ORGANIC LETTERS

2002
Vol. 4, No. 25
4387−4390

UCS1025A and B, New Antitumor Antibiotics from the Fungus *Acremonium* Species

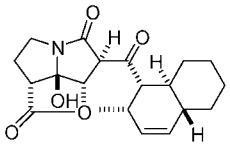
Tsutomu Agatsuma,*,† Tsutomu Akama,† Shinji Nara,† Shigeki Matsumiya,‡ Ryuichiro Nakai,† Harumi Ogawa,† Shizuo Otaki,‡ Shun-ichi Ikeda,† Yutaka Saitoh,† and Yutaka Kanda*,‡

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6 Asahimachi, Machida, Tokyo 194-8533, Japan, and Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi, Sunto, Shizuoka 411-8731, Japan

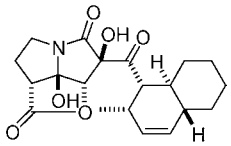
tsutomu.agatsuma@kyowa.co.jp

Received September 17, 2002

ABSTRACT



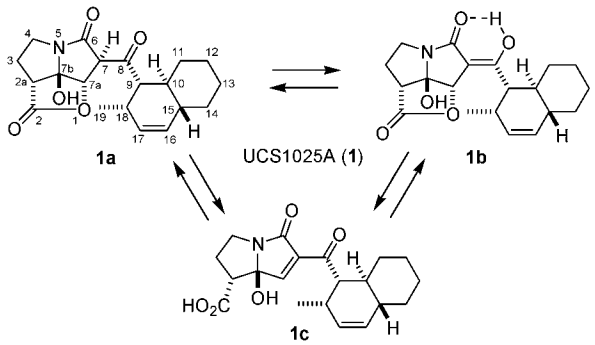
UCS1025A (**1a**)



UCS1025B (**2**)

UCS1025A and B, novel pentacyclic polyketides with an unprecedented furopyrrolizidine skeleton, were isolated from the fungus *Acremonium* sp. KY4917. The structures and stereochemistry were elucidated by a combination of two-dimensional NMR and X-ray crystallographic analysis. UCS1025A showed unique chemical equilibria involving three tautomeric isomers and exhibited antimicrobial activity and antiproliferative activity against human tumor cell lines.

In the course of our screening program of microbial extracts for new antitumor antibiotics, two novel compounds, UCS1025A (**1**) and B (**2**), were discovered in the fermentation broth of the fungus *Acremonium* sp. KY4917.[1] Structure elucidation of these compounds led to the identification of novel pentacyclic polyketide-derived structures containing an unprecedented furopyrrolizidine skeleton. One of these compounds, UCS1025A (**1**), showed antiproliferative activity against human tumor cell lines as well as antimicrobial activity.[2] We describe herein the structures, stereochemistry, and chemical properties of UCS1025A (**1**) and B (**2**).



1a UCS1025A (**1**) **1b**

1c

The molecular formula of **1** was established as $C_{20}H_{25}NO_5$ by HRFAB-MS analysis (*m*/*z* 360.1799 $[M + H]^+$, calcd for $C_{20}H_{26}NO_5$ *m*/*z* 360.1811). The UV spectrum of **1**

† Tokyo Research Laboratories.

‡ Pharmaceutical Research Institute.

(1) Nakai, R.; Ogawa, H.; Asai, A.; Ando, K.; Agatsuma, T.; Matsumiya, S.; Akinaga, S.; Yamashita, Y.; Mizukami, T. *J. Antibiot.* **2000**, *53*, 294−296.

(2) Recently, UCS1025A (**1**) was identified as a novel telomerase inhibitor. Telomeres, the repetitive sequences of six base pairs TTAGGG in the chromosome ends, are essential for stable chromosome maintenance and are maintained by the ribonucleoprotein enzyme telomerase. Since telomerase activity appears to be necessary for the proliferation of most tumor cells, antitumor agents based on telomerase inhibition may potentially provide an effective chemotherapy. Details of full accounts of the inhibitory activity of UCS1025A (**1**) against telomerase will be reported elsewhere.

10.1021/ol026923b CCC: $22.00
Published on Web 11/21/2002

showed an absorption band at λ_{max}^{MeOH} 260 (ϵ 7100) nm, while the IR spectrum exhibited absorption bands for hydroxyl (ν_{max} 3440 cm^{-1}) and carbonyl (ν_{max} 1670, 1790 cm^{-1}) groups. Of interest is the tautomeric phenomenon involving two isomers (**1a** and **1b**) as seen by ^1H NMR but no longer present after allowing **1** to stand in $CDCl_3$ for 7 days at room temperature. The structure elucidation was therefore carried out mainly by use of the NMR spectral data of the resulting single isomer **1a**. The ^{13}C NMR spectrum of **1a** displayed twenty carbon signals, which were classified into one methyl, six methylene, seven methine, two olefinic, and four quaternary carbons, including three carbonyl and one quaternary sp^3 carbons as judged by the DEPT and HSQC data, supporting the molecular formula obtained from HRFAB-MS analysis. The $^1H-^1H$ spin systems and one-bond $^1H-^{13}C$ connectivities were analyzed by DQF−COSY and HSQC data, respectively. The following $^1H-^1H$ spin systems were assigned by the interpretation of DQF−COSY spectrum: $H_3$19−H18−(H17−H16−)H9−H10−$H_2$11 (**A**) and H2a−$H_2$3−$H_2$4 (**B**). Although analysis of the high-field region of the ^1H NMR spectrum was difficult as a result of the high degree of overlap in the methylene proton signals at δ 1.33 and 1.77 ppm, the chemical shift values of corresponding carbon signals (C-11, δ 29.9 ppm; C-12, δ 26.46 ppm; C-13, δ 26.52 ppm; C-14, δ 32.9 ppm; assignments of C-12 and C-13 may be interchanged) indicated the presence of contiguous tetramethylene system, $H_2$11−$H_2$12−$H_2$13−$H_2$14 (**C**). The $^1H-^1H$ correlation between H-10 (δ 1.51 ppm) and H_{ax}-11 (δ 0.89 ppm, apparently the isolated methylene proton signal in fragment **C**) and HMBC correlations from H-16 (δ 5.41 ppm) to C-10 (δ 36.8 ppm), C-15 (δ 42.2 ppm) and C-14 (δ 32.9 ppm) allowed the extension of the fragment **A** and **C** to form a 2-methyloctahydronaphthalene (2-methyldecalin) system (**I**).

Aliphatic polyketide compounds containing decalin moieties are widely distributed in fungal or actinomycetes metabolites.[3] In particular, the 2-methyloctahydronaphthalene system was found in solanapyrones, phytotoxins isolated from phytopathogenic fungus *Alternaria solani*.[4] Comparison of the reported NMR data of solanapyrone D (*trans*-decalin) with those of **1a** resulted in good agreement, supporting this partial structure. The relative stereochemistry of the *trans*-decalin unit was further supported by the NOESY correlations of H-10 (δ 1.51 ppm) with H_3-19 (δ 0.79 ppm) and H-9 (δ 3.18 ppm) with H-15 (δ 1.77 ppm). Although the $^1H-^1H$ spin system of H2a−$H_2$3−$H_2$4 (**B**) was readily discerned in the DQF−COSY spectrum, those of H-7 (δ 4.05 ppm) and H-7a (δ 4.746 ppm) were obscured due to their weak vicinal coupling ($^3J_{H-7,H-7a} \approx 0$ Hz). One-dimensional differential NOE experiments revealed the presence of the weak NOE between H-7 and H-7a, indicating the vicinal relationship of these protons. The molecular formula of **1** indicated nine double-bond equivalents. The presence of a 2-methyloctahydronaphthalene system (three degrees of unsaturation) and three carbonyl groups (δ 208.5, 167.1, and 174.5 ppm) accounted for six degrees of unsaturation, requiring the tricyclic system for the remaining structure of **1a**. The only quaternary sp^3 carbon resonance of **1a** appeared in a relatively low field (δ 101.0) suggesting that this carbon could be bearing both oxygen and nitrogen atoms. Additionally, the deuterium isotope-induced ^{13}C shifts experiments ($CDCl_3-CD_3OD-CD_3OH$) revealed that the hydroxyl group (δ 4.754 ppm) should be attached to this carbon. The H-7 (δ 4.05 ppm) of **1a** showed HMBC correlations to C-6 amide carbonyl (δ 167.1 ppm), C-8 ketone-type carbonyl (δ 208.5 ppm), C-7a oxymethine (δ 80.3 ppm), and C-7b (δ 101.0 ppm), while the H-7a (δ 4.746 ppm), identified as the lactone methine proton by the corresponding downfield shift of C-7a (δ 80.3 ppm), showed correlations to C-6, C-7b, C-2a methine (δ 47.7 ppm), and C-2 lactone carbonyl carbon (δ 174.5 ppm). The nitrogen atom should be placed adjacent to the C-4 carbon in fragment **B** to account for the H_2-4 (δ 3.36, 3.84 ppm) and C-4 (δ 41.8 ppm) chemical shifts. These data suggested that the most probable structure for the remaining tricyclic system would be 7b-hydroxy-octahydrofuropyrrolizin-2,6-dione (**II**) as shown in Figure 1.

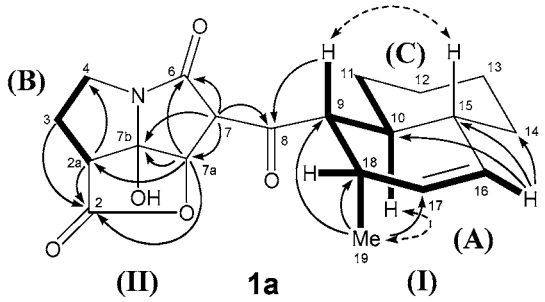


Figure 1. Structure of UCS1025A (**1a**) with COSY-defined spin systems (bold lines), selected NOESY (dashed arrows), and HMBC (solid arrows) correlations.

Partial structures **I** and **II** thus revealed were connected through the carbonyl group by the strong HMBC correlations of H-7 (δ 4.05 ppm) and H-9 (δ 3.18 ppm) to C-8 carbonyl carbon (δ 208.5 ppm). The total planar structure of **1a**, including the partial relative stereostructure of the decalin system, was thus proposed as shown in Figure 1.

The structure of **1b** was deduced as the enol form of **1a** by interpreting the ^1H and ^{13}C chemical shift data (8-OH, δ 11.78 ppm; C-7, δ 102.2 ppm; C-8, δ 179.1 ppm), which was further verified by the HMBC spectral data. The relative stereochemistry of the decalin unit could not be directly correlated to that of the furopyrrolizidine unit because of their separation by C-8 carbonyl group. To establish the stereochemistry as well as confirm the proposed structure of **1**, an X-ray crystallographic analysis was carried out. Colorless needlelike crystals of **1** were grown in a mixture of *n*-hexane/acetone, and the result showed that UCS1025A (**1**) mainly existed as the enol isomer **1b** in crystal packing. Although the stereochemical information at C-7 of the ketone isomer **1a** could not be obtained from the X-ray crystal-

(3) For example, see: Li, J. Y.; Strobel, G.; Harper, J.; Lobkovsky, E.; Clardy, J. *Org. Lett.* **2000**, *2*, 767−770.
(4) Oikawa, H.; Yokota, T.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1989**, 1284−1285.

lographic analysis, the small coupling constant value and weakly observed NOE between H-7 and H-7a indicated α-H configuration at C-7 (anti relationship of H-7 and H-7a). Examination of Dreiding models suggested that α-H isomer is consistent with the small coupling constant value between H-7 and H-7a due to the H7−C7−C7a−H7a dihedral angle value of approximately 90°. Further evidence for an α-H configuration at C-7 was obtained from the molecular modeling studies of 7β- or 7α-acetyl-7b-hydroxy-octahydrofuropyrrolizin-2,6-dione (Figure 2). Modeling studies (ab initio, HF/3-21G*)[5] indicated that the dihedral angle value of more stable α-H isomer (ΔE = 13.23 kcal/mol) is 83.4°, which is in good agreement with the weak vicinal coupling of H-7 and H-7a in **1a**.

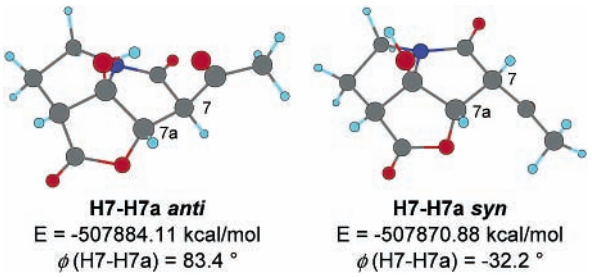
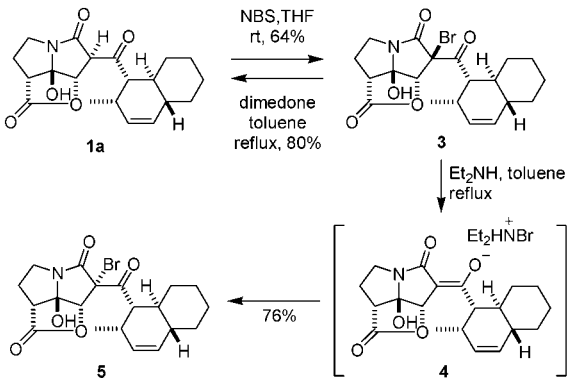


Figure 2. Molecular modeling of 7β- or 7α-acetyl-7b-hydroxy-octahydrofuropyrrolizin-2,6-dione (ab initio, HF/3-21G*).

Bromination of UCS1025A (**1**) with NBS in THF afforded 7-bromo-UCS1025A (**3**), which was further treated with diethylamine in toluene to give thermodynamically more stable 7-epimeric isomer **5** (Scheme 1). The X-ray crystallographic analysis of **5** established the absolute stereochemistry of **1** as well as **5** (Figure 3). Treatment of **3** with dimedone[6] in toluene at reflux temperature resulted in the recovery of **1** in 80% yield, indicating that base-catalyzed inversion at C-7 occurred via *N*-bromoammonium enolate **4** as the intermediate. The similar base-catalyzed isomerization was reported in the chemical transformation of (8a*R*)-8a-bromoalbomitomycin A to the corresponding (8a*S*)-isomer.[7]

Scheme 1



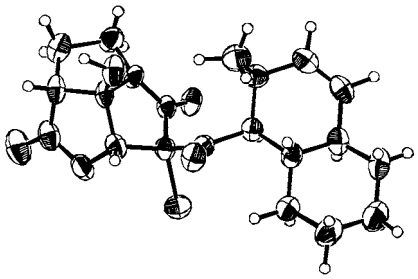


Figure 3. ORTEP drawing of **5**.

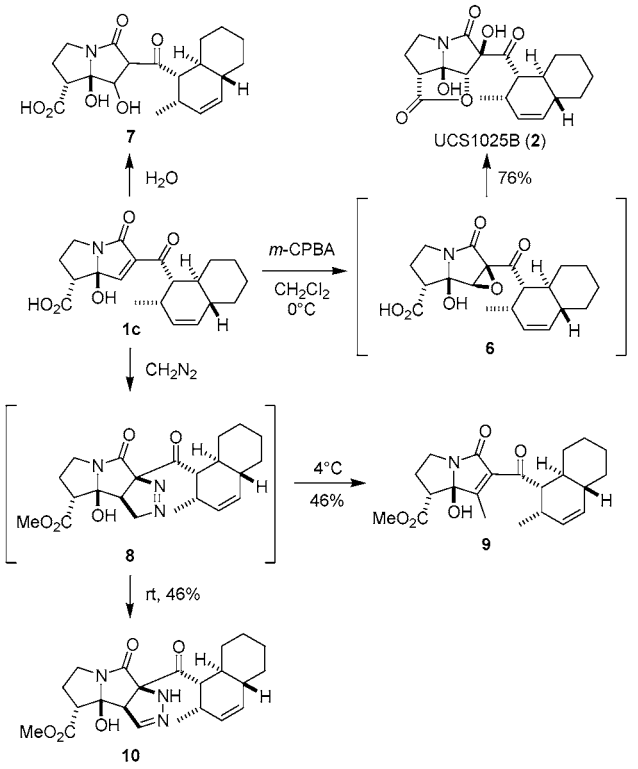
HRFAB-MS analysis (*m*/*z* 376.1757 $[M + H]^+$, calcd for $C_{20}H_{26}NO_6$ *m*/*z* 376.1760) of UCS1025B (**2**) provided the molecular formula of $C_{20}H_{25}NO_6$, which was supported by the ^1H and ^{13}C NMR data. The ^1H NMR spectrum of **2** showed a pattern similar to that of **1a**, implying that **2** could be a congener with one additional oxygen atom. Absence of the ketone-enol tautomerization observed in **1** and comparison of the ^{13}C chemical shift values for C-7 (**1a**, δ 66.4 ppm; **2**, δ 85.0 ppm) strongly suggested the 7-hydroxyl structure for **2**, which was further confirmed by HMBC spectral data. The X-ray crystallographic analysis established the relative stereostructure of **2**, exhibiting the kinetically favored β-OH configuration at C-7 position for **2** in contrast to the thermodynamically favored α-H configuration for **1a**. Treatment of UCS1025A (**1**) with 1 equiv of *m*-CPBA afforded UCS1025B (**2**) in a moderate yield (76%, Scheme 2). This result indicated the involvement of enedione carboxylic acid **1c** as the third isomer of **1** as well as the kinetically controlled β-epoxide **6** as the reaction intermediate, though we were unable to isolate **6**. The stereochemical correlation between **1** and **2** was thus completed. The presence of enedione isomer **1c** was verified in the following manner: the various two-dimensional NMR spectral data of UCS1025A (**1**) dissolved in a 4:1 phosphate buffer (100 mM Na_2HPO_4−KH_2PO_4, pH 7.16)−D_2O mixture were recorded, displaying the extremely downfield-shifted olefinic proton (δ 7.93, H-7a) and carbon (δ 155.5, C-7a) resonances, which supported the enedione structure for **1c**. In these NMR experiments, a trace amount of minor congener **7** was detected. The structure of **7** was determined as the 7a-hydroxyl derivative, which could be generated by the hydration at C-7a of **1c**. The structures of **1c** and **7** were fully supported by the HMBC data. The high susceptibility of enedione **1c** to the nucleophilic attack was further represented by the reaction with diazomethane: treatment of UCS1025A (**1**) with excess amount of ethereal

(5) Molecular modeling studies were performed using SPARTAN version 5.0 run on a Silicon Graphics OCTANE workstation. Molecules were initially built in SPARTAN; the resultant conformers were subjected to restricted Hartree−Fock ab initio calculations using the 3-21G* basis set, and the energies of the conformers were recorded.

(6) Arai, H.; Kasai, M. *J. Org. Chem.* **1993**, *58*, 4151−4152.

(7) Kasai, M.; Kono, M.; Ikeda, S.; Yoda, N.; Hirayama, N. *J. Org. Chem.* **1992**, *57*, 7296−7299.

Scheme 2



diazomethane in methanol at 4 °C afforded the 7a-methyl derivative **9**.[8] Although 1-pyrazoline **8** could not be isolated, **8** was considered to be an intermediate because of the isolation of isomerized 2-pyrazoline **10**[9] under the same reaction condition at room temperature. The structures of **9** and **10** were confirmed by their two-dimensional NMR and FAB-MS data.

Of biogenetic interest is the presence of a unique furopyrrolizin-2,6-dione system not previously found in other fungal metabolites.[10] To date, several natural products containing 3-acyl-5-hydroxy-3-pyrrolin-2-one moieties closely related to **1c** have been reported such as oteromycin,[11] ZG-1494α,[12] and talaroconvolutins.[13] These compounds are a relatively rare class of natural products and could be biogenetically related to tetramic acids, which are believed to be derived from polyketide precursors and amino acids.[14] However, the presence of a furopyrrolizidine system in UCS1025A, which seems to be derived from a polyketide precursor, glycine, and an unknown C_4 unit (or aspartic acid and unknown C_2 unit), is quite outstanding from other related compounds. Another biogenetic feature is the construction of the decalin system, which may involve an enzymatic Diels−Alder reaction as revealed in the biosynthetic studies of solanapyrones[15] or lovastatin.[16]

Acknowledgment. We thank Dr. M. Yoshida and her staff in our laboratories for measurements of the NMR and FAB-MS data.

Supporting Information Available: Experimental procedures and characterization for compounds **1a**−**c**, **2**, **3**, **5**, **7**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL026923B

(8) Norman, B. H.; Paschal, J.; Vlahos, C. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1183−1186.

(9) Jefford, C. W.; Sledeski, A. W.; Boukouvalas, J. *Helv. Chim. Acta* **1989**, *72*, 1362−1370.

(10) Recently, CJ-16,264, a pyrrolizidinone antibiotic closely related to **1a**, was reported by a Pfizer group. However, this compound differs from **1a** because of the presence of tetramethyl-octahydronaphthalene system and the absence of ketone−enol tautomerization. The relative configuration of CJ-16,264 was determined on the basis of NOE experiments and proposed to have *cis*-decalin and H-7, H-7a-syn configurations in furopyrrolizidine system. These stereochemical features are quite different from **1a**. Sugie, Y.; Hirai, H.; Kachi-Tonai, H.; Kim, Y.-J.; Kojima, Y.; Shiomi, Y.; Sugiura, A.; Sugiura, A.; Suzuki, Y.; Yoshikawa, N.; Brennan, L.; Duignan, J.; Huang, L. H.; Sutcliffe, J.; Kojima, N. *J. Antibiot.* **2001**, *54*, 917−925.

(11) Singh, S. B.; Goetz, M. A.; Jones, E. T.; Bills, G. F.; Giacobbe, R. A.; Herranz, L.; Stevens-Miles, S.; Williams, Jr., D. L. *J. Org. Chem.* **1995**, *60*, 7040−7042.

(12) West, R. R.; Ness, J. V.; Varming, A.-M.; Rassing, B.; Biggs, S.; Gasper, S.; Mckernan, P. A.; Piggott, J. *J. Antibiot.* **1996**, *49*, 967−973.

(13) Suzuki, S.; Hosoe, T.; Nozawa, K.; Kawai, K.; Yaguchi, T.; Udagawa, S. *J. Nat. Prod.* **2000**, *63*, 768−772.

(14) Marfori, E. C.; Bamba, T.; Kajiyama, S.; Fukusaki, E.; Kobayashi, A. *Tetrahedron* **2002**, *58*, 6655−6658.

(15) Oikawa, H.; Kobayashi, T.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748−8756.

(16) Auclair, K.; Sutherland, A.; Kennedy, J.; Witter, D. J.; Van den Heever, J. P.; Hutchinson, C. R.; Vederas, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 11519−11520.