Synthesis and Antimalarial Activity of Mallatojaponin C and Related Compounds

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Supporting Information

ABSTRACT: The phloroglucinol mallotojaponin C (1) from Mallotus oppositifolius, which was previously shown by us to have both antiplasmodial and cytoidal activities against the malaria parasite Plasmodium falciparum, was synthesized in three steps from 2,4,6-trihydroxyacetophenone, and various derivatives were synthesized in an attempt to improve the bioactivity of this class of compounds. Two derivatives, the simple prenylated phloroglucinols 12 and 13, were found to have comparable antiplasmodial activities to that of mallotojaponin C.

The phloroglucinol mallotojaponin C (1) was first isolated from Mallotus oppositifolius Muell. Arg., a member of the Euphorbiaceae family, and was shown to have both cytostatic and cytoidal activity against the chloroquine/mefloquine-resistant Dd2 strain of Plasmodium falciparum. The related compounds methylated mallotojaponin C (2), mallotophenone (3), and mallotojaponin B (4) were also obtained, but had less potent antimalarial activity than 1, indicating the importance of the alkenyl side chain and the phenolic hydroxy substituents at C-2 and C-6 for antiplasmodial activity. Many monomeric phloroglucinols have been investigated for their antimalarial activity, and some additional dimeric compounds from Mallotus oppositifolius have been recently shown to have trypanocidal and antileishmanial activities. This paper describes the syntheses and biological evaluations of mallotojaponin C and various derivatives that were reported in 2015 in a Ph.D. dissertation and complements the results in an excellent recent paper by Cariou and Dubois et al.

RESULTS AND DISCUSSION

Synthesis of Mallotojaponin C and Analogues. The prenylated monomeric phloroglucinols 11−14 and 16−18 (Scheme 1) were synthesized by prenylation of either 2,4,6-trihydroxyacetophenone (15) or 2,6-dihydroxy-4-methoxyacetophenone (10) (Scheme 1). Prenylation of the precursors 10 and 15 was achieved by reaction of the phenol with either sodium hydroxide or lithium hydroxide and the appropriate alkenyl halide in 80% aqueous ethanol at room temperature; the solvent was chosen based on a previous study of the effect of solvent on the C-alkylation of phloroglucinols and on trials of various solvents for the synthesis of 11,16−18 The use of sodium hydroxide led to higher yields of 11, 12, 16, and 17 than the use of lithium hydroxide. Compounds 11,16−18,19,20 have previously been synthesized, and their structures were confirmed by MS and by comparison of their 1H and 13C NMR spectra with previously published spectra. Thus, treatment of 10 with 3,3-dimethylallyl bromide (prenyl bromide) in 80% aqueous ethanol gave the monoprenylated product 11 in 33% yield. Alkylation of 10 with geranyl bromide and farnesyl bromide under the same conditions yielded the corresponding geranylated and farnesylated products 12 and 13, with the yields decreasing with increasing chain length. Purification of the final product was carried out in each case by reversed-phase chromatography over C18-silica gel. Purification of 11 over silica gel in the presence of 0.1% formic acid yielded the hydroxylated product 14.

Alkylation of 15 with prenyl, geranyl, and farnesyl bromides gave the alkylated derivatives 16−18 in low to moderate yields. The synthesis of dimeric phloroglucinols such as mallotojaponin C (1) required the coupling of the monomeric...
compounds 10–12, 14, and 16−17. The initial coupling conditions used were based upon previous work with similar substrates,21−23 but it was apparent that the alkene moiety was readily hydrated under the standard acidic conditions. Most of the previously published work was on derivatives not containing an alkene moiety, so this problem did not arise in these cases. The coupling conditions were thus optimized by a study of synthetic conditions for compound 8, and various solvents, catalysts, and reaction durations were investigated. The best conditions were found to be the use of acetonitrile as solvent and a small amount of concentrated sulfuric acid as catalyst to reduce hydration of the alkene. These conditions allowed the reaction to be completed in less than 30 min, and this short reaction time also reduced the amount of hydration. Although the reaction yield was low, the desired product could easily be purified. These conditions were then applied to the synthesis of mallatojaponin C (1) and compounds 5−9. The latter compounds were prepared in an attempt to improve upon the antiparasitic activity of the coupled compounds and specifically to investigate the effect of the length of the alkenyl chain and the substituent at C-5 on the bioactivity of the resulting compounds. In the case of the known compound 1 the structure was confirmed by comparison of spectroscopic data with the published data,2 and the structures of the other compounds were established by analysis of their spectroscopic data.

The synthesis of other derivatives was attempted by replacing formaldehyde with various aldehydes. The synthesis of the p-fluorophenyl derivative 19 initially appeared to be successful, as judged by its 1H NMR spectrum, consistent with its assigned structure, and the compound showed promising bioactivity, with an IC50 value in the 2−4 μM range. Unfortunately, the compound proved to be unstable on storage in the freezer and decomposed before MS data could be obtained and an accurate IC50 value could be determined. Attempts to synthesize other proposed derivatives were unsuccessful, presumably because of the stability of the secondary benzylic carbocation that would be formed in an acid-catalyzed retrocoupling reaction.
The potencies against the two different strains of *P. falciparum* show similar trends, but the Dd2 strain is more sensitive to these compounds than the FcB1/Columbia strain.

For the dimeric phloroglucinols (1, 5–9), it was found that the prenyl side chain maximized the bioactivity, with the bis-prenylated natural product 1 having the best potency. Both the monoprenylated compound 4 and the bisgeranylated compound 6 were less potent than 1 against the Dd2 strain. Methylation at the 4-position is also very important. Thus, the bisgeranyl compound 6, with methylation at both the 4- and 4′-hydroxy groups, has an *IC₅₀* value of 1.7 ± 0.8 μM, while the corresponding unmethylated compound 8 is much less potent, with an *IC₅₀* value greater than 20 μM. This difference may be partly due to polarity, since the hydrated derivative 7 is much less active than the unhydrated natural product 1.

The situation is somewhat different with the monomeric phloroglucinol derivatives. With these compounds potency increases as the alkynyl side chain increases in length from prenyl to geranyl to farnesyl in both the 4- and 4'-methylated series (compounds 11–13) and the unmethylated series (compounds 16–18). As with the dimeric phloroglucinols, the compounds in the 4-methylated series are more potent than those in the unmethylated series, but the differences are smaller. Thus, the unmethylated farnesyl compound 18 is only about 9-fold less potent than its methylated analogue 13.

Finally, the excellent potencies of the monomeric compounds 12 and 13 are worth noting. Both compounds show submicromolar activities, with the farnesylated compound 13 having an *IC₅₀* value of 0.56 μM against the Dd2 strain, comparable to the dimeric lead compound 1. It is thus conceivable that further explorations in this chemical space could yield relatively simple compounds with significant antiparasitic activities.

### Experimental Section

#### General Experimental Procedures

Melting points were obtained using a Büchi B-540 melting point apparatus. UV–vis spectra were recorded on a Shimadzu UV-1201 spectrophotometer. NMR spectra were recorded on Varian 400 or Bruker Avance 500 MHz spectrometers. Mass spectra were obtained with an Agilent 6220 LC-TOF-MS. Preparatory HPLC separations were performed using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A controller, and a Varian Dynamax C18 column (250 × 10 mm). Semi-preparative HPLC separations were performed using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A system controller, and a Phenomenex Luna C18 column (250 × 10 mm). Bioactive compounds were checked for purity by analytical HPLC analysis using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A system controller, and a Phenomenex Luna C18 column (250 × 4.6 mm). Compounds 10 and 15 were purchased from Indofine Chemical Company and tested for bioactivity without further purification. Compounds 2 and 3 were isolated from *M. oppositifolius*, and compound 4 was previously synthesized from 1. Geranyl bromide was purchased from Alfa Aesar, and prenyl and farnesyl bromides were purchased from Sigma-Aldrich.

#### Standard Procedure A for Synthesis of Alkylated Phloroglucinols 11–14 and 16–18.

The following general procedure was used. Compound 10 or 15 (1 equiv) was dissolved in a solution of 80% EOH (40 mL) containing LiOH or NaOH (2 equiv). Prenyl, geranyl, or farnesyl bromide (1.1 equiv) was added, and the reaction mixture was stirred for 16 h at rt. The reaction mixture was acidified with 3 N HCl and extracted with CH₂Cl₂ (3 × 50 mL). The organic solution was washed with saturated aqueous NaCl (50 mL) and dried with MgSO₄, and the solvent was removed under reduced pressure. The residue was purified utilizing C₁₈ HPLC (MeOH/H₂O gradient with 0.1% formic acid) to yield the alkylated phloroglucinol (11–14, 16–18).

#### Synthesis of 11. Standard procedure A was used with NaOH to convert 10 (495 mg, 2.72 mmol) to 11 (228 mg, 0.91 mmol, 33%).

#### Compound 11: UV (MeOH) *λ*<sub>max</sub> (log ε) 215 (4.06), 288 (4.09), 333 (3.36) nm; *¹H* NMR (MeOH-d₄, 400 MHz) δ 1.60 (3H, s, H-5'), 1.71 (3H, s, H-4'), 2.60 (3H, s, -COCH₃), 3.15 (2H, d, *J* = 7.2 Hz, H-1'), 3.78 (3H, s, -OCH₃), 5.10 (1H, m, H-2'), 5.96 (1H, s, H-5); *¹³C* NMR (MeOH-d₄, 100 MHz) δ 17.8, 22.0, 55.8, 90.9, 106.0, 108.9, 124.3, 131.1, 162.3, 163.5, 165.0, 205.0; HRESIMS [M + H]<sup>+</sup> *m/z* 251.1274 (calcld for C₁₀H₁₇O₄Na⁺); [M + Na]<sup>+</sup> *m/z* 273.1114 (calcld for C₁₀H₁₇NaO₄+).

#### Synthesis of 12. Standard procedure A was used with LiOH as base to convert 10 (150 mg, 0.82 mmol) to 12 (78 mg, 0.25 mmol, 30%). When NaOH was used as base, the yield of 12 from 100 mg (0.55 mmol) of 10 was 37 mg (0.12 mmol, 22%).

#### Compound 12: UV (MeOH) *λ*<sub>max</sub> (log ε) 216 (4.16), 289 (4.14), 333 (3.38) nm; *¹H* NMR (MeOH-d₄, 400 MHz) δ 1.60 (3H, s, H-5'), 1.87 (3H, s, H-10'), 1.87 (3H, s, H-9'), 2.08 (4H, H-4', H-5'); 2.67 (3H, s, -COCH₃), 3.33 (2H, d, *J* = 7.0 Hz, H-1'), 3.81 (3H, s, -OCH₃), 5.06 (1H, m, H-6'), 5.19 (1H, m, H-6), 6.02 (1H, s, H-5); *¹³C* NMR (MeOH-d₄, 100 MHz) δ 16.2, 17.7, 21.4, 25.7, 26.3, 32.9, 39.7, 55.7, 91.8, 105.4, 121.6, 123.7, 131.9, 163.1, 203.7; HRESIMS [M + H]<sup>+</sup> *m/z* 319.1896 (calcld for C₁₉H₂₇O₄Na⁺); [M + Na]<sup>+</sup> *m/z* 341.1707 (calcld for C₁₉H₂₇NaO₄+).

#### Synthesis of 13. Standard procedure A was used with LiOH as base. The yield of 13 from 100 mg (0.55 mmol) of 10 was 61 mg (0.16 mmol, 29%).

#### Compound 13: UV (MeOH) *λ*<sub>max</sub> (log ε) 213 (4.21), 288 (4.14), 333 (3.38) nm; *¹H* NMR (MeOH-d₄, 400 MHz) δ 1.60 (3H, s, H-10'), 1.67 (3H, s, H-9'), 1.87 (3H, s, H-8'), 2.08 (4H, H-4', H-5'); 2.67 (3H, s, -COCH₃), 3.33 (2H, d, *J* = 7.0 Hz H-1'), 3.81 (3H, s, -OCH₃), 5.06 (1H, m, H-6'), 5.19 (1H, m, H-6), 6.02 (1H, s, H-5); *¹³C* NMR (MeOH-d₄, 100 MHz) δ 16.2, 17.7, 21.4, 25.7, 26.3, 32.9, 39.7, 55.7, 91.8, 105.4, 121.6, 123.7, 131.9, 163.1, 203.7; HRESIMS [M + H]<sup>+</sup> *m/z* 387.2535 (calcld for C₂₃H₃₆O₄⁺); [M + Na]<sup>+</sup> *m/z* 409.2346 (calcld for C₂₃H₃₆NaO₄-).
Hg, H-1), 5.16 (1H, m, H-2), 3.79 (2H, s, H-5a), 5.05 (2H, m, H-6, H-10), 3.15 (4H, d, HCOCH3), 2.67 (2H, s, H-3), 1.59 (6H, s, H-4), 1.68 (2H, m, H-9), 1.59 (6H, s, H-5a). 13C NMR (CDCl3, 75 MHz) δ 16.7, 17.0, 25.7, 26.2, 32.8, 39.7, 94.7, 104.8, 105.9, 107.9, 124.8, 131.1, 132.4, 133.0, 134.3, 160.4, 162.5, 163.4, 203.1; HRESIMS [M + H]+ m/z 399.1050 (calcd for C23H33O8Na+ 399.2369). Synthesis of Mallotojaponin C (1). Standard procedure B was used. With LiOH as base. The yield of 1 from 253 mg (1.5 mmol) of 15 was 65 mg (0.043 mmol, 27%).

**Compounds 18 and 19.** Light yellow powder; UV (MeOH) λmax (log ε) 214 (4.21), 291 (4.19) nm; 1H NMR (CD3OD, 400 MHz) δ 1.54 (3H, s, H-15′), 1.56 (3H, s, H-14′), 1.65 (3H, s, H-13′), 1.78 (3H, s, H-12′), 1.97 (8H, m, H-3′, H-5’-15′), 2.89 (1H, m, H-6′), 2.95 (3H, s, COCH3), 3.18 (2H, d, J = 7.1 Hz, H-1′), 3.29 (1H, m, H-4′), 4.84 (3H, s, H-5a), 5.71 (1H, m, H-2′, 6′), 5.89 (1H, d, J = 7.1 Hz, H-1′). 13C NMR (CDCl3, 100 MHz) δ 14.7, 14.9, 16.2, 20.3, 24.6, 26.0, 26.3, 31.4, 39.4, 39.4, 93.3, 104.1, 106.5, 123.3, 123.9, 124.1, 130.0, 133.0, 134.0, 140.6, 162.4, 162.5, 204.3, 201.3; HRESIMS [M + H]+ m/z 373.2366 (calcd for C27H33O8Na+, 373.2733) [M + Na]+ m/z 395.2169 (calcd for C28H35NaO8Na+ 395.2193).

**Synthesis of Mallotojaponin C (1).** Standard procedure B was used with 3 mL of CH3CN and HCl instead of H2SO4. The yield of 1 was 53.7 mg (0.011 mmol, 11%). Attempts at recrystallizing the product in MeOH/H2O were unsuccessful and resulted in decomposition before MS data could be obtained.

**Compound 22.** UV (MeOH) λ max (log ε) 208 (4.27), 230 (4.17), 292 (4.11) nm; 1H NMR (CDCl3, 400 MHz) δ 1.60 (6H, s, H-1′, H-10′), 1.68 (6H, s, H-9′, H-9), 1.83 (6H, s, H-8′, H-8), 2.11 (8H, m, H-4′, H-4′-15′), 2.67 (2 × COCH3, 6H, s), 3.41 (4H, d, J = 6.7 Hz, H-1′-15′), 3.79 (2H, s, H-5a), 5.05 (2H, m, H-6, H-10), 5.21 (2H, m, H-2′, H-2′), 16.2, 17.7, 25.6, 25.7, 32.7, 39.6, 39.7, 93.9, 105.7, 121.4, 123.4, 132.2, 140.7, 204.2.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00347.

*1H and 13C NMR spectra of compounds 5–9, 11–14, and 16–18, 1H NMR spectra of 1 and 5, and HPLC chromatograms of 11–13 and 16–18 (PDF)*

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**Notes**

The authors declare no competing financial interest.

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