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Gallic Esters of Sucrose as a New Class of Antioxidants

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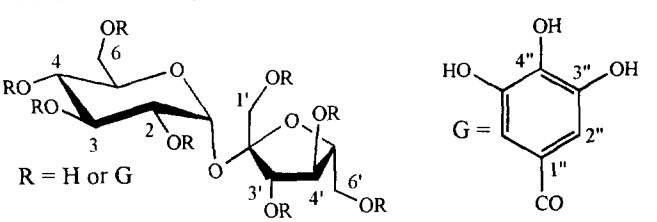
Abstract: 4 analogs of naturally occurring gallotannins have been synthesized upon acylation of sucrose by 2, 3, 6 and 8 galloyl groups. In a simple test using the DPPH radical, the antioxidant activity of such esters appears proportional to the number of the galloyl units.

Gallotannins are plant polyphenols displaying a polyol core acylated by gallic acid (3,4,5-trihydroxybenzoic acid) units.[1] They are intermediates in the biosynthesis of the much more complex ellagitannin family. The general ability of hydrolyzable tannins to bind proteins underlies their role in the traditional tannage of animal hides, the chemical defense of plants against herbivores and pathogenic microbes, the promising anticancer and antiviral properties of some ellagitannins... In addition, the galloyl units make hydrolyzable tannins efficient antioxidants able to trap reactive oxygen species (superoxide, hydroxy and alkylperoxy radicals, singlet dioxygen) typically involved in cardiovascular diseases and cancer.[2] Interestingly, the antioxidant efficiency in the galloylglucose series seems roughly proportional to the number of galloyl units. The most common polyol core of gallotannins is by far D-glucose although other monosaccharides (D-hamamelose, D-fructose, D-xylose) and non sugar polyols such as quinic and shikimic acids have also been found. Remarkably, five monogalloylsucroses (2-, 6-, 1'-, 4'- and 6'-galloylsucroses) were extracted from commercial rhubarbs produced in China and North Korea.[3]

In this paper, we report the straightforward chemical synthesis of 6,6'-digalloylsucrose (SG_2), 3',4',6'-trigalloysucrose (SG_3), 1',2,3,3',4',6'-hexagalloylsucrose (SG_6) and octagalloylsucrose (SG_8). The efficiency of



SG_2: R = G at O6 and O6' SG_3: R = G at O3', O4' and O6'

SG_6: R = G at O1', O2, O3, O3', O4' and O6' SG_8: R = G

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these gallotannin analogs in trapping radicals is then estimated and compared to that of methylgallate.

Synthesis. When a solution of sucrose and DMAP (2 equiv.) in DMF - NEt_3 (2:1) is slowly added with a solution of 3,4,5-tribenzylgalloylchloride (8 equiv.) in CH_2Cl_2 at 0°C and stirred overnight at room temperature, a 51% yield of protected **SG_8** is obtained after purification on silica gel. Debenzylation by hydrogenolysis in EtOH - THF (1:1) using Pd/C as a catalyst followed by purification on C_{18}-silica gel afforded **SG_8** as a pink powder (mass: 40 mg, yield: 97%).[4] Acylation by 3,4,5-tribenzylgallic acid using the DCC - DMAP method only resulted in complex mixtures of partially acylated products probably because of the relatively low reactivity of the 3,4,5-tribenzylgallic acid - DCC ester which could even be isolated and characterized. Digalloylation of sucrose by 3,4,5-tribenzylgallic acid (2.6 equiv.) was achieved in DMF under typical Mitsunobu conditions using PPh_3 (2.7 equiv.) and DIAD (2.8 equiv.). The protected 6,6'-digalloylsucrose, obtained in 35% yield after chromatography on silica gel, was then quantitatively debenzylated to give **SG_2** (mass: 60 mg).[4]

The 4,6-mono- and 1',2,4,6-diisopropylidene derivatives of sucrose[5] were acylated (NEt_3 - DMAP - DMF or CH_2Cl_2) by 6 and 4.5 equiv. of 3,4,5-tribenzylgalloylchloride, respectively. The 3-OH group of the diisopropylidene derivative did not react under these conditions. The protected trigalloyl- and hexagalloylsucrose derivatives were thus obtained in 52% and 48% yield, respectively. Selective acid hydrolysis of the isopropylidene groups was achieved in HBF_4 - H_2O - THF (1:5:500) at 35°C (yield: 70-75%). No significant hydrolysis of the glycosidic linkage occurred under these mild conditions. In the final step, hydrogenolysis of the benzyl groups and subsequent purification on C_{18}-silica gel afforded **SG_3** (mass: 40 mg) and **SG_6** (mass: 70 mg) in high yields (> 90%).[4]

Oxidation by DPPH. DPPH (diphenylpicrylhydrazyl) is a highly coloured commercially available radical widely used for a rough estimation of the ability of antioxidants to trap potentially damaging one-electron oxidants.[6] In particular, antioxidants can be characterized by their stoichiometry *i.e.* the number of DPPH molecules reduced by one molecule of antioxidant. In order to achieve a more quantitative description, the following simple model was used. An antioxidant of stoichiometry N is represented as N independent *sub*-units AH which all transfer a H atom to DPPH with the same second-order rate constant k. Hence, the following equations can be used in the curve-fitting of the kinetic traces featuring the decay of the DPPH visible absorption band:

$A=A_0\,[DPPH]/c_0$

$-d[AH]/dt = -d[DPPH]/dt = k[AH][DPPH]$

$C = Nc$

A: visible absorbance at time t, A_0: initial absorbance, c: initial antioxidant concentration, c_0: initial DPPH concentration, C: initial concentration of antioxidant *sub*-unit AH.

Large DPPH:galloyl unit ratios (4-7) were used in the experiments in order to obtain reliable values for the antioxidant stoichiometry. This procedure gave satisfying curve-fittings ($r > 0.99$, Scientist program, MicroMath, USA) and accurate values for N and k (Table 1). Values for the stoichiometry per galloyl unit (n) are also reported.

Values for N and n show that the galloyl units of the gallotannins synthesized in this work remain fully accessible to DPPH. The stoichiometry of these antioxidant grows in proportion of the number of galloyl units. No saturation, which could be due to steric hindrance, is observed. By contrast, the average rate constant is highly sensitive to the number of galloyl units. Such differences in the k values may reflect intra- and/or intermolecular stacking interactions (as well as possible hydrogen bonding) between the galloyl units. For instance, self-association of the most galloylated tannins is manifested by the significant shifts in the wavelength of absorption maximum (from 273 to 278 nm for SG_6, from 272 to 280 nm for SG_8) which are recorded when the tannin concentration is varied from 10^{-6} to 10^{-5} M in methanol.

Table 1. Efficiency of Methylgallate ($5x10^{-5}$ M) and the Gallic Esters of Sucrose ($5\text{-}25x10^{-6}$ M) in Trapping DPPH ($2x10^{-4}$ M) in Methanol at 25°C. For definition of k, N and n, *see text*.

antioxidant	methylgallate	SG_2	SG_3	SG_6	SG_8
k ($M^{-1} s^{-1}$)	1737 (± 60)	377 (± 6)	3589 (± 74)	270 (± 2)	2082 (± 52)
N	2.42 (± 0.02)	5.30 (± 0.03)	7.04 (± 0.02)	15.56 (± 0.03)	18.22 (± 0.07)
n	2.42	2.65	2.35	2.59	2.28

The gallotannins synthesized in this work from two cheap natural sources, gallic acid and sucrose, could be more efficient as antioxidants than naturally occurring gallotannins because of a larger number of reactive galloyl units in their structure. They could thereby find applications in pharmacology and the food industry.

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4. NMR signals were assigned from ^{1}H-^{1}H (COSY) and ^{1}H-^{13}C (HMQC, HMBC) correlations.

SG$_2$ ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 7.09 (2 H, *s*, H2'' on G unit at O6'); 7.04 (2 H, *s*, H2'' on G unit at O6); 5.45 (1 H, *d*, J = 3.6 Hz, H1); 4.97 (3H, H6'b, H6a, H6b); 4.55 (1 H, *d*, J = 12.1 Hz, H6'a); 4.23 (1H, *m*, H5); 4.20 (1H, *d*, J = 8.3 Hz, H3'); 4.15 (1H, *t*, J = 8.3 Hz, H4'); 4.07 (1H, *m*, H5'); 3.80 (1H, *t*, J = 9.3 Hz, H3); 3.68 (2H, *s*, H1'); 3.50 (1H, *dd*, J = 9.3, 3.6 Hz, H2); 3.41 (1H, *t*, J = 9.3 Hz, H4). ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm) = 168.7, 168.5 (C=O); 146.7 (C3''); 140.1 (C4''); 121.6 (C1''); 110.6 (C2''); 106.0 (C2'); 93.8 (C1); 81.2 (C5'); 79.1 (C3'); 77.4 (C4'); 74.9 (C3); 73.5 (C2); 72.4 (C5); 72.0 (C4); 67.4 (C6); 65.3 (C6'); 64.1 (C1'). Mass (FAB, positive mode): *m/z* = 669.1310 (MNa^+)

SG$_3$ ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 7.12 (2 H, *s*, H2'' on G unit at O3'); 7.09 (2 H, *s*, H2'' on G unit at O6'); 7.05 (2 H, *s*, H2'' on G unit at O4'); 5.91 (1H, *d*, J = 7.0 Hz, H3'); 5.82 (1H, *t*, J = 7.0 Hz, H4'); 5.55 (1H, *d*, J = 3.6 Hz, H1); 4.74 (1H, *dd*, J = 12.0, 7.3 Hz, H6'a); 4.67 (1H, *dd*, J = 12.0, 4.4 Hz, H6'b); 4.53 (1H, *ddd*, J = 7.3, 7.0, 4.4 Hz, H5'); 4.02 (1H, *m*, H5); 4.00 (1H, *d*, J = 11.5 Hz, H6a); 3.82 (1H, *dd*, J = 11.5, 3.9 Hz, H6b); 3.77 (1H, *d*, J = 12.5 Hz, H1'a); 3.72 (1H, *t*, J = 9.5 Hz, H4); 3.68 (1H, *d*, J = 12.5 Hz, H1'b); 3.53 (1H, *t*, J = 9.5 Hz, H3); 3.50 (1H, *dd*, J = 9.5, 3.6 Hz, H2). ^{13}C-NMR (125 MHz, CD_3OD): δ = 168.6-167.5 (C=O); 146.7 (C-3''); 140.4 (C4''); 120.9 (C1''); 110.7 (C2'');106.3 (C2'); 93.8 (C1); 80.4 (C5'); 77.7 (C3'); 77.5 (C4'); 74.9 (C4); 74.8 (C5); 73.3 (C2); 71.3 (C3); 66.5 (C6'); 64.8 (C1'); 62.4 (C6).
Mass (FAB, positive mode): *m/z* = 821.1411 (MNa^+)

SG$_6$ ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 7.19 (2 H, *s*, H2'' on G unit at O3'); 7.13 (2 H, *s*, H2'' on G unit at O6'); 7.06 (2 H, *s*, H2'' on G unit at O1'); 7.03 (2x2 H, 2*s*, H2'' on G units at O3 and O4'); 6.94 (2 H, *s*, H2'' on G unit at O2); 6.11 (1H, *d*, J = 3.5 Hz, H1); 6.04 (1H, *t*, J = 8.4 Hz, H4'); 5.94 (1H, *d*, J = 8.4 Hz, H3'); 5.82 (1H, *t*, J = 9.8 Hz, H3); 5.09 (1H, *dd*, J = 9.8, 3.5 Hz, H2); 4.71 (1H, *dd*, J = 12.3, 3.2 Hz, H6'a); 4.65 (1H, *dd*, J = 12.3, 6.1 Hz, H6'b); 4.55 (1H, *m*, H5'); 4.40 (1H, *d*, J = 11.9 Hz, H1'a); 4.25 (1H, *d*, J = 11.9 Hz, H1'b); 4.21 (1H, broad *d*, J = 9.8 Hz, H5); 4.07 (1H, *t*, J = 9.8 Hz, H4); 4.05 (1H, *d*, J = 12.1 Hz, H6a); 3.95 (1H, *dd*, J = 12.1, 2.8 Hz, H6b). ^{13}C-NMR (125 MHz, CD_3OD): δ = 169.0-167.6 (C=O); 146.6 (C3''); 140.6-140.2 (C4''); 121.3-120.5 (C1''); 110.7 (C2'');104.8 (C2'); 91.4 (C1); 79.6 (C5'); 77.0 (C3'); 75.6 (C4'); 75.2 (C5); 74.3 (C3); 72.7 (C2); 69.1 (C4); 65.8 (C6'); 65.5 (C1'); 61.7 (C6).
Mass (FAB, positive mode): *m/z* = 1277.1731 (MNa^+)

SG$_8$ ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 7.31 (2 H, *s*, H2'' on G unit at O3'); 7.14 (2 H, *s*, H2'' on G unit at O6); 7.06 (2 H, *s*, H2'' on G unit at O6'); 7.05, 7.04 (2x 2 H, 2*s*,. H2'' on G units at O1' and O4'); 6.95 (2 H, *s*, H2'' on G unit at O2); 6.90 (2 H, *s*, H2'' on G unit at O4); 6.87 (2 H, *s*, H2'' on G unit at O3); 6.10 (1H, *d*, J = 3.5 Hz, H1); 5.93 (1H, *t*, J = 10.0 Hz, H3); 5.91 (1H, *t*, J = 6.1 Hz, H4'); 5.85 (1H, *d*, J = 6.1 Hz, H3'); 5.70 (1H, *t*, J = 10.0 Hz, H4); 5.40 (1H, *dd*, J = 10.0, 3.5 Hz, H2); 4.73 (1H, *dd*, J = 13.9, 8.3 Hz, H6'a); 4.67 (1H, broad *d*, J = 13.9 Hz, H6'b); 4.62 - 4.60 (3H, H5', H5, H6a); 4.52 (1H, *d*, J = 11.8 Hz, H1'a); 4.42 (1H, *d*, J = 13.0 Hz, H6b); 4.39 (1H, *d*, J = 11.8 Hz, H1'b). ^{13}C-NMR (125 MHz, CD_3OD): δ = 168.4-167.2 (C=O); 146.9-146.5 (C3''); 140.9-140.2 (C4''); 121.5-120.4 (C1''); 110.7 (C2''); 105.9 (C2'); 92.3 (C1); 80.7 (C5'); 78.1 (C3'); 77.3 (C4'); 72.3 (C2, C3); 70.9 (C5); 69.7 (C4); 66.3 (C1'); 65.9 (C6'); 63.0 (C6).
Mass (FAB, positive mode): *m/z* = 1581.1851 (MNa^+)

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