Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 5355–5359
Bioorganic & Medicinal Chemistry

# Precise structural elucidation of dehydroaltenusin, a specific inhibitor of mammalian DNA polymerase α

Shinji Kamisuki,[a] Shunya Takahashi,[b] Yoshiyuki Mizushina,[c] Kengo Sakaguchi,[a,d] Tadashi Nakata[e] and Fumio Sugawara[a,d,*]

[a]*Department of Applied Biological Science, Tokyo University of Science, Noda, Chiba 278-8510, Japan*
[b]*RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan*
[c]*Department of Nutritional Science, Kobe-Gakuin University, Kobe, Hyogo 651-2180, Japan*
[d]*Genome and Drug Research Center, Tokyo University of Science, Noda, Chiba 278-8510, Japan*
[e]*Department of Chemistry, Tokyo University of Science, Shinjuku, Tokyo 162-8601, Japan*

Received 8 July 2004; revised 23 July 2004; accepted 23 July 2004
Available online 19 August 2004

**Abstract**—The X-ray crystal structure of dehydroaltenusin, a specific inhibitor of mammalian DNA polymerase α, has previously been reported. We show that dehydroaltenusin exists in an equilibrium mixture of two tautomers possessing γ-lactone or δ-lactone in polar solvents by NMR experiments. Acetylation of dehydroaltenusin afforded two types of diacetates and two types of monoacetate, possessing γ-lactone or δ-lactone, respectively. The inhibitory activities of these acetate derivatives against DNA polymerase α were all much weaker than that of dehydroaltenusin.


## 1. Introduction

Dehydroaltenusin was first isolated from mycelium extracts of *Alternaria tennuis* and *A. kikuchiana* by Rosett et al.[1] The structure was initially proposed to be a γ-lactone derivative of β-resorcylic acid monomethylether based on the chemical and spectroscopic data[2] and later revised to **1** possessing a δ-lactone ring by the X-ray diffraction method (Fig. 1).[3] Fuska et al. have reported the complete assignments of $^{1}H$ and $^{13}C$ NMR signals of **1** in $CDCl_3$ and shown that its structure was consistent with the crystal structure.[4] Compound **1** was reported to inhibit the calmodulin-dependent activity of the myosin light chain kinase (MLCK).[5]

We have also isolated **1** from *Acremonium* sp. 98H02B04-1 (2) and shown it to be a specific inhibitor of mammalian DNA polymerase α with an $IC_{50}$ value of 0.68 μM.[6] Fifteen eukaryotic DNA polymerases (α, β, γ, δ, ε, ζ, η, θ, ι, κ, λ, μ, σ, ϕ, ν) have been reported.[7,8] DNA polymerase α is an essential enzyme for DNA replication and subsequently for cell division.[9] Aphidicolin, a well-known DNA polymerase α inhibitor, has been very useful for studying the DNA replication system.[10] Unfortunately, this agent also inhibits the enzymatic activities of other DNA polymerase enzymes (δ and ε) involved in replication. However, compound **1** specifically inhibits DNA polymerase α, but not DNA polymerase δ and ε, making it a more useful than aphidicolin as an experimental tool. We have also reported that **1** suppresses cell proliferation of the human gastric cell line NUGC-3 by inhibiting DNA polymerase α.[11] Specific inhibitors of mammalian DNA polymerase α are not only useful as molecular tools to study the biological functions of this enzyme in vivo, but are potentially useful chemotherapeutic agents in cancer treatment. Recently, we have completed the total synthesis of **1**.[12] In this report, the tautomerism of **1** in polar solvents

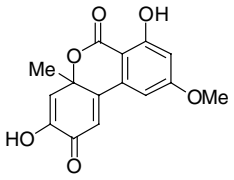



**Figure 1.** Structure of dehydroaltenusin (**1**).

*Keywords*: Dehydroaltenusin; Tautomer; DNA polymerase α; Enzyme inhibitor.
* Corresponding author. Tel.: +81-4-7124-1501(3400); fax: +81-4-7123-9767; e-mail: sugawara@rs.noda.tus.ac.jp

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.07.047

is discussed. We have also acetylated dehydroaltenusin and examined the inhibitory activities of the resultant acetates against different DNA polymerases.

## 2. Results and discussion

### 2.1. Tautomerism of dehydroaltenusin in polar solvents

Fuska et al. have revealed that the structure of dehydroaltenusin in $CDCl_3$ was consistent with the reported crystal structure.[4] We have independently conducted NMR analyses to study the conformation of **1** in various solvents. The $^1H$ and $^{13}C$ NMR data of **1** in $CDCl_3$ (Fig. 2a) were well matched with those of Fuska et al. However, in polar solvents (DMSO-$d_6$ and $CD_3OD$) **1** gave a complicated $^1H$ NMR spectra comprising signals derived from two compounds (Fig. 2b and c). By 2D NMR analyses (COSY, HMQC, HMBC, and INADEQUATE) in DMSO-$d_6$ (Fig. 3), the structure of the minor and major component was determined to be dehydroaltenusin (**1**) and the corresponding γ-lactone **1a**, respectively. The structure of **1a** was originally proposed for natural dehydroaltenusin by Coombe et al.[2] The assignments of $^1H$ and $^{13}C$ NMR signals for each compound are summarized in Table 1. In the COSY spectrum, long-range coupling between H-3′ (δ 6.28 ppm) and Me-2′ (δ 1.56 ppm) in **1a** suggested that Me-2′ should be an olefinic methyl group. Furthermore, the characteristic signal deemed to be due to the spiro carbon was observed at δ 82.6 ppm in the $^{13}C$ NMR spectrum. The γ-lactone structure in **1a** was also supported by the HMBC correlation between H-6 (δ 6.29 ppm) and C-1′ (δ 82.6 ppm) (Fig. 3), and correlation between C-1 (δ 152.8 ppm) and C-1′ in the INADEQUATE spectrum.

As shown in Figure 2a–c, except for $CDCl_3$, a mixture of **1** and **1a** was observed in the $^1H$ NMR spectra for all the other solvents used in this study. The ratios of **1a/1** in the various solvents are summarized in Table 2. These results suggested that dehydroaltenusin exists in an equilibrium mixture of two tautomers **1** and **1a** in polar solvents such as MeOH, DMSO, $H_2O$, and pyridine,

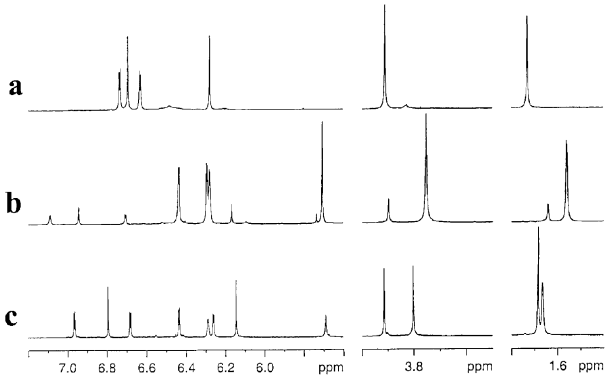



**Figure 2.** $^1H$ NMR spectra of dehydroaltenusin in (a) $CDCl_3$, (b) DMSO-$d_6$, and (c) $CD_3OD$.

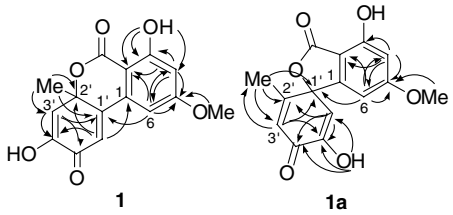



**Figure 3.** HMBC correlation of dehydroaltenusin in DMSO-$d_6$.

**Table 1.** $^1H$ and $^{13}C$ NMR data of dehydroaltenusin in DMSO-$d_6$

| Position | 1 | | 1a | |
|---|---|---|---|---|
| | δH (*J* in Hz) | δC | δH (*J* in Hz) | δC |
| 1 | | 135.6 | | 152.8 |
| 2 | | 99.9 | | 104.7 |
| 3 | | 163.5 | | 158.7 |
| 4 | 6.71 (d, 2.3) | 104.0 | 6.44 (d, 1.9) | 102.9 |
| 5 | | 166.2 | | 166.7 |
| 6 | 7.09 (d, 2.3) | 104.2 | 6.29 (d, 1.9) | 98.0 |
| 7 | | 167.1 | | 166.9 |
| 1′ | | 150.0 | | 82.6 |
| 2′ | | 79.4 | | 155.4 |
| 3′ | 6.17 (s) | 116.9 | 6.28 (q, 1.4) | 127.2 |
| 4′ | | 147.8 | | 181.6 |
| 5′ | | 181.3 | | 148.3 |
| 6′ | 6.94 (s) | 123.0 | 5.71 (s) | 114.1 |
| OMe-3 | 3.90 (s) | 56.4 | 3.75 (s) | 56.2 |
| Me-2′ | 1.64 (s) | 29.2 | 1.56 (d, 1.4) | 16.6 |
| OH-3 | 11.15 (s) | | 11.02 (s) | |
| OH-4′ | 9.56 (s) | | | |
| OH-5′ | | | 9.51 (s) | |

Chemical shifts are expressed δ ppm.

**Table 2.** The ratios of **1a/1** in various solvents

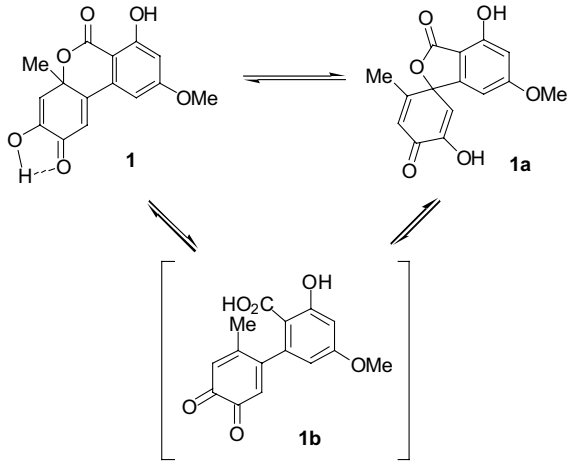
| Solvents | Ratio of **1a/1**[a] |
|---|---|
| $CDCl_3$ | 0 |
| $C_6D_6$ | 0 |
| $CD_2Cl_2$ | 0.1 |
| $CD_3OD$ | 1 |
| Pyridine-$d_5$ | 2 |
| $D_2O$ | 4 |
| DMSO-$d_6$ | 5 |

[a] Each ratio was estimated by $^1H$ NMR experiments.

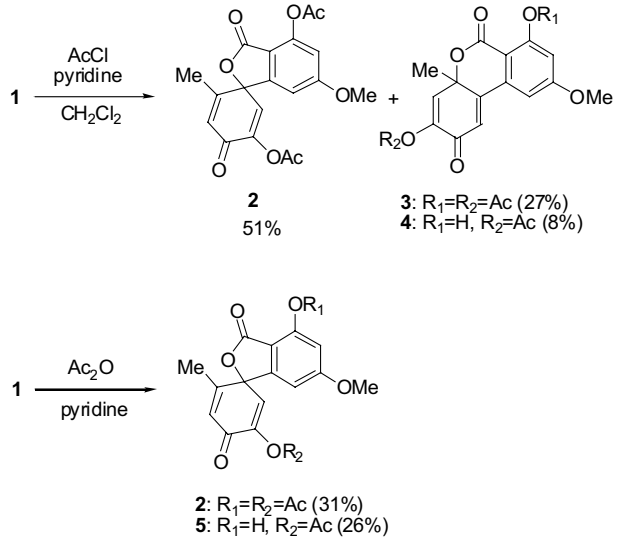
whereas the δ-lactone form **1** is the predominant species in $CHCl_3$, $CH_2Cl_2$, and benzene as well as the crystal state.[3] We considered that the tautomerism might occur either through a proton transfer assisted by the intramolecular hydrogen bond, or via the possible *o*-quinone intermediate **1b** as illustrated in Scheme 1.

### 2.2. Acetylation of dehydroaltenusin

In order to isolate the two isomeric lactones, acetylation (acetyl chloride–pyridine) of dehydroaltenusin in $CH_2Cl_2$ was attempted. After work-up followed by column chromatography on silica gel, the corresponding




**Scheme 1.** Equilibrium of dehydroaltenusin in polar solvents.




**Scheme 2.** Acetylation of dehydroaltenusin.

diacetates **2** and **3**, and monoacetate **4** were obtained in 51%, 27%, and 8% yield, respectively (Scheme 2). In addition, dehydroaltenusin was reacted with acetic anhydride in pyridine to give diacetate **2** and monoacetate **5** in 31% and 26% yield, respectively (Scheme 2). The $^{1}H$ and $^{13}C$ NMR spectra of **2** and **3** were similar to those of **1a** and **1** except for the resonances attributed to the acetyl groups. In the COSY spectrum of **2**, the long-range coupling between H-3′ ($\delta$ 6.30 ppm) and Me-2′ ($\delta$ 1.73 ppm) suggested that **2** was the diacetyl derivative of the **1a** form. We observed a characteristic carbonyl vibration (1778 $cm^{-1}$) in the IR spectrum of **2**, corresponding to the acetyl group and the γ-lactone ring. A band at 1724 $cm^{-1}$ in the IR spectrum of **3**, characteristic of the carbonyl vibration of the δ-lactone ring, confirmed the structure of the diacetyl derivative of the δ-lactone **1**. These results support the structures of **1** and **1a**. It has been reported that acetylation (acetic anhydride–pyridine) of **1** yielded diacetate possessing the structure of **3**.[11,13,14] However, this diacetate corresponds to **2** rather than **3**, because the NMR data reported[13,14] was identical to that of **2**.

The NMR spectra of **4** and **5** were similar to those of **1** and **1a** except for the resonances attributed to the acetyl groups. The $^{1}H$ NMR spectrum of **4** gave a signal of OH-3 at $\delta$ 11.22 ppm, corresponding to the monoacetyl derivative at position 4′ of **1**. In the COSY and HMBC spectra of **5**, the long-range coupling between H-3′ ($\delta$ 6.29 ppm) and Me-2′ ($\delta$ 1.74 ppm), together with the long-range correlation between the acetyl proton ($\delta$ 2.28 ppm) and C-5′ ($\delta$ 145.3 ppm) were observed. These results suggested that **5** was the monoacetyl derivative at position 5′ of **1a**.

### 2.3. DNA polymerase inhibitory studies and discussion

To investigate structure–activity relationship, we performed DNA polymerase assays for dehydroaltenusin (**1**) and its acetyl derivatives **2**–**5** as described previously.[15,16] The dose–response curves for each compound against DNA polymerase α and β are shown in Figure 4. All the acetate derivatives inhibited the activity of DNA polymerase α in a dose-dependent manner. The 50% inhibitory concentration values ($IC_{50}$) of **1**–**5** against DNA polymerase α were 0.68, 25, 13, 16, and 28 μM, respectively. The inhibitory activities of monoacetates **4** and **5** against DNA polymerase α were 20-fold weaker than for **1**, indicating that OH-4′ of **1** is important for inhibition. Since the inhibitory activity of diacetates **2** and **3** were similar to those of monoacetates **4** and **5**, OH-3 of **1** does not appear to be necessary for the inhibition of DNA polymerase α. The enone moiety of **1** can be reduced to give altenuene, which has been reported to show no inhibitory activity against DNA polymerase α.[11] This result indicates that the enone moiety of **1** is essential for inhibition of DNA polymerase α. As shown in Table 2, dehydroaltenusin appears to exist in an equilibrium mixture **1** and **1a** in $H_2O$, but this tautomerism cannot occur in the acetate derivatives and in altenuene.[11] These results suggest that the equilibrium between **1** and **1a** might be important for inhibition of DNA polymerase α. Since the quinone **1b** seems to be a chemically active species, the inhibition may be caused by **1b** and not **1** or **1a** (Scheme 1).

## 3. Conclusion

We revealed that dehydroaltenusin exists in an equilibrium mixture between δ-lactone form **1** and γ-lactone form **1a** in polar solvents. Acetylation of dehydroaltenusin afforded diacetate **2** and monoacetate **5** corresponding to the **1a** form, and diacetate **3** and monoacetate **4** corresponding to the **1** form. The inhibitory activities of these four acetate derivatives against DNA polymerase α were much weaker than that of dehydroaltenusin. The OH-4′ of **1** was found to be important for the inhibitory activity. Furthermore, the equilibrium between **1** and **1a** may be important for the inhibition of DNA polymerase α. The results presented in this paper

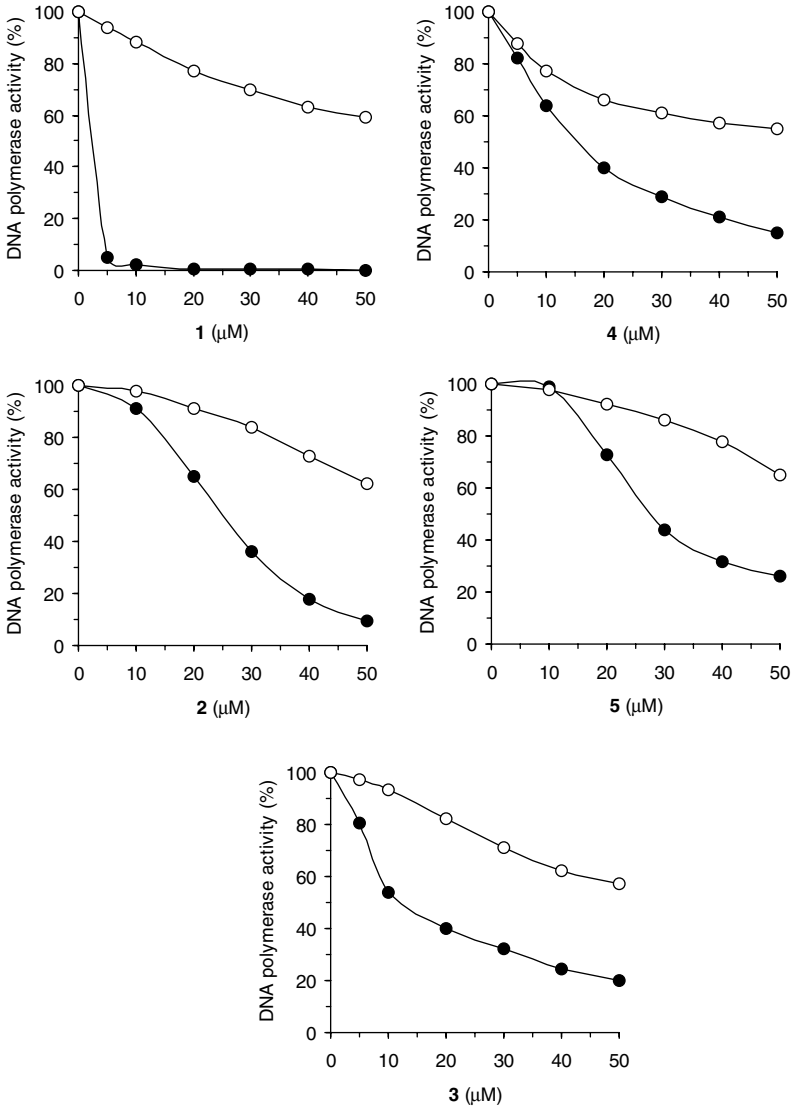



**Figure 4.** Dose–response curves of compound **1**–**5**. Inhibitory activities against DNA polymerase α and β are shown as closed and open circles, respectively. DNA polymerase activity in the absence of compound was taken as 100%.

will assist in understanding the mechanism of inhibition of DNA polymerase α by dehydroaltenusin.

## 4. Experimental section

### 4.1. General procedures

$^{1}H$ and $^{13}C$ NMR spectra were recorded at 400 or 600 MHz with Bruker DRX-400 or DRX-600 spectrometers, using tetramethylsilane as the internal standard. IR spectra were recorded with a HORIBA FREEX-ACT-II FT-720 spectrophotometer. High-resolution mass spectra were obtained on an Applied Biosystems QSTAR Mass Spectrometer using the electron spray ionization (ESI) method. Column chromatography was performed on a Kanto silica gel (spherical, neutral; 40–50 μm). Merck precoated silica gel 60 $F_{254}$ 0.25 mm thickness was used for analytical thin-layer chromatography.

### 4.2. Acetates 2, 3, and 4

To a stirred mixture of **1** (25.9 mg, 0.0899 mmol) and pyridine (0.018 mL, 0.224 mmol) in $CH_2Cl_2$ (5 mL) was added dropwise acetyl chloride (0.016 mL, 0.224 mmol) at 0 °C. The reaction was stirred at the same temperature for 20 min. After addition of water, the mixture was extracted with EtOAc. The extracts were washed with water, dried, and concentrated. Chromatography on silica gel with benzene–diisopropyl ether (1:1) as the eluent yielded **2** (17.0 mg, 51%), **3** (9.0 mg, 27%), and **4** (2.5 mg, 8%) as white solids.

Compound **2**; mp 137–139 °C {hexane–EtOAc (40:1)}; IR (KBr) 2981, 2951, 1778, 1685, 1666, 1616, 1369, 1315, 1200, 1146, 976 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.73 (3H, d, $J = 1.4$ Hz), 2.28 (3H, s), 2.42 (3H, s), 3.87 (3H, s), 6.30 (1H, q, $J = 1.4$ Hz), 6.36 (1H, s), 6.61 (1H, d, $J = 2.0$ Hz), 6.77 (1H, d, $J = 2.0$ Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 17.0, 20.3,

20.6, 56.5, 82.6, 103.6, 110.4, 111.7, 127.5, 130.8, 145.4, 150.2, 150.8, 155.2, 165.8, 167.0, 168.3, 168.4, 178.3; HRMS calcd for $C_{19}H_{16}O_8Na$ $[M + Na]^+$ 395.0737, found 395.0731. Anal. found: C, 61.34; H, 4.54. Calcd for $C_{19}H_{16}O_8$: C, 61.29; H, 4.33.

Compound **3**; mp 165–167 °C {hexane–EtOAc (20:1)}; IR (KBr) 2978, 2935, 1770, 1724, 1682, 1662, 1623, 1604, 1196, 1153, 1065 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.75 (3H, s), 2.31 (3H, s), 2.39 (3H, s), 3.94 (3H, s), 6.69 (1H, s), 6.75 (1H, s), 6.82 (1H, d, $J = 2.5$ Hz), 7.03 (1H, d, $J = 2.5$ Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 20.4, 21.1, 28.7, 56.1, 77.4, 107.8, 109.1, 112.7, 122.8, 133.8, 136.4, 144.4, 150.2, 154.2, 159.2, 164.9, 168.2, 169.3, 178.1; HRMS calcd for $C_{19}H_{16}O_8Na$ $[M + Na]^+$ 395.0737, found 395.0750.

Compound **4**; mp 183–186 °C {hexane–EtOAc (40:1)}; IR (KBr) 3444, 2978, 2935, 1759, 1685, 1674, 1658, 1628, 1608, 1215, 1161, 1076 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.78 (3H, s), 2.32 (3H, s), 3.91 (3H, s), 6.63 (1H, d, $J = 2.3$ Hz), 6.65 (1H, s), 6.74 (1H, d, $J = 2.3$ Hz), 6.77 (1H, s), 11.22 (1H, s); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 20.4, 29.0, 56.0, 78.5, 99.7, 103.7, 104.3, 122.7, 133.2, 134.8, 144.6, 149.9, 164.8, 166.5, 166.8, 168.2, 178.0; HRMS calcd for $C_{17}H_{14}O_7Na$ $[M + Na]^+$ 353.0631, found 353.0629.

### 4.3. Acetates 2 and 5

To a stirred solution of **1** (6.0 mg, 0.0208 mmol) in pyridine (0.7 mL) was added dropwise acetic anhydride (0.3 mL) at room temperature. The reaction was stirred at the same temperature for 55 h. After addition of water, the mixture was concentrated. Chromatography on silica gel with toluene–EtOAc (4:1 → 1:1) as the eluent yielded **2** (2.4 mg, 31%) and **5** (1.8 mg, 26%) as white solids.

Compound **5**; mp 142–145 °C {hexane–EtOAc (40:1)}; IR (KBr) 3444, 2989, 2951, 1763, 1685, 1666, 1616, 1373, 1200, 1153, 980 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.62 (3H, d, $J = 1.4$ Hz), 2.22 (3H, s), 3.76 (3H, s), 6.31 (1H, d, $J = 2.0$ Hz), 6.37 (1H, q, $J = 1.4$ Hz), 6.48 (1H, d, $J = 2.0$ Hz), 6.80 (1H, s), 11.21 (1H, br s); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 17.1, 20.4, 56.3, 84.2, 100.2, 102.8, 103.7, 127.5, 130.2, 145.3, 149.2, 154.5, 158.1, 168.2, 168.3, 170.2, 178.3; HRMS calcd for $C_{17}H_{14}O_7Na$ $[M + Na]^+$ 353.0631, found 353.0631.

## Acknowledgements

This work was supported in part by Grant-in-Aid for JSPS Fellows from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Chemical Biology Project (RIKEN). We express our thanks to Ms. E. Satake (Tokyo University of Science) for the elemental analyses.

## References and notes

1. Rosett, T.; Sankhala, R. H.; Stickings, C. E.; Taylor, M. E. U.; Thomas, R. *Biochem. J.* **1957**, *67*, 390–400.
2. Coombe, R. G.; Jacobs, J. J.; Watson, T. R. *Aust. J. Chem.* **1970**, *23*, 2343–2351.
3. Rogers, D.; Williams, D. J.; Thomas, R. *J. Chem. Soc., Chem. Commun.* **1971**, 393.
4. Fuska, J.; Proksa, B.; Uhrin, D.; Marvanova, L.; Sturdikova, M. *Acta Biotechnol.* **1991**, *11*, 73–76.
5. Nakanishi, S.; Toki, S.; Saitoh, Y.; Tsukuda, E.; Kawahara, K.; Ando, K.; Matsuda, Y. *Biosci. Biotech. Biochem.* **1995**, *59*, 1333–1335.
6. Mizushina, Y.; Kamisuki, S.; Mizuno, T.; Takemura, M.; Asahara, H.; Linn, S.; Yamaguchi, T.; Matsukage, A.; Hanaoka, F.; Yoshida, S.; Saneyoshi, M.; Sugawara, F.; Sakaguchi, K. *J. Biol. Chem.* **2000**, *275*, 33957–33961.
7. Hubscher, U.; Maga, G.; Spadari, S. *Ann. Rev. Biochem.* **2002**, *71*, 133–163.
8. Marini, F.; Kim, N.; Schuffert, A.; Wood, R. D. *J. Biol. Chem.* **2003**, *278*, 32014–32019.
9. Kornberg, A.; Baker, T. A., 2nd ed. In *DNA Replication*; Freeman: New York, 1992; Vol. 6, pp 197–225.
10. Ikegami, S.; Taguchi, T.; Ohashi, M. *Nature* **1978**, *275*, 458–460.
11. Kamisuki, S.; Murakami, C.; Ohta, K.; Yoshida, H.; Sugawara, F.; Sakaguchi, K.; Mizushina, Y. *Biochem. Pharmacol.* **2002**, *63*, 421–427.
12. Kamisuki, S.; Takahashi, S.; Mizushina, Y.; Hanashima, S.; Kuramochi, K.; Kobayashi, S.; Sakaguchi, K.; Nakata, T.; Sugawara, F. *Tetrahedron* **2004**, *60*, 5695–5700.
13. Ayer, W. A.; Racok, J. S. *Can. J. Chem.* **1990**, *68*, 2085–2094.
14. Jabbar, A.; Shresta, A. P.; Hasan, C. M.; Rashid, M. A. *Nat. Prod. Sci.* **1999**, *5*, 162–164.
15. Mizushina, Y.; Tanaka, N.; Yagi, H.; Kurosawa, T.; Onoue, M.; Seto, H.; Horie, T.; Aoyagi, N.; Yamaoka, M.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biochim. Biophys. Acta* **1996**, *1308*, 256–262.
16. Mizushina, Y.; Yoshida, S.; Matsukage, A.; Sakaguchi, K. *Biochim. Biophys. Acta* **1997**, *1336*, 509–521.