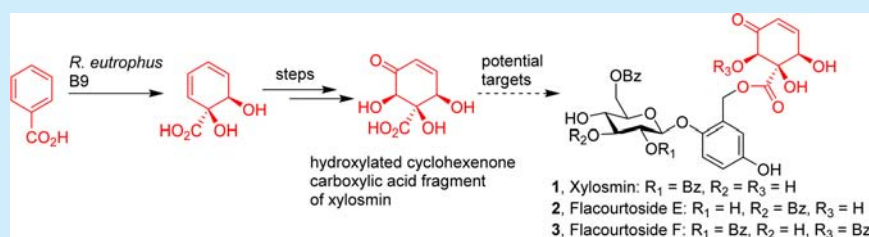


A Formal Approach to Xylosmin and Flacourtosides E and F: Chemoenzymatic Total Synthesis of the Hydroxylated Cyclohexenone Carboxylic Acid Moiety of Xylosmin

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S Supporting Information



ABSTRACT: The hydroxylated cyclohexenone carboxylic acid moiety of xylosmin was synthesized in eight steps from benzoic acid. The key steps in the synthesis involved the enzymatic dihydroxylation of benzoic acid by the whole cell fermentation with *Ralstonia eutrophus* B9, and Henbest epoxidation. Early attempts led to the synthesis of a C6 epimer of the methyl ester of the hydroxylated cyclohexenone carboxylic acid moiety. The absolute stereochemistry of an advanced intermediate was confirmed by X-ray crystallography. Complete characterization of the previously reported but not fully characterized hydroxylated cyclohexenone carboxylic acid is provided.

Chikungunya and dengue viruses infect roughly 500 million people per year, predominantly in the tropical zone of the eastern hemisphere. Chikungunya and dengue fevers are generally characterized by symptoms such as headaches, fever, fatigue, vomiting, nausea, skin rashes, and blood-thinning and can be fatal.^{1–3}

No effective vaccines are available, but broad-spectrum antivirals have had some effect in tackling the overarching virus, although the greatest levels of success have been observed in addressing the associated symptoms, usually through either retroactive or preventative measures. Phenolic glycosides, found in the *flacourtia ramontchi* plant of Madagascar, have shown replicative inhibition against these arboviral diseases. Inhibitory plant extracts containing glycosylated derivatives of 2-hydroxymethylhydroquinone as a common motif have been shown to be effective against these viral diseases (Figure 1).^{4–7}



Figure 1. Naturally occurring phenolglycosides found in the *flacourtia* plant genus.⁷

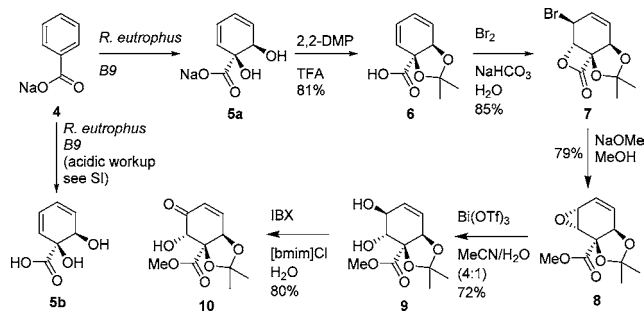
In 2013, the cyclohexenone carboxylate portion of xylosmin was reported as the product of base-catalyzed hydrolysis of a compound related to xylosmin 1 (having a free hydroxymethyl group on the monosaccharide residue, Figure 1).⁵ No physical or spectral data, other than the optical rotation (reported as +13.2 (c 0.07, MeOH) and +12.6 (c 0.06, MeOH)) were provided for the free carboxylic acid. In this paper, we report the chemoenzymatic synthesis, starting from benzoic acid, of the hydroxylated cyclohexenone carboxylic acid fragment common to xylosmin (1),⁶ flacourtoside E (2), and flacourtoside F (3) (Figure 1).⁷

Our initial approach to the target cyclohexenone fragment employed the enzymatic dihydroxylation of benzoic acid as shown in Scheme 1. The use of arene dioxygenases for desymmetrization of common aromatic compounds has gained prominent utility in the synthesis of natural products. Toluene and other arene dioxygenases that are overexpressed in *E. coli* vectors are frequently employed in chemoenzymatic syntheses, and this topic has been reviewed on several occasions.⁸ Less common metabolites are the *ipso*-diols of type 5a and 5b⁹ produced by dihydroxylation of benzoic acids with benzoate dioxygenase through the whole cell fermentation with *Ralstonia eutrophus* B9.¹⁰ The *ipso*-diol 5a,^{10,11} derived from sodium benzoate 4, was protected as its corresponding acetone 6 and carefully treated with bromine to provide the β -lactone 7, as

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Scheme 1. Synthesis of Enone 10 from Sodium Benzoate



shown in Scheme 1. A similar protocol was reported by Banwell in a formal approach to the C-ring of vinblastine.¹²

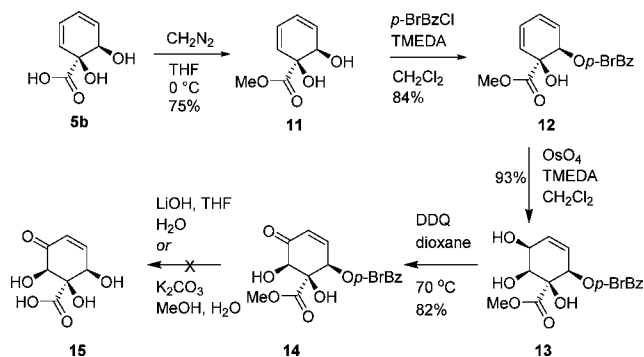
Treatment of 7 with sodium methoxide in methanol generated methyl ester 8 from lactone 7 and provided, through the intramolecular displacement of the bromide by the intermediate alkoxide, epoxide 8. Cyclohexenyl epoxide 8 was considered as a putative precursor to the conduritol carboxylate unit found in compounds 1–3.

A mild epoxide opening was achieved with catalytic Bi(OTf)₃ in acetonitrile and water,^{13,14} leading to opening of the cyclohexenyl epoxide at the allylic position without the loss of either the ester or acetonide protecting groups. The selective oxidation of the allylic alcohol in diol 9 proved to be more difficult than initially expected. Surprisingly, oxidation with manganese dioxide always led to the 1,2-diketone product and not to the desired hydroxyenone 10. Other oxidants (DDQ, IBX, PIFA) either proved ineffective or led to complex mixtures. Ultimately, when IBX was dissolved in the ionic liquid [bmim]Cl¹⁵ and water, the selective oxidation of the allylic alcohol to the desired enone 10 was achieved. The expected epimerization of the C6 hydroxyl failed under both Mitsunobu conditions and base catalysis. The reported conditions for hydrolysis of the phenolglycosides (Ba(OH)₂, 80 °C)⁵ failed as well and led only to extensive decomposition.

To approach the synthesis of the natural stereoisomer a new strategy was devised that utilized *ipso*-diol 5b, which was converted to methyl ester 11.^{11,16} Protection of the distal alcohol (with reference to the carboxylate) in 11 as a *p*-bromobenzoate was followed by the hydroxyl-directed Donohoe osmylation¹⁷ to furnish triol 13 in a stereo- and regioselective fashion, Scheme 2.

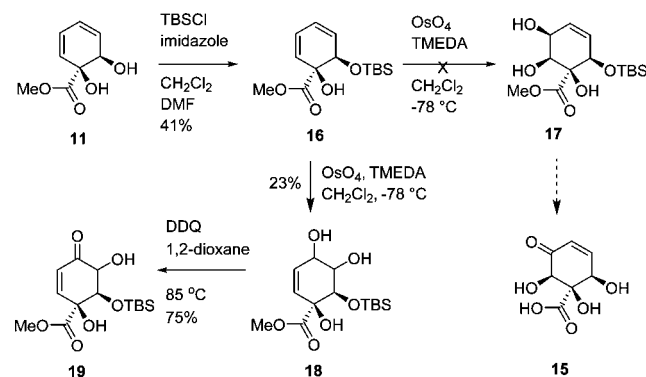
Oxidation of 13 with IBX in ionic liquid failed, in contrast to the successful oxidation of 9; however, the allylic alcohol in 13 was cleanly oxidized to the desired enone 14 with DDQ.

Scheme 2. Synthesis of Diester 14 from Diol 5b



Unfortunately, the original plan for the double deprotection of 14 in the final step failed and the treatment of 14 under the reported conditions [Ba(OH)₂, or NaOMe/MeOH] resulted only in decomposition to an aromatic product. Because of this turn of events, the same sequence was executed again with a silyl protecting group instead of the benzoate at C2, as shown in Scheme 3.

Scheme 3. Failed Attempts toward the Synthesis of Enone 15

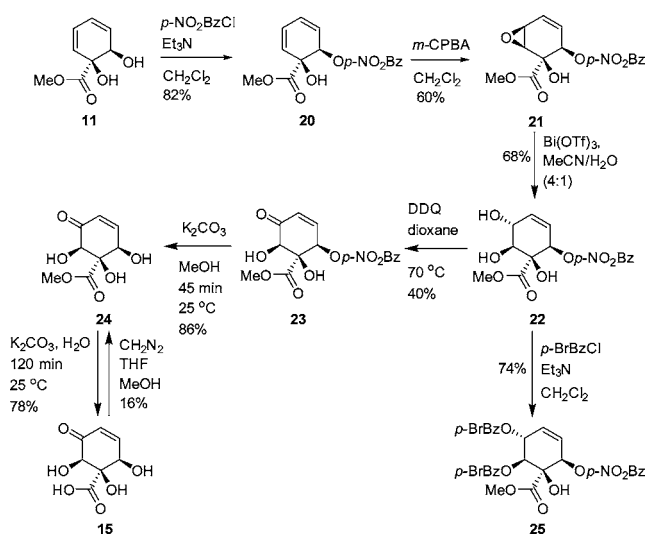


The secondary silyl ether was synthesized by a previously published sequence¹⁸ and subjected to Donohoe dihydroxylation. Unfortunately, this dihydroxylation afforded the trihydroxy regioisomer 18, as opposed to the regioisomer of type 17 as was previously observed. In order to confirm the assignment of structure 18, the allylic alcohol was oxidized to afford enone 19. The ¹H NMR spectrum of 19 did not show the expected ABX pattern of the olefinic signals and the proton at C2, but rather the presence of two clean doublets (δ 6.90 and 6.19, J = 9.9 Hz) corresponding to the olefinic hydrogens of the enone and therefore confirming the assignment of regiochemistry as that shown in 19. The relative stereochemistry of the alcohol moieties of compounds 18 and 19 was not further investigated, but it may be assumed that these would be anti- to the TBS group, with the oxidation proceeding through a classic Upjohn mechanism, instead of the hydrogen bond-controlled Donohoe pathway.

In light of these observations, we decided to return to an approach based on the use of benzoyl protecting groups. To this end, the *p*-nitrobenzoyl ester 20 was produced from ester 11, as shown in Scheme 4. Benzoyl ester 20 was then subjected to a Henbest epoxidation to afford epoxide 21,¹⁹ which was treated with Bi(OTf)₃ as previously described to effect the epoxide opening.

In order to confirm the absolute stereochemistry of triol 22, this compound was subjected to benzoylation with *p*-bromobenzoyl chloride to afford the tetraester 25. This compound was analyzed by X-ray crystallography. The structure (Figure 2) confirmed the absolute stereochemistry of 22 as shown in Scheme 4. Triol 22 was then subjected to selective oxidation with DDQ to furnish enone 23. In contrast to previous observations involving the failure to hydrolyze *p*-bromobenzoate 14, the *p*-nitrobenzoate ester in enone 23 was smoothly hydrolyzed with potassium carbonate (0.5 equiv) in methanol over 45 min to afford 24, the methyl ester of the hydroxylated cyclohexenone carboxylic acid. Further hydrolysis (K₂CO₃, 0.5 equiv, H₂O, rt, 2 h) provided the desired hydroxylated cyclohexenone carboxylic acid 15 as a solid [mp = 135–137 °C (methanol/pentane, 1:3); $[\alpha]_D^{22}$ –49.4 (c = 0.5,

Scheme 4. Synthesis of 15, the Hydroxylated Cyclohexenone Carboxylic Acid Moiety of Xylosmin



methanol)]. This compound was not fully characterized in the original report,⁵ and therefore this work represents the first synthesis of the hydroxylated cyclohexenone carboxylic acid moiety of xylosmin.

The previous attempts at the base-catalyzed isomerization of the C6 stereogenic center in enone **10** proved unsuccessful. Nevertheless, in order to ascertain that the stereochemical integrity of the C6 stereogenic center remained unchanged during the base hydrolysis of **24**, the freshly prepared free acid **15** was converted back to the methyl ester **24** with diazomethane. This ester was found to be identical (*vide* NMR and optical rotation) with the material used in the final hydrolysis.

Acid **15**, the hydroxylated cyclohexenone carboxylic acid fragment of xylosmin, was synthesized, and the absolute stereochemistry of ester **25** was determined to be as reported for the natural product (the structure of xylosmin was confirmed by X-ray crystallography⁶). Several approaches were investigated to prepare the hydroxylated cyclohexenone carboxylic acid **15**, and the successful one was based on the

directed epoxidation of *p*-nitrobenzoate ester **20** and subsequent manipulations that included mild conditions for the hydrolysis of the ester moieties. Carboxylic acid **15** is not a very stable compound (degradation occurs at room temperature with notable decomposition within 12 h or so; degradation proceeds at a slower rate even at $-80\text{ }^{\circ}\text{C}$). In the original report,⁵ no data were provided for the free carboxylic acid except for the optical rotation of $+13.2$ (c 0.07, MeOH) and $+12.6$ (c 0.06, MeOH). The hydrolysis of compound **26** (Figure 3), closely related to xylosmin and

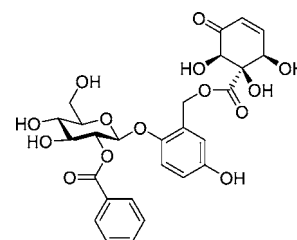


Figure 3. Free hydroxyl derivative of xylosmin **26**.⁵

having a levorotatory optical rotation, under acidic conditions was reported⁵ to give D-glucose as confirmed by the optical rotation value ($[\alpha]_D^{25} +50.5$ (c 0.10, H_2O) of the sugar obtained.

The basic hydrolysis of **26**, as reported in the literature,⁵ provided material, assumed to be the hydroxylated cyclohexenone carboxylic acid fragment, **15**, with a dextrorotatory optical rotation. Our experience with the hydrolysis of esters **14**, **23**, and **24** raises some questions whether the hydroxylated cyclohexenone carboxylic acid fragment was ever obtained under the harsh basic hydrolysis conditions reported. In addition, as the sign of the optical rotation of the carboxylic acid **15** obtained by synthesis is opposite to that reported for the material obtained by isolation, we may conclude that the material reported⁵ from the basic hydrolysis of **26** was simply contaminated with the free sugar. The authors of the original report confirmed by correspondence that they obtained no other analytical or spectral data for the free carboxylic acid.

Based on the X-ray confirmation of structure **25** as shown, and given the fact that epimerization of the hydroxyl group at the α -position in ketones of type **14**, **23**, and **24** was not

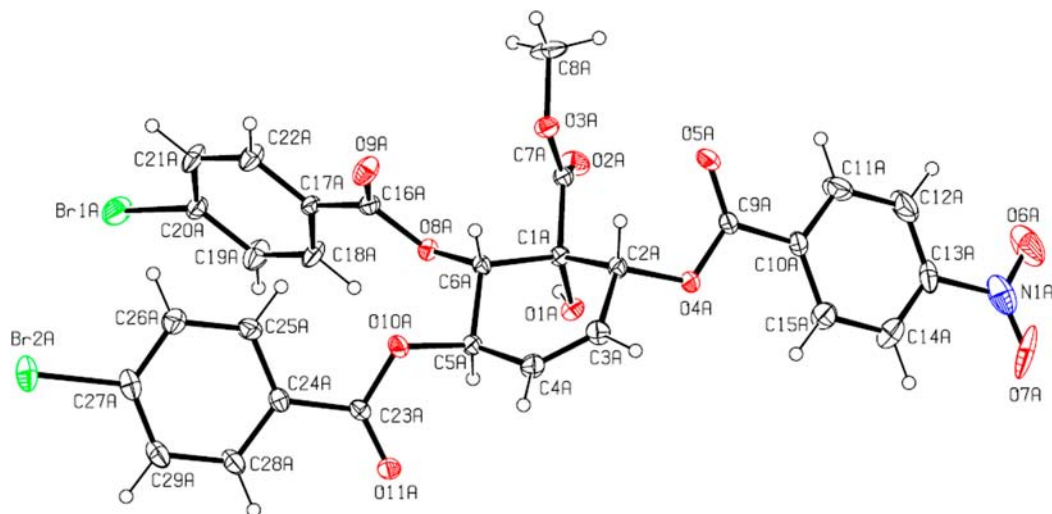


Figure 2. Crystal structure of ester **25**.

detected, we are reasonably certain that **15** was attained with the correct absolute stereochemistry, as that determined for the natural product.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.orglett.7b00194](https://doi.org/10.1021/acs.orglett.7b00194).

Experimental and spectral data are provided for the compounds **5b**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, and **24** (PDF)

X-ray data is provided for **25** (CIF)

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Notes

The authors declare no competing financial interest.

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