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Enantioselective Synthesis of (+)-Chamaecypanone C, a Novel Microtubule Inhibitor**

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Abstract

A number of bicyclo[2.2.2]octenone-containing natural products have been isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana* (Figure 1) including the Diels-Alder adducts[1] obtunone (**1**),[2] chamaecypanone C (**2**),[3] and the [4+2] dimer (+)-**3**.[2],[4] Compound (+)-**2** was shown to exhibit potent cytotoxicity against several human cancer cells including human oral epidermoid carcinoma (KB) (IC_{50} = 190 nM).[3] The biosynthesis of **2** was proposed[3] to occur *via endo* [4+2] cycloaddition between 1-hydroxymentha-3,5-dien-2-one **4** (Figure 2) and 1,3-*bis*-arylcyclopenta-1,3-diene **5**, followed by oxidation to an enone in accord with literature reports of cyclopentadienes as biosynthetic precursors to natural products.[1b] An alternative possibility involving the corresponding cyclopentadienone **6** as dienophile may also be considered in light of known biosyntheses involving reactive cyclopentadienones.[5] Herein, we report a concise synthesis of both enantiomers of chamaecypanone C involving a retro-DA/DA cascade of dimer **3**, obtained utilizing copper-mediated asymmetric oxidative dearomatization,[6] as well as biological studies documenting that the cytotoxic action of (+)-**2** involves mitotic arrest as a consequence of its binding in the colchicine site of tubulin.

Keywords

cycloaddition; total synthesis; natural product; cyclopentadienone; microtubule inhibitor

Inspired by literature reports of tandem retro-DA/DA reactions of dimers derived from *o*-quinols and masked *o*-benzoquinones (MOBs), [7], [8] we first evaluated reactions between the readily accessible dimer (−)-**3**[6] and *N*-phenylmaleimide (**7**) under thermolytic conditions in different solvents (Table 1). Although reactions in toluene (entry 1) and chlorobenzene (entry 2) generated the desired cycloadduct **8** in moderate to good conversion

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(12 h), reactions in mesitylene at 150 °C were found to give both excellent conversion and isolated yield of **8** in 1.5 h (entries 3 and 4).

Using these optimized conditions, a number of representative dienophiles were thermolyzed in the presence of dimer (−)-**3** in mesitylene (Table 2). Reactions with methyl vinyl ketone (MVK, **9**, entry 1), 2,3-dihydrofuran **10** (entry 2), and indene **11** (entry 3) successfully generated bicyclo[2.2.2]octenones **12** to **14** in good to excellent yields, which underscores the reactivity of *o*-quinols as both normal and inverse demand dienes. The observed regioselectivity for products of **12–14** is in agreement with that reported for related cyclohexadienones (MOBs).[7c,d] Reaction with β-myrcene (**15**, entry 4) smoothly generated an inseparable 1:1 mixture of *ent*-obtunone (**1**) and a decalin product, both of which were acetylated to afford **16** and **17**.[9] Hydrolysis of **16** (aq. NaOH/MeOH) afforded optically pure product *ent*-**1**.[2], [10] Furthermore, cyclopentadiene dimer **18** (entry 5) was found to be very reactive, affording [4+2] adduct **19** as a single diastereomer in nearly quantitative yield.[7a] In contrast, reaction with cyclopentadienone dimer **20** (entry 6) produced **21** in moderate yield, probably due to side reactions of **20** at high temperature including decarbonylation.[11]

Based on our ability to trap (6*S*)-**4** with a number of dienophiles, we proceeded to evaluate both cyclopentadienes and cyclopentadienones for the synthesis of **2**. Accordingly, we targeted a single starting material for preparation of both precursors. Starting from the known *bis*-arylcyclopentene derivative **22**,[12] allylic oxidation using selenium dioxide afforded alcohol **23** as major product (50%) along with small amount of enone **24** (Scheme 1). Although diaryl cyclopentadienes[13] were detected by GC-MS under acid-catalyzed dehydration conditions (*cat*. MP-TsOH, toluene, 110 °C, 1 h),[14] all attempts to isolate pure product **25**, or trap it with reactive dienophiles (e.g. maleic anhydride, tetracyanoethylene (TCNE)) failed. Moreover, thermolysis of the crude mixture from either dehydration of **23** or base-promoted elimination of the derived mesylate derivative with dimer **3** also did not afford the desired cycloadduct **26**.

Alternatively, allylic alcohol **23** could be efficiently converted to cyclopentenone **24** using IBX as oxidant[15] (Scheme 2). After extensive experimentation, it was found that DDQ oxidation of **24**[16] in the presence of dimer **3** afforded the desired cycloadduct **27** in good yield. The *endo* stereochemistry of cycloadduct **27** was unambiguously assigned by NOE experiments.[10] The transformation presumably proceeds *via* initial formation of the reactive cyclopentadienone **28** from cyclopentenone **24**.[17] Unfortunately, all efforts to isolate either the cyclopentadienone monomer or derived dimers in control experiments have thus far failed. Finally, treatment of **27** with BBr_3 effected smooth demethylation to afford (−)-chamaecypanone C (*ent*-**2**) (86%). To the best of our knowledge, this is the first example of generation of a 2,4-diarylcyclopentadienone and its usage in natural product synthesis.[18] The instability and high reactivity of the diarylcyclopentadienone intermediate[19] is likely due to the relief of antiaromaticity upon cycloadditionas suggested by Harmata and coworkers.[20]

In a similar manner, we prepared (+)-chamaecypanone C (**2**, Scheme 3). Hydrogenation of **29** quantitatively generated 2,4-disubstituted phenol **30**. An asymmetric hydroxylation-α –ketol rearrangement-dimerization sequence[6] afforded (+)-dimer **3** in moderate yield over two steps (>99% *ee*) which was further elaborated into (+)-chamaecypanone C (53%, two steps from enone **24**). Synthetic **2** was confirmed to be identical with data reported for natural chamaecypanone C by comparison of ^1H and ^{13}C NMR spectra, mass spectrum, IR, and $[\alpha]_D$, thus confirming its absolute configuration.[10]

Both enantiomers of **2** were tested in the NCI 60-cell single dose assay at 10^{-5} M. Confirming earlier studies on the natural product[3], (+)-**2** inhibited tumor cell growth by an average of 71%, while (−)-**2** had no effect. (+)-**2** was then tested in a dose response format, where it displayed robust selectivity with a mean GI_{50} value of 0.21 μM. COMPARE analysis of the data[21] at the TGI level suggested that (+)-**2** might act through interference with tubulin function, as high correlations were seen to the data for seven established tubulin inhibitors.[10] Examination of this hypothesis using an *in vitro* tubulin polymerization assay[22] found this to be the case, with an IC_{50} of 2.0±0.1 μM, while the (−)-enantiomer had no effect at 40 μM. (+)-**2** was also tested for inhibition of colchicine binding[23] where it had moderate activity at 50 μM with 5 μM [^{3}H]colchicine and 1 μM tubulin. Finally, we confirmed that (+)-**2** had effects in cells consistent with its inhibitory effects on tubulin assembly. Cytotoxic concentration of (+)-**2** arrested cells in mitosis concordant with inhibition of cell growth (Figure 3) and caused the disassembly of the intracellular microtubule network (Figure 4). [24]

In conclusion, we have accomplished total syntheses of both (+)- and (−)-chamaecypanone C. The key transformation involves Diels-Alder cycloaddition between an *in situ*-generated diarylcyclopentadienone and a chiral *ortho*-quinol derived from a retro-Diels-Alder reaction of its dimeric form. Initial biological studies indicate that (+)-chamaecypanone C is a potent tumor cell growth inhibitor[3] acting primarily through inhibition of tubulin polymerization. Further studies on preparation of (+)-chamaecypanone C analogues using a retro-DA/DA cascade, as well as biological evaluation of these compounds, are currently in progress and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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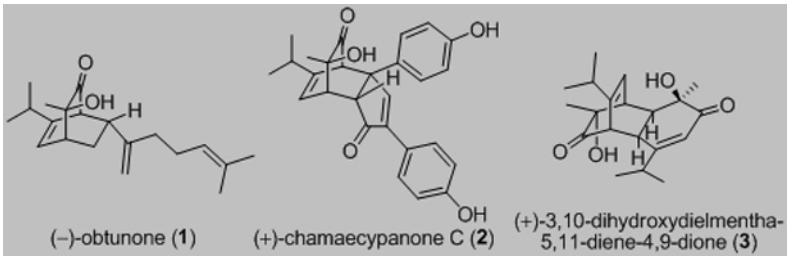


Figure 1.
Representative Bicyclo[2.2.2]octenone-Containing Natural Products

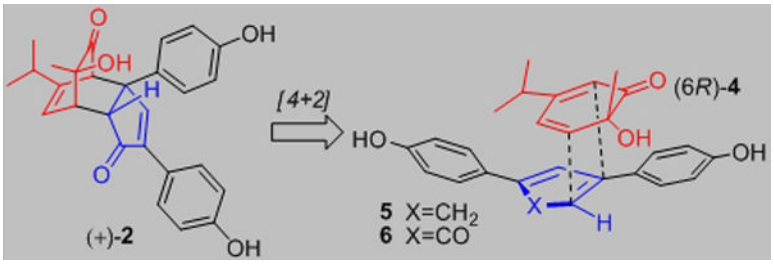


Figure 2.
Plausible Biosyntheses of Chamaecypanone C

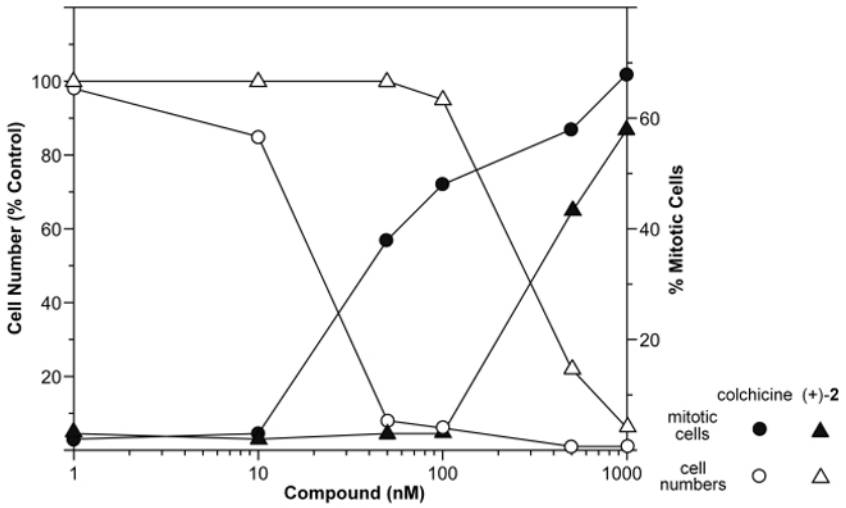


Figure 3.

Human Burkitt lymphoma CA46 cells, obtained from the American Type Tissue Collection, were grown in suspension culture for 24 h at 37 °C in a humidified, 5% CO_2 atmosphere. The medium was RPMI 1640 supplemented with 5% fetal bovine serum. Initially, the culture medium contained 20,000 cells/mL. For determination of cell growth, the increase in cell number was determined, with the cells counted in a Beckman Coulter model Z1 particle counter. For determination of mitotic cells, cells were harvested by centrifugation, briefly swollen in a hypotonic solution, fixed on a glass slide, and stained with Giemsa. The percentage of cells with condensed chromosomes was determined.

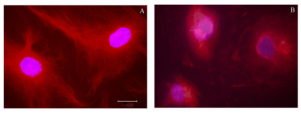
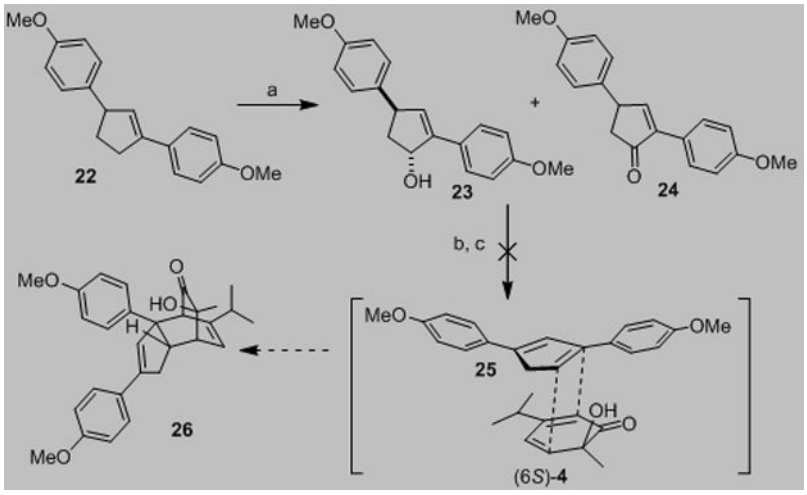


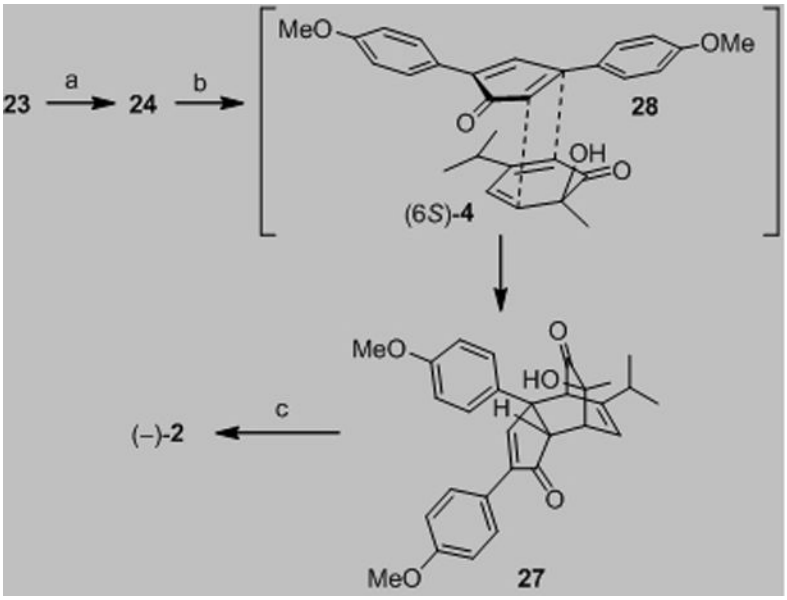
Figure 4.

Disruption of intracellular microtubule network by chamaecypanone C. *Potorus tridactylis* PtK2 kidney epithelial cells were obtained from the American Type Tissue Collection and were cultured in Minimal Essential Medium supplemented with 10% fetal bovine serum, 1 mM glutamine, and 1 mM sodium pyruvate. The cells were grown to confluence, disrupted by trypsinization, and seeded at about 35,000 cells into each compartment of a Chambered Coverglass System from Nunc with either no compound (A) or 0.5 μM chamaecypanone C (B) added to the culture medium. Following growth for 16 h at 37 °C in a humidified, 5% CO_2 atmosphere, the cells on the coverglass were fixed with − 20 °C acetone, washed with phosphate-buffered saline, and stained with a monoclonal antibody to β-tubulin conjugated to the fluorescent dye Cy3 (Sigma product C4585), following instructions provided by the manufacturer. The coverglass was mounted on a slide with Antifade Mounting Solution and examined in a Nikon Eclipse E800 microscope with a 100x oil objective and using appropriate epifluorescence accessories. Images were captured with a Spot digital camera. The scale bar shown in panel A represents 20 μm.



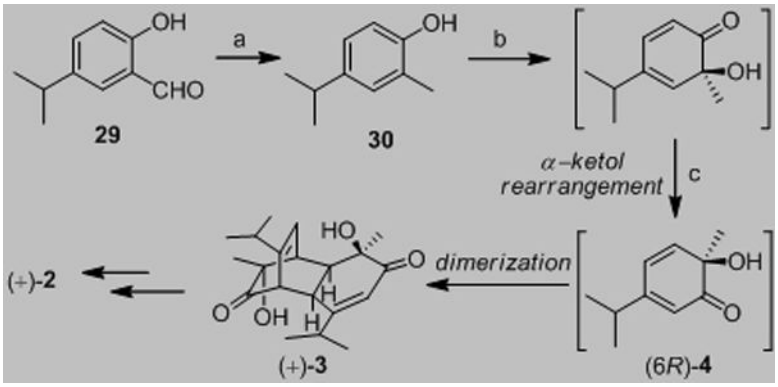
Scheme 1.

a) 0.5 equiv SeO_2, 2 equiv TBHP, DCE, 60 °C, 2 h, 50% (**23**), and 5% (**24**); b) cat. MP-TsOH, toluene, 110 °C, 1 h; or Martin sulfurane, CH_2Cl_2, r.t., 0.5 h; c) 0.2 equiv (−)-**3**, mesitylene, 150 °C. TBHP=*tert*-butyl hydroperoxide, DCE=1,2-dichloroethane.



Scheme 2.

a) 2.0 equiv IBX, toluene/DMSO (1 M, 2:1), 50 °C, 30 min, 90%; b) 1.5 equiv (−)-**3**, 2.0 equiv DDQ, *o*-dichlorobenzene, 150 °C, 1 h, 61%; c) 8.0 equiv BBr_3, CH_2Cl_2, −78 °C to rt, 4 h, 86%. DMSO=dimethyl sulfoxide, DDQ=2,3-dichloro-5,6-dicyanobenzoquinone.

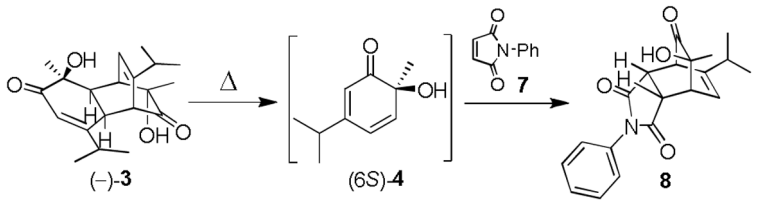


Scheme 3.

a) H-Cube® (Pd/C), H_2 (40 bar), MeOH (0.03 M), 50 °C, 0.5 mL/min, quantitative; b) 1.0 equiv LiOH · H_2O, EtOH/toluene, azeotrope; $Cu(CH_3CN)_4PF_6$ (2.2 equiv.), (−)-sparteine (2.3 equiv), 3Å molecular sieves, O_2, THF, −78 °C, 16 h; c) benzene, reflux, 12 h, 47% (2 steps). THF=tetrahydrofuran.

Table 1

Optimization of the Retro-DA/DA Cascade



(−)-**3** (6*S*)-**4** **8**

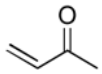
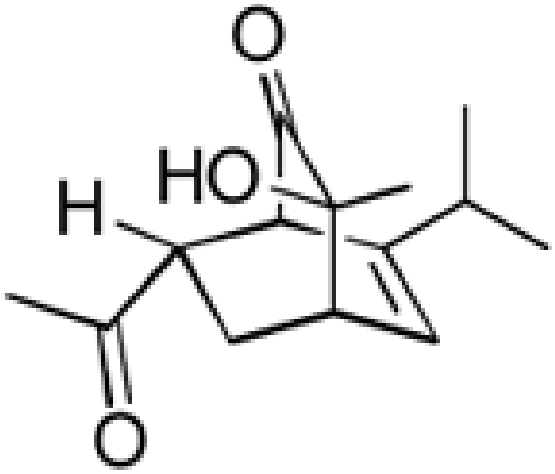
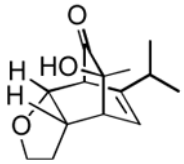
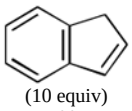
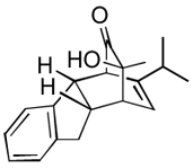
Entry	solvent	temp. (°C)	dienophile 7 (equiv.)	time (h)	conversion[a] (%)
1	toluene	110	5	12	69
2	chlorobenzene	130	5	12	92
3	mesitylene	150	5	1.5	>99 (98)[b]
4	mesitylene	150	3	1.5	>99 (97)[b]

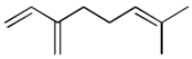
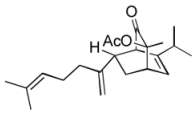
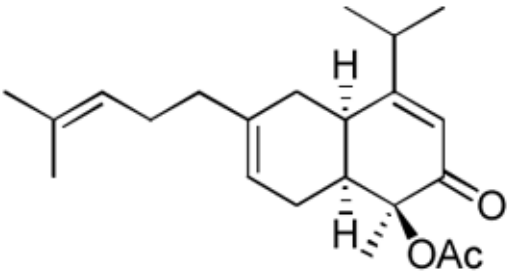
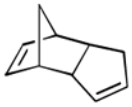
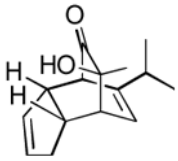
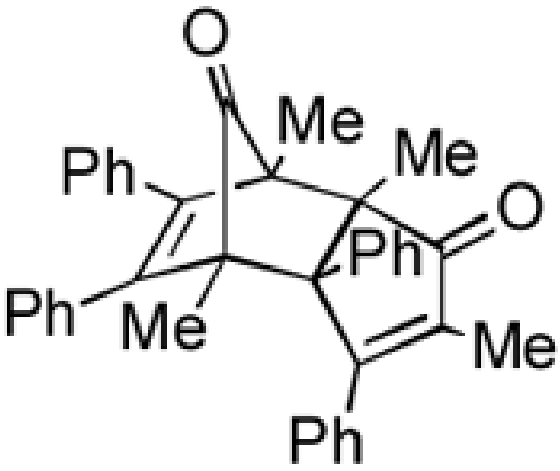
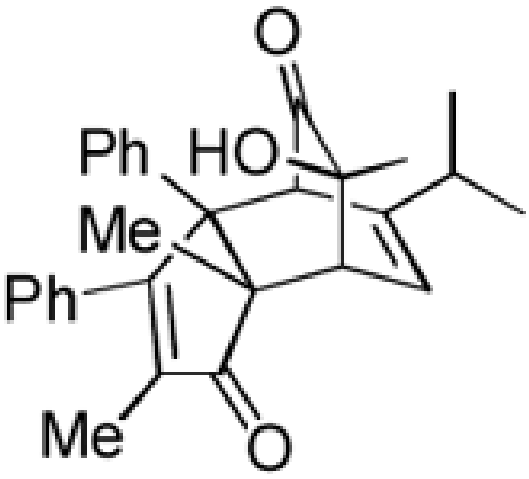
[a] Conversion based on ^{1}H-NMR analysis of **8** and starting material (−)-**3**;

[b] Isolated yield of **8** in parenthesis.

Table 2

Tandem retro-DA/DA reactions using bicyclooctenone (−)-**3**[a]

entry	Dienophile(eqiv)	cycloadduct	Time(h)	Yi
1	 (5 equiv) **9**	 **12**[c]	3	
2	 (20 equiv) **10**	 **13**	12	
3	 (10 equiv) **11**	 **14**	3	

entry	Dienophile(eqiv)	cycloadduct	Time(h)	Yi
4[d]	 (10 equiv) **15**	 **16**	4	
		 **17**	4	
5	 (5 equiv) **18**	 **19**	4	
6	 (2.5 equiv) **20**	 **21**	5	

[a] Reaction conditions: dimer (−)-**3**, dienophile, mesitylene, 150 °C;

[b] Isolated yield after column chromatography;

[c] Approximately 6% of an inseparable minor product was observed by ^{1}H-NMR;

[d] Acetylation required for product separation.