The first total synthesis of calabricoside A

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Abstract—Quercetin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl(1→2)]-7-O-β-D-glucopyranoside (calabricoside A), a new flavonol triglycoside isolated from the aerial parts of Putoria calabrica showing strong radical scavenging activity, was synthesized through a combination of phase-transfer-catalyzed C-3 glycosylation and AgOTf promoted homogeneous C-7 glycosylation in CH2Cl2.

Flavonoid and other polyphenol glycosides are widespread ubiquitous natural product from fruits, vegetables, red wines and teas. Many flavonoids show biological activities important in the growth and development of plants, and more interestingly, represent potential drug candidates having antimicrobial, anticancer and antioxidant properties. Polyphenol-rich diets are often advocated to lower the risk of developing cardiovascular diseases and cancers and some of flavonoid glycosides are currently used for the treatment of various vascular diseases. Despite of the wide occurrence and biological importance of flavonol and other polyphenol glycosides, synthetic efforts towards efficient preparation of this group of natural products are surprisingly rarely reported. The major challenge for the synthesis of these flavonoid glycosides is that standard high yielding glycosylation reactions, catalyzed by trimethylsilyl triflate (TMSOTf), boron trifluoride etherate (BF3·Et2O) or N-iodo succinimide (NIS), are not generally applicable for the formation of C-3 glycosylated products, as many of these, such as catechin, are very sensitive in acidic medium. Furthermore, regioselective glycosylation, especially multi-glycosylation of flavonoids or polyphenols, is often complicated by low yields and multiple stereo chemical outcomes.

Scheme 1. Retrosynthetic analysis.

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Calabricoside A (quercetin 3-O-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside)-7-O-β-D-glucopyranoside), a new flavonol triglycoside isolated from the aerial parts of *Putoria calabrica*, shows strong radical scavenging activity with an IC₅₀ value of 0.25 μM determined by formyl-methionyl-leucyl-phenylalanin (FMLP) stimulated human polymorphonuclear neutrophils (PMNs). Here we describe the first total synthesis of calabricoside A.

We envisioned that calabricoside A (1) could be constructed from the easily prepared glucosyl donor 2, suitably 7,4'-methoxybenzyl (PMB) ether protected quercetin 4 and a disaccharide donor 5 (Scheme 1).

Commercially available quercetin was converted into 7,4'-di-O-methoxybenzylated 4 in three steps and in 31% overall yield, employing a similar procedure to that developed by Jurd, i.e. acetylation of quercetin with acetic anhydride in pyridine; regioselective benzylation of C-7 and C-4' with 4-methoxybenzyl chloride and K₂CO₃ in refluxing acetone; and deacetylation with 10% aqueous NaOH. Disaccharide bromide 5 was prepared through conventional glycosylation and protecting group manipulation (Scheme 2). To this end, rhamnopyranosyl trichloroacetimidate 6 was condensed with allyl 3,4-di-O-isopropylidene-β-L-arabinopyranoside (7) giving disaccharide 8 in 92% yield. The isopropylidene of 8 was readily cleaved using 80% HOAc in THF at 50°C (→9), the free hydroxyl groups were then acetylated with acetic anhydride in pyridine (→10). Removal of allyl group from 10 was carried out smoothly with PdCl₂ in 90% HOAc–NaOAc system to give hemiacetal 11 in excellent yield (90% from 8). We then tried to convert 11 to 5 via acetylation (Ac₂O in pyridine) and bromination (HBr in HOAc). However, bromination appeared to be very slow and by-products were observed on TLC when the starting material had been completely consumed, thus only a modest yield of 5 was obtained. To improve the synthesis of 5, hemiacetal 11 was first transformed into 4-nitrobenzoyl derivative 12 with 4-nitrobenzoyl chloride and PMB in acetonitrile in good yield (Scheme 2).

**Scheme 2.** Reagents and conditions: (a) TMSOTf, CH₂Cl₂, 92%; (b) 80% HOAc, THF, 50°C; (c) Ac₂O, Pyr; (d) PdCl₂, 90% HOAc, NaOAc, 90% from 8; (e) p-NO₂BzCl, Pyr; (f) HBr, HOAc, 87% from 11; (g) 0.15 M aq. K₂CO₃, CHCl₃, TBAB, 50°C, >78% for 13; 11% for 17; (h) Pd(OH)₂-C, H₂, 93%; (i) K₂CO₃, DMF, 6%; (j) AgOTf, CH₂Cl₂, 52%; (k) NaOMe, MeOH, 91%.
ride in pyridine, then subjected to bromination, with HBr in HOAc, affording disaccharide bromide 5 was thus furnished in 87% yield from 11. Phase-transfer-catalyzed (PTC)\textsuperscript{8,10} coupling reaction of 5 and 4 was first conducted in 1.25 M aqueous KOH–CHCl\textsubscript{3} system at 65°C, gave 13 in 30% yield. Encouraged by a recent report from Han and Yu\textsuperscript{9} 5 and 4 was condensed in 0.15 M aqueous K\textsubscript{2}CO\textsubscript{3}–CHCl\textsubscript{3} system in the presence of tetrabutylammonium bromide (TBAB) at 50°C giving the desired 13 in >78% isolation yield. The proton coupling (J=6.7 Hz) observed for the anomeric proton of arabinosyl H-1 (δ 5.76 ppm) demonstrates that the disaccharide has the expected α-linkage to the quercetin aglycone.\textsuperscript{18} Moreover, the cross peak from arabinosyl H-1 (δ 5.76 ppm) to quercetin C-3 (δ 135.6 ppm) in the HMBC spectra confirmed that the glycosylation took place at the C-3 position of quercetin. No β-anomer was detected under these glycosylation conditions. After acetylation of remaining 5,3'-diol of 13 (+14), 7,4'-methoxybenzyl groups were cleanly removed by hydrogenation over 20% Pd(OH)\textsubscript{2} on-charcoal at 1 atm pressure. Surprisingly, an inseparable mixture (3:1 based on \textsuperscript{1}H NMR spectra) was isolated in 93% yield. Acetylation of this mixture affording a single compound suggesting that the mixture came from 3,4'-acetyl migration in the quercetin residue. The \textsuperscript{1}H NMR spectra showed H-5' at 6.62 ppm in major fraction while at 7.07 ppm in the minor one, indicating 4'-OAc component 16 is the dominant in the mixture. It was reported that the glycosylation or sulfonation of the catechol moiety makes the residual H-atom (OH-C(3') or OH-C(4')) less reactive.\textsuperscript{5} We took advantage of this report to directly glycosylate 16/15 with donor 2a under the PTC conditions as described in the preparation of 13, affording the mixture 17, which was acetylated giving 18 in a yield of 11%. We rationalized that the poor solubility of 2a and 16/15 in this two-phase solvent system might be responsible for the low glycosylation yield. Thus, the same reaction was conducted in dry DMF with anhydrous K\textsubscript{2}CO\textsubscript{3} as base at rt. However, much lower yield of 18 (6%) was obtained, probably due to the easy loss of acetyl groups from flavonoid under these coupling conditions.\textsuperscript{19} This reaction could be significantly improved by condensing trichloroacetimidate donor 2b and 16/15 in CH\textsubscript{2}Cl\textsubscript{2} using 1 equiv. of AgOTf catalyst, furnishing 18 in 52% yield after acetylation. The observed J\textsubscript{1,2} value (8.4 Hz) for the glucose residue in \textsuperscript{1}H NMR spectra and the cross peak from glucose H-1\textsuperscript{H} (5.31 ppm) to quercetin C-7 (162.5 ppm) in the HMBC spectrum clearly indicates α-glycosylation took place at C-7. Finally, removal of all acyl protection groups using catalytic amount of NaOMe in MeOH followed by purification on Sephadex LH20 using MeOH as eluent furnished target molecule 1 (91%). All the data recorded for 1 were identical to those previously reported.\textsuperscript{13}

In summary, we have described the first total synthesis of calabricoside A from 7,4'-di-O-methoxybenzyl quercetin in 12 steps and 24.7% overall yield. The phase transfer catalyzed glycosylation of quercetin C-3 was proved to be very efficient using 0.15 M aqueous K\textsubscript{2}CO\textsubscript{3} and 1 equiv. of TBAB in chloroform at 50°C, while further glycosylation of C-7 was more practical in CH\textsubscript{2}Cl\textsubscript{2} using trichloroacetimidate as donor and AgOTf as catalyst. The present exploration should be valuable to the preparation of multi-glycosylated flavonol glycosides.\textsuperscript{21,22}

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**References**


19. Physical data for some new compounds. 8: [\(\alpha\)]\textsubscript{D}\textsuperscript{5} = +139° (c 1, CHCl\textsubscript{3}), 1.33 (d, 3 H, J 6.2 Hz, H-6\textsuperscript{H}), 1.37, 1.55 (2 s, 6 H, 2 CH\textsubscript{3}), 3.87 (dd, 1 H, J 3.4, 8.0 Hz, H-2\textsuperscript{H}), 4.00–4.07 (m, 3 H), 4.22–4.31 (m, 3 H), 4.44 (dd, 1 H, J 5.6, 8.0 Hz, H-3\textsuperscript{H}), 4.98 (d, 1 H, J 3.4 Hz, H-1\textsuperscript{H}), 5.27–5.31 (m, 1 H, CH\textsubscript{3}–CH=CH-C=CH-C=CH\textsubscript{2}), 5.33 (d, 1 H, J 1.6, H-1\textsuperscript{H}), 5.41–5.46 (dd, 1 H, CH\textsubscript{3}–CH=CH-C=CH-C=CH\textsubscript{2}), 5.64 (t, 1 H, J 10.0 Hz, H-4\textsuperscript{H}), 5.79 (dd, 1 H, J 1.6, 3.5 Hz, H-2\textsuperscript{H}), 5.88 (dd, 1 H, J 3.5, 10.0 Hz, H-3\textsuperscript{H}), 5.96–6.06 (m, 1 H, CH\textsubscript{3}–CH=CH-C=CH-C=CH\textsubscript{2}), 7.24–8.11 (m, 15 H, Ph). 13: [\(\alpha\)]\textsubscript{D}\textsuperscript{5} = –23° (c 0.3, CHCl\textsubscript{3}), 1.12 (d, 3 H, J 6.4 Hz, H-6\textsuperscript{H}), 2.12, 2.23 (2 s, 6 H, 2 Ac), 3.60 (dd, 1 H,
$J$ 1.5, 12.9 Hz, H-5a), 3.82, 3.83 (2 s, 6 H, 2 CH$_3$O), 3.86 (dd, 1 H, J 3.2, 12.9 Hz, H-5b), 4.28 (dd, 1 H, J 6.7, 9.6 Hz, H-2), 4.68–4.73 (m, 1 H, H-3II), 5.06 (s, 2 H, MeOPhCH$_3$), 5.08, 5.12 (2 d, 2 H, J 12.8 Hz, MeOPhCH$_3$), 5.20 (dd, 1 H, J 9.6, 3.5 Hz, H-3I), 5.25–5.28 (m, 1 H, H-4), 5.35 (d, 1 H, J 1.1 Hz, H-1I), 5.60 (dd, 1 H, J 1.1 Hz, H-2I), 5.66 (t, 1 H, J 9.6 Hz, H-4II), 5.73 (br s, 1 H, 3-0H), 5.76 (d, 1 H, J 6.7 Hz, H-1I), 5.97 (dd, 1 H, J 3.3, 9.6 Hz, H-4I), 6.45 (d, 1 H, J 2.1 Hz, H-6), 6.50 (d, 1 H, J 2.1 Hz, H-8), 6.92–6.96 (m, 4 H, Ar), 7.02 (d, 1 H, J 9.5 Hz, H-5), 7.23–7.60 (m, 13 H, Ar), 7.67 (dd, 1 H, J 2.0, 9.5 Hz, H-6'), 7.84–8.10 (m, 7 H, H-2' and Ar), 12.65 (s, 1 H, 5-OH). MALDITOF-MS Calcd for C$_{67}$H$_{67}$O$_{32}$: 1216.36; Found 1239 (M$+$Na)$^+$. 16: 1.26 (d, 3 H, J 6.3 Hz, H-6III), 2.07, 2.23, 2.28, 2.50 (4 s, 12 H, 4 Ac), 3.66 (dd, 1 H, J 1.0, 12.4 Hz, H-5a$^-$), 3.85 (dd, 1 H, J 2.1, 12.4 Hz, H-5b$^-$), 4.24 (dd, 1 H, J 7.0, 9.3 Hz, H-2$^-$), 4.78, 4.85 (m, 1 H, H-5IV), 5.22 (dd, 1 H, J 3.5, 9.3 Hz, H-3$^-$), 5.27 (br s, 1 H, H-4$^-$), 5.37 (d, 1 H, J 1.0 Hz, H-1IV), 5.63 (dd, 1 H, J 1.0, 3.3 Hz, H-2IV), 5.71 (d, 1 H, J 7.0 Hz, H-1$^-$), 5.73 (t, 1 H, J 10.1 Hz, H-4$^-$), 6.02 (dd, 1 H, J 3.3, 10.1 Hz, H-3$^-$), 6.37 (d, 1 H, J 1.9 Hz, H-6), 6.50 (d, 1 H, J 1.9 Hz, H-8), 6.62 (d, 1 H, J 8.4 Hz, H-5$^-$), 7.24–8.13 (m, 17 H, H-2',6' and Ar). MALDITOF-MS Calcd for C$_{67}$H$_{67}$O$_{32}$: 1060.26; Found 1083 (M$+$Na)$^+$. 18: $[a]_D^{25}$ +38° (c 1.6, CHCl$_3$), 1.15 (d, 3 H, J 6.3 Hz, H-6III), 2.03, 2.05, 2.06, 2.07, 2.08, 2.21, 2.27, 2.34, 2.47 (9 s, 27 H, 9 Ac), 3.55 (dd, 1 H, J 1.0, 12.9 Hz, H-5a$^-$), 3.78 (dd, 1 H, J 3.0, 12.9 Hz, H-5b$^-$), 3.91–3.94 (m, 1 H, H-5III), 4.11 (dd, 1 H, J 7.1, 9.2 Hz, H-2$^-$), 4.15 (dd, 1 H, J 2.4, 12.4 Hz, H-6aIII), 4.30 (dd, 1 H, J 5.0, 12.4 Hz, H-6bIII), 4.70–4.75 (m, 1 H, H-5IV), 5.14–5.20 (m, 3 H, H-2IV, H-4IV, H-3$^-$), 5.23 (br s, 1 H, H-4$^-$), 5.29 (d, 1 H, J 1.3 Hz, H-1IV), 5.31 (d, 1 H, J 8.4 Hz, H-1III), 5.34 (t, 1 H, J 9.3 Hz, H-3III), 5.56–5.59 (m, 2 H, J 7.1, 1.3, 3.6 Hz, H-1$^-$ and H-2$^-$), 5.69 (t, 1 H, J 10.0 Hz, H-4IV), 5.96 (dd, 1 H, J 3.6, 10.0 Hz, H-3IV), 6.85 (d, 1 H, J 2.1 Hz, H-6), 7.10–8.11 (m, 19 H, H-8, H-5, H-2', H-6' and Ar). MALDITOF-MS Calcd for C$_{71}$H$_{68}$O$_{32}$: 1432.4; Found 1455.3 (M$+$Na)$^+$. I: $[a]_D^{21}$ −129° (c 1, MeOH); 1.07 (d, 1 H), 3.35–3.55 (m, 6 H), 3.68–3.95 (m, 8 H), 4.12 (dd, 1 H, J 6.3, 7.4 Hz, H-2IV), 5.06 (d, 1 H, J 8.0 Hz, H-1IV), 5.10 (br s, 1 H, H-1IV), 5.59 (d, 1 H, J 6.3 Hz, H-1$^-$), 6.48, 6.74 (2 d, 2 H, J 1.6 Hz, H-6, H-8), 6.90 (d, 1 H, J 8.3 Hz, H-5), 7.59 (dd, 1 H, J 2.0, 8.3 Hz, H-6'), 7.66 (d, 1 H, J 2.0 Hz, H-2'); $^{13}$C NMR (CD$_3$OD): 17.4, 62.1, 65.5, 68.5, 70.0, 71.2, 72.3 (2 C), 73.0, 74.0, 74.5, 77.1, 77.8, 78.2, 96.2, 100.9, 101.3, 101.8, 102.4, 107.5, 116.4, 117.0, 123.1 (2 C), 135.5, 146.1, 150.0, 157.9, 158.6, 163.0, 164.8, 179.7. MALDITOF-MS Calcd for C$_{152}$H$_{178}$O$_{120}$: 742.2; Found 765 (M$+$Na)$^+$, 781 (M$+$K)$^+$.