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Structural Reassignment of Cytosporolides A–C via Biomimetic Synthetic Studies and Reinterpretation of NMR Data

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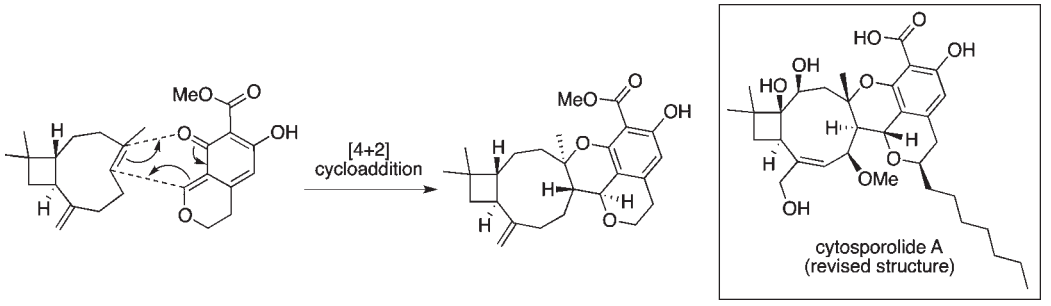
ABSTRACT



A structure revision for the recently isolated fungal meroterpenoids, cytosporolides A–C, is suggested based on biosynthetic speculation and reinterpretation of existing spectroscopic data. The structure revision is supported by a biomimetic synthetic study, featuring a [4 + 2] cycloaddition reaction between a presumed *o*-quinone methide intermediate and β-caryophyllene.

Cytosporolides A–C (**1**–**3**, Figure 1) are three meroterpenoids isolated from *Cytospora* sp., a fungus found in a soil sample collected at high altitude on the Tibetan plateau.[1] **1**–**3** Showed modest antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumonia*. The stuctures **1**–**3** were proposed on the basis of NMR studies by Che et al. The most striking feature of the proposed structures is a highly unusual 9-membered peroxylactone ring, which is fused to a caryophyllene-derived bicyclo[7.2.0]undec-4-ene system. Furthermore, an aromatic ring is embedded within the 9-membered peroxylactone ring of the proposed structures **1**–**3**. The molecular framework of the proposed structures is thus analogous to a [6]metacyclophane. [6]Metacyclophanes are relatively unstable and difficult to synthesize due to ring strain, which forces the aromatic ring to adopt a nonplanar conformation. The proposed cytosporolide structures **1**–**3** would therefore be expected to be highly strained molecules. Although several cyclophanes have been isolated as natural products, such as cavicularin[2] and haouamine A,[3] we believed that the highly strained peroxylactone ring system of the proposed structures of the cytosporolides warranted further investigation and, possibly, structural reassignment.[4]

Cytosporolides A–C were coisolated from *cytospora* sp. with the known caryophyllene derived natural product fuscoatrol[5] (**4**), which has been previously isolated from a marine fungus and whose structure has been unambiguously assigned via X-ray crystallography. Che et al. assigned the structures **1**–**3** for the cytosporolides A–C based on NMR studies and proposed a biosynthesis involving cycloaddition of a polyketide derived peroxy radical cation species to fuscoatrol. In our view, this biosynthetic proposal is

(1) Li, Y; Niu, S; Sun, B; Liu, S; Liu, X; Che, Y *Org. Lett.* **2010**, *12*, 3144.

(2) Toyota, M.; Yoshida, T.; Kan, Y.; Takaoka, S.; Asakawa, Y. *Tetrahedron Lett.* **1996**, *37*, 4745.

(3) Garrido, L.; Zubia, E.; Ortega, M. J.; Salva, J. *J. Org. Chem.* **2003**, *68*, 293.

(4) For a review of structural reassignment of natural products, see: Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012.

(5) Smetanina, O. F.; Kuznetsova, T. A.; Gerasimenko, A. V.; Kalinovsky, A. I.; Pivkin, M. V.; Dmitrenok, P. C.; Elyakov, G. B. *Russ. Chem. Bull.* **2004**, *53*, 2643.

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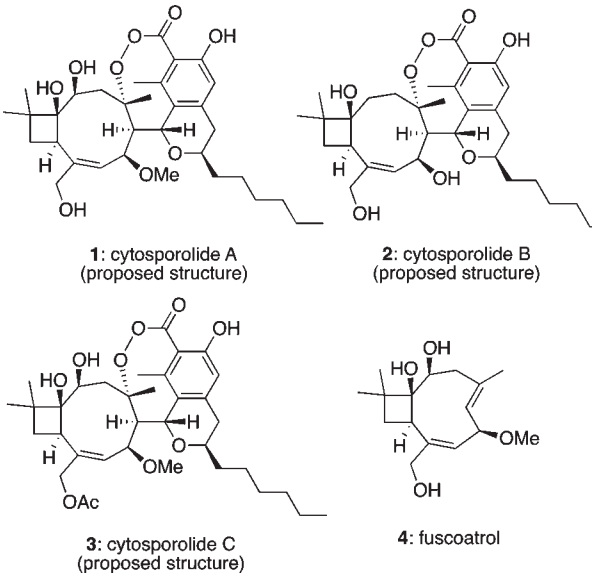


Figure 1. Originally proposed structures of cytosprolides A−C and fuscoatrol.

implausible, and the proposed structures **1**−**3** are unlikely to be correct due to the high degree of ring strain present in the peroxylactone ring system.

A key part of the assignment by Che et al. involved the chemical shift of C-8 (δ_C 87.5), which was observed to be significantly downfield compared to C-8 (δ_C 74.2) of the related caryophyllene-derived terpenoid 6-hydroxypunctaporonin (**5**, Figure 2). This difference in chemical shift led Che et al. to propose a peroxide bond between C-8 and C-23. However, no further evidence for the peroxylactone ring was given. Based in part on biosynthetic speculation, we considered that a more likely structure for the cytosporolides A−C might contain a 6-membered aryl ether ring, similar to those found in the caryophyllene-derived natural products guajadial[6] (**6**) and psidial A[7] (**7**), rather than the 9-membered peroxylactone ring proposed by Che at al. Indeed, the ^{13}C NMR chemical shifts of the oxygenated C-4 carbon atoms of guajadial (δ_C 84.3) and psidial A (δ_C 88.0) are very similar to that of C-8 of cytosporolide A (δ_C 87.5). The biosynthesis of guajadial (**6**) and psidial A (**7**) was proposed to involve a [4 + 2] cycloaddition of an *o*-quinone methide to *β*-caryophyllene, a pathway that has recently been supported by an elegant biomimetic synthesis by Lee et al.[8]

Our suggested revised structures of cytosporolides A−C (**8**−**10**), with 6-membered aryl ether rings replacing the 9-membered peroxylactone rings of **1**−**3**, are shown in Figure 3. These structures correlate well with the NMR data of Che et al. Importantly, the revised structures

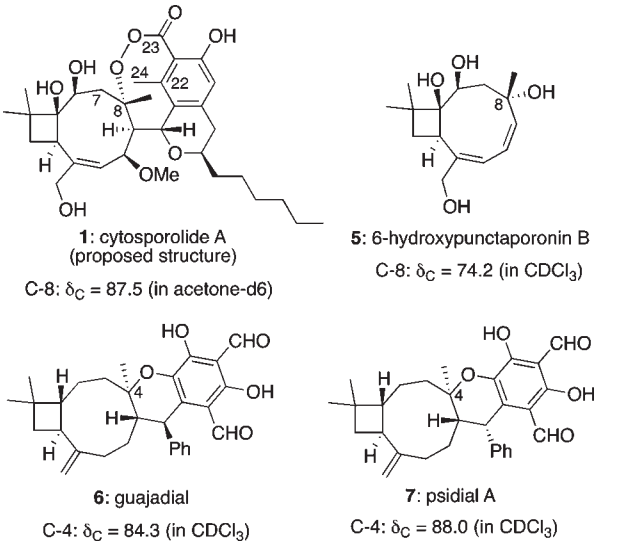


Figure 2. Some related caryophyllene-derived natural products.

require an extra CH_2 group in the long alkyl chain instead of the C-24 Me group of **1**−**3**. This fits the NMR data of Che et al., who assigned the C-24 Me group of **1** to a broad singlet at δ_H 1.28 in the ^1H NMR spectrum. This resonance is very low for an aryl Me group. Furthermore, the δ_H 1.28−1.30 region of the ^1H NMR spectrum is heavily congested due to overlapped CH_2 resonances of the akyl side chain, so an extra CH_2 group in this region, rather than an aryl Me group, is a more plausible assignment. Close inspection of the 2D NMR spectra of Che et al. for cytosporolide A reveals that the claimed HMBC correlation between the proposed C-24 Me group at 1.28 ppm and C-22 at 151.0 ppm has been erroneously reported. However, the same HMBC spectrum does show a distinct correlation between the C-7 proton at 3.08 ppm and C-22, which would only be possible for our reassigned structure **8**. This HMBC correlation would be impossible for Che's originally proposed structure **1**. The remaining structural assignments for cytosporolide A made by Che et al. appear to be sound on the basis of their 1D and 2D NMR data, with the overall bond connectivity elucidated by COSY, HMQC, and HMBC spectra, and with the relative stereochemistry assigned by NOESY spectra.

The revised structures **8**−**10** are further supported by the very close similarity between the aromatic regions of the ^{13}C NMR spectra of cytosporolides A−C and berkelic acid[9] (**11**), a spiroketal natural product isolated from an extremophilic *Penicillium* fungus found in the Berkeley Pit Lake.

The originally proposed cytosporolide structures were speculated to arise in Nature via an unusual cycloaddition of a peroxy radical cation to fuscoatrol derivatives to generate the 9-membered peroxylactone ring. The revised

(6) Yang, X.-L.; Hsieh, K.-L.; Liu, J.-K. *Org. Lett*. **2007**, *9*, 5135.
(7) Fu, H.-Z.; Luo, Y.-M.; Li, C.-J.; Yang, J.-Z.; Zhang, D.-M. *Org. Lett*. **2010**, *12*, 656.
(8) Lawrence, A. L.; Adlington, R. M.; Baldwin, J. E.; Lee, V.; Kershaw, J. A.; Thompson, A. L. *Org. Lett*. **2010**, *12*, 1676.
(9) Stierle, A. A.; Stierle, D. B.; Kelly, K. *J. Org. Chem*. **2006**, *71*, 5357.

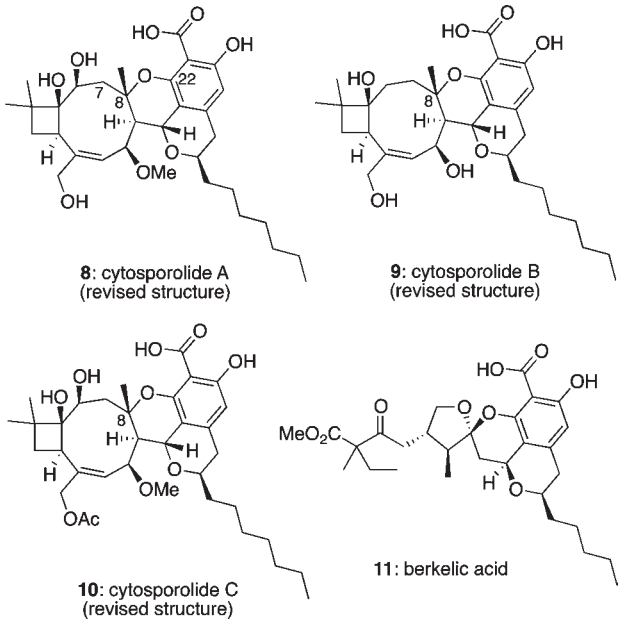


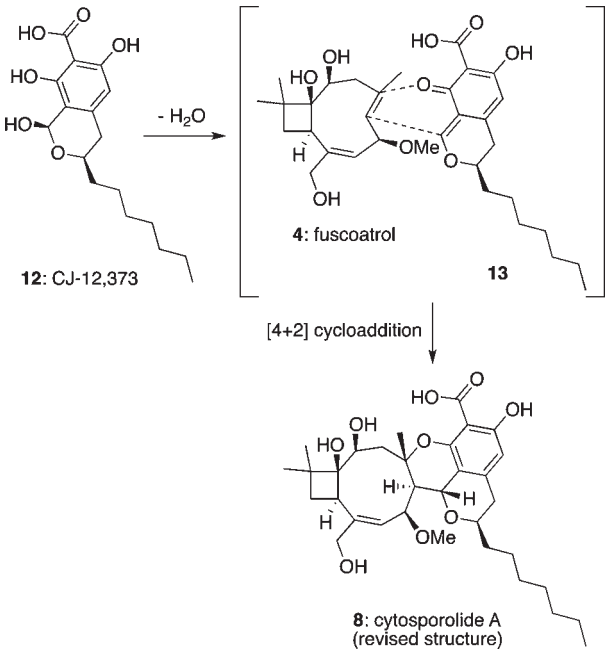
Figure 3. Revised structures of cytosporolides A–C and berkelic acid.

structures of the cytosporolides could be biosynthesized by a far more feasible pathway (Scheme 1). In this case, dehydration of the known fungal metabolite CJ-12,373[10] (**12**) could generate the *o*-quinone methide[11] **13**. Cycloaddition of this reactive intermediate to fuscoatrol would then generate the revised structure of cytosporolide A (**8**). A related biosynthesis of berkelic acid (**11**), involving cycloaddition of an *o*-quinone methide to an enol ether, has been the subject of a recent biomimetic synthesis by De Brabander et al.[12]

As part of our continuing interest in biomimetic reactions of *o*-quinone methides,[13] and to lend support to the proposed biosynthesis of the revised cytosporolide structures, we conducted a brief biomimetic synthetic study (Scheme 2). Aryl triflate **15** was prepared via reaction of methyl 2,4,6-trihydroxy benzoate with Tf_2O according to a published procedure.[12] Stille coupling of **15** with tributylvinyltin gave **16** in good yield (75%), which was then benzylated under standard conditions to give **17** (91%). Epoxidation of **17** with *m*CPBA in CH_2Cl_2 gave **18** in 55% yield. Hydrogenation of **18** then simultaneously cleaved the benzyl ethers and ring-opened the epoxide to give **19** (86%).

Alcohol **19** was then treated with TFA and an excess of $HC(OEt)_3$ at room temperature (Scheme 3). Following

Scheme 1. Proposed Biosynthesis of Cytosporolide A



addition of β-caryophyllene (**22**), the reaction mixture was stirred at 100 °C for 18 h, which generated the cycloadduct **23** as a single diasteroisomer in 53% yield. Presumably this three-component cascade reaction proceeds via initial formation of the cyclic acetal **20**, followed by elimination of EtOH to give the *o*-quinone methide intermediate **21**. [4 + 2] cycloaddition of **21** to **22** would then generate **23** via either a pericyclic Diels–Alder reaction or perhaps a stepwise ionic process. Overall this reaction forms two new 6-membered rings and three new stereocenters in one step, with complete stereoselectivity. Basic hydrolysis of the methyl ester of **23** then gave carboxylic acid **24** in 57% yield.

Importantly, the ^{13}C NMR chemical shift of C-8 of **24** was found to be 88.6 ppm, which is in good agreement with the value recorded for C-8 of the cytosporolides. Furthermore, the aromatic region of the ^{13}C NMR spectrum of **24** shows an excellent correlation with the cytosporolide spectra (see Supporting Information for a full comparison). The relative configuration of **24** was established via X-ray crystallography.[14] The stereochemical configurations at C-8, C-9, and C-16 of **23** were found to be opposite to that found in the cytosporolides. This fact can be rationalized by invoking addition of *o*-quinone methide **21** to either the $\alpha\alpha$ or $\beta\alpha$ conformations of β-caryophyllene,[15] whereas in the biosynthesis of cytosporolide A the *o*-quinone methide **13** might add to the favored $\beta\beta$ conformation of fuscoatrol.[5] The IR spectrum of the carboxylic acid **24**

(10) Inagaki, T.; Kaneda, K.; Suzuki, Y.; Hirai, H.; Nomura, E.; Sakakibara, T.; Yamauchi, Y.; Huang, L.; Norcia, M.; Wondrack, L. M.; Sutcliffe, J. A.; Kojima, N. *J. Antibiot.* **1998**, *51*, 112.

(11) For a review of *o*-quinone methide chemistry, see: Van De Water, R. W.; Pettus, T. R. *Tetrahedron* **2002**, *58*, 5367.

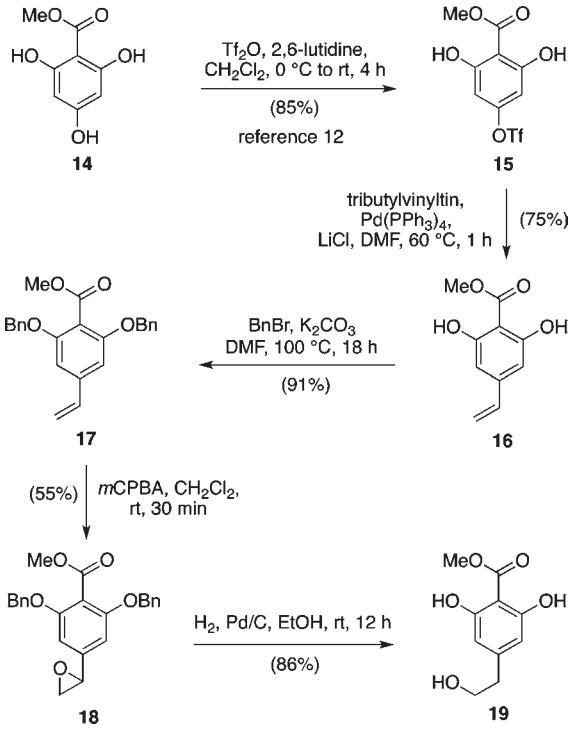
(12) Bender, C. F.; Yoshimoto, F. K.; Paradise, C. L.; De Brabander, J. K. *J. Am. Chem. Soc.* **2009**, *131*, 11350.

(13) (a) George, J. H.; Baldwin, J. E.; Adlington, R. M. *Org. Lett.* **2010**, *12*, 2394. (b) George, J. H.; Hesse, M. D.; Baldwin, J. E.; Adlington, R. M. *Org. Lett.* **2010**, *12*, 3532.

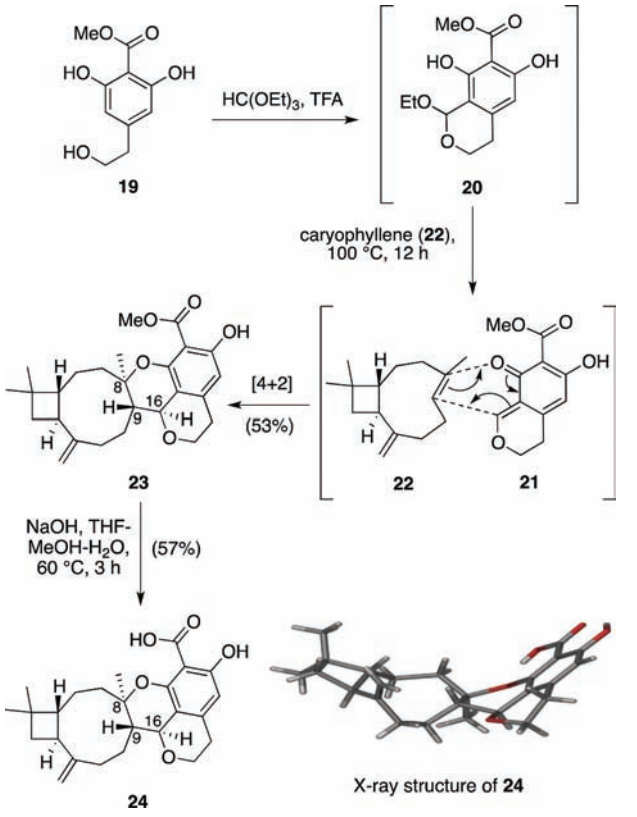
(14) Crystallographic data for the structure of **24** have been deposited with the Cambridge Crystallographic Data Centre (CCDC 829815). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

(15) Clericuzio, M.; Alagona, G.; Ghio, C.; Toma, L. *J. Org. Chem.* **2000**, *65*, 6910.

Scheme 2. Synthesis of *o*-Quinone Methide Precursor **19**



Scheme 3. Synthesis of **24** via a Biomimetic [4 + 2] Cycloaddition



shows a C=O stretch at 1694 cm^{-1}. This is in good agreement with the IR data for the cytosporolides A–C, which were reported by Che et al. to have C=O stretches in the range 1690–1694 cm^{-1}. Furthermore, berkelic acid (**11**) has an identical carboxylic acid functional group to **24** and the revised cytosporolide structures (**8**–**10**), and it has a C=O stretch of 1693 cm^{-1}. This excellent correlation in IR data between the model compound **24**, berkelic acid, and the cytosporolides is good evidence that all of these compounds contain an aryl carboxylic acid with an *ortho*-hydroxy substituent.

In conclusion, we have proposed that the structures of the cytosporolides A–C have been incorrectly assigned, and we suggest that the revised structures **8**–**10** more accurately represent these natural products. In part, this reassignment was inspired by biosynthetic considerations. Re-evaluation of NMR data supported the argument, with instructive comparisons made between the ^{1}H and ^{13}C chemical shift data for the cytosporolides with the related compounds guajadial (**6**) and berkelic acid (**11**). Finally, a biomimetic cycloaddition of an *o*-quinone methide onto *β*-caryophyllene generated a simplified cytosporolide analogue via a three-component cascade reaction.

Acknowledgment. We thank Dr. Chris Sumby and Mr. Witold Bloch (University of Adelaide) for assistance with X-ray crystallography studies and Prof. Yongsheng Che (Institute of Microbiology, Chinese Academy of Sciences) for the provision of NMR spectra.

Supporting Information Available. Synthetic procedures and analytical data for compounds **14**–**19** and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.