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Total Synthesis and Antifungal Activity of 9-Methoxystrobilurin L as the Originally Proposed 1,4-Benzodioxan Structure

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Abstract—Total synthesis of both enantiomers of 9-methoxystrobilurin L as the originally proposed 1,4-benzodioxan structure was successfully achieved. The ^{1}H and ^{13}C NMR spectra of synthesized 9-methoxystrobilurin L were compared with those of a naturally-occurring sample. It was strongly indicated that naturally-occurring 9-methoxystrobilurin L has not the originally reported 1,4-benzodioxan structure but a 1,5-benzodioxepin structure, the same as previously reported 9-methoxystrobilurin K. Antifungal activities of the synthesized compounds toward several typical fungi were also examined, and they were less active than 9-methoxystrobilurin K.

Introduction

9-methoxystrobilurin L was isolated as a new analogue of β-methoxyacrylate antibiotics from mycelial culture of basidomycete *Favolaschia pustulosa* by Xenova's group in 1996.[1] The compound exhibits strong antifungal activities toward several typical fungi by inhibiting a mitochondrial respiration pathway as other β-methoxyacrylates (MOAs) do.[2] In addition, the compound shows remarkable cytostatic activity toward human Burkitt's lymphoma derived cell lines (Jijoye) at very low concentration. The ^{1}H and ^{13}C NMR spectra of 9-methoxystrobilurin L were very similar to those of 9-methoxystrobilurin K (**2**) previously reported by Anke et al.;[3] however, it was assigned as an original 1,4-benzodioxepin structure **1**. On the other hand, Blunt and Munro isolated a compound showing almost the same ^{1}H and ^{13}C NMR spectra as **1** or **2** from another culture in 1996, and claimed the 1,5-benzodioxepin structure **3** based on the assignment of the HMBC spectrum in $CDCl_3$ for these compounds.[4] Recently, Anke and Steglich formally revised the structure of 9-methoxystrobilurin K to **3** by comparison of a synthesized 1,5-benzodioxepin substructure with the degradation product of natural strobilurin K.[5] Further, a total synthesis of 9-methoxystrobilurin K **3** was recently achieved and its 1,5-benzodioxepin structure was synthetically confirmed in our previous paper.[6]

According to these results, 9-methoxystrobilurin L also seemed to have the same 1,5-benzodioxepin structure; however, it should be confirmed synthetically for future study of structure–activity relationships. In addition, 1,4-benzodioxan structure **1** was also expected to show strong biological activities because strobilurin E (**4**) having a similar 1,4-benzodioxan structure indicated strong antifungal and cytostatic activities (Fig. 1).[7] In this paper, we would like to report the asymmetric total synthesis of both enantiomers of 9-methoxystrobilurin L as the originally proposed 1,4-benzodioxan structure and its antifungal activities.

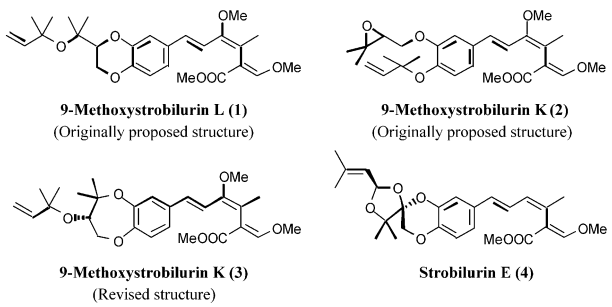

Figure 1. Cytostatic strobilurin analogues.

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Synthetic Plan

A retrosynthesis of 9-methoxystrobilurin L is shown in Scheme 1.

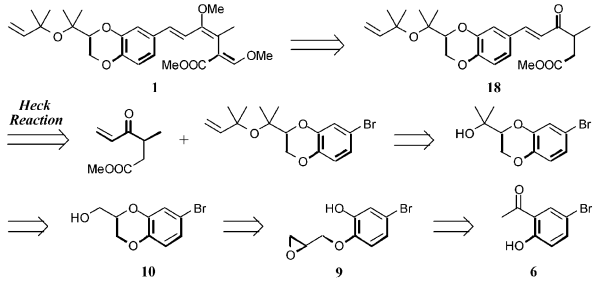
A construction of the relatively unstable conjugated triene moiety was to be performed at the last stage of total synthesis. Therefore, an enone-type intermediate **18** was decided as the first target molecule. Further, an optically active 1,4-benzodioxan **10** would be constructed by 6-*exo* selective intramolecular cyclization of epoxyphenol **9**. The epoxyphenol **9**, in turn, would be prepared from 2′-hydroxy-5′-bromoacetophenone **6** and chiral epichlorohydrin via Baeyer–Villiger oxidation.

Synthesis

The reaction of 2′-hydroxy-5′-bromoacetophenone **6** with optically active epichlorohydrin **5** was carried out by Augstein's α-selective coupling method[8] and the desired ether **7** was obtained in 68% yield (Scheme 2).

The Baeyer–Villiger oxidation of **7** proceeded smoothly to afford the corresponding epoxy ester **8**. Interestingly, the desired 6-*exo* cyclization subsequently proceeded on hydrolysis of the epoxy ester **8** for the preparation of cyclization precursor **9**. The thus-obtained primary alcohol **10** having a 1,4-benzodioxan-ring was oxidized to the carboxylic acid, which led to the corresponding methyl ester **11**. Treatment of the ester **11** with an excess of methylmagnesium bromide gave a tertiary alcohol **12** in good yield. The optical purity of **12** was determined by HPLC analysis as 93% ee.[9] A direct ether synthesis using the thus-obtained tertiary alcohol **12** with isoprene under acidic conditions failed due to the exclusive polymerization of the diene. Therefore, stepwise synthesis was next carried out. The α-alkoxy-α-methyl ester **13** was prepared by treatment of the alcohol **12** with sodium hydride and alkylation of the resulting alkoxide with methyl α-bromopropionate. Further, an enolate anion generated from **13** and sodium bis(trimethylsilyl)amide was methylated by the addition of methyl iodide. The prepared α,α-dimethyl-α-alkoxy ester **14** was reduced to the alcohol and subsequently oxidized to the corresponding aldehyde **15**. Terminal olefination of the aldehyde **15** utilizing Wittig reaction gave the aryl bromide **16**, and the desired tertiary–tertiary ether linkage was successfully constructed.

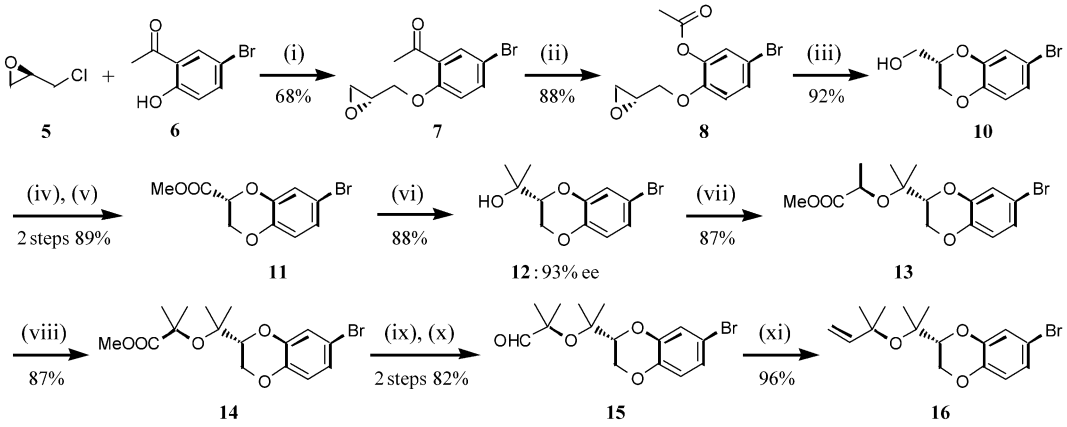
The Heck reaction of the aryl bromide **16** with a vinyl ketone **17**[10] proceeded smoothly to give the desired enone-type intermediate **18** (Scheme 3). The ester **18** was hydrolyzed to the corresponding carboxylic acid, which led to the methylenolether **19** by treatment with potassium *tert*-butoxide and dimethyl sulfate. Further, the β-methoxyacrylate moiety was constructed by our previously reported method,[10] and total synthesis of (*R*)-9-methoxystrobilurin L (**1*R***) was successfully achieved. The enantiomer (**1*S***) was also synthesized by the same procedure.



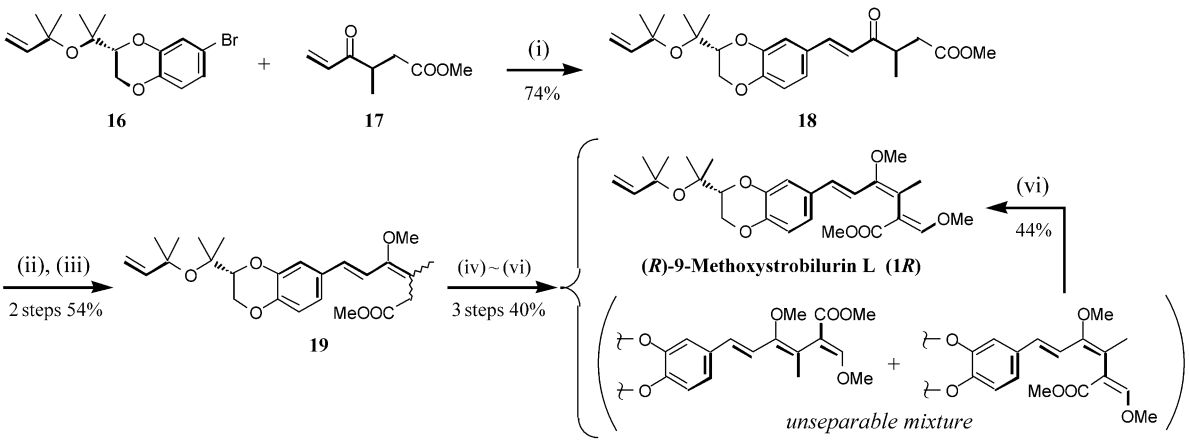
Scheme 1. Retrosynthesis of 9-methyoxystrobilurin L.

Spectral Comparison

The ^1H and ^{13}C NMR spectra of synthesized 9-methoxystrobilurin L (**1**)[11] were compared with those of a natural sample reported by Xenova's group[1,13] (Typical ^1H NMR data are summarized in Table 1). The present study made it clear that 9-methoxystrobilurin L was not the 'originally reported' 1,4-benzodioxan structure because of the obvious differences in the chemical shifts on its ^1H and ^{13}C NMR spectra in CD_3OD. On the other hand, a greater spectral similarity between natu-

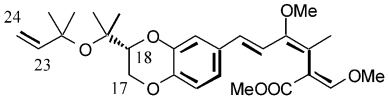


Scheme 2. Synthesis of 1,4-benzodioxan including tertiary–tertiary ether linkage (*R*-form). Reaction conditions: (i) KOh, EtOH–H_2O, reflux; (ii) mCPBA, $CHCl_3$, reflux; (iii) NaOH aq–THF, reflux; (iv) $KMnO_4$, KOH, H_2O, rt; (v) CN_2N_2, Et_2O, rt, 20 min; (vi) MeMGBr (excess), THF, rt, 1 h; (vii) NaH, methyl 2-bromopropionate, 0 °C; (viii) NaHMDS, MeI, THF, −78 °C; (ix) DIBAH, CH_2Cl_2, −78 °C; (x) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −60 °C; (xi) $Ph_3P{=}CH_2$, DMSO–THF, rt.

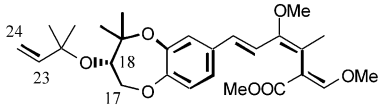


Scheme 3. Synthesis of (*R*)-9-methoxystrobilurin L. Reaction conditions: (i) 10 mol% $Pd(OAc)_2$, PPh_3, Et_3N, 100 °C; (ii) NaOH aq–MeOH, rt then HCl; (iii) KO^tBu, Me_2SO_4, DMF, −45 to −15 °C; (iv) NaH, HCOOMe, rt; (v) K_2CO_3, Me_2SO_4, HCOOMe, rt; (vi) hν (λ: 365 nm), acetone–benzene, rt.

Table 1. ^1H NMR spectra of 9-methoxystrobilurins L and K[a,b]



9-Methoxystrobilurin L (1) (Synthesized)



9-Methoxystrobilurin K (3)

Compound	9-Methoxystrobilurin L		9-Methoxystrobilurin K
Position	Natural	Synthesized	Synthesized
18	3.70 (m)	3.84 (dd, J=2.0, 8.8 Hz)	3.69 (dd, J=3.4, 7.6 Hz)
17a,17b	4.00 (dd, J=7.5, 12.4 Hz) 4.20 (dd, J=3.1, 12.3 Hz)	3.97 (dd, J=8.8, 11.2 Hz) 4.55 (dd, J=2.0, 11.2 Hz)	3.98 (dd, J=7.6, 12.5 Hz) 4.20 (dd, J=3.4, 12.5 Hz)
23	5.90 (dd, J=10.0, 17.4 Hz)	6.07 (dd, J=10.7, 17.6 Hz)	5.93 (dd, J=11.0, 17.7 Hz)
24a,24b	5.19 (dd, J=0.7, 10.0 Hz) 5.22 (dd, J=0.7, 18.0 Hz)	5.00 (dd, J=1.0, 10.7 Hz) 5.13 (dd, J=1.0, 17.6 Hz)	5.17 (dd, J=0.9, 10.7 Hz) 5.23 (dd, J=0.9, 17.7 Hz)

[a]CD_3OD was used as the solvent.
[b]Tetramethylsilane was used as the internal standard.

Table 2. Antifungal activities of synthesized (*R*)- and (*S*)-9-methoxystrobilurin L

Compound	Diameter of inhibition zone (mm)[a,b]					
	Conc. (μg/disk)	*Penicillium citrium*	*Aspergillus fumigatus*	*Fusarium solani*	*Candida albicans*	*Saccharomyces cerevisiae*
Nystatin (positive control)	10	10	12	—	16	17
	10	—	8	—	10	8
(**1*R***)	1	—	±	—	±	±
	0.1	—	±	—	—	—
	10	—	8	—	8	8
(**1*S***)	1	—	8	—	—	—
	0.1	—	±	—	—	—
	10	—	10	25*	22	25
(**3**)	1	—	8	19*	17	20
	0.1	—	±	—	—	±

[a]The diameter of each inhibition zone (mean values of two samples) was measured after 48 h incubation.
[b]—, Not effective; ±, slightly effective; *, incomplete inhibition.

rally-occurring 9-methoxystrobilurin L and synthesized 9-methoxystrobilurin K[12] was clearly demonstrated.

Biological Assay

Antifungal activities of both synthesized enantiomers of 9-methoxystrobilurin L toward several typical fungi examined by the disk diffusion method using potato dextrose agar are shown in Table 2.

Interestingly, both synthesized enantiomers of 9-methoxystrobilurin L were unexpectedly less active than 9-methoxystrobilurin K and nystatin (positive control) toward all of the examined fungi.

Conclusion

The originally proposed 1,4-benzodioxan structure of 9-methoxystrobilurin L was successfully synthesized; however, its structural assignment was clearly ruled out by obvious differences in ^{1}H and ^{13}C NMR spectra. In conclusion, it was strongly indicated that naturally-occurring 9-methoxystrobilurin L should be the same compound as 9-methoxystrobilurin K previously isolated by two other groups. In addition, both synthesized enantiomers of the 1,4-benzodioxan structure were less effective than 9-methoxystrobilurin K. This result is in contrast to the strong antifungal activity of strobilurin E (**4**) having a similar 1,4-benzodioxan structure and indicated that the geometry of the hindered ether-type side chain moiety on the 1,4-benzodioxan ring is very influential in the biological activities of these compounds. Further extensive studies for the synthesis and design of novel β-methoxyacrylate antibiotics having a more effective side chain moiety are now in progress.

References and Notes

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11. Physical data of synthesized 9-methoxystrobilurin L (**1**): ^{1}H NMR (δ, 500 MHz, CD_3OD) 1.31 (s, 3H), 1.36 (s, 6H), 1.44 (s, 3H), 1.84 (s, 3H), 3.65 (s, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 3.78 (dd, 1H, $J=2.0$, 8.8 Hz), 3.94 (dd, 1H, $J=8.8$, 11.2 Hz), 4.48 (dd, 1H, $J=2.0$, 11.2 Hz), 5.00 (dd, 1H, $J=1.0$, 10.7 Hz), 5.13 (dd, 1H, $J=1.0$, 17.6 Hz), 6.12 (dd, 1H, $J=10.7$, 17.6 Hz), 6.34 (d, 1H, $J=15.9$ Hz), 6.54 (d, 1H, $J=16.1$ Hz), 6.74 (d, 1H, $J=8.3$ Hz), 6.81 (dd, 1H, $J=2.0$, 8.3 Hz), 6.84 (d, 1H, $J=2.0$ Hz), 7.48 (s, 1H); ^{13}C NMR (δ, 125.6 MHz, CD_3OD) 16.5, 23.8, 26.9, 30.09, 30.11, 52.0, 59.8, 62.4, 66.4, 77.2, 78.1, 80.9, 111.5, 112.1, 115.5, 118.0, 120.7, 120.9, 128.5, 132.3, 144.8, 145.3, 148.7, 154.0, 161.4, 169.8; $[\alpha]_D^{28}=72.9$ (**1*R***), ($c=0.402$, $CHCl_3$), −71.5 (**1*S***), ($c=0.442$, $CHCl_3$); HRMS calcd for $C_{27}H_{36}O_7$ (M^+) 472.2461, found 472.2446 (**1*R***), 472.2452 (**1*S***).
12. Physical data of synthesized 9-methoxystrobilurin K (**3**):[6] ^{1}H NMR (δ, 500 MHz, CD_3OD) 1.22 (s, 3H), 1.32 (s, 3H), 1.32 (s, 3H), 1.38 (s, 3H), 1.85 (s, 3H), 3.63 (s, 3H), 3.69 (dd, 1H, $J=3.4$, 7.6 Hz), 3.70 (s, 3H), 3.83 (s, 3H), 3.98 (dd, 1H, $J=7.6$, 12.5 Hz), 4.20 (dd, 1H, $J=3.4$, 12.5 Hz), 5.17 (dd, 1H, $J=0.9$, 10.7 Hz), 5.23 (dd, 1H, $J=0.9$, 17.7 Hz), 5.93 (dd, 1H, $J=11.0$, 17.7 Hz), 6.36 (d, 1H, $J=15.9$ Hz), 6.54 (d, 1H, $J=15.9$ Hz), 6.80 (d, 1H, $J=8.2$ Hz), 6.88 (d, 1H, $J=2.1$ Hz), 6.94 (dd, 1H, $J=2.1$, 8.2 Hz), 7.49 (s, 1H); ^{13}C NMR (δ, 125.6 MHz, CD_3OD) 16.5, 22.6, 26.3, 26.7; $[\alpha]_D^{24}=-8.87$ ($c=0.860$, $CHCl_3$); HRMS calcd for $C_{27}H_{36}O_7$ (M^+) 472.2461, found 472.2456.
13. Physical data of natural 9-methoxystrobilurin L:[1] ^{1}H NMR (δ, 400 MHz, CD_3OD) 1.21 (s, 3H), 1.32 (s, 3H), 1.32 (s, 3H), 1.39 (s, 3H), 1.85 (s, 3H), 3.62 (s, 3H), 3.70 (s, 3H), 3.70 (m, 1H), 3.82 (s, 3H), 4.00 (dd, 1H, $J=7.5$, 12.4 Hz), 4.20 (dd, 1H, $J=3.1$, 12.3 Hz), 5.19 (dd, 1H, $J=0.7$, 10.0 Hz), 5.22 (dd, 1H, $J=0.7$, 18.0 Hz), 5.90 (dd, 1H, $J=10.0$, 17.4 Hz), 6.39 (d, 1H, $J=15.0$ Hz), 6.54 (d, 1H, $J=15.0$ Hz), 6.80 (d, 1H, $J=8.4$ Hz), 6.84 (d, 1H, $J=2.1$ Hz), 6.97 (dd, 1H, $J=2.1$, 8.2 Hz), 7.44 (s, 1H); ^{13}C NMR (δ, 100 MHz, CD_3OD) 16.5, 22.6, 26.4, 26.8, 28.2, 52.2, 59.9 62.6, 72.8, 77.4, 77.7, 82.9, 111.9, 114.9, 119.6, 121.3, 121.5, 122.9, 123.7, 128.3, 134.5, 145.3, 147.9, 152.2, 154.3, 161.6, 169.9.