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Structure–Activity Relationships among DNA-Gyrase Inhibitors. Synthesis and Antimicrobial Evaluation of Chromones and Coumarins Related to Oxolinic Acid

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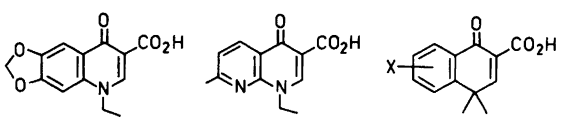
In the course of synthesizing 7-methoxychromone-3-carboxylic acid (*5a*) and 6,7-methylenedioxychromone-3-carboxylic acid (*5b*), as 1-oxa analogues of oxolinic acid (*1*), we investigated three different routes to chromone-3-carboxylates. Acid *5b* was most effectively achieved via a Kostanecki-Robinson reaction. Acid *5a* was obtained by NBS oxidation of the corresponding aldehyde *14a*, which was synthesized by an improved method utilizing a difluorodioxaborin intermediate *13*. Reaction between diethyl ethoxymethylenemalonate (*9a*) and 3-hydroxyanisol (*10*) has been studied under thermal and Lewis acid catalyzed conditions. Upon heating, small amounts of ethyl ester of chromone *5a* (*4a*), corresponding coumarin *6a*, and uncyclized addition product *9b* were found. No significant antimicrobial activity of the compounds tested was revealed.

Antibacterial agents of the oxolinic acid (*1*) and nalidixic acid (*2*) type possess a specific activity against DNA gyrase and have found a use in human medicine in the treatment of urinary tract infections. Extensive structure activity studies in this class of compounds have been made.[1] Recently, we reported the synthesis and antimicrobial activity of a series of carbocyclic analogues (*3*).[2] This study clearly showed that replacement of the *N*-Et moiety of *1* by a Me–C–Me grouping diminishes activity against both bacteria and the semi-purified enzyme by several orders of magnitude. As a continuation of our studies on the importance of the nitrogen atom, we now report the synthesis and evaluation of some chromone (*4,5*) and coumarin (*6–8*) analogues.



1 *2* *3*

It was reasoned that nitrogen, with its pronounced electron releasing character, was in an excellent position in these molecules (*1*) to contribute to electrophilicly driven reactions with the unsaturated β-ketocarboxylic acid system of the A ring (Fig. 1). On the other hand, the dimethylcarba analogues (*3*) could not do so. The 1-oxa analogues lie somewhere between these two extremes and therefore seemed worthy of study.

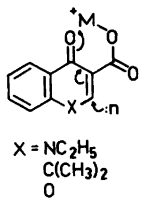


Fig. 1. Putative mechanism of action of DNA-gyrase inhibitors involves complexation of a metal cation possibly to facilitate Michael attack by bio-nucleophile.

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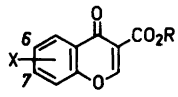
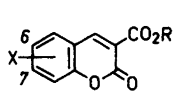
CHEMISTRY

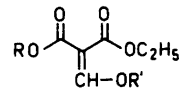
Compounds such as *1* and *2* can be made by reaction of the corresponding arylamine and diethyl ethoxymethylenemalonate (*9a*) at high temperatures.[1] A corresponding reaction between an activated phenol and *9a* would, in principle, be a simple way to the corresponding chromones (*e.g.*, *4*). However, such reactions have been studied and are claimed to lead to coumarins (*6*) in low yields.[3] This reaction was recently improved upon by Bissell through use of





R	X		
Et	7-OCH_3	*4a*	*6a*
Et	6,7-OCH_2O	*4b*	*6b*
H	7-OCH_3	*5a*	*7a*
H	6,7-OCH_2O	*5b*	*7b*
H	H	*5c*	*7c*

Lewis acid catalysis.[4] However, the inherent low yields in the above reactions and the possibility of formation of chromone via initial addition-elimination of the double bond in *9a* in analogy with reaction with anilines, prompted us to reinvestigate this process to see if the desired chromones could be formed in this way. We chose to study the reaction of 3-hydroxyanisole (*10*) and *9a* since the desired product (*4a*) has the methoxy group in the C-7 position known to be optimal for bioactivity based on structure-activity relationships established for quinolonecarboxylic acids.[1] Furthermore, a *meta*-substitution pattern is necessary for efficient ring closure to either a chromone or a coumarin system. We were indeed able to isolate a small amount (3 %) of chromone *4a* from the very complex reaction mixture resulting from thermal reaction of *9a* and *10*. However, to further complicate matters, the melting point (117–118 °C) of *4a* so obtained was identical to that reported for *6a* by Bissell resulting from a similar Lewis acid catalyzed reaction of *9a* and *10*.[4] Since it is well known to be difficult to differentiate between chromones and coumarins,[5] we felt it necessary to synthesize *6a* by an unambiguous Knoevenagel reaction and to repeat Bissell's reaction employing, however, a simplified work up. The coumarins *6a* obtained by the two routes had melting points of 134 °C in accordance with earlier reports[6] and were identical to each other. We were, however, not able to detect any appreciable amounts of chromone *4a* by TLC or NMR in the crude product of the $ZnCl_2$ catalyzed Bissell reaction. In extending our work, we found that a similar thermal condensation of *9a* and *15* led to no isolable quantity of chromone *4b* but gave only small amounts of coumarin *6b* instead. When desired, the latter coumarin (*6b*) was preferably made by the Lewis acid catalyzed reaction which proceeded in this case in 42 % yield.



9a. R=R′=Et
9b. R=Et, R′=3-$MeOC_6H_4$
9c. R=3-$MeOC_6H_4$, R′=Et

In the thermal reaction of *10* and *9a* we also isolated an uncyclized addition product in 10 % yield. ^1H NMR showed two sets of ethoxy signals, which clearly indicated that the structure was *9b* and not that of acylation product *9c*. The latter (*9c*) has the option of *Z*/*E* isomerism which should result in at least a small amount of two more sets of ethoxy signals. Furthermore, *9b* did not form either *4a* or *6a* upon refluxing for 2 h with one equivalent of $ZnCl_2$ in ethanol, but rather underwent ethanolysis to *10* and *9a*. Hydrogenation of *9b* in methanol gave diethyl methylmalonate, which confirms the structure to be *9b i. e.*, the first intermediate in the formation of chromone *4a*.

We also attempted a Lewis acid ($ZnCl_2$) catalyzed condensation of *10* and *9a* in diphenyl ether at 200 °C, conditions similar to those successfully applied to the synthesis of 4-hydroxyquinoline-3-carboxylic esters.[7] The main compounds formed were coumarin *6a* and a symmetrical coumarinocoumarin *11* formed by *O*-acylation of *10* with *6a*, ring closure, and air-oxidation. The other alternatives, shown in Fig. 2, the chromonochromone and the unsymmetrical chromonocoumarin, can be ruled out primarily based upon the lack of a retro Diels-Alder fragment in the mass spectra (*vide infra*).[5] A trace amount of *11* dissolved in DMSO and the ^1H NMR of this solution showed a characteristic non-bonded interaction between the C-1 H and the C-12 H

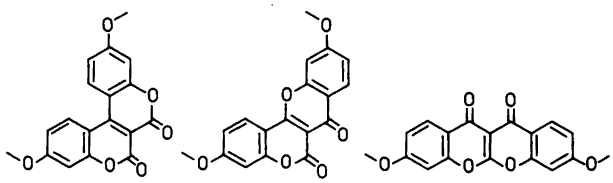


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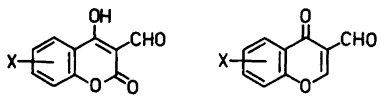
Fig. 2. Three possible [1]benzopyrano[1]benzopyran products from the reaction between diethyl ethoxymethylenemalonate and 3-methoxyphenol.

causing a down-field shift to 8.32 ppm similar to that observed in 3,4-benzophenanthrene.[8,9] The larger *ortho* coupling constant (J=9.5 Hz) is also consistent with this type of annelation.[9] Furthermore, the unsubstituted chromonochromone and chromonocoumarin, made from bis(2-hydroxybenzoyl)methane,[10] have CO absorption frequencies in IR significantly lower than that of the coumarinocoumarin *11*.

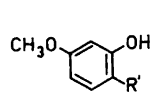
Basic hydrolysis of the coumarin esters gave *7a* and *7b*. However, when chromone-3-carboxylates (*4*) are subjected to basic conditions they rearrange, via initial Michael attack of hydroxide, to give 3-formyl-4-hydroxycoumarins (*8*).[11] We hydrolyzed *4a* with hydrochloric acid to the known acid *5a* and with base to *8a* because the



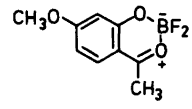
X=7-OCH_3	*8a*	*14a*
X=6,7-OCH_2O	*8b*	*14b*

enolic system in *8a* is acidic[11] enough to render it an interesting structure, related to *7a*, to investigate biologically.

An alternative route to chromone-3-carboxylic acids *via* the corresponding aldehyde has been devised by Nohara *et al.*[12] However, the Vilsmeier-Haack type reaction with acetophenone *12* and DMF-$POCl_3$ is reported to give the desired aldehyde *14a* in very low yield (6 %),[12] which proved to be the case in our hands also. Similarly, we were only able to obtain aldehyde *14b* in low yield (7 %) from *16*. This is consistent with the reported low yield reaction of 4,5-dimethoxy-2-hydroxyacetophenone (4 %).[12] To overcome the problems of using this type of activated acetophenone as starting materials, we next investigated the method reported by Reynolds *et al.* for the



10. R′=H
12. R′=$COCH_3$



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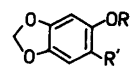
preparation of 3-formylbenzo[*h*]-chrom-4-one from 2,2-difluoro-4-methylnaphtho[2,1-*e*]-1,3,2-dioxaborin.[13]

Thus *12* was reacted with boron trifluoride etherate in acetic anhydride to give the difluorodioxaborin *13* in 85 % yield. Treatment of *13* with Vilsmeier reagent in DMF yielded aldehyde *14a* in 94 % yield. Oxidation of *14a* was then accomplished by *N*-bromosuccinimide followed by quenching with water in accordance with Machida *et al.*[14] to give *5a*. We were not, however, able to oxidize the methylenedioxy



	R	R′
15	H	H
16	H	$COCH_3$
17	CH_2Ph	$COCH_3$
18	CH_2Ph	$COCH_2CO_2Et$
19	H	$COCH_2CO_2Et$

aldehyde *14b* to produce useful quantities of the acid despite exploration of various conditions.

Therefore, an alternative route from the same acetophenone *16*, following the outline of Okumura's synthesis of *5c*,[15] was employed. Phenol *16* was benzylated to *17* and reacted with diethyl carbonate and sodium hydride to give *18* in good yield. Following deprotection to *19*, Kostanecki-Robinson type reaction with acetic formic anhydride/sodium formate in THF gave *4b* in high yield. Acidic and basic hydrolysis of *4b* as

Table 1. Structure elucidation by ^{1}H NMR and mass spectrometry.

No.	δ(ppm) C-5 H	C-2 H, C-4 H, or CHO	Prominent MS fragments; *m/z* (rel. intensity)
6a	7.51	8.50	248(M, 53 %), 148(M−CO_2Et+H−CO, 25 %)
4a	8.16	8.57	248(M, 10 %), 151(retro DA+H, 24 %), 150(4 %)
6b	6.91	8.42	262(M, 100 %), 162(M−CO_2Et+H−CO, 68 %)
4b	7.58	8.55	262(M, 17 %), 165(25 %), 164(retro DA, 14 %)
7a	7.50	8.86	220(M, 85 %), 176(M−CO_2, 100 %), 148(M−CO_2−CO, 68 %)
5a	8.23	8.92	220(M, 3 %), 176(M−CO_2, 100 %), 150(retro DA, 11 %)
8a	7.94	9.97	220(M, 54 %), 192(M−CO, 100 %), 150(retro DA, 98 %)
7b	7.10	8.80	234(M, 46 %), 190(M−CO_2, 32 %), 162(M−CO_2−CO, 100 %)
5b	7.58	8.89	234(M, 13 %), 190(M−CO_2, 100 %), 164(retro DA, 31 %)
8b	7.31	9.97	234(M, 26 %), 206(M−CO, 27 %), 164(retro DA, 100 %)
14b	7.59	8.45 10.38	218(M, 8 %), 190(M−CO, 100 %), 164(retro DA, 40 %)

described above gave *5b* and *8b*, respectively.

The problems often encountered in distinguishing chromones and coumarins[5] lead us to comment on the structural proofs of *4–8* and *14*. Table 1 outlines some of the important features found in their ^{1}H NMR and mass spectra. The C-5 H has a pronounced downfield shift in the chromones and in the 4-hydroxycoumarins (*8*) an intermediate effect is observed. In the mass spectra, the retro Diels-Alder fragmentation is of great diagnostic value for the chromones whereas the ring contraction by elimination of carbon monoxide is more characteristic for the coumarins. In the case of *8*, a marked retro Diels-Alder fragmentation is observed due to the tautomerism with the 2-hydroxy-3-formyl-chromone.

BIOLOGY

The *in vitro* antimicrobial assays were carried out by the standard agardilution/streak technique with a highest test level of 250 μg/ml.[16] The compounds *4b, 5a, 5b, 6a, 6b, 7a, 7b, 7c, 8a* and *8b* were tested on inhibition of *Staphylococcus aureus* (ATCC 13709), *Escherichia coli* (ATCC 9637), *Salmonella gallinarum* (ATCC 9184), *Klebsiella pneumoniae* AD (ATCC 10031), *Mycobacterium smegmatis* (ATCC 607) and *Candida albicans* (ATCC 10231). However, no appreciable inhibition was observed with the exception of *8a*, which inhibited *Staph. aureus* and *M. smegmatis* at the level of 12.5 μg/ml. These results further underline the unique properties of the *N*−Et moiety in antimicrobial agents such as *1* and *2*.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded on a Beckman 33 spectrophotometer. Mass spectra were conducted on a Hitachi Perkin-Elmer RMS-4 mass spectrometer (EI, 70 eV). ^{1}H NMR spectra were recorded on a Varian FT 80-A spectrometer with tetramethylsilane as internal standard. Microanalyses were performed on a Hewlett-Packard 185B at the University of Kansas and the results are within ±0.4 % of the theoretical values.

Ethyl 6,7-methylenedioxycoumarin-3-carboxylate (*6b*). A solution of sesamol (*15*, 2.76 g, 0.02 mol), diethyl ethoxymethylenemalonate (*9a*, 6.48 g, 0.03 mol) and dry $ZnCl_2$ (3.4 g, 0.025 mol) in 10 ml EtOH was refluxed for 23 h. After addition of aqueous HCl, the mixture was extracted several times with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to give 5.8 g of a residue. Repeated recrystallization from EtOH/EtOAc afforded 2.20 g (42 %) pure coumarin *6b*, m.p. 192.5–194 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (t), 4.39 (q), 6.12 (s, OCH_2O), 6.80 (s), 6.91 (s), 8.42 (s, 4-H); MS (EI): *m/z* (rel. int.) 262 (M, 100), 234 (M−CO, 8), 217 (M−C_2H_5O, 77), 206 (30), 205 (23), 190 (M−$CO_2C_2H_5$+H, 84), 162 (190-CO, 68), 133

(52); Anal. $C_{13}H_{10}O_6$: C, H.

Ethyl 7-methoxycoumarin-3-carboxylate (*6a*) was prepared in analogy with the above procedure from 3-methoxyphenol (*10*) and *9a* (1:1 ratio) in 9 % yield, m.p. 133–134 °C (lit.[4] 25 %, m.p. 117 °C). It was also prepared according to Rangaswami *et al.*[6a] from 2-hydroxy-4-methoxybenzaldehyde and diethylmalonate in 64 % yield, m.p. 134–135 °C (lit.[6a,6b] 134 °C). ^{1}H NMR ($CDCl_3$): δ 1.40 (t), 3.90 (s), 4.40 (q), 6.82 (m, 8-H), 6.87 (m, 6-H), 7.51 (d, J=8.1 Hz, 5-H), 8.50 (s, 4-H); MS (EI): *m/z* (rel. int.) 248 (M, 53), 220 (M–CO, 4), 203 (M–C_2H_5O, 100), 176 (M–$CO_2C_2H_5$+H, 86), 148 (176–CO, 25).

6,7-Methylenedioxycoumarin-3-carboxylic acid (*7b*). The ester *6b* (262 mg, 1 mmol) was stirred in 5 ml 2 M NaOH and 10 ml EtOH for 3.5 h at room temperature. The ethanol was evaporated and the yellow suspension was acidified with conc. HCl and stirred for 4 h. The precipitate was filtered and washed with water. Recrystallization from EtOH/acetone afforded 205 mg (88 %) acid, m.p. 275–276 °C. ^{1}H NMR ($CDCl_3$/CD_3CN): δ 6.18 (s, OCH_2O), 6.95 (s, 8-H), 7.10 (s, 5-H), 8.80 (s, 4-H); MS (EI): *m/z* (rel. int.) 234 (M, 46), 206 (M–CO, 10), 205 (23), 190 (M–CO_2, 32), 162 (M–CO_2–CO, 100), 161 (86), 134 (11), 133 (24), 132 (16), Anal. $C_{11}H_6O_6$: C, H.

7-Methoxycoumarin-3-carboxylic acid (*7a*) was prepared by alkaline hydrolysis of the ethyl ester *6a*, m.p. 192–194 °C (lit.[6a,6b] 195 °C); ^{1}H NMR ($CDCl_3$): δ 3.96 (s), 6.9 and 7.0 (overlapping d and dd, $J_{6,8}$=2.4 Hz, 6-H and 8-H), 7.50 (d, J=8.5 Hz, 5-H), 8.86 (s, 4-H), 12.20 (br s, OH); MS (EI): *m/z* (rel. int.) 220 (M, 85), 203 (17), 192 (M–CO, 8), 177 (49), 176 (M–CO_2, 100), 149 (15), 148 (M–CO_2–CO, 68), 134 (38), 133 (99).

Coumarin-3-carboxylic acid (*7c*) was prepared by alkaline hydrolysis of the methyl ester, m.p. 189–191 °C (lit.[17] 191–192 °C).

Ethyl 7-methoxychromone-3-carboxylate (*4a*). A. A mixture of 3-methoxyphenol (*10*, 1.24 g, 0.01 mol) and diethyl ethoxymethylenemalonate (*9a*, 6.49 g, 0.03 mol) was heated at 170 °C for 2 days. TLC showed a complex reaction mixture containing at least 6 components including a small amount of coumarin (*6a*). Most of the excess of malonate was distilled off in vacuo and the residue separated by flash chromatography on SiO_2 with 3 l (1:3) and 1 l (1:1) EtOAc–hexane to give *4a* and *9b*. The chromone *4a* was isolated in 2.9 % yield, m.p. 117–118 °C. ^{1}H NMR ($CDCl_3$): δ 1.38 (t), 3.90 (s), 4.38 (q), 6.86 (d, J=2.3 Hz, 8-H), 6.99 (dd, J=8.9 and 2.3 Hz, 6-H), 8.16 (d, J=8.9 Hz, 5-H), 8.57 (s, 2-H); MS (EI): *m/z* (rel. int.) 248 (M, 10), 203 (M–C_2H_5O, 27), 176 (M–$CO_2C_2H_5$+H, 100), 151 (retro Diels-Alder +H, 24), 150 (4), 148 (1.6): Peak match: Found *m/z* 248.06666. Calc. for $C_{13}H_{12}O_5$ 248.06839.

Open adduct 9b was isolated in 10 % yield. ^{1}H NMR ($CDCl_3$): δ 1.30 and 1.34 (two t), 3.79 (s), 4.26 and 4.35 (two q), 6.6 (m), 7.27 (ca. dd, J=8.4 Hz, 5-H), 7.87 (s). MS (EI): *m/z* (rel. int.) 294 (M, 79), 249 (M–C_2H_5O, 64), 248 (48), 220 (34), 203 (55), 192 (15), 177 (14), 176 (19), 175 (27), 149 (13), 148 (27), 135 (100); Anal. $C_{15}H_{18}O_6$: C, H.

B. A mixture of *10* (2.48 g, 20 mmol) and *9a* (8.64 g, 40 mmol) was heated for 12 h at 205 °C. Work-up as above gave 2.2 % *4a* and 6.8 % *9b*.

Hydrogenation of 9b. The adduct *9b* (100 mg, 0.4 mmol) was hydrogenated over 5 % Pd/C (0.05 g) in 50 ml MeOH at 0.28 MPa for 1.5 h. CH_2Cl_2 was added and the solution was filtered and evaporated. The residue was dissolved in CH_2Cl_2 and filtered through SiO_2 plug and eluted with CH_2Cl_2 to remove phenol *10*. Evaporation gave 47 mg (68 %) diethyl methylmalonate. ^{1}H NMR ($CDCl_3$): δ 1.27 (t), 1.40 (d), 3.43 (q), 4.21 (q), MS (EI): *m/z* (rel. int.) 174 (M, 6), 129 (M–OC_2H_5, 100).

6H,7H-3,10-Dimethoxy[1]benzopyrano[3,4-c]-[1]benzopyran-6,7-dione (*11*). A mixture of 3-methoxyphenol (*10*, 1.24 g, 10 mmol), diethyl ethoxymethylenemalonate (*9a*, 3.24 g, 15 mmol), $ZnCl_2$ (100 mg, 0.7 mmol) and 6 g diphenyl ether was heated to 200 °C for 1 h. After the first 10 min a sudden precipitate developed. After cooling, hexane was added and the solvent decanted. The precipitate proved largely insoluble in most solvents (EtOAc, CH_2Cl_2, Et_2O). The precipitate was triturated with hot EtOH, filtered and washed with EtOH and finally Et_2O to give 0.30 g (0.9 mmol) weakly yellow precipitate, m.p. 317–319 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 3.95 (s), 7.1 (m, 2- and 11-H), 7.14 (br s, 4- and 9-H), 8.32 (d, J=9.5 Hz, 1- and 12-H); IR (KBr): 1765, 1605 cm^{-1}; MS (EI): *m/z* (rel. int.) 324 (M, 100), 323 (43), 296 (M–CO, 27), 281 (M–CO–CH_3, 17), 268 (M–CO–CO, 59), 253 (M–CO–CO–CH_3, 32), 238 (11), 237 (52). Peak match: *m/z* Found 324.0641. Calc. for $C_{18}H_{12}O_6$ 324.0633.

2,2-Difluoro-7-methoxy-4-methylbenzo[e]-1,3,2-dioxaborin (*13*). 2-Hydroxy-4-methoxyacetophenone (*12*, 100 g, 0.6 mol) and boron trifluoride etherate (150 ml, 1.2 mol) were added to 230 ml acetic anhydride. The solution was heated at 100 °C for 1 h. The precipitate was collected and washed with Et_2O three times to give 109.7 g (85 %) of *13*, m.p. 170 °C; ^{1}H NMR ($CDCl_3$): δ 2.70 (s, CH_3), 3.93 (s, OCH_3), 6.53

(d, J=2.2 Hz, 8-H), 6.60 (dd, J=2.2 and 8.8 Hz, 6-H), 7.70 (d, J=8.8 Hz, 5-H). Anal. $C_9H_9BF_2O_3$: C, H.

7-Methoxychromone-3-carboxaldehyde (*14a*). To 50 ml DMF at 0 °C was added $POCl_3$ (6.5 ml, 70 mmol) followed by difluorodioxaborin *13* (5 g, 23 mmol). The solution was heated at 100 °C for 1 h, cooled and poured into 600 ml ice water. After storing in refrigerator the precipitate was collected, washed with cold water and dried. Recrystallization from DMF yielded 4.5 g (94 %) of *14a*, m.p. 188 °C (lit.[11] 188–190 °C); ^{1}H NMR ($CDCl_3$): 3.95 (s), 7.01 (d, J=2.2 Hz, 8-H), 7.06 (dd, J=2.2 and 8.8 Hz, 6-H), 8.25 (d, J=8.8 Hz, 5-H), 8.47 (s, 2-H), 10.40 (s, CHO). Anal. $C_{11}H_8O_4$: C, H.

6,7-Methylenedioxychromone-3-carboxaldehyde (*14b*) was prepared in analogy with Nohara *et al.*[12] A solution of 2-hydroxy-4,5-methylenedioxyacetophenone[18] (*16*, 541 mg, 3 mmol) in 15 ml dry DMF was cooled in an ice bath and freshly distilled $POCl_3$ (0.92 ml, 10 mmol) was injected under Ar over 1 h. The mixture was stirred at 0 °C for 2 h and at room temperature over night and then poured into ice water. The aqueous phase was extracted with EtOAc, dried ($MgSO_4$) and evaporated. Flash chromatography on SiO_2 with 25 % EtOAc in hexane afforded 48 mg (7 %) aldehyde, m.p. 227–230 °C; ^{1}H NMR ($CDCl_3$): δ 6.15 (s), 6.92 (s, 8-H), 7.59 (s, 5-H), 8.45 (s, 2-H), 10.38 (s, CHO); MS (EI): *m/z* (rel. int.) 218 (M, 8), 190 (M–CO, 100), 189 (51), 164 (retro-Diels-Alder, 40); Peak match: Found *m/z* 218.02034. Calc. for $C_{11}H_6O_5$ 218.02147.

2-Benzyloxy-4,5-methylenedioxyacetophenone (*17*). A mixture of 2-hydroxy-4,5-methylenedioxyacetophenone[18] (*16*, 895 mg, 5 mmol), benzyl bromide (1 ml, 8.4 mmol) and K_2CO_3 (1.32 g, 9.5 mmol) in 30 ml dry acetonitrile was refluxed overnight. TLC ($FeCl_3$ spray) indicated some unreacted phenol so more benzyl bromide (0.35 ml) and K_2CO_3 (0.45 g) were added and reflux was continued another night. After evaporation of the solvent, water was added and the aqueous phase extracted with Et_2O. The ethereal layer was washed with aqueous NaOH, dried ($MgSO_4$) and evaporated (1.64 g). Flash chromatography on SiO_2 with 25 % EtOAc in hexane gave 693 mg (52 %) crystalline product. Recrystallization of an analytical sample from EtOAc–hexane gave *17*, m.p. 117–118 °C. ^{1}H NMR ($CDCl_3$): δ 2.53 (s), 5.10 (s, CH_2Ph), 5.96 (s, OCH_2O), 6.57 (s, 3-H), 7.33 (s, 6-H), 7.39 (s, Ph); MS (EI): *m/z* (rel. int.) 270 (M, 6.6), 228 (4.4), 227 (2.6), 165 (2.7), 91 (100); Anal. $C_{16}H_{14}O_4$: C, H.

Ethyl (2-benzyloxy-4,5-methylenedioxybenzoyl)acetate (*18*): A mixture of *17* (615 mg, 2.27 mmol), diethyl carbonate (0.51 ml, 6 mmol) and NaH (50 % oil dispersion, 0.38 g, 8 mmol) in 25 ml THF was refluxed overnight. TLC showed complete conversion. The mixture was cooled and 1 ml HOAc and C_6H_6 were added. After evaporation the residue was taken up in Et_2O, washed with water and brine. Drying (Na_2SO_4) and evaporation gave a residue which was recrystallized from Et_2O to give 620 mg (80 %) pure product, m.p. 93–94.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.26 (t), 3.91 (s, $COCH_2CO$), 4.07 (q), 5.11 (s, CH_2Ph), 5.97 (s, OCH_2O), 6.53 (s, 3-H), 7.38 (s, Ph), 7.39 (s, 6-H); MS (EI): *m/z* (rel. int.) 342 (M, 1.9), 296 (M–C_2H_5OH, 4.8), 165 (4.7), 164 (6.3), 91 (100); Anal. $C_{19}H_{18}O_6$: C, H.

Ethyl (2-hydroxy-4,5-methylenedioxybenzoyl)acetate (*19*): A solution of *18* (439 mg, 1.28 mmol) in 250 ml EtOH was hydrogenated over 5 % Pd/C (0.3 g) for 25 min at 0.25 MPa at room temperature. Filtration and evaporation gave a residue which was dissolved in Et_2O and filtered through a small SiO_2 plug. Evaporation, addition of CH_2Cl_2 and evaporation gave 323 mg (100 %) of an oil which crystallized, m.p. 61–63 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (t), 3.86 (s, $COCH_2CO$), 4.22 (q), 5.98 (s, OCH_2O), 6.43 (s, 3-H), 6.98 (6-H), 12.64 (s, OH); MS (EI): *m/z* (rel. int.) 252 (M, 18), 206 (M–C_2H_5OH, 48), 165 (55), 164 (100); Anal. $C_{12}H_{12}O_6$: C, H.

Ethyl 6,7-methylenedioxychromone-3-carboxylate (*4b*). A mixture of *19* (194 mg, 0.77 mmol), sodium formate (68 mg, 1 mmol) and acetic formic anhydride (0.25 ml, ca. 1.75 mmol) in 1.5 ml dry THF was stirred at room temperature under Ar. TLC showed incomplete conversion after 4 h and another 0.25 ml acetic formic anhydride was injected, and the stirring continued overnight. The mixture was poured into ice water and extracted twice with CH_2Cl_2. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated to give 190 mg (94 %) of a white solid. Recrystallization from Et_2O gave 151 mg (75 %), m.p. 177–179 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (t), 4.39 (q), 6.11 (s, OCH_2O), 6.87 (s, 8-H), 7.58 (s, 5-H), 8.55 (s, 2-H); MS (EI): *m/z* (rel. int.) 262 (M, 17), 217 (M–C_2H_5O, 20), 190 (M–$CO_2C_2H_5$+H, 100), 189 (24), 165 (25), 164 (retro Diels-Alder, 14), 133 (4); Anal. $C_{13}H_{10}O_6$: C, H.

7-Methoxychromone-3-carboxylic acid (*5a*). A. The ester *4a* (15 mg, 0.060 mmol) was refluxed in 4 ml 50 % aqueous HCl for 15 minutes and then diluted with some water and filtered. The filter cake was washed with H_2O, EtOH and finally Et_2O. Drying gave 8 mg (61 %) pure acid 5a, m.p. 205–206.5 °C, with same m.p. after recrys-

tallization from acetone–hexane (lit.[14] 212–215 °C). By extraction of the aqueous filtrate with CH_2Cl_2 another 4.8 mg (36 %) acid was obtained.

B. To a suspension of aldehyde *14a* (5 g, 25 mmol) in 150 ml alcohol free $CHCl_3$ was added *N*-bromosuccinimide (9 g, 50 mmol) and the mixture was illuminated by a flood lamp for 2 h with gentle refluxing. After cooling the solution was extracted with aqueous $NaHCO_3$. The aqueous extract was acidified and extracted twice with CH_2Cl_2. After drying and evaporation the residue was recrystallized from acetonitrile to give 1.67 g (30 %) acid *5a*, m.p. 198–200 °C.

The acids prepared by the two routes were identical. ^1H NMR ($CDCl_3$): δ 3.96 (s), 6.99 (d, J=2.2 Hz, 8-H), 7.13 (dd, J=2.2 and 8.9 Hz, 6-H), 8.23 (d, J=8.9 Hz, 5-H), 8.92 (s, 2-H), 13.64 (s, OH); MS (EI) *m/z* (rel. int.): 220 (M, 3), 176 (M–CO_2, 100), 151 (7), 150 (retro Diels-Alder, 11), 134 (25). Peak match: Found *m/z* 220.03682. Calc. for $C_{11}H_8O_5$ 220.03711.

6,7-Methylenedioxychromone-3-carboxylic acid (*5b*): The ester *4b* (26 mg, 0.10 mmol) was refluxed in 2 ml 50 % aqueous HCl for 5 min and then diluted with ice water and filtered. The precipitate was washed with water and finally Et_2O. Drying gave 16 mg (68 %) pure acid, m.p. 232–233 °C. ^1H NMR ($CDCl_3$): δ 6.20 (s, OCH_2O), 7.00 (s, 8-H), 7.58 (s, 5-H), 8.89 (s, 2-H), 13.69 (s, OH); MS (EI): *m/z* (rel. int.) 234 (M, 13), 190 (M–CO_2, 100), 189 (52), 164 (retro Diels-Alder, 31), 165 (6); Peak match: Found *m/z* 234.01749. Calc. for $C_{11}H_6O_6$: 234.01638; Anal. $C_{11}H_6O_6$: C, H.

4-Hydroxy-7-methoxycoumarin-3-carboxaldehyde (*8a*): A mixture of *4a* (64 mg, 0.26 mmol), 2 ml 2 M NaOH and 10 ml EtOH was stirred at room temperature for 2 h. The ethanol was evaporated and aqueous $NaHCO_3$ was added and the aqueous phase was washed with Et_2O twice. After acidification with H_2SO_4 the aqueous phase was extracted with CH_2Cl_2. Drying (Na_2SO_4) and evaporation gave 53 mg (93 %) orange *8a*. Recrystallization from EtOAc gave an analytical sample, m.p. 215–217 °C (dec.). ^1H NMR ($CDCl_3$): δ 3.92 (s), 6.78 (d, J=2.3 Hz, 8-H), 6.91 (dd, J=2.3 and 8.8 Hz, 6-H), 7.94 (d, J=8.8 Hz, 5-H), 9.97 (s, CHO), 14.5 (br, OH); IR ($CHCl_3$): 1720 (CHO), 1615 (pyrone CO); MS (EI): *m/z* (rel. int.) 220 (M, 54), 192 (M–CO, 100), 151 (80), 150 (retro Diels-Alder, 98), 122 (38), 107 (26); Peak match: Found *m/z* 220.03651. Calc. for $C_{11}H_8O_5$ 220.03711.

4-Hydroxy-6,7-methylenedioxycoumarin-3-carboxaldehyde (*8b*). A mixture of *4b* (47 mg, 0.18 mmol), 5 ml 2 M NaOH and 5 ml EtOH was stirred at room temperature for 45 min. The ethanol was evaporated and the residue was acidified with aqueous HCl and extracted twice with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4) and evaporated to give 37 mg (89 %). Trituration with hot EtOH gave 32 mg (76 %) pink product, m.p. 270 °C (dec.). ^1H NMR ($CDCl_3$): δ 6.14 (s, OCH_2O), 6.79 (s, 8-H), 7.31 (s, 5-H), 9.97 (s, CHO); MS (EI): *m/z* (rel. int.) 234 (M, 26), 206 (M–CO, 27), 164 (retro Diels-Alder, 100), 136 (164-CO, 18); Peak match: Found *m/z* 234.01692. Calc. for $C_{11}H_6O_6$ 234.01638; Anal. $C_{11}H_6O_6$: C, H.

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