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Synthesis and antibiotic activity of the tricyclic furo[3,2-*c*] isochromen-2-trione unit of the pyranonaphthoquinones

Darío A. Bianchi,[a] Emma G. Sutich[b] and Teodoro S. Kaufman[a,*]

[a]*Instituto de Química Orgánica de Síntesis (CONICET-UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, República Argentina*
[b]*Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, República Argentina*

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Abstract—The elaboration and biological activity of **15**, containing the proposed pharmacophore for the antibiotic activity of the pyranonaphthoquinones, are reported. The synthetic strategy involved acid-catalyzed lactonization of mandelate **17** for isochroman ring formation, in combination with a Wittig–oxa-Michael functionalization of isochroman-3-ol derivative **20**, a lactonization involving configurational inversion of a benzylic alcohol and a final AgO oxidation. Compound **15** showed activity against *Staphylococcus aureus* and *Bacillus subtilis* with MIC of 64 and 32 μg/mL, respectively.

The 3,3a,5,9b-tetrahydro-furo[3,2-*c*]isochromen-2-trione moiety (**1**) is a key structural element of a number of biologically important natural products, mainly of fungal origin. Some representative compounds displaying this framework include the antifungal agent kalafungin (**2**),[1] arizonin A1 (**3**), a kalafungin-type antibiotic isolated from *Actinoplanes arizonaensis*,[2a] frenolicin B (**4**), an interesting anticoccidial agent from *Streptomyces roseofulvus* AM-3867[2b] and medermycin (**5**), a platelet aggregation inhibitor[3] from *Streptomyces tanashiensis* K73,[4] with potent anticancer activity as well as highly active against Gram positive microorganisms, including *Staphylococcus* and *Bacillus* (Fig. 1).

Other pyranonaphthoquinones displaying this feature are the novel antibiotic mederrhodin A (**6**) produced by recombinant DNA techniques,[5] the antimicrobial griseusin A (**7**) from *Streptomyces griseus* K-63,[6a,b] nanaomycin D (**8**) the enantiomer of kalafungin, the unique human platelet aggregation inhibitor Sch 38519 (**9**) from *Thermomonospora* spp,[6c] (+)-granaticin (**10**), an RNA synthesis inhibiting compound from *Streptomyces*

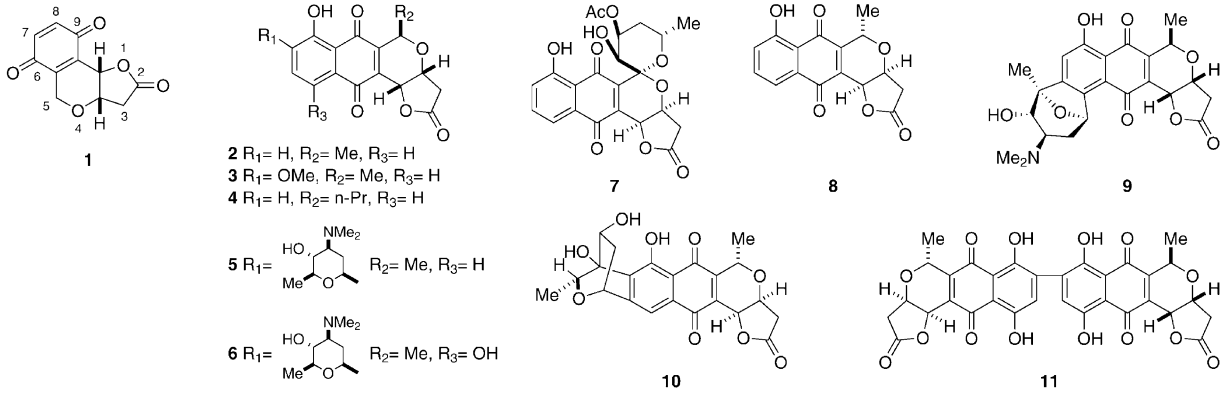

Figure 1.

* Corresponding author. Tel./fax: + 54-341-437-0477; e-mail: tkaufman@fbioyf.unr.edu.ar

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olivaceous[7] and γ-actinorhodin (**11**), produced by *Streptomyces coelicolor*[8a] and representative of the dimeric pyranonaphthoquinone antibiotics, which also include some of the crisamycins.[8b] The wide therapeutic potential of these natural products and the significant synthetic challenge represented by their complex structures have attracted considerable attention from the organic chemistry community and different approaches for their partial or total syntheses have been reported.[9]

The 3,3a,5,9b-tetrahydro-furo[3,2-*c*] isochromen-2-trione moiety, characteristic of these pyrano naphthoquinone natural products, has been elaborated in various imaginative ways; Giles and co-workers have recently disclosed an intramolecular version of the Mukaiyama reaction capable of yielding isochromans,[10a] which was conveniently modified to furnish the furo[3,2-*c*]isochromen-2-one system,[10b] while the groups of Kraus and Semmelhack[11] have designed a palladium mediated alkoxy-carbonylation of suitably functionalized allylic alcohols and Brimble and co-workers have devised for this purpose an interesting, and very effective furo[3,2-*b*]naphtho[2,1-*d*]furan oxidative rearrangement,[9a,12] all of which complement earlier developments made by the teams of Kraus[13] and Li,[14] involving intramolecular additions of oxygen-bearing functional groups to intermediate species carrying conjugated double bonds.

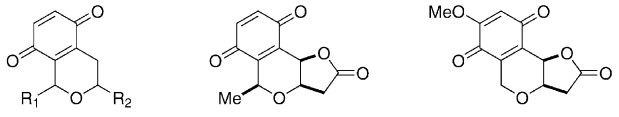
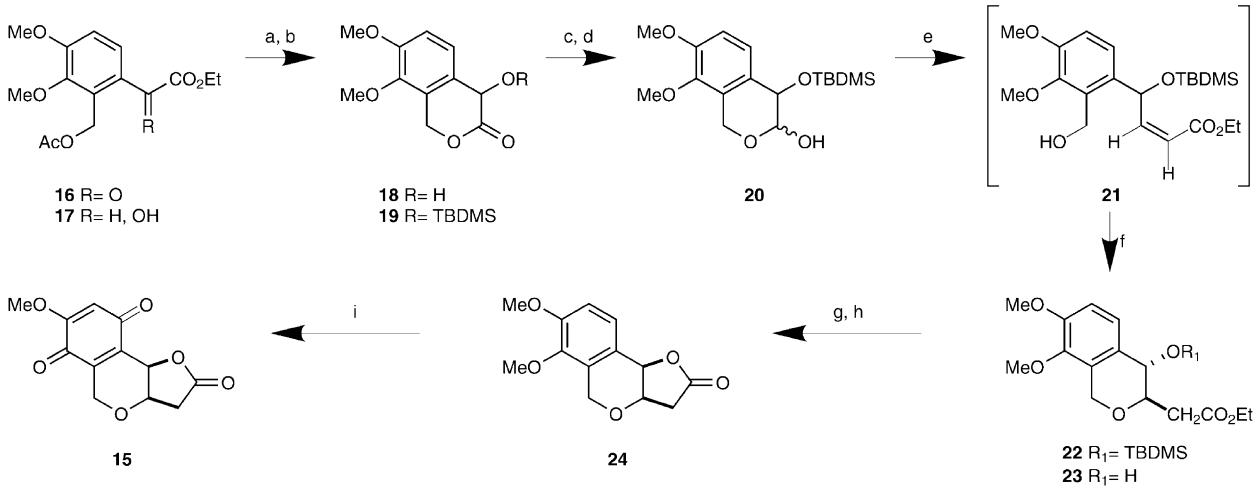


Figure 2.

Experiments with several naphthocyclinones, polycyclic pyranonaphthoquinones structurally related to the actinorhodins, demonstrated their inhibitory properties against Gram positive bacteria, suggesting that the minimal structure **12** (Fig. 2) is required for activity;[1c,15] their benzylic oxygen functionality was also linked to bioactivity.[16] In addition, benzoisochroman-5,8-diones of general structure **13** were prepared from condensation of 1,4-dimethoxy-β-phenethyl alcohols with aldehydes followed by oxidative demethylation of the resultant pyrans,[17] and tricyclic lactones such as **14** have also been synthesized,[10b] but their activity has not been tested.

We report here the elaboration of 3,3a,5,9b-tetrahydro-furo[3,2-*c*]isochromen-2-trione **15** from the easily available glyoxylate **16**,[18] employing an acid-catalyzed lactonization and a one-pot Wittig–oxa-Michael sequence for isochroman ring formation and functionalization, followed by a mesylate-assisted lactonization sequence for the elaboration of the five-membered lactone; we also disclose results of the antimicrobial activity of **15** and its aromatic precursor against *Staphylococcus aureus* and *Bacillus subtilis*.

To access **15**, glyoxylate **16** was selectively reduced with sodium cyanoborohydride in EtOH furnishing mandelate **17** which, when submitted to camphorsulfonic acid-promoted lactonization in MeOH, smoothly afforded α-hydroxylactone **18** as the sole product in 89% isolated yield, as shown in Scheme 1. Garner and Ramakanth observed that during the Wittig olefination of α-hydroxy carbonyls the starting material can rearrange, furnishing other carbonyl substances, which in turn are able to undergo olefination, leading to diminished yields of the desired product.[19] Therefore, **18** was protected as the TBDMS ether **19** under conventional conditions, and this was then carefully and efficiently reduced to lactol **20** with 1.1 equivalents of DIBAL-H in toluene at −78 °C.[20]



Scheme 1. Reagents and conditions: (a) $NaCNBH_3$, $AcOH_{gl}$, EtOH, rt, overnight (96%); (b) CSA (0.5 equiv), MeOH, 40 °C, 6 h (89%); (c) TBDMSCl, imidazole, DMAP, DMF, rt, 4 h (87%); (d) DIBAL-H, toluene, −78 °C, 10 min (93%); (e) PPh_3=$CHCO_2Et$, MeCN, reflux, 40 min; (f) K′BuO (0.1 equiv), 2 min (76%, overall); (g) TBAF, THF, rt, 1 h (90%); (h) TEA, CH_2Cl_2, rt, overnight (80%); (i) AgO, 6 N HNO_3, 5 min (55%).

Submission of **20** to olefination with (carbethoxy methylene) triphenylphosphorane in refluxing MeCN gave intermediate ester **21** as the sole product, to which the *Z*-configuration was attributed based on analysis of the chemical shifts and coupling constants of the diagnostic benzylic (δ 5.71, dd, $J = 0.6$ and 11.2 Hz) and olefinic protons α- and β- to the carbonyl moiety [δ 6.60 (dd, $J = 0.6$ and 9.0 Hz) and δ 6.47 (dd, $J = 9.0$ and 11.2 Hz), respectively]. Without purification, **21** was treated in situ with potassium *tert*-butoxide, selectively providing isochroman ester **22** in 70% yield from **19**. The expected *trans* stereochemistry of the substituents in **22**, probably formed through a chair-like transition state, agreed with literature reports[21] and was further confirmed by the value of $J_{H3-H4} = 8.1$ Hz. Interestingly, Martín and co-workers have recently shown in analogous systems that when the *E*-isomers undergo oxa-Michael cyclization, *cis*-substituted pyran derivatives are preferentially obtained, while cyclization of the *Z*-olefins give rise mainly to the *trans*-substituted oxacycles.[22]

Protection of the benzylic alcohol in **18** proved to be crucial to the success of the sequence, because it prevented hydroxyl group participation in side reactions[23] during the lactone reduction and Wittig olefination steps and also improved stereochemical control of the oxa-Michael product. Simultaneous experiments carried out without protection of **18** evidenced difficulties in accomplishing the reduction of this hydroxylactone and lead to a 1.15:1 mixture of **23** and **24** in 16% overall yield.

Next, exposure of silyl ether **22** to tetrabutyl ammonium fluoride resulted in mild and efficient desilylation and isolation of the expected alcohol **23**, which failed to undergo a retro-Michael–Michael addition–lactonization sequence upon treatment with potassium *tert*-butoxide or with camphorsulfonic acid in toluene at 70 °C.[9c] Therefore, an alternative lactonization strategy was devised, consisting in a one pot reaction of **23** with MsCl in CH_2Cl_2–triethylamine containing one equivalent of KOH; this smoothly afforded isochroman-γ-lactone **24** in 80% yield, presumably by carboxylate displacement of an intermediate mesylate.

Finally, oxidation of **24** to quinone–lactone **15** was undertaken. While treatment of **24** with ceric ammonium nitrate gave no reaction at room temperature and heating at 40 °C rapidly conducted to a series of highly unstable products, its exposure to AgO in 6N HNO_3 smoothly effected the desired transformation, furnishing **15** in 55% isolated yield.[24,25]

Both tricyclic lactones **24** and **15** were submitted to antibiotic assays against *S. aureus* ATCC 29213 and *B. subtilis*. In the disk assay, it was observed that compound **24** showed very poor activity, while **15** proved to display dose-dependent antibiotic activity against both bacterial strains.[26]

Compounds **15** and **24** were then subjected to the determination of their minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) in liquid media, with results (Table 1) indicating that both lactones are capable of inhibiting bacterial growth, being **15** more potent than **24**. These observations confirm the previous supposition regarding the structure of the pharmacophore of the pyranonaphthoquinone antibiotics and underscore the importance of the quinone functionality for their antibiotic activity.

Table 1. MIC and MBC of tricyclic lactones **15** and **24** against *Staphylococcus aureus* and *Bacillus subtilis*[27]

Compd[a]	*S. aureus*		*B. subtilis*	
	MIC	MBC	MIC	MBC
15	64	>128	32	128
24	128	>128	128	>128

[a] MIC and MBC are given in μg/mL.

In conclusion, a facile synthesis of the 3,3a,5,9b-tetrahydro-furo[3,2-*c*] isochromen-2-trione **15**, which embodies the tricyclic ring system found in many pyranonaphthoquinone natural products with antibiotic activity, was achieved from glyoxylate–acetate **16**. Key steps of the synthesis were acid-catalyzed lactonization of mandelate **17**, Wittig–oxa-Michael functionalization of isochroman-3-ol **20**, lactonization of **23** with configurational inversion and final AgO-mediated oxidation. Compound **15** proved to be active against *S. aureus* and *B. subtilis*.

Acknowledgements

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24. All new compounds were fully characterized by IR, ^{1}H and ^{13}C NMR, as well as microanalysis or high resolution mass spectra.
25. Data of **15**: Yellowish oil; IR (ν_{max}): 2940, 1780, 1680, 1590, 1340, 1230, 1140, 1050, 960, 900 and 730 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.71 (d, 1H, $J = 17.8$ Hz, H-3), 2.91 (dd, 1H, $J = 4.8$ and 17.7 Hz, H-3), 3.86 (s, 3H, OMe), 4.31 (dd, 1H, $J = 1.9$ and 18.7 Hz, H-5_{ax}), 4.33 (dd, 1H, $J = 2.3$ and 4.8 Hz, H-3a), 4.78 (d, 1H, $J = 18.7$ Hz, H-5_{eq}), 5.11 (dd, 1H, $J = 1.9$ and 2.3 Hz, H-9b) and 6.02 (s, 1H, H-8); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 36.66 (C-3), 56.37 (OMe), 60.99 (C-5), 68.57 (C-9b), 72.24 (C-3a), 107.46 (C-8), 132.94 (C-5a), 141.82 (C-9a), 158.38 (C-7), 173.51 (C-2), 180.00 (C-6) and 183.83 (C-9). HREIMS *m*/*z*: 250.04807; calcd for $C_{12}H_{10}O_6$: 250.04774.
26. Bacterial suspensions (10^8 CFU/mL) were prepared and spread over Mueller–Hinton agar plates. After 3 min at rt, 6 mm disks (prepared by adding known amounts of test compound in MeOH and allowing to dry at rt) with various amounts of test substance were placed at distances of 24 mm to each other and incubated at rt for periods of 15 or 120 min, after which they were incubated at 37 °C for 24 h, when inhibition haloes were measured.
27. Working solutions (256 μg/mL) were prepared by dilution of stock solutions (2000 μg/mL in MeOH) with Mueller–Hinton broth. The bacterial innoculum was a 1/10 dilution of a bacterial suspension (grown overnight in brain hearth broth), adjusted to 0.5 in the Mc Farland turbidity scale. Serial dilutions of the working solutions were added to different tubes containing the bacterial suspension and the tubes were incubated during 24 h at 37 °C. MIC is the minimum concentration of the substance which avoids production of turbidity, while MBC is the minimum concentration of the tested substance which kills at least 99% of the bacterial population.