives high yields of crystalline 3-methylbenzopyrans of type **7** containing the morpholine or pyrrolidine group. Hydrolysis of **7b** or **7c** in aqueous acetic acid yields a crystalline mixture of the *trans-trans* alcohol **7d** and the *cis-trans* isomer **8**. Unlike ithe benzopyranylamines with a 3,4,5-trimethoxyphenyl substituent, the analogs with a 4-hydroxy-3,5-dimethoxyphenyl nucleus prepared in this investigation showed little or no activity in the NIC *in vitro* tests. Thus, further studies on benzopyrans with this nucleus have not been pursued. The formation of benzopyrans of type **7b** from the base **3b** may be rationalized by assuming that the enamine present in excess from a morpholine-propionaldehyde reaction (a) (Scheme 1) reacts preferentially (c) (Scheme 1) with the *o*-quinone methide formed by dissociation of the Mannich base **3b** in solution (b) (Scheme 1).

Scheme 1



EXPERIMENTAL

The nmr spectra were determined in dimethyl sulfoxide with TMS as the internal standard on a Nicolet NT-WB 200 FT instrument at 200 MHz (^{13}C). Analyses were performed in a commercial laboratory. Melting points are uncorrected.

Mannich Base **3b**.

A solution of sesamol (13.8 g), 4-hydroxy-3,5-dimethoxybenzaldehyde (18.2 g) and 2-aminopyridine (9.4 g) in methanol (30 ml) was heated under reflux for 5 hours. The crystalline product, which separated on cooling, was recrystallized from acetone-methanol to give the base **3b** as colorless needles, mp 172-173° (29.4 g).

Anal. Calcd. for $C_{21}H_{20}N_2O_6$: C, 63.6; H, 5.1; N, 7.1. Found: C, 63.5; H, 5.2; N, 6.9.

Mannich Base **3c**.

A mixture of sesamol (1.38 g), 4-hydroxy-3,5-dimethoxybenzaldehyde (1.82 g) and 2-aminopyrimidine (0.95 g) in methanol was heated under reflux for 4 hours and cooled. The crystalline product was collected and recrystallized from acetone to give the base **3c** as cream colored needles, mp 183-184° (1.4 g).

Anal. Calcd. for $C_{20}H_{19}N_3O_6$: C, 60.5; H, 4.8; N, 10.6. Found: C, 60.4; H, 4.8; N, 10.6.

Warmed with acetic anhydride and pyridine this product formed a *diacetate* which crystallized from acetone-methanol as colorless glistening needles, mp 175-176°.

Anal. Calcd. for $C_{24}H_{23}N_3O_8$: C, 59.9; H, 4.8; N, 8.7. Found: C, 60.0; H, 4.8, N, 8.6.

4-Hydroxy-3-[(6-hydroxy-1,3-benzodioxol-5-yl)(4-hydroxy-3,5-dimethoxyphenyl)methyl]-2(5*H*)-furanone **4c**.

A solution of base **3b** (34 g) and tetronic acid (34 g) in methanol (200 ml) was heated to boiling for 15 minutes and allowed to cool. The crystalline product which separated (32 g) was collected and recrystallized from acetone-methanol to give **4c** as colorless needles, mp 197-198°; ^{1}H nmr: δ 3.66 (2 OCH_3), 4.64 (CH_2), 5.23 (CH), 5.82 (d, J = 1 Hz and 5.87, d, J = 1 Hz, OCH_2O) 6.38 (ArH), 6.43 (2 ArH), 6.66 (ArH), 8.17 (OH), 8.95 (OH), 11.27 (OH).

Anal. Calcd. For $C_{20}H_{18}O_9$, C 59.7; H, 4.5. Found: C, 60.0; H, 4.7.

A solution of the above product (15 g) in acetic anhydride (50 ml) and pyridine (5 ml) was heated in a steam-bath for 5 minutes and diluted with water. The gummy product was collected and dissolved in warm, wet methanol. On cooling the diacetate crystallized. Recrystallized from acetone-methanol diacetate **4d** separated as colorless needles, mp 233-234° (17 g); ^{1}H nmr: δ 2.16 ($COCH_3$), 2.31 ($COCH_3$), 3.67 (2 OCH_3), 4.63 (d, J = 16 Hz, and 4.71, d, J = 16 Hz, CH_2), 5.10 (CH), 6.01 (OCH_2O), 6.57 (2 ArH), 6.68 (ArH), 6.79 (ArH), 11.82 (br s, OH).

Anal. Calcd. for $C_{24}H_{22}O_{11}$: C, 59.3; H, 4.6. Found: C, 59.3; H, 4.8.

A mixture of the diacetate **4d** (10 g), methyl iodide (20 ml), potassium carbonate (45 g) and acetone (150 ml) was heated under reflux for 8 hours and filtered. The solid residue was suspended in water and the insoluble material was collected and added to the residue obtained on removing the acetone solvent from the reaction filtrate. Recrystallized from acetone-methanol the methylated diacetate **4e** was obtained as colorless, solvated needles, mp 137-138°; when recrystallized from benzene, **4e** has mp 156-157° (9.8 g); ^{1}H nmr: δ 2.16 ($COCH_3$), 2.23 ($COCH_3$) 3.68 (2 OCH_3), 3.88 (OCH_3), 5.07 (m, CH_2, CH), 6.01 (OCH_2O), 6.53 (2 ArH), 6.63 (ArH), 6.74 (ArH).

Anal. Calcd. for $C_{25}H_{24}O_{11}$: C, 60.0; H, 4.8. Found: C, 60.1; H, 5.1.

Methylation of **4d** with dimethyl sulfate in the place of methyl iodide led to the formation of **4e** as well as minor amounts of a second product, **5**. Thus, a mixture of the diacetate **4d** (10 g), dimethyl sulfate (5 g), potassium carbonate (25 g) and acetone (100 ml) was heated under reflux for 5 hours. The solvent was then removed and the residue was treated with water. The insoluble product was washed with dilute aqueous acetic acid and crystallized from acetone to give **4e** (7.9 g, mp 156-157° after recrystallization from benzene). The acetone filtrate from **4e** was concentrated to small bulk and diluted with methanol. The minor product crystallized. Recrystallized from acetone-methanol **5** was obtained as colorless needles, mp 183-184° (0.99 g); ^{1}H nmr: δ 2.25 ($COCH_3$), 3.42 (OCH_3), 3.67 (d, J = 2 Hz, CH), 3.73 (2 OCH_3), 4.25 (d, J = 2 Hz), 4.45 (d, J = 10 Hz, and 4.64, d, J = 10 Hz, CH_2), 5.93 (d, J = 1 Hz, and 5.97, d, J = 1 Hz, OCH_2O), 6.42 (ArH), 6.68 (ArH), 6.78 (2 ArH).

Anal. Calcd. for $C_{23}H_{22}O_{10}$: C, 60.2; H, 4.8. Found: C 59.9; H, 4.8.

6,9-Dihydro-9-(4-hydroxy-3,5-dimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*]furo[3,4-*b*][1]benzopyran-8-one **1b**.

A suspension of the methylated diacetate **4e** (4 g) in boiling methanol (40 ml) was slowly treated with 10% aqueous sodium hydroxide (8 ml). A clear solution was obtained after one ml of the sodium hydroxide solution had been added. At the end of the addition, approximately 3 minutes, a crystalline product rapidly separated. Heating was continued for a further 2 minutes and water (40 ml) was added. The solid product was collected, washed with dilute aqueous acetic acid, and recrystallized from acetone-methanol to give the unsaturated lactone **1b** as colorless, soft needles, mp 226-227° (2.2 g); ^{1}H nmr; δ 3.72 (2 OCH_3), 4.75 (CH), 5.01 (d, J = 16 Hz, and 5.14, d, J = 16 Hz, CH_2), 5.98 (d, J = 1 Hz, and 6.03, d, J = 1 Hz, OCH_2O), 6.49 (2 ArH), 6.68 (ArH), 6.92 (ArH), 8.24 (br s, OH).

Anal. Calcd. for $C_{20}H_{16}O_8$: C, 62.5; H, 4.2. Found: C, 62.4; H, 4.4.

Warmed with acetic anhydride and a drop of pyridine, the above product gave the monoacetate **1c**. This crystallized from acetone-methanol as colorless glistening needles, mp 232-233°; ^{1}H nmr: δ 2.21 ($COCH_3$), 3.70 (2 OCH_3), 4.90 (CH), 5.12 (CH_2), 6.03 (OCH_2O), 6.64 (2 ArH), 6.73 (ArH), 6.96 (ArH).

Anal. Calcd. for $C_{22}H_{18}O_9$: C, 62.0; H, 4.3. Found: C, 62.0; H, 4.3.

A mixture of the lactone **1b** (1 g), ethyl bromoacetate (2 g), potassium carbonate (5 g) and acetone (20 ml) was refluxed for 8 hours and filtered. Evaporation of the solvent from the filtrate gave a residue which crystallized from wet methanol (0.95 g). Recrystallized from acetone-methanol, **1d** was obtained as colorless, soft needles, mp 134-135°; ^{1}H nmr: δ 1.19 (t, J = 7 Hz, CH_3), 3.72 (2 OCH_3), 4.14 (q, J = 7 Hz, CH_2), 4.46 (CH_2), 4.82 (CH), 5.03 (d, J = 16 Hz, and 5.16, d, J = 16 Hz, CH_2), 5.99 (d, J = 1 Hz, and 6.05, d, J = 1 Hz, OCH_2O), 6.54 (2 ArH), 6.72 (ArH), 6.94 (ArH).

Anal. Calcd. for $C_{24}H_{22}O_{10}$: C, 61.3; H, 4.7. Found: C, 61.3; H, 4.9.

Lactone **6**.

Aqueous sodium hydroxide (5 ml, 10%) was added to a suspension of the methylated acetate **4b** (1.5 g) in acetone (5 ml) and methanol (5 ml) at boiling. The acetate dissolved almost at once. The solution was heated for 10 minutes, diluted with water (10 ml) and acidified with hydrochloric acid. The gummy product which separated was collected, washed with water and aqueous sodium bicarbonate solution and crystallized from acetone-

methanol. The lactone **6** was obtained as colorless needles, mp 214-215° (0.79 g); ^{1}H nmr: δ 1.32 (CH_3), 1.39 (CH_3), 3.60 (OCH_3), 3.73 (2 OCH_3), 4.78 (d, J = 1 Hz, CH), 5.04 (d, J = 1 Hz, and 5.21, d, J = 1 Hz, CH_2), 5.92 (d, J = 1 Hz, and 6.05, d, J = 1 Hz, OCH_2O), 6.68 (2 ArH), 6.83 (ArH), 6.91 (ArH); ^{13}C nmr: δ 25.6 (CH_3), 26.7 (CH_3), 37.2 (CH), 55.7 (2 OCH_3), 58.8 (OCH_3), 70.1 (CH_2), 88.9 (CH), 102.0 (C), 102.8 (OCH_2O), 104.8 (2CH), 108.8 (CH), 115.7 (C), 136.0 (C), 139.8 (C), 143.2 (C), 144.81 (C), 144.83 (C), 146.9 (C), 152.9 (2 C), 169.8 (C), 170.1 (C).

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.1; H, 5.3. Found: C, 63.0; H, 5.4.

1-[7,8-Dihydro-7-methyl-8-(4-hydroxy-3,5-dimethoxyphenyl)-6*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-6-yl]morpholine **7b**.

Morpholine (20 g) was added to a suspension of the base **3b** (20 g) in methanol (250 ml) and the mixture was refluxed for 10 minutes. Propionaldehyde (20 g) was then added and the mixture was heated under reflux for 1.5 hours. The base gradually dissolved. The product subsequently began to crystallize. After cooling, the product was collected and washed well with methanol (18.1 g). Recrystallized from acetone, the morpholine derivative **7b** separated as colorless needles, mp 220-221°; ^{1}H nmr: δ 2.25 (CH), 2.67 (m, NCH_2), 2.92 (m, NCH_2), 3.56 (d, J = 12 Hz, CH), 3.62 (m, CH_2OCH_2), 3.74 (2 OCH_3), 4.43 (d, J = 12 Hz, CH), 5.83 (d, J = 1 Hz, and 5.86, d, J = 1 Hz, OCH_2O), 5.95 (ArH), 6.40 (3 ArH), 8.27 (br s, OH).

Anal. Calcd. for $C_{23}H_{27}NO_7$: C, 64.3; H, 6.3. Found: C, 64.1; H, 6.5.

Warmed with acetic anhydride and pyridine the morpholine product **7b** formed an acetate. This product crystallized from acetone-methanol as colorless needles, mp 196-197°.

Anal. Calcd. for $C_{25}H_{29}NO_8$: C, 63.7; H, 6.2. Found: C, 63.4; H, 6.4.

Other amines reacted similarly with **3b**. Thus, for example, a mixture of the base **3b** (1 g), pyrrolidine (1 g) and propionaldehyde (1 ml) was heated under reflux for 1.5 hours. Colorless crystals separated. Recrystallized from acetone-methanol the pyrrolidine product **7c** was obtained as colorless, soft needles, mp 209-210° (0.8 g).

Anal. Calcd. for $C_{23}H_{27}NO_6$: C, 66.8; H, 6.6. Found: C, 66.9; H, 6.6.

A solution of the morpholine compound **7b** (10 g) in acetic acid (5 ml) and water (10 ml) was heated on a steam bath and slowly diluted with water (30 ml). Colorless crystals began to separate. After 1.5 hours the crystals were collected and recrystallized from acetone-methanol to give an alcoholic product as colorless needles, mp 203-204° (8.4 g). The ^{1}H nmr spectrum of this product indicated that it was a mixture of the *trans-trans* compound **7d** (about 30%) and the *cis-trans* isomer **8** (70%); ^{1}H nmr of the *trans-trans* isomer: δ 0.85 (d, J = 7 Hz, CH_3), 1.95 (m, CH), 3.56 (d, J = 12 Hz, CH), 3.72 (2 OCH_3), 4.94 (d, J = 12 Hz, CH), 5.83 (OCH_2O), 5.99 (ArH), 6.40 (m, 3 ArH), 6.98 (br s, OH), 8.20 (br s, OH); *cis-trans* isomer ^{1}H nmr: δ 0.78 (d, J = 7 Hz, CH_3), 2.10 (m, CH), 3.60 (d, J = 12 Hz, CH), 3.72 (2 OCH_3), 5.32 (d, J = 2 Hz, CH), 5.88 (OCH_2 O), 6.05 (ArH), 6.40 (m, 3 ArH), 6.98 (br s, OH), 8.20 (br s, OH).

Anal. Calcd. for $C_{19}H_{20}O_7$: C, 63.3; H, 5.6. Found: C, 63.2; H, 5.7.

Acknowledgments.

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[6] The NCI has assigned the following NSC numbers to compounds: **1a** (618862), **1b** (669380), **1c** (669381), **6** (665485), **7a** (666217), **7b** (671165).