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## Absolute Configuration of Curacin A, a Novel Antimitotic Agent from the Tropical Marine Cyanobacterium *Lyngbya majuscula*

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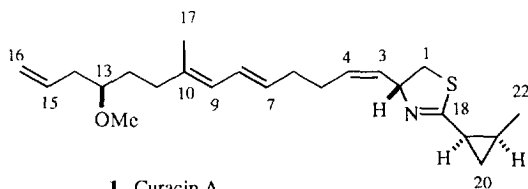
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**Abstract:** Curacin A is a structurally novel antimitotic agent isolated from the Caribbean cyanobacterium *Lyngbya majuscula*. Its planar structure has been previously determined from a spectroscopic investigation. Here, we define the complete relative and absolute configuration of curacin A by comparison of products obtained from chemical degradation of the natural product with the same substances prepared by synthesis. Curacin A is shown to have 2*R*, 13*R*, 19*R*, 21*S* absolute configuration.

Marine cyanobacteria (blue-green algae) are rich in structurally unique and biologically active secondary metabolites, some of which have potential in treating human disease.<sup>1</sup> Recently, the isolation of curacin A (**1**), a thiazoline-containing lipid with potent antiproliferative activity, was reported from a Curaçao (Caribbean) collection of *Lyngbya majuscula*.<sup>2</sup> In the original report, as well as in continuing studies,<sup>3</sup> it was found that curacin A exerts its biological effects in cells by inhibiting the polymerization of tubulin and thereby inhibiting microtubule formation. Of the two well characterized drug-binding sites on microtubules, the vinca alkaloid site and the colchicine site,<sup>4</sup> curacin A has been found to bind to the latter with high affinity.<sup>2</sup> This result is surprising, as these two drugs show little obvious structural homology. Crucial to developing a more complete appreciation for the nature of the interaction between curacin A and its biomolecular target, and as an essential precondition for efforts aimed at its synthesis, we undertook a degradative approach to define its relative and absolute stereochemistry. Here we report our results which establish that curacin A possesses 2*R*, 13*R*, 19*R*, 21*S* configuration.

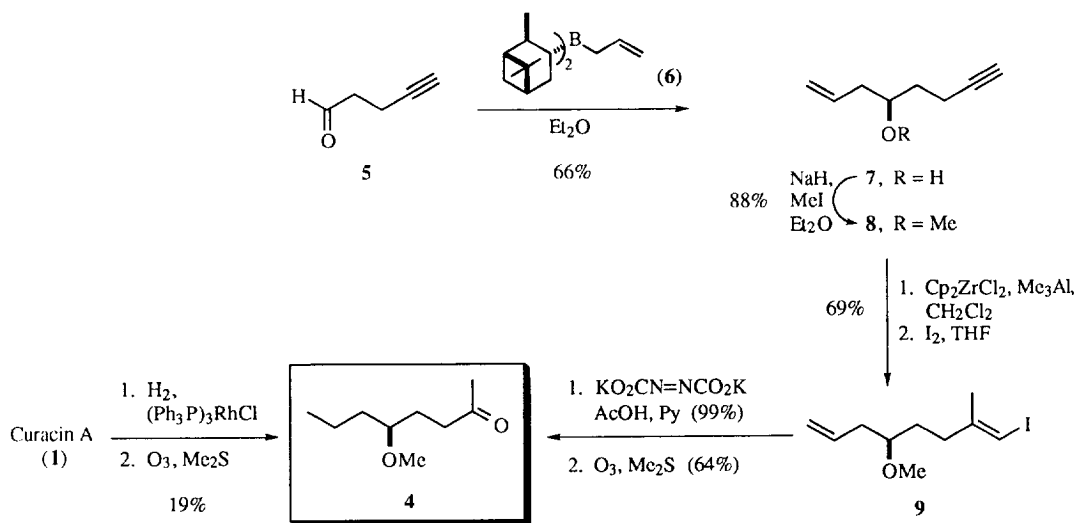
The strategy for determination of the C13 stereochemistry in **1** hinged upon ozonolysis of the C9-C10 olefin to produce a methyl ketone. However, when curacin A was subjected to ozonolysis directly, we were unable to recover a fragment deriving from the C9-C17 region. Suspecting that the C15-C16 olefin was responsible for this undesired reactivity, curacin A was partially hydrogenated with Wilkinson's catalyst to yield a mixture of 15,16-dihydrocuracin A (**2**) and 3,4,15,16-tetrahydrocuracin A (**3**) by GC-MS analysis. This mixture was ozonized and the ozonide was reduced with excess dimethyl sulfide to yield, following chromatography, 5-methoxyoctan-2-one (**4**).<sup>5</sup> The latter was fully characterized by <sup>1</sup>H and <sup>13</sup>C NMR and GC-MS,<sup>6</sup> and showed  $[\alpha]_D^{23} +14.2^\circ$  (*c* 0.22, CDCl<sub>3</sub>). Elucidation of the *R* configuration of this fragment was achieved through comparison with (+)-**4** obtained by enantiospecific synthesis.



1. Curacin A
2. 15,16-Dihydrocuracin A
3. 3,4,15,16-Tetrahydrocuracin A

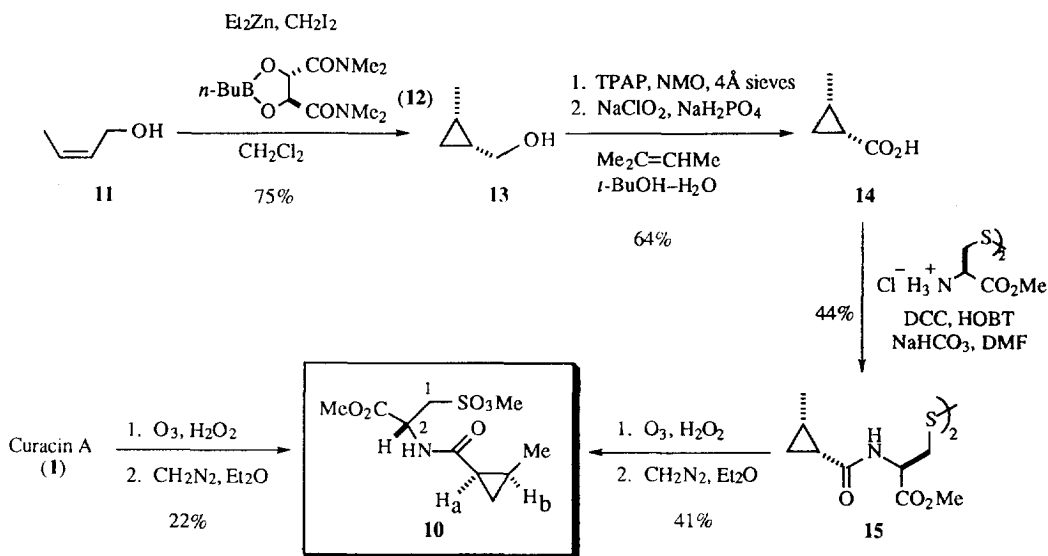
Allylation of 4-pentynal (**5**), prepared by Swern oxidation of the corresponding alcohol, with the salt-free allylborane **6** derived from (-)-diisopinylcamphylmethoxyborane<sup>7</sup> gave (5*R*)-1-octyn-7-en-5-ol (**7**) in 95% ee. After conversion of **7** to its methyl ether **8**, the latter was treated with dicyclopentadienylzirconium dichloride in the presence of trimethylaluminum and then with iodine, to yield the *E*-iodooctadiene **9**.<sup>8</sup> Selective hydrogenation of the monosubstituted olefin of **9** was accomplished with diimide<sup>9</sup> and the resultant alkene was ozonized to afford, after reductive workup, (5*R*)-methoxyoctan-2-one (**4**),  $[\alpha]_{\text{D}}^{25} +15.0^\circ$  (*c* 0.36,  $\text{CDCl}_3$ ). This substance was identical by comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR and GC-MS with the material obtained by degradation of curacin A.

The strategy for determining the configuration of the C2, C19, and C21 stereocenters in curacin A originally envisioned oxidative cleavage of the C3-C4 olefin and recovery of a methylcyclopropyl- and carboxyl-substituted thiazoline that would be compared with synthetic substances of known chirality. However, ozonolysis of curacin A ( $-78^\circ$ ,  $\text{CHCl}_3$ , 2 min), followed by oxidative workup ( $\text{H}_2\text{O}_2$ ,  $45^\circ$ , 16 hr) and then reaction with excess  $\text{CH}_2\text{N}_2$  in  $\text{Et}_2\text{O}$ , gave after flash chromatography and HPLC, methyl sulfonate derivative **10**,  $[\alpha]_{\text{D}}^{25} -17.1^\circ$  (*c* 0.12, MeOH) (22% overall yield).<sup>10</sup> The sulfonate **10**, of molecular constitution  $\text{C}_{10}\text{H}_{17}\text{NO}_6\text{S}$ , displayed IR stretching absorptions for ester ( $1738\text{ cm}^{-1}$ ), amide ( $1637\text{ cm}^{-1}$ ) and sulfonate ( $1353, 1170\text{ cm}^{-1}$ ) functionalities. The methylcyclopropyl and  $\text{H}_2-1 \rightarrow \text{H}-2 \rightarrow \text{NH}$  spin systems were readily evident in the  $^1\text{H}$  NMR spectrum of **10**, as were two ester methyl groups at  $\delta$  3.88 and  $\delta$  3.82. By HMBC,<sup>11</sup> we were able to connect the latter signal to a  $\delta$  169.2 carbonyl which in turn showed 3-bond coupling to the C1 methylene protons, thereby providing assignment of the two methyl groups. Combination of these two partial structures through the remaining elements of the molecular formula, "CO", completed the structure determination of this degradation product (**10**).



This assignment was confirmed and the absolute configuration of **10** was established by an asymmetric synthesis from *cis* crotyl alcohol (**11**). The latter, prepared by hydrogenation of 2-butyne-1-ol over Lindlar's catalyst, was cyclopropanated with diethylzinc and diiodomethane in the presence of the *n*-butylboron complex of (5*S*)-(-)-*N,N,N',N'*-tetramethyltartaramide (**12**) to give **13** in >95% ee.<sup>12</sup> Oxidation of **13**, first with

perruthenate<sup>13</sup> and then with sodium chlorite,<sup>14</sup> afforded (1*R*,2*S*)-2-methylcyclopropanecarboxylic acid (**14**) which was coupled with (*R*)-(-)-cystine dimethyl ester dihydrochloride using DCC and HOBT.<sup>15</sup> The resulting disulfide **15** was ozonized and the crude product was treated with diazomethane to furnish **10**, [ $\alpha$ ]<sub>D</sub><sup>25</sup> -21.4° (*c* 0.21, MeOH), identical by comparison of <sup>1</sup>H and <sup>13</sup>C NMR and GC-MS with the corresponding substance obtained by degradation of curacin A. A stereoisomer of **10** derived from (1*S*,2*R*)-2-methylcyclopropanecarboxylic acid, prepared with the tartaramide complex antipodal to **12**, was distinguishable by <sup>1</sup>H NMR, <sup>13</sup>C NMR and GC-MS from the sulfonate derived from **1**. Careful analysis of the <sup>1</sup>H NMR spectrum of **10** in d<sub>6</sub>-benzene confirmed that *cis* configuration of the cyclopropane had been retained. It was shown that H<sub>a</sub> and H<sub>b</sub> are each *cis* (*J*=8 Hz) to the same proton of the geminal pair and are therefore *cis* to each other.



These results fully define the absolute configuration of curacin A (**1**). It is noteworthy that curacin A possesses *R* configuration at the  $\alpha$ -amino center (C2), a fact consistent with the hypothesis that the thiazoline portion of the molecule is derived from *L*-cysteine.<sup>2</sup>

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## References and Notes

- Ireland, C.M.; Copp, B.R.; Foster, M.P.; McDonald, L.A.; Radisky, D.C.; Swersey, J.C. In "Marine Biotechnology, Volume 1, Pharmaceutical and Bioactive Natural Products." D.H. Attaway and O.R. Saborsky, Eds., Plenum Press, New York, 1993, pp. 1-43.
- Gerwick, W.H.; Proteau, P.J.; Nagle, D.G.; Hamel, E.; Blokhin, A.; Slate, D. *J. Org. Chem.* **1994**, *59*, 1243-1245.
- Hamel, E.; Blokhin, A.V.; Nagle, D.G.; Yoo, H.-D.; Gerwick, W.H. *Drug Dev. Res.* (in press).
- a) Sackett, D.L. *Pharmacol. Ther.*, in press; b) Hamel, E. *Pharmacol. Ther.* **1992**, *55*, 31-51.
- Purification of **4** was achieved by silica gel flash chromatography using a stepwise gradient [eluted in 25% Et<sub>2</sub>O/pentane (v/v)] followed by HPLC (dual 10- $\mu$ m Alltech Versapak Si columns; 300 x 4.1 mm; 10% (v/v) EtOAc in hexanes; UV detection at 254 nm; flow rate 2.5 mL/min) to give a colorless oil (4.0 mg, 25.3  $\mu$ mol, 19% overall yield).
- 5-Methoxyoctan-2-one (4)*. IR  $\nu_{\text{max}}^{\text{film}}$ : 2958, 2932, 2886, 2874, 1717, 1457, 1361, 1205, 1164, 1129, 1092 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.30 (s, 3H, -OCH<sub>3</sub>), 3.16 (ddd, 1H, J = 6, 5, 5, H-5), 2.50 (m, 2H, H-3), 2.15 (s, 3H, H-1), 1.79-1.87 (m, 1H), 1.67 (6-lines, 1H), 1.50 (m, 1H), 1.30-1.41 (m, 3H), 0.92 (t, 3H, J = 7.0, H-1); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  208.92 (C2), 79.75 (C5), 56.39 (-OCH<sub>3</sub>), 39.28, 35.59, 29.94, 27.33, 18.52, 14.21 (C8); GC EIMS 70 eV *m/z* (rel. int.): 143 [M - CH<sub>3</sub>]<sup>+</sup> (6), 126 (2), 115 (100), 100 (13), 87 (39), 83 (38), 72 (28), 71 (18), 55 (35).
- Racherla, U.S.; Brown, H.C. *J. Org. Chem.* **1991**, *56*, 401-404.
- Negishi, E.-i.; Van Horn, D.E.; King, A.O.; Okukado, N. *Synthesis* **1979**, 501-502.
- Hamersma, J.W.; Snyder, E.I. *J. Org. Chem.* **1965**, *30*, 3985-3988.
- Methyl Sulfonate 10*. HR EIMS obs M<sup>+</sup> 279.0775, 0.2 mmu dev. for C<sub>10</sub>H<sub>17</sub>NO<sub>6</sub>S; IR  $\nu_{\text{max}}^{\text{film}}$ : 3340, 1738, 1637, 1536, 1353, 1275, 1182, 1170, 996, 833 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.62 (bd, 1H, J  $\approx$  6, N-H), 4.90 (dt, 1H, J = 7.3, 4.8, 4.7, H-2), 3.88 (s, 3H, H-10), 3.82 (s, 3H, H-9), 3.79 (d, 2H, J = 4.8, H-1), 1.55 (m, 1H, H-5), 1.26 (m, 1H, H-7), 1.16 (d, 3H, J = 6.1, H-8), 0.95 (m, 2H, H-6); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$  171.80, 169.16, 56.04, 53.23, 50.12 (C1), 48.81, 20.29 (C5), 15.68 (C7), 13.00 (C6), 11.97 (C8); GC EIMS 70 eV *m/z* (rel. int.): 279 [M]<sup>+</sup> (7), 264 [M - CH<sub>3</sub>]<sup>+</sup> (1), 248 [M - OCH<sub>3</sub>]<sup>+</sup> (1), 220 [M - CO<sub>2</sub>CH<sub>3</sub>]<sup>+</sup> (18), 198 (43), 196 [M - C<sub>5</sub>H<sub>7</sub>O]<sup>+</sup> (9), 184 [M - SO<sub>3</sub>CH<sub>3</sub>]<sup>+</sup> (4), 164 (9), 138 (36), 83 [C<sub>5</sub>H<sub>7</sub>O]<sup>+</sup> (100), 55 (29).
- Bax, A.; Summers, M.F. *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.
- Charette, A.B.; Juteau, H. *J. Am. Chem. Soc.* **1994**, *116*, 2651-2652.
- Griffith, W. P.; Ley, S.V.; Whitcombe, G.P.; White, A.D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625-1627.
- a) Lindgren, B.O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888-890; b) Kraus, G.A.; Taschner, H.J. *J. Org. Chem.* **1980**, *45*, 1175-1176.
- Windridge, G.C.; Jorgensen, E.C. *J. Am. Chem. Soc.* **1971**, *93*, 6318-6319.

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