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# *O*-Methylglucogalloyl esters: Synthesis and evaluation of their antimycotic activity

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**Abstract**—The two anomers of *O*-methyl gluco-2,3-digalloyl esters were synthesized and their antimycotic activity toward yeasts of biomedical importance was evaluated. When used at subinhibitory concentration and regardless of stereochemistry at the anomeric carbon, these compounds enhance the antimycotic activity of Amphotericin B.


Gallic acid (GA) and its derivatives are widespread natural molecules having an important role in food and pharmaceutical industry.

Previous studies evaluated the biological activities of some *n*-alkyl esters of gallic acid (propyl, octyl, etc.). These non-natural molecules, currently used as antioxidant additives, exhibited an antimycotic activity toward some yeasts,[1] and were noticed to be able to increase the antimycotic activity of some antibiotics, such as Amphotericin B (AmB),[2] a polyenic molecule that, in spite of its low stability, is currently used for the treatment of human mycoses.[3]

Although the protecting action of glucogalloyl derivatives against cardiovascular diseases[4,5] is a well-known phenomenon, their antimycotic activity toward yeasts of biomedical relevance has never been critically evaluated.

With this background, a chemical and biological program was undertaken to synthesize and evaluate the antimycotic activity of new glucogalloyl esters. The initial targets chosen were the *O*-methylgluco-2,3-digalloyl esters for their relative low molecular weight, their lead-

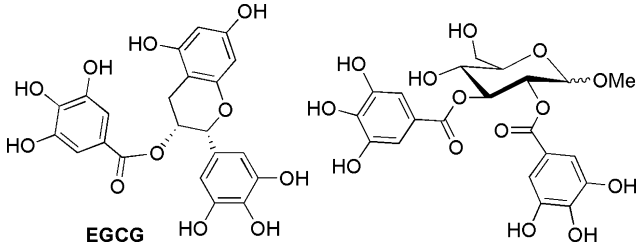


**Figure 1.** EGCG and *O*-methyl gluco-2,3-galloyl ester skeletons.

form to more complex glucogalloyl and ellagitannins derivatives and steric similarity with epigallocatechin gallate (EGCG) (Fig. 1) that is known to exhibit a biological activity towards living yeast cell systems.[6]

The synthetic strategy started with the preparation of suitable protected gallic acid **1**. Commercially available methyl gallate (MG) was reacted with dichlorodiphenyl methane to give the expected cyclic dioxolane **2** that was hydrolyzed to the corresponding acid **3** with LiOH in MeOH/$H_2O$ followed by silylation with an excess of TBDMSCl and DIPEA at both the acid and the residual phenolic position.[7] The silyl ester **4** was selectively deprotected to **1** by acid hydrolysis with AcOH in THF/$H_2O$ (Scheme 1).[8]

As far as the sugar moiety is concerned, a 2:1 mixture of α- and β-*O*-methylglucosides **5**, protected on the posi-

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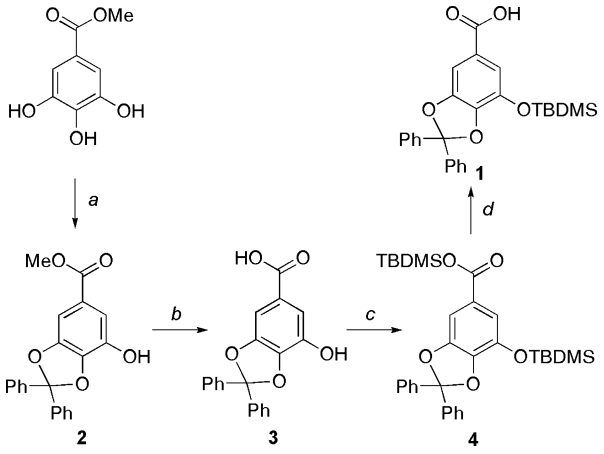


**Scheme 1.** Synthesis of galloyl acid derivative **1**. Reagents and conditions: (a) $Ph_2CCl_2$ 180 °C, 83%; (b) LiOH, MeOH/$H_2O$ reflux, 97%; (c) TBDMSCl, DIPEA, DMF, 98%; (d) AcOH, THF/$H_2O$, 80%.

tions 4 and 6 as benzylidene derivatives, were used. The esterification reaction of **1** with **5** was initially carried out by activating the acid 'via' the formation of the corresponding acyl chloride, but low yields (22%) of esters **6** were isolated. The poor result obtained prompted us to carry on a modified Steglich esterification reaction.[9] This procedure allowed the isolation of reasonable yields of a mixture of galloyl esters **6** which were also isolated as single anomers by skilled flash chromatography[10] (Scheme 2).

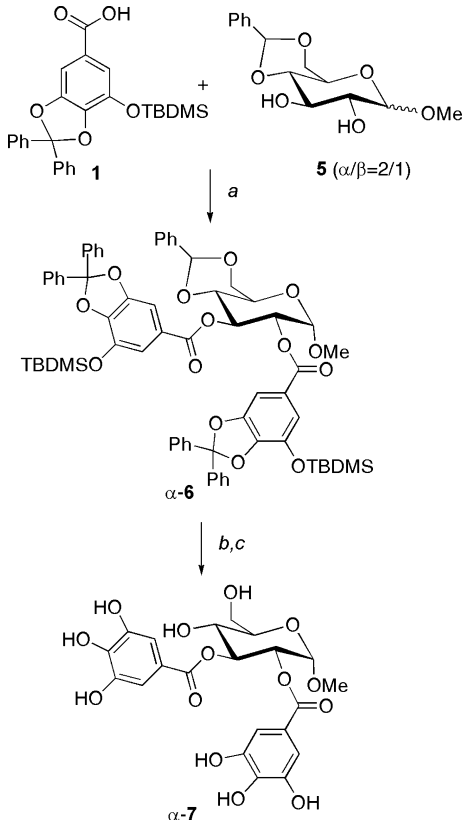
The deprotection of the hydroxyl groups was achieved in two steps by desilylation, with TBAF/AcOH to prevent ester hydrolysis, followed by catalytic hydrogenation of the dioxolane ring of both the aromatic and the sugar moieties.[11] Reverse phase preparative thin-layer chromatography (TLC) allowed the purification of α- and β-*O*-methylgluco-2,3-galloyl derivatives **7**, from a very small amount of partially protected intermediates, as reported in Scheme 2 where, for simplicity, only the major α-anomer has been depicted.[10]

The identification of the two anomers was performed by NMR, UV–vis, and MS techniques.[10]

The HPLC-DAD analysis of galloyl derivatives **7**, in Figure 2, shows the different retention times, the high purity (>98%), and the eventual separation of the two anomers **6**, performed by flash chromatography during the synthetic pathway (Scheme 2, Fig. 2).

In preliminary tests aimed at evaluating the biological properties of the glucogalloyl esters, a mixture of α- and β-anomers **7** exhibited only a slight and temporary antimycotic activity toward *Candida glabrata* DBVPG 3828.

When AmB[13] was used in association with a subinhibitory concentration of glucogalloyl compounds **7** (Table 1), an increased antimycotic activity of the above polyenic antibiotic was observed, under form of a clear decrease of its minimal inhibitory concentration (MIC).[14] This effect was particularly relevant when *Issatchenkia*



**Scheme 2.** Synthesis of α- and β-*O*-methylgluco-2,3-digalloyl esters **7**. Reagents and conditions: (a) DCC, DMAP, DMAP·HCl, $CH_2Cl_2$, rt, 24 h, flash chromatography, [α-**6** (26%), β-**6** (16%), α/β-**6** (13%)]; (b) TBAF/AcOH, THF, rt, 5 h, 85%; (c) $H_2$, Pd/C, THF, rt, 17 h (three cycles), 27%.

*orientalis* (teleomorphic state of *Candida kruseii*) DBVPG 6782, a well-known yeast pathogen,[15] was used as target strain (Table 1). In addition, our studies indicated that either mixture or pure α-**7** or β-**7** anomers did not exhibit significantly different effects on the activity of AmB (Fig. 3).

Concerning the possible mechanism(s) of such behavior, some hypotheses can be proposed. Apparently, the more significant may concern the well-known interaction between antioxidant molecules (such as galloyl derivatives) and AmB.[2,16]

It is generally accepted that the damaging action of AmB toward yeast cells originates from its binding to sterols incorporated in cell membranes,[3] even though no clear relationship has been so far found between this first step and the expression of biological activity. Although some evidence suggests that yeast cell lethality does not originate exclusively from changes in membrane permeability, the binding of AmB to sterols results in a disorganization of cell membranes, through the formation of specific pores constituted of small aggregates of AmB and sterols, which causes a loss of protons and monovalent cations.[3]

Several reports indicate that oxidation-dependent events may be involved in AmB antimycotic activity.[2,16]

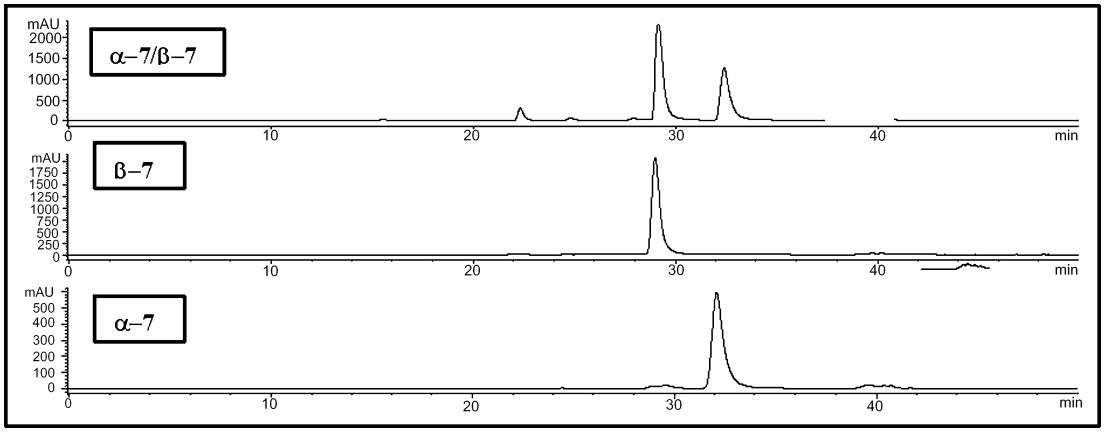



**Figure 2.** HPLC-DAD chromatograms of a mixture of α-7and β-7 and the isolated pure compounds.

**Table 1.** MICs (μg/mL) of mixture α-7/β-7 alone, AmB alone, or AmB in association with mixture α-7/β-7 (49.8 μg/mL), gallic acid (GA) (34.0 μg/mL), and methyl gallate (MG) (36.8 μg/mL)

| | MICs | | | | |
|---|---|---|---|---|---|
| | α-7/β-7 | AmB | AmB in association with | | |
| | | | α-7/β-7 | GA | MG |
| *C. albicans*[12] | 1940 | 1.06 | 0.05 | 0.09 | 0.09 |
| *C. glabrata*[12] | >4670 | 1.06 | 0.05 | 1.00 | 0.09 |
| *I. orientalis*[12] | >4670 | >100 | 2.05 | 8.00 | 45.0 |

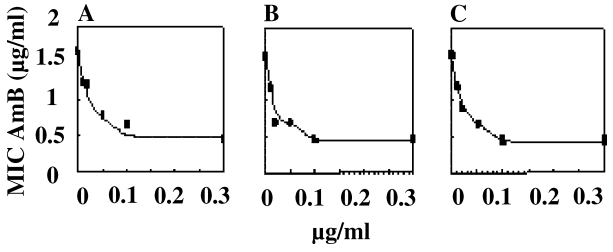



**Figure 3.** MICs (μg/mL) of AmB in association with increasing concentrations of: mixture α/β-7 (A) or pure α-7 (B) or β-7 (C). Target strain: *C. glabrata* DBVPG 3828.

Because AmB injury to yeast cells could be modulated by the extracellular presence of compounds exhibiting antioxidant action, it has been postulated that these molecules could act as simple protective agents toward polyene oxidation, thus resulting in an increased AmB stability.[2,16] Therefore, a possible explanation of the observed synergistic (or additive) effect of glucogalloyl molecules on AmB may be related to the presence of tri-hydroxy, substituted aromatic rings in the structure of glucogalloyl derivatives **7** which clearly can exert an antioxidant action.

To verify the above hypothesis, MICs of AmB in association with either the mixture α-7/β-7 or with gallic acid (GA) or methyl gallate (MG) (both at double molar concentration, to have the same amount of antioxidant moiety) were compared. Because both GA and MG revealed an apparently lesser effect on AmB activity (under form of higher MICs) (Table 1), the existence of additional effect(s) (other than the antioxidant action) of glucogalloyl derivatives **7** on AmB molecule may be hypothesized.

In conclusion, the present study shows that glucogalloyl derivatives **7** could be used for enhancing the antimycotic activity of AmB toward yeasts of biomedical relevance. Accordingly, the use of reduced concentrations of known antibiotics in association with subinhibitory concentrations of glucogalloyl derivatives could be proposed for the treatment of yeast infections, thereby potentially reducing the effects of high dosage of antibiotic exposure. This finding is of interest for its potential exploitation in pharmaceutical sciences.

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10. Experimental procedure for the preparation of α-7 and β-7 is as follows: To a solution of methylglucoside **5**, acid **1** (2.2 equiv), DMAP (1.1 equiv), and DMAP·HCl (1.1 equiv) in dry $CH_2Cl_2$, a solution of DCC (2.2 equiv) in dry $CH_2Cl_2$ was added. The mixture was purged with $N_2$ and stirred for 19 h at rt, then cooled at −18 °C for 30 min and filtered through celite. The organic phase was washed with HCl 3%, brine, and $H_2O$, dried over $Na_2SO_4$, and concentrated. Purification of the residue by flash chromatography (hexane/ethyl acetate = 10:1) afforded pure protected galloyl derivatives α-**6** (26%), β-**6** (16%) and a combination of α + β fraction (13%). To a solution of α-**6** (or β-**6**) in THF, a 1/1 mixture of *n*-$Bu_4NF$/acetic acid (3 equiv) was added and the mixture was stirred at rt for 5 h, then diluted with EtOAc, and poured into 1 M $H_3PO_3$. The organic phase was washed with $H_2O$ and brine, dried over $Na_2SO_4$, filtered and concentrated to provide the desilylated galloyl derivative which was hydrogenated over Pd/C 10% in THF. The hydrogenation was repeated twice to complete the deprotection. The gray–green glassy solid obtained was triturated with three portions of $Et_2O$ and hexanes and purified by a reverse phase C-18 preparative TLC. α-7. $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ (ppm): 3.47 (s, 3H), 3.62–3.71 (m, 2H), 3.76–3.79 (m, 1H), 4.28 (td, $J$ = 0.8, 6.4 Hz, 1H), 4.90 (s, 8H), 5.01–5.07 (m, 2H, H), 5.65–5.72 (m, 1H), 7.00 (s, 2H), 7.06 (s, 2H). $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ (ppm): 55.6, 67.9, 69.8, 73.6, 74.3, 98.6, 110.3, 110.4, 120.6, 121.4, 140.0, 140.1, 146.3, 146.4, 167.6, 168.2. MS *m*/*z* (%): 497(100, $M^-$), 345(65), 169(60), 125(9). β-7. $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ (ppm): 3.54 (s, 3H), 3.65–3.71 (m, 1H), 3.76–3.84 (m, 2H), 4.67 (d, $J$ = 8.0 Hz, 1H), 4.91 (s, 8H), 5.11 (dd, $J$ = 8.0, 9.6 Hz, 1H), 5.42 (dd, $J$ = 9.2 and 9.6 Hz, 1H), 6.98 (s, 2H), 7.02 (s, 2H). $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ (ppm) 57.3, 62.3, 69.8, 73.4, 77.0, 78.1, 103.2, 110.26, 110.32, 120.9, 121.1, 139.8, 139.9, 146.3, 146.34, 167.2, 167.9. MS *m*/*z* (%): 497(100, $M^-$), 345(65), 169(60), 125(9).
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