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# The First Synthesis and Antifungal Activities of 9-Methoxystrobilurin-type β-Substituted β-Methoxyacrylate

Hiromi Uchiro, Koh Nagasawa, Tomoya Kotake, Daiju Hasegawa, Aya Tomita and Susumu Kobayashi*

*Faculty of Pharmaceutical Sciences, Tokyo University of Science, 12 Ichigayafunagawara-machi, Shinjuku-ku, Tokyo 162-0826, Japan*

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**Abstract**—The first synthesis of 9-methoxystrobilurin-type β-substituted MOAs was successfully achieved. A chiral oudemansin-type β-substituted MOA was also synthesized utilizing Mukaiyama's asymmetric aldol reaction. Antifungal activities of the synthesized compounds against several representative fungi were examined by disk-diffusion assay. As a result, unique and superior antifungal properties of 9-methoxystrobilurin-type β-substituted MOAs compared with those of oudemansin-type analogue were clearly revealed.


## Introduction

β-Methoxyacrylate antibiotics (MOAs) represented by strobilurins and oudemansins are highly potent antifungal compounds and have, therefore, been applied to agricultural disinfectants in many countries.[1] In addition, novel 9-methoxystrobilurin-type analogues were recently isolated as strong growth inhibitors toward human-derived tumor cell lines.[2–4] Pharmaceutical application studies of these new types of MOAs in the antifungal, antitumor, and antimalarial fields based on their SAR studies are currently investigated.[5–7] These known type MOAs have an α-substituted β-methoxyacrylate moiety as a common pharmacophoric substructure. On the other hand, several β-substituted-type β-methoxyacrylates such as cystothiazoles[8] and melithiazoles[9] were recently isolated from nature (Fig. 1). Both of these new β-substituted MOAs include oudemansin-type *syn*-9-methoxy-10-methyl substructures at their 9-10-position; however, this saturated linkage seems not ideal for their antifungal properties. Previous SAR studies apparently indicated that the potent antifungal activities were usually observed in the derivatives having strobilurin-type or aromatic-type unsaturated linkage at the 9-10-position.[1,10] From this point of view, Anke and Steglich proposed the importance of orthogonal arrangement of the pharmacophoric MOA moiety and the molecular plane extending from the aromatic ring to 9-10 unsaturated linkage for the antifungal activity of α-substituted

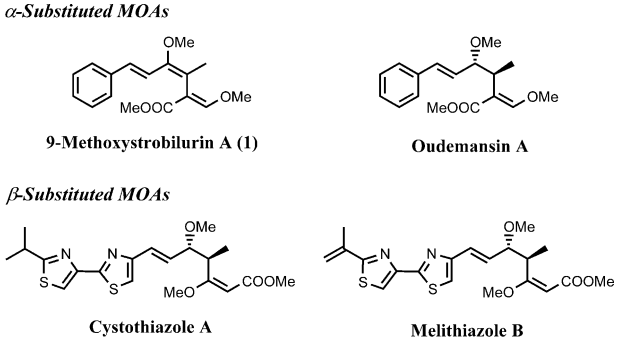


**Figure 1.** Two types of naturally-occurred β-methoxyacrylates (MOAs).

MOAs.[11] In this paper, we would like to describe the first synthesis of 9-methoxystrobilurin-type β-substituted β-methoxy acrylates which have not yet been discovered from nature. In addition, we also would like to reveal the pharmacological superiority of the 9-methoxy strobilurin-type β-substituted MOAs in their antifungal activities over the oudemansin-type analogue.

## Synthetic Strategy

Our synthetic strategy for 9-methoxystrobilurin-type β-substituted β-methoxyacrylates is shown in Scheme 1. An efficient synthetic route based on the aldol reaction of α, β-unsaturated aldehydes **2** with a dianion generated from 3-ketovaleric acid methyl ester **3** was designed. The central methyl enol ether moiety on the target

*Corresponding author. Tel. +81-3-3260-8848; fax: +81-3-3260-8848; e-mail: kobayash@ps.kagu.sut.ac.jp

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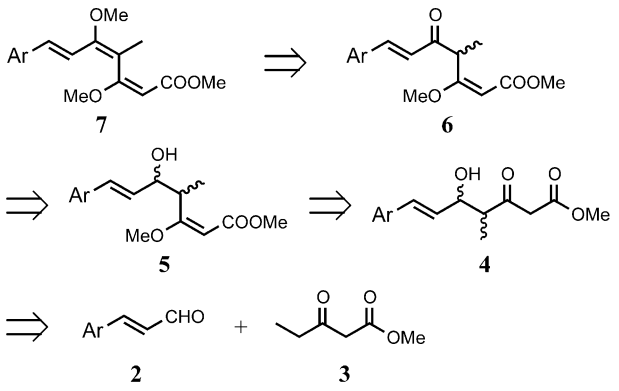


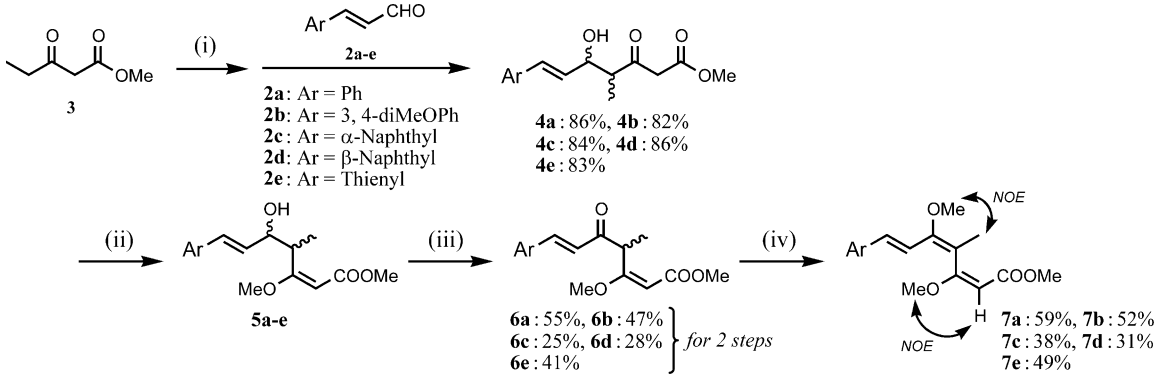
**Scheme 1.** Retrosynthesis of 9-methoxystrobilurin-type β-substituted β-methoxyacrylates.

molecule **7** could be easily constructed on enone-type intermediate **6**. The intermediate **6** would be prepared by the direct methyl enol ether formation starting from the aldol adduct **4** and successive oxidation of the remaining secondary hydroxyl group of intermediate **5**.

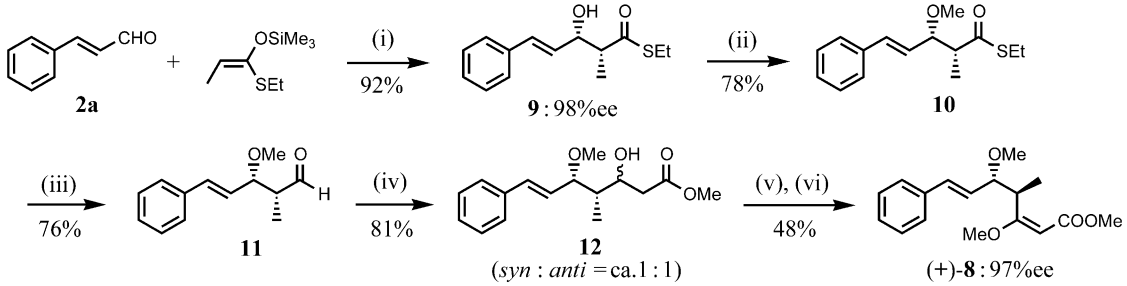
## Synthesis

The aldol reaction of cinnamaldehyde **2a** with a dianion generated from methyl 3-ketovalerate **3** was first carried out. The desired γ-adduct **4a** was obtained in 86% yield, and no undesirable α-adduct was formed. The direct methyl enol ether formation on the adduct **4a** was performed by use of 2 equivalents of potassium *tert*-butoxide and dimethyl sulfate in dimethylformamide, and the desired intermediate **5a** was successfully obtained. The remaining secondary hydroxyl group of **5a** was then oxidized by Dess–Martin periodinane to give the corresponding enone **6a** (55% yield for two steps). Finally, the desired 9-methoxystrobilurin-type β-substituted MOA **7a**[14] was obtained in 59% yield by the addition of 2 equivalents of potassium hexamethyldisilazide (KHMDS) and methyl triflate in THF–HMPA (4:1) at −78 °C. Several analogues modified on the aromatic moiety **7b–e**[14] were also synthesized from the corresponding α,β-unsaturated aldehydes **2b–e**. The stereochemistries of tri- and tetra-substituted olefin moieties of **7a–e** were respectively determined by NOE measurements as shown in Scheme 2.

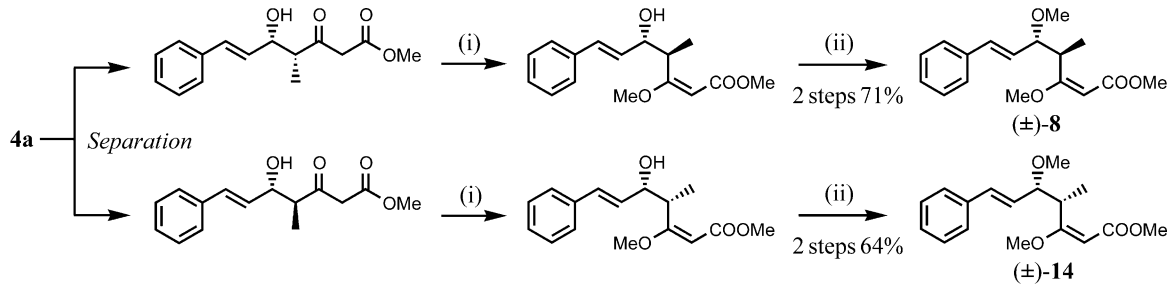
An optically active oudemansin-type β-substituted MOA (+)-**8** was synthesized from chiral aldol adduct **9** which was prepared by Mukaiyama's asymmetric aldol reaction[12] in 98% ee as shown in Scheme 3. The hydroxyl group of **9** was methylated by diazomethane-boron trifluoride etherate to give the intermediate ether **10**. The thiolester moiety of **10** was reduced by DIBAL to the corresponding aldehyde **11**. The second aldol reaction of the aldehyde **11** with the lithium enolate of methyl acetate was performed, and the resulting hydroxyl group of the aldol adduct **12** was oxidized to give the corresponding β-ketoester **13** (the structure is not shown). The desired optically active β-substituted MOA (+)-**8**[14] was prepared by *O*-methylation of a sodium enolate generated from β-ketoester **13**. The optical purity of (+)-**8** was



**Scheme 2.** Synthesis of 9-methoxystrobilurin-type β-substituted MOAs. (i) NaH, $^{n}$BuLi, THF, 0 °C, 30 min; (ii) $^{t}$BuOK, $Me_2SO_4$, DMF, 0 °C to rt, 1.5 h; (iii) Dess–Martin periodinane, $CH_2Cl_2$, rt, 30 min; (iv) KHMDS, MeOTf, THF–HMPA (4:1), −78 °C, 10 min.



**Scheme 3.** Synthesis of chiral oudemansin-type β-subsituted MOAs. (i) $Sn(OTf)_2$, , $^{n}Bu_2Sn(OAc)_2$, $CH_2Cl_2$, −78 °C, 3 h; (ii) $CH_2N_2$, $BF_3{\cdot}OEt_2$, $Et_2O$, 0 °C to rt, 1 h; (iii) DIBAL, $CH_2Cl_2$, −78 °C, 30 min; (iv) AcOMe with LDA, THF, −78 °C, 1 h; (v) Dess–Martin periodinane, $CH_2Cl_2$, rt, 1 h; (vi) $^{t}$BuOK, $Me_2SO_4$, DMF, 0 °C, 3 h.




**Scheme 4.** Synthesis of racemic oudemansin-type and *epi*-oudemansin-type-β-substituted MOAs. (i) $^t$BuOK, $Me_2SO_4$, DMF, 0 °C, 3 h; (ii) $CH_2N_2$, $BF_3{\cdot}OEt_2$, $Et_2O$, 0 °C to rt, 3 h.

**Table 1.** Antifungal activities of β-substituted β-methoxyacrylates

| Compd | Conc (μg/disk) | Diameter of inhibition zone (mm)[a,b] | | | | |
|---|---|---|---|---|---|---|
| | | Pc | Af | Fs | Ca | Sc |
| Nystatin (positive control) | 10 | 10 | 16 | — | 16 | 18 |
| **1** | 10 | 33 | 38 | — | 42*i* | 44*i* |
| | 1 | 23 | 28 | — | 32*i* | 39*i* |
| | 0.1 | 19 | 16 | — | ± | 13*i* |
| **7a** | 10 | 42 | 36 | 30*i* | 32*i* | 46*i* |
| | 1 | 17 | 17 | 22*i* | 27*i* | 30*i* |
| | 0.1 | — | — | — | — | — |
| **7b** | 10 | 33 | 12 | — | ± | 18*i* |
| | 1 | 18 | 8 | — | — | 12*i* |
| | 0.1 | — | — | — | — | — |
| **7c** | 10 | — | — | — | — | ± |
| | 1 | — | — | — | — | — |
| | 0.1 | — | — | — | — | — |
| **7d** | 10 | 13 | 16 | 27*i* | 14*i* | 21*i* |
| | 1 | — | 10 | 19*i* | 8*i* | 15i |
| | 0.1 | — | — | — | — | — |
| **7e** | 10 | 40 | 29 | — | 32*i* | 49*i* |
| | 1 | 21 | 20 | — | 16*i* | 31*i* |
| | 0.1 | ± | — | — | — | — |
| (+)-**8** | 10 | 28 | 23 | — | 20*i* | 43*i* |
| | 1 | ± | — | — | — | 25*i* |
| | 0.1 | — | — | — | — | — |
| (±)-**8** | 10 | 26 | 23 | — | 14*i* | 39*i* |
| | 1 | ± | — | — | — | ± |
| | 0.1 | — | — | — | — | — |
| (±)-**14** | 10 | — | — | — | — | — |
| | 1 | — | — | — | — | — |
| | 0.1 | — | — | — | — | — |

Pc, *Penicilium ctrinum* R-3703; Af, *Aspergillus fumigatus* R-1301; Fs, *Fusarium solani* R-2800; Ca, *Candida albicans* IFO 1594; Sc, *Saccharomyces cerevisae* IAM 4861.
[a]The diameter of each inhibition zones (mean value of two samples) were measured after 48 hr incubation.
[b]—, not effective, ±, slightly effective, *i*, incomplete inhibition.

determined to be 97%ee by chiral HPLC analysis (column: Daicel Chiralcel OD; eluent: hexane/2-propanol = 50/1).

Racemic oudemansin-type and *epi*-oudemansin-type β-substituted MOAs (±)-**8** and (±)-**14** were also synthesized as shown in Scheme 4. The diastereomers of racemic aldol adduct *syn*-**4a** and *anti*-**4a** were separated by column chromatography, and each diastereomer was respectively transformed to the desired β-methoxyacrylates (±)-**8** and (±)-**14**[14] via methyl enol ether formation and sequential 9-*O*-methylation.

## Antifungal Activity

Antifungal activities of the synthesized compounds toward several pathogenic or non-pathogenic fungi were examined by disk-diffusion assay (Table 1). 9-Methoxystrobilurin-type β-substituted MOAs bearing unsubstituted benzene ring **7a** exhibited strong antifungal activity similar to that of 9-methoxystrobirulin A (**1**) which is the corresponding α-substituted MOA. In addition, **7a** also showed fungicide activity against drug-resistant *Fusarium solani* although it was still in an incomplete manner.[13] The optically active and racemic oudemansin-type analogues (+)-**8** and (±)-**8** were apparently less effective than **7a**, and no significant effect between molecular chirality and their antifungal property was observed. The racemic *epi*-oudemansin-type analogue was almost ineffective to all fungi tested. On the other hand, aromatic-modified compounds **7b–d** were inferior to **7a** except for a thiophene analogue **7e**. These results are in sharp contrast to those of previously reported 9-methoxystrobilurin-type α-substituted MOAs.[6]

## Conclusion

In conclusion, the first synthesis of 9-methoxystrobilurin-type β-substituted MOAs was successfully achieved and their unique and superior antifungal properties were also revealed. Further extensive SAR studies of 9-methoxystrobilurin-type β-substituted MOAs to develop a more effective antifungal compound, and synthetic studies of 9-methoxystrobilurin-type analogues of natural β-substituted MOAs are now in progress.

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13. The compound **7a** produced relatively large inhibition zones toward *Fusarium solani* during 12–48 h incubation; however, the zones became gradually obscure and were lost after 96 h.
14. Physical data of synthesized compounds: **7a**: $^1$H NMR ($CDCl_3$, 500 MHz) δ 1.99 (s, 3H), 3.63 (s, 3H), 3.73 (s, 3H), 3.74 (s, 3H), 5.22 (s, 1H), 6.53 (d, 1H, $J$ = 15.9 Hz), 6.78 (d, 1H, $J$ = 15.9 Hz), 7.22 (t, 1H, $J$ = 7.3 Hz), 7.30 (dd, 2H, $J$ = 7.3, 7.9 Hz), 7.37(d, 1H, $J$ = 7.9 Hz); EI–MS 288 ($M^+$); **7b**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 1.98 (s, 3H), 3.63 (s, 3H), 3.73 (s, 3H), 3.73 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 5.21 (s, 1H), 6.39 (d, 1H, $J$ = 15.8 Hz), 6.73 (d, 1H, $J$ = 15.8 Hz), 6.81 (d, 1H, $J$ = 8.3 Hz), 6.88 (dd, 1H, $J$ = 1.8, 8.3 Hz; EI–MS 348 ($M^+$); **7c**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.03 (s, 3H), 3.65 (s, 3H), 3.72 (s, 3H), 3.84 (s, 3H), 5.22 (s, 1H), 6.59 (d, 1H, $J$ = 15.9 Hz), 7.52 (d, 1H, $J$ = 15.9 Hz), 7.34–8.17 (m, 7H); EI–MS 338 ($M^+$); **7d**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.01 (s, 3H), 3.63 (s, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 5.25 (s, 2H), 6.65 (d, 1H, $J$ = 15.8 Hz), 6.95 (d, 1H, $J$ = 15.9 Hz), 7.36–7.81 (m, 7H); EI–MS 338 ($M^+$); **7e**: $^1$H NMR ($CDCl_3$, 500 MHz) δ 1.97 (s, 3H), 3.63 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 5.21 (s, 1H), 6.34 (d, 1H, $J$ = 15.3 Hz), 6.89 (d, 1H, $J$ = 15.3 Hz), 6.96 (dd, 1H, $J$ = 3.1, 4.9 Hz), 6.99 (d, 1H, $J$ = 3.1 Hz), 7.16 (d, 1H, $J$ = 4.9 Hz); EI–MS 294 ($M^+$); (+)-**8**: $^1$H NMR ($CDCl_3$, 500 MHz) δ 1.21 (d, 3H, $J$ = 7.0 Hz), 3.31 (s, 3H), 3.57 (s, 3H), 3.66 (s, 3H), 3.74 (dd, 1H, $J$ = 8.2, 8.2 Hz), 4.20 (dq, 1H, $J$ = 7.0, 8.2 Hz), 4.94 (s, 1H), 6.07 (dd, 1H, $J$ = 8.2, 15.9 Hz), 6.47 (d, 1H, $J$ = 15.9 Hz), 7.23 (t, 1H, $J$ = 7.2 Hz), 7.30 (dd, 1H, $J$ = 7.2, 7.3 Hz), 7.34 (d, 1H, $J$ = 7.3 Hz); EI–MS 290 ($M^+$); $[\alpha]_D^{26} = +239$ (*c* 1.0, $CHCl_3$); (±)-**14**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 1.04 (d, 3H, $J$ = 7.0 Hz), 3.26 (s, 3H), 3.66 (s, 3H), 3.68 (s, 3H), 3.83 (dd, 1H, $J$ = 8.5, 9.2 Hz), 4.21 (dq, 1H, $J$ = 7.0, 9.2 Hz), 5.08 (s, 1H), 6.05 (dd, 1H, $J$ = 8.5, 15.9 Hz), 6.56 (d, 1H, $J$ = 15.9 Hz), 6.88 (d, 1H, $J$ = 15.8 Hz), 7.25 (t, 1H, $J$ = 7.3 Hz), 7.33 (dd, 1H, $J$ = 7.3, 7.3 Hz), 7.40 (d, 1H, $J$ = 7.3 Hz); EI–MS 290 ($M^+$).