Structure of FR 900482, a Novel Antitumor Antibiotic from a *Streptomyces*

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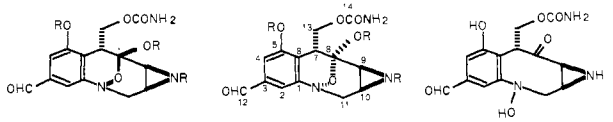
FR 900482, recently isolated from *Streptomyces sandaensis* No. 6897,[1] is a novel antibiotic with exceptionally potent antitumor activity. Herein we report structure **1** for this natural product which exists as a mixture of two stereoisomers **A** and **B** in tautomeric equilibrium attributable to a unique hydroxylamine hemiketal functionality.



A B C

1 R = H
2 R = Ac

FR 900482 (**1**) was isolated as a white powder; $C_{14}H_{15}N_3O_6$; mp ~175 °C dec; $[\alpha]^{23}_D$ -26.5° (*c* 1.0, 0.1 N HCl).[2] The ^{13}C and ^1H NMR spectra[3] and the TLC behavior[4] revealed that **1** is constituted of two isomers (**A**:**B** = ca. 2:1). Acetylation of **1** (Ac_2O/pyr) gave two triacetates, **2A** (7%) and **2B** (79%).[5,6] Detailed NMR analysis ($CDCl_3$) of the major product **2B** including H-H COSY, C-H COSY, and NOE experiments argued the presence of a 3,4,5-trisubstituted benzaldehyde [δ_C 190.3 (d, C-12), 149.7 (s, C-1), 149.4 (s, C-5), 136.2 (s, C-3), 124.2 (s, C-6), 119.0 (d, C-4), 115.5 (d, C-2); δ_H 9.90 (s, 12-H), 7.14 (d, J = 1.3 Hz, 2-H), 7.31 (d, J = 1.3 Hz, 4-H); NOE, 12-H ↔ 4-H, 12-H ↔ 2-H], a hemiketal or hemiaminal [δ_C 96.4 (s, C-8)], an aziridine [δ_C 40.2 (d, C-9, $^1J_{CH}$ = 178 Hz), 31.6 (d, C-10, $^1J_{CH}$ = 175 Hz); δ_H 3.41 (d, J = 6.3 Hz, 9-H), 2.84 (dd, J = 6.3, 2 Hz, 10-H)], and a carbamoyloxymethyl [δ_C 155.9 (s, C-14), 63.2 (t, C-13); δ_H 4.40 (dd, J = 12, 7 Hz, 13-H_a), 4.35 (dd, J = 12, 3.8 Hz, 13-H_b), 4.72 (br s, NH_2); IR of **1**, 1690 cm^{-1}] function.[7]

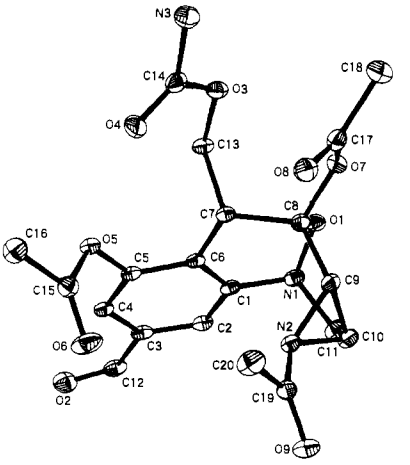
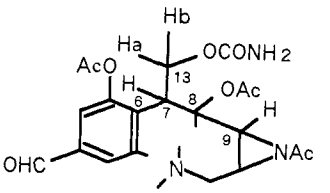


Figure 1. ORTEP drawing of **2B**.

The spectra further revealed that 10-H of the aziridine was coupled (J = 2 Hz) to a methylene proton (11-H_a) bearing nitrogen [δ_C 52.9 (t), δ_H 4.06 (dd, J = 15, 2 Hz)], and 13-H_2 of the carbamoyloxymethyl group were both coupled (J = 7 and 3.8 Hz, respectively) to a methine proton (7-H) [δ_C 39.8 (d); δ_H 3.89 (dd, J = 7, 3.8 Hz)].

A reasonable linkage of the partial structures was obtained by the following COLOC experiment.[8] The data showed that C-6 was long-range coupled to 7-H and 13-H_b, suggesting the linkage of C-6 and C-7. Observation of couplings between C-8 and both 13-H_2 necessitates the bonding of C-7 and C-8. C-8 exhibited a further coupling to 9-H, which requires the bonding of C-8 and C-9. Hence the partial structure a was derived for the triacetate **2B** except for the position of the remaining one oxygen atom.



a

Since the available chemical and spectral data did not readily distinguish among possible structures for FR 900482, X-ray crystal analysis was undertaken by using crystals of **2B**: orthorhombic, space group $P2_12_12_1$ with a = 17.724 (1), b = 11.355 (1), and c = 10.381 (1) Å. The structure was determined by the direct method (MULTAN 78) and successive least-squares and Fourier synthesis. Parameters were refined by using anisotropic temperature factors to R = 0.042 for 1881 reflections. A perspective drawing of the final X-ray model of **2B** is given in Figure 1. The structure of the triacetate was thus defined to be **2B**.

The spectral characteristics of the minor triacetate **2A** resemble those of **2B**,[9] indicating that **2A** is a stereoisomer of **2B**. A reasonable presumption on the basis of these data is that the minor triacetate **2A** is an epimer of **2B**. This assignment was further supported by observation of a NOE between 13-H_2 (δ 4.82) and 9-H (δ 3.06) in the ^1H NMR spectrum; on the other hand, **2B** exhibited a NOE between 7-H and 9-H.[10]

(1) Kiyoto, S.; Shibata, T.; Yamashita, M.; Komori, T.; Okuhara, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.*, in press.

(2) SIMS, m/z 322 (M^+ + 1). Anal. Calcd for $C_{14}H_{15}N_3O_6 \cdot H_2O$: C, 49.56; H, 5.05; N, 12.38. Found: C, 49.73; H, 4.83; N, 12.52. UV (MeOH) 236 nm (ε 19 200), 281 (6100), 330 (2200); IR (KBr) 3600–3000, 1690, 1580 cm^{-1}.

(3) ^{13}C NMR (D_2O) and ^1H NMR (D_2O) attributable to **1A**: δ_C 197.8 (d, C-12), 161.9 (s, C-14), 159.0 (s, C-5), 150.9 (s, C-1), 138.6 (s, C-3), 122.0 (s, C-6), 116.7 (d, C-2), 112.7 (d, C-4), 96.0 (s, C-8), 63.6 (t, C-13), 56.2 (t, C-11), 46.3 (d, C-7), 32.5 (d, C-9), 32.0 (d, C-10); δ_H 9.74 (s, 12-H), 7.08 (d, J = 1.3 Hz, 4-H), 7.05 (d, J = 1.3 Hz, 2-H), 5.13 (dd, J = 11, 6 Hz, 13-H), 4.68 (dd, J = 11, 1 Hz, 13-H), 3.79 (d, J = 3.5 Hz, 11-H_2), 3.52 (dd, J = 6, 1 Hz, 7-H), 2.72 (d, J = 6.5 Hz, 9-H), 2.69 (dd, J = 6.5, 3.5 Hz, 10-H). ^{13}C NMR (D_2O) and ^1H NMR (D_2O) assignable to **1B**: δ_C 198.0 (d, C-12), 162.3 (s, C-14), 157.7 (s, C-5), 152.3 (s, C-1), 138.5 (d, C-3), 122.0 (s, C-6), 115.3 (d, C-2 or C-4), 113.7 (d, C-4 or C-2), 96.4 (s, C-8), 64.8 (t, C-13), 55.3 (t, C-11), 42.8 (d, C-7), 40.4 (d, C-9), 28.2 (d, C-10); δ_H 9.75 (s, 12-H), 7.12 (d, J = 1.3 Hz, 4-H), 6.96 (d, J = 1.3 Hz, 2-H), 4.66 (dd, J = 11.5, 5.5 Hz, 13-H), 4.45 (dd, J = 11.5, 2 Hz, 13-H), 3.86 (dd, J = 14.5, 2 Hz, 11-H), 3.63 (d, J = 14.5 Hz, 11-H), 3.42 (dd, J = 5.5, 2 Hz, 7-H), 2.89 (d, J = 7 Hz, 9-H), 2.51 (dd, J = 7, 2 Hz, 10-H).

(4) TLC: $CHCl_3$–MeOH (4:1) R_f 0.20 (major) and 0.45 (minor); *i*-PrOH–H_2O (9:1) R_f 0.55 (major) and 0.65 (minor).

(5) Since **1** was recovered from both triacetates on treatment with aqueous $NaHCO_3$, there was no structural alteration of the molecule during the acetylation.

(6) **2A**: EIMS, 447 (M^+); oil. **2B**: EIMS, 447 (M^+); mp 207–208 °C.

(7) The triacetyl groups were observed at δ_C 180.3 (s), 169.1 (s), 168.5 (s), 22.9 (q), 21.7 (q), 21.0 (q) and δ_H 2.40 (s, 3 H), 2.24 (s, 3 H), 1.98 (s, 3 H).

(8) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331.

(9) ^{13}C NMR ($CDCl_3$) δ 190.2 (d), 182.0 (s), 168.2 (s), 167.8 (s), 155.6 (s), 149.8 (s), 148.3 (s), 136.1 (s), 122.3 (s), 118.9 (d), 118.0 (d), 97.4 (s), 60.4 (t), 53.6 (t), 38.8 (d), 34.4 (d), 33.9 (d), 23.4 (q), 22.2 (q), 21.3 (q); ^1H NMR ($CDCl_3$) δ 9.90 (s, 1 H), 7.41 (d, J = 1.3 Hz, 1 H), 7.27 (d, J = 1.3 Hz, 1 H), 4.88 (dd, J = 5.6, 3 Hz, 1 H), 4.82 (m, 2 H), 4.64 (br s, 2 H), 4.02 (dd, J = 15, 1.6 Hz, 1 H), 3.74 (dd, J = 15, 5.8 Hz, 1 H), 3.06 (d, J = 5.8 Hz, 1 H), 3.03 (dt, J = 5.8, 1.6 Hz, 1 H), 2.40 (s, 3 H), 2.28 (s, 3 H), 2.22 (s, 3 H).

(10) In the **A** form, the aziridine 9-H is situated in a position close to the carbamoyloxymethyl group, while in the **B** form, the corresponding aziridine proton is located near the 7-methine proton.

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It is apparent that FR 900482 consists of the two tautomers with structures **1A** and **1B** which interconvert most likely via an intermediary keto form **C**. In the ^{1}H NMR spectrum of **1**, the major isomer showed a NOE between 9-H and one (δ_H 4.68) of 13-H_2, while, in the minor one, a NOE was observed between 7-H and 9-H, indicating that, contrary to the case of the triacetates, the major isomer has the **A** form.[11] The tautomer **1A** is possibly favored by an intramolecular hydrogen bonding between the aziridine NH and the bridged oxygen.

FR 900482 is quite unique in containing a hydroxylamine function whose hydroxy group participates in a hemiketal moiety. The structure of FR 900482 is of interest in comparison with those of the mitomycin family of antibiotics. Thus, FR 900482 resembles mitomycins by having an aziridine and a carbamoyloxymethyl group but differs by lacking a quinonoid structure. FR 900482 exhibits powerful antitumor activity in a variety of transplantable murine tumors including mitomycin-resisted P388.[12]

Supplementary Material Available: Tables of fractional coordinates and thermal parameters, anisotropic thermal parameters, bond lengths, and bond angles (4 pages). Ordering information is given on any current masthead page.

(11) In an acidic solution, the equilibrium of **1** lies far toward A. The NMR spectra showed that in D_2O–DCl **1** exists nearly quantitatively in the **1A** form: ^{13}C NMR (D_2O–DCl) δ 195.4 (d), 159.1 (s), 156.3 (s), 147.4 (s), 136.6 (s), 118.3 (s), 114.3 (d), 111.2 (d), 90.8 (s), 60.7 (t), 49.4 (t), 44.1 (d), 36.3 (d, 2 C); ^{1}H NMR (D_2O–DCl) δ 9.67 (s, 1 H), 7.0 (s, 2 H), 5.21 (dd, J = 11, 6 Hz, 1 H), 4.6 (d, J = 11 Hz, 1 H), 4.16 (d, J = 17 Hz, 1 H), 4.04 (dd, J = 17, 5 Hz, 1 H), 3.74 (m, 2 H), 3.65 (d, J = 6 Hz, 1 H).

(12) Antitumor activities in mice in comparison with those of mitomycin C (MMC) (shown by the highest T/C values). P388 (ip/ip, D1-5): **1**, 10.0 (mg/kg), T/C 305 (%); MMC, 1.0, 153. MMC-resisted P388 (ip/ip, D1), **1**, 18.0, 155; MMC, 3.2, 114. L1210 (ip/ip, D1-9): **1**, 10.0, 305; MMC, 1.0, 200. B16 (ip/ip, D1-9): **1**, 10.0, 350; MMC, 1.0, 223. Details will be reported: Shimomura, K.; Hirai, O.; Mizota, T.; Matsumoto, S.; Mori, J.; Shibayama, F.; Kikuchi, H. *J. Antibiot.*, in press.

Reaction of Aluminum Atoms with Ethylene: Formation of a Metallocyclopentane by Cyclodimerization of Ethylene at Low Temperatures[1]

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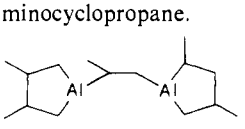
Kasai[2,3] has undertaken a detailed electron spin resonance (ESR) study of the reaction of aluminum atoms with ethylene in inert gas matrices at temperatures below 40 K and has concluded that addition to the C=C double bond does not occur but that a π complex is formed. The free electron of this species is believed to reside mainly in the Al $3p_y$ orbital lying parallel to the C=C bond with some spin population in the π^* orbital of the ethylene.

Skell and Wolf[4] somewhat earlier found that Al atoms and propene at 77 K give, upon deuteriolysis, products indicative of trivalent aluminum σ-bonded intermediates,[5] e.g., **1**, which contain two alumino-substituted cyclopentane rings linked by a C_3 fragment. It was concluded that **1** is formed by ring opening of the initially formed aluminocyclopropane.



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(1) Issued as NRCC No. 27614.
(2) Kasai, P. H.; McLeod, D., Jr. *J. Am. Chem. Soc.* **1975**, *97*, 5609-5611.
(3) Kasai, P. H. *J. Am. Chem. Soc.* **1982**, *104*, 1165-1172.
(4) Skell, P. S.; Wolf, L. R. *J. Am. Chem. Soc.* **1972**, *94*, 7919-7920.
(5) McGlinchey, M. J.; Skell, P. S. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 195-199.

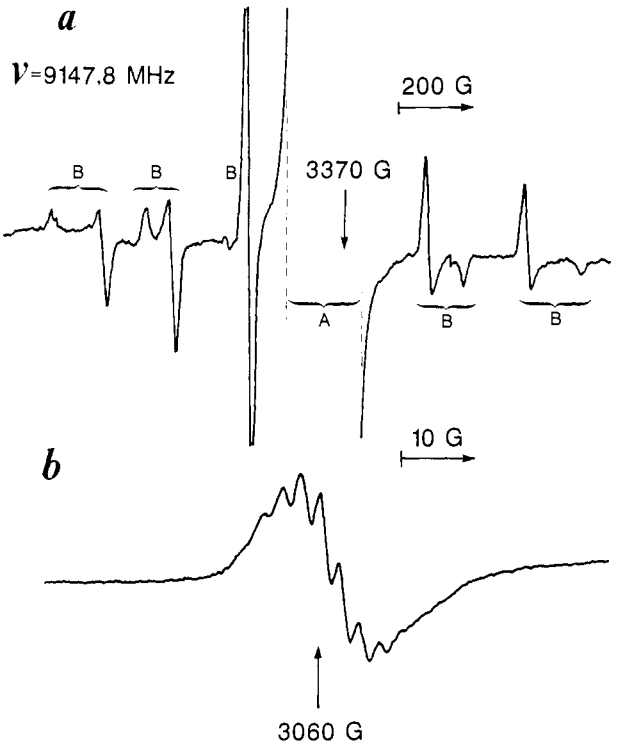

Figure 1. (a) ESR spectrum given by Al atoms and ethylene in cyclohexane at 77 K. (b) ESR spectrum of the $M_I = +^1/_2$ line of species B at 210 K in adamantane.

We have recently shown that aluminum atoms react with buta-1,3-diene in hydrocarbon matrices at 77 K to form an alumino-substituted allyl and through a cheleotropic cycloaddition to give an aluminocyclopentene in which the aluminum atom has adopted a hybridized sp^2 orbital configuration.[6] In this latter species two of the sp^2 orbitals are used to covalently bond the aluminum to the terminal carbon atoms with the unpaired electron located mainly in the remaining sp^2 orbital. The 33% Al 3s contribution to the SOMO produces a large Al hyperfine interaction of 640 MHz. In this context we were interested to see whether the use of higher temperatures, hydrocarbon matrices, and the experimental conditions of the rotating cryostat might result in a σ-bonded species even in the case of ethylene, and we report here the results of our studies, which show that cyclodimerization to form aluminocyclopentane does indeed occur.

The rotating cryostat[7] was used to react aluminum atoms, produced in a tungsten coil furnace, with newly deposited ethylene molecules trapped on the cold surface (77 K) of a continuously renewed matrix of adamantane or cyclohexane on the spinning drum containing liquid nitrogen. The resulting deposits were examined by ESR after transfer from the drum still at 77 K and under high vacuum.

The ESR spectrum shown in Figure 1a has two paramagnetic species A and B. Species A gives the more intense spectrum and will be fully discussed in a later publication; here we note only that its ESR parameters at low temperatures are similar to those reported by Kasai[2,3] and assigned to the monoligand π complex Al[C_2H_4]. The absorption lines of species **B** are a textbook example of a species with axially symmetric g and A tensors. Its ESR parameters $a_{\parallel}$ = 798.3 MHz, $a_{\perp}$ = 635.2 MHz, $g_{\parallel}$ = 1.9986, and $g_{\perp}$ = 1.9970 are similar to those of aluminocyclopentene, suggesting that it has a structure similar to the product from Al atoms and buta-1,3-diene.[6]

Small hyperfine interactions were discernable on the aluminum lines, especially on the $M_I = ^1/_2$ line, which became more evident

(6) Chenier, J. H. B.; Howard, J. A.; Tse, J. S.; Mile, B. *J. Am. Chem. Soc.* **1985**, *107*, 7290-7294.
(7) Buck, A. J.; Mile, B.; Howard, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 3381-3387. Bennett, J. E.; Mile, B.; Ward, B.; Thomas, A. *Adv. Phys. Org. Chem.* **1970**, *8*, 1-77.

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