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Synthetic Studies on the Natural Product Myrsinoic Acid F Reveal Biologically Active Analogues

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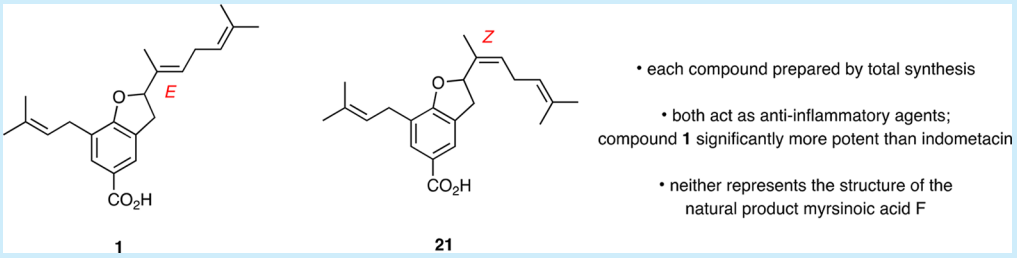
Supporting Information



ABSTRACT: The synthesis of the structure, **1**, assigned to the anti-inflammatory natural product myrsinoic acid F is reported together with a means for preparing its *Z*-isomer **21**. While neither of these compounds corresponds to the natural product, both of them are anti-inflammatory agents (as determined using a mouse ear edema assay) with congener **1** being notably more potent than the widely prescribed NSAID indometacin.

In 2002, Hirota and co-workers reported[1] the isolation of a terpeno-*p*-hydroxybenzoic acid and certain cyclic analogues from the methanolic extracts of the leaves and twigs of *Myrsine seguinii*, a shrub found in a range of countries, including China, Japan, Cambodia, Vietnam, and Myanmar. These compounds were evaluated as anti-inflammatory agents, and the most active proved to be the one named myrsinoic acid F (MA-F) and for which the cyclic structure **1** (Figure 1) was assigned on the basis of various NMR and other spectroscopic analyses. MA-F and its congeners may exert their anti-inflammatory effects by inhibiting DNA polymerase λ.[2]

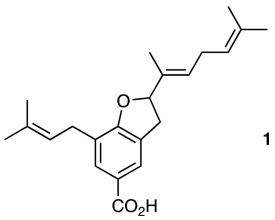
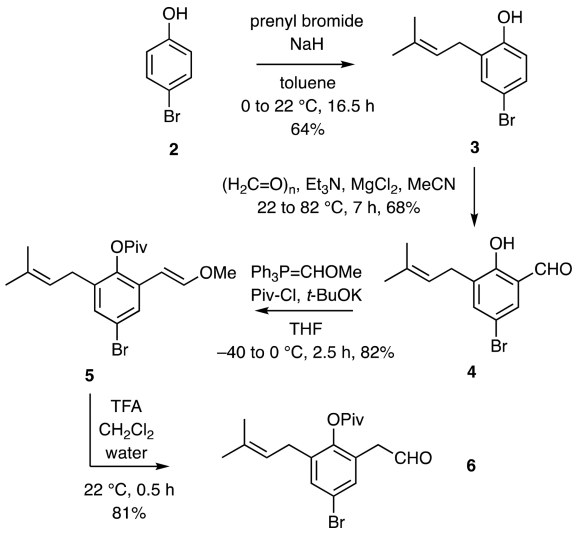


Figure 1. Structure assigned to the anti-inflammatory natural product myrsinoic acid F.

In the intervening period, MA-F and its various cometabolites, which can also be obtained from certain other plant sources,[3] have been identified as HB-EGF inhibitors[4] (and hence represent potential anticancer agents), as key elements of formulations for treating halitosis,[5] as antimicrobial agents,[6] and as compounds with notable antileishmanial properties.[7] Given these pronounced activities and the absence of studies on the preparation of any cyclic members of the myrsinoic acid class,[8] we sought to prepare compound **1**. Herein, we report the outcomes of such studies.

The opening stages of the route we established to access compound **1** are shown in Scheme 1. This involved the *C*-prenylation of *p*-bromophenol (**2**) under conditions defined by

Scheme 1. Synthesis of Aldehyde 6 from *p*-Bromophenol (2)



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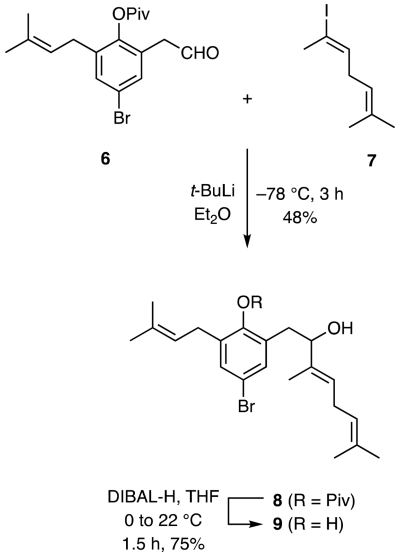
Bates[9] and thus affording compound **3** in 64% yield. Formylation of phenol **3** using paraformaldehyde in the presence of triethylamine/magnesium chloride under the conditions reported by Skattebøl[10] then gave the salicylaldehyde **4** (68%) that was subjected to treatment with in situ generated methoxymethylenetriphenylphosphorane and pivaloyl chloride (Piv-Cl)/potassium *t*-butoxide to give the styrenyl ether **5** (82%), acid catalyzed hydrolysis of which afforded the arylacetaldehyde **6** (81%).

The completion of the installation of the geranyl-like substructure associated with target **1** is shown in Scheme 2 and

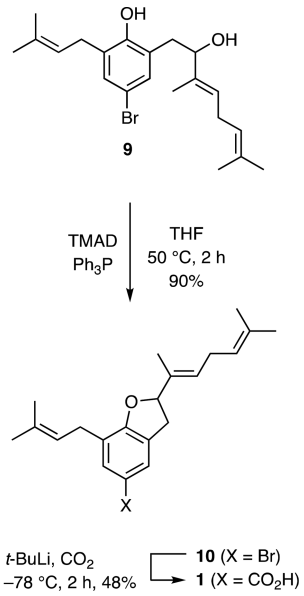
Scheme 2. Completing the Installation of the Geranyl-Type Substructure Associated with Target 1



involved reacting an ethereal solution of the readily prepared alkenyl iodide **7**[11] with *t*-butyllithium (*t*-BuLi) and then adding the resulting alkenyl lithium to aldehyde **6** and so affording, after aqueous workup, the 2°-alcohol **8** (48%) as a clear, light-yellow oil. Reduction of this last compound with di-*iso*-butylaluminum hydride (DIBAL-H) resulted in cleavage of the associated ester moiety, thus affording phenol **9** in 75% yield.

The conversion of phenol **9** into target **1** was readily achieved by the pathway shown in Scheme 3. Thus, the former compound was engaged in an intramolecular Mitsunobu reaction using *N,N,N,N*-tetramethylazodicarboxamide (TMAD) in the presence of triphenylphosphine,[12] thereby affording the anticipated dihydrobenzofuran **10** in 90% yield.[13] Finally, compound **10** was treated with *t*-BuLi and the resulting aryllithium was then trapped with carbon dioxide. By such means, and after acidic work up, the target benzoic acid **1** was obtained in 48% yield. All of the spectral data acquired on compound **1** were in complete accord with the assigned structure. Of particular note was the observation of a NOE between the resonances due to the oxymethine and proximate olefinic protons, although the quantitation of this proved difficult because of the proximity of the signals in question. Disappointingly, a comparison of the ^{1}H and ^{13}C NMR data acquired on compound **1** with those reported[1] for MA-F revealed significant differences [see the Supporting Information (SI) for tabulated comparisons of the ^{13}C and ^{1}H NMR spectral data sets]. These discrepancies persisted even with variations in the pH of the medium in which the NMR spectra of carboxylic acid **1** were recorded.

Scheme 3. Final Steps in the Synthesis of Target 1



On the basis of the foregoing, we considered the possibility that the *Z*-isomeric form of compound **1** might represent the true structure of MA-F. Accordingly, a route to this compound was devised. This started (Scheme 4) with the monocrotoylation of *p*-bromophenol **2** in the presence of sodium hydride, thus affording compound **11** in 86% yield. Reaction of the latter compound with $VO(acac)_2$ in the presence of *tert*-butylhydroperoxide (TBHP)[14] resulted in the coformation of the chromatographically inseparable pyran **12** and the isomeric dihydrobenzofuran **13** (67% combined yield), each of which was obtained as a mixture of diastereoisomers and, presumably, as a consequence of the cyclization of the initially formed epoxides. Oxidation of this mixture of four compounds with the Dess–Martin periodinane (DMP)[15] afforded the corresponding ketones, the major one, *viz*. compound **14** (78%), being obtained in pure form after flash column chromatography. Reduction of compound **14** using sodium borohydride afforded a ca. 1:1 mixture of the diastereoisomeric forms of compound **13** (99%) that could be regioselectively iodinated using molecular iodine in the presence of Ag_2SO_4 to afford the dihalide **15** (80%) that, upon oxidation with DMP, then gave the ketone **16** (78%), the structure of which was confirmed by single-crystal X-ray analysis (see SI for details). Attempts to effect the iodination of ketone **14** and thus form compound **16** directly only led to complex mixtures of products.

The three-step conversion of ketone **16** into the target *Z*-configured olefin is shown in Scheme 5 and involved first subjecting the former compound to a regioselective Suzuki–Miyaura cross-coupling reaction with boronate ester **17**,[16] thus affording the expected prenylated product **18** (82%). Reaction of compound **18** with the readily obtained ylide **19** in diethyl ether at −40 to 0 °C then gave the *Z*-configured olefin **20** in 72% yield. Finally, sequential treatment of compound **20** with *t*-BuLi then carbon dioxide gave, after acid workup, the target olefin **21** in 88% yield. Compounds **19**, **20**, and **21** were each contaminated with ca. 15% of the isomer incorporating a terminal double bond (see SI for details).

All the spectral data acquired on acid **21** were in complete accord with the assigned structure. Notable differences between the NMR spectra of compound **21** and its *E*-isomer **1** include a

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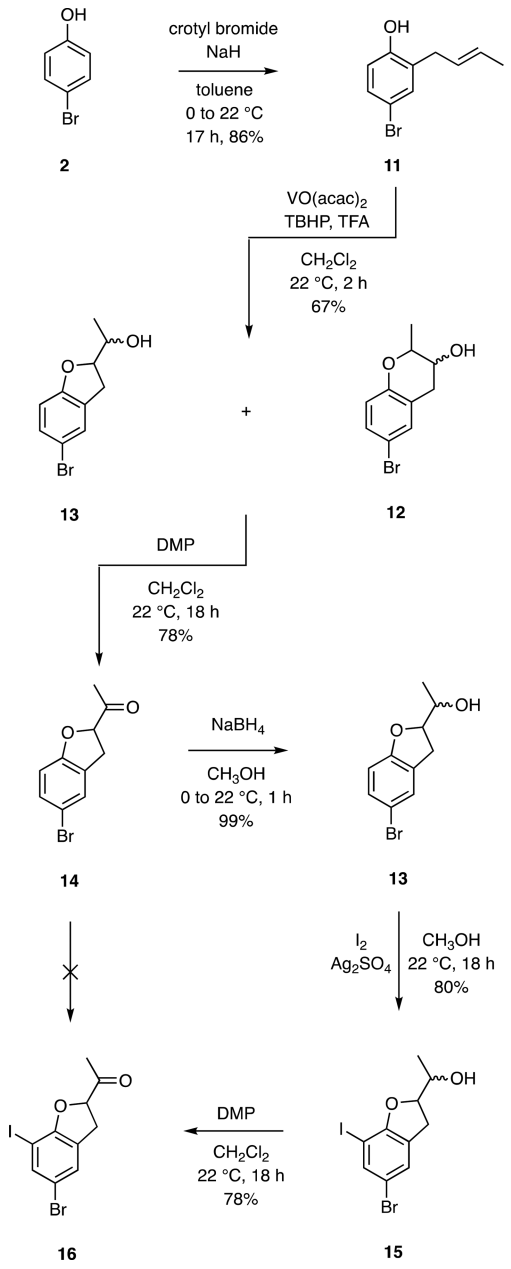
Scheme 4. Preparation of the Dihydrobenzofuran 16 from *p*-Bromophenol (2)



much higher field resonance due to the oxymethine carbon in the former compound (81.5 vs 88.8 ppm) and a significantly lower field shift for the signal due to the methyl group associated with the proximate double bond (17.1 vs 11.0). While all these data clearly support the acquisition of compound **21** by the means just described, they do not match those reported[1] for MA-F (see the SI for a tabulated comparison of the relevant NMR data sets). Accordingly, the true structure of MA-F remains unclear at the present time.

The likely structural similarity of compounds **1** and **21** reported here to MA-F prompted their biological evaluation. In particular, these compounds were subjected to a TPA-induced mouse ear edema assay[17] using the NSAID indometacin[18] as positive control. These tests reveal, as shown in Table 1, that the synthetically derived materials **1** and **21** are also anti-inflammatory agents with the former showing an inhibition

Scheme 5. Completion of the Synthesis of Target 21

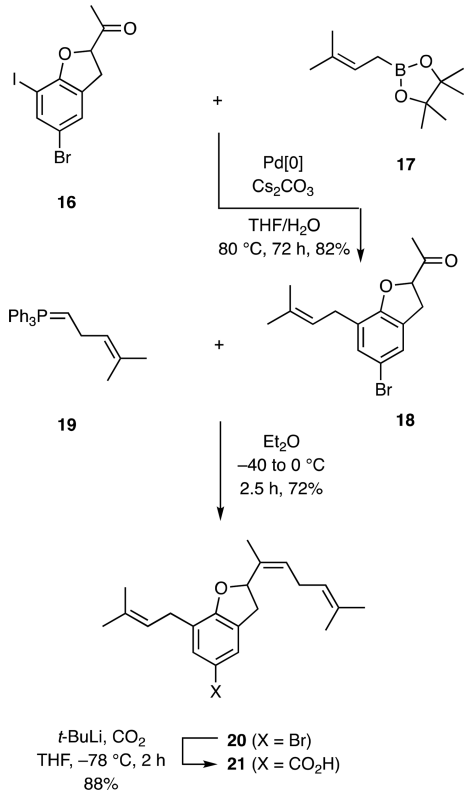


Table 1. Outcomes of the Biological Evaluation of Compounds 1, 15, 16, and 21 in a TPA-Induced Mouse Ear Edema Assay[a]

compound	concentration (μM)	inhibition rate (%)
blank		0
indometacin	0.56	9.9
	1.4	43.1
1	0.56	42.1
	1.4	49.0
15	0.56	2.9
	1.4	4.5
16	0.56	2.0
	1.4	0.3
21	0.56	6.5
	1.4	14.6

[a]A sample (0.56 or 1.4 μM) of the test compound was applied to one mouse ear, and after 0.5 h, TPA (0.5 μg) was applied to both ears of the mouse. The edema was evaluated after 7 h, the cited inhibition rate being determined as detailed in the SI. Five mice were used for each experiment.

rate (IR) significantly greater than the control at the two micromolar concentrations employed.[19,20]

The studies outlined above suggest that cyclic analogues of terpeno-*p*-hydroxybenzoic acids exhibit "tunable" anti-inflammatory effects and are thus worthy of further investigation as sources of potential therapeutic agents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01558.

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Experimental procedures, spectroscopic data, copies of the NMR spectra of compounds **1**, **3**−**6**, **7**, precursor to **8**−**11**, **13**−**16**, **18**, and precursor to **19**, **20**, **21** (PDF)

Accession Codes

CCDC 1842970 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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(19) Compounds **1** and **21** are both slowly oxidized on exposure to air (ca. 50% decomposition at −5 °C over 2 months) and were, therefore, stored under argon, including while being shipped for biological evaluation. As such, the reported biological activities need to be taken as indicative of anti-inflammatory activity rather than defining such properties in a truly quantitative manner. Mass spectrometric analyses suggest the decomposition pathways involve the incorporation of oxygen (rather than dehydrogenation to form benzofurans).

(20) Compounds **1** and **21** were tested as racemates. Since the observed anti-inflammatory activities could arise from a single enantiomer, the IR values for the relevant homochiral forms may be higher than cited in Table 1.

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