A51493A, A NOVEL ANTHRACYCLINONE ANTIBIOTIC PRODUCED BY A STRAIN OF *STREPTOMYCES HUMIFER*

JONATHAN W. PASCHAL, JOHN L. OCCOLOWITZ, STEPHEN H. LARSEN, LAVERNE D. BOECK and FREDERICK P. MERTZ

Lilly Research Laboratories,
Eli Lilly and Company,
Lilly Corporate Center,
Indianapolis, Indiana 46285, U.S.A.

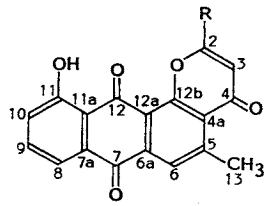
(Received for publication November 4, 1988)

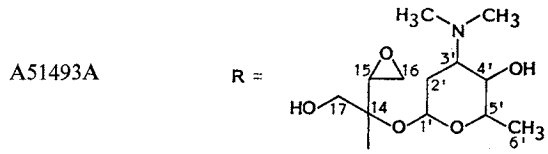
During the process of screening actinomycetes for novel antimicrobial substances, a strain of *Streptomyces humifer* was found to produce a previously unreported antibiotic, A51493A, with *in vitro* activity against Gram-positive bacteria. Spectroscopic analysis has established that A51493A is structurally related to the anthracyclinones rubiflavinone C-1 and C-2[1].

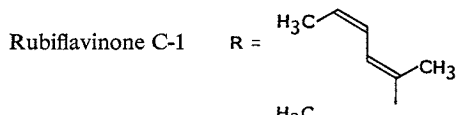
Culture A51493 grew well on both complex and defined media. Trace amounts of white aerial hyphae were occasionally produced. Fragmentation similar to that described for *S. humifer* occurred when the culture was grown in liquid media[2]. A51493 utilized glucose, galactose, fructose, inositol, mannitol, and raffinose. Starch was hydrolyzed. No melanoid pigments were produced. Sugars present in the whole cells were glucose and ribose. Hydrolyzed whole cells contained LL-diaminopimelic acid. Using methods described by SHIRLING and GOTTLIEB[3], culture A51493 was determined to be a strain of *S. humifer*.

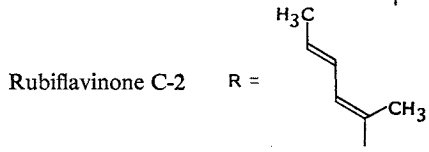
Fermentations were conducted for 5~7 days at 30°C in fully baffled vessels of conventional design with a total capacity of 165 liters. They contained 115 liters of a medium composed of potato dextrin 3.5%, ammonium tartrate 0.26%, NZ-Amine A 0.2%, KCl 0.1%, $MgCl_2 \cdot 6H_2O$ 0.02%, Na_2SO_4 0.2%, sodium glycerophosphate 0.025%, $CuSO_4 \cdot 5H_2O$ 0.0003%, $MnCl_2 \cdot 6H_2O$ 0.0008%, $ZnSO_4 \cdot 7H_2O$ 0.00008% and $CaCO_3$ 0.3% in deionized water. Dissolved oxygen was maintained at 45% of air saturation with 0.34 atmospheres of internal vessel pressure, and the pH was controlled at 6.8~7.0. Antibiotic production was initially monitored by a conventional disc-plate agar diffusion technique employing *Micrococcus luteus* as the test organism. Qualitative determinations of A51493A were made with a TLC system using Merck-Darmstadt

Fig. 1. The structure of A51493A and rubiflavinones C-1 and C-2.









silica gel plates developed in chloroform - methanol (3 : 1). Chromatograms were bioautographed vs. *M. luteus*. The Rf value in this system was 0.3.

100 liters of filtered broth were extracted with chloroform. After being concentrated *in vacuo*, the extract, containing 13.3 g solids, was subjected to silica gel column chromatography. The column was washed with 4 liters of $CHCl_3$, then eluted with a solvent system advancing from chloroform to chloroform - methanol (9 : 1) *via* a linear gradient. Fractions were evaluated on the basis of bioassay vs. *M. luteus* and by HPLC (column: Millipore/Waters μBondapak C18 (3.9×300 mm); solvent: CH_3CN - H_2O (35 : 65) containing 0.2% TEA; flow rate: 1.5 ml/minute; detector: UV (254 nm)). Fractions containing A51493A were combined and concentrated to a yellow oil (580 mg). The oil was dissolved in 75 ml warm methanol which, when cooled slowly, gave A51493A as pale yellow needles (315 mg) which char at 210~216°C. The compound gave a single HPLC peak with a retention time of 7.0 minutes.

Electron impact mass spectrum (EI-MS) was obtained on a CEC 21-110 mass spectrometer, and accurate mass (high resolution) measurement experiments were run on a Varian-Mat 731 mass spectrometer. NMR data was obtained in $CDCl_3$ on a Bruker WM270 equipped with an Aspect 3000 computer.

The mass spectrum of A51493A provided the

Table 1. The ^{13}C and ^1H NMR data for A51493A and ^1H comparison of rubiflavinones C-1 and C-2[1] nucleus in $CDCl_3$.

Position	A51493A		Rubiflavinone C-1	Rubiflavinone C-2
	^{13}C	^1H	^1H	^1H
2	165.90	—	—	—
3	112.46	6.72	6.45	6.42
4	178.67	—	—	—
4a	126.58	—	—	—
5	150.03	—	—	—
6	125.82	8.08	8.05	8.04
6a	135.96	—	—	—
7	181.54	—	—	—
7a	132.19	—	—	—
8	119.15	7.83	7.84	7.84
9	136.50	7.68	7.69	7.68
10	125.34	7.36	7.35	7.37
11	162.66	—	—	—
11a	116.67	—	—	—
12	187.34	—	—	—
12a	119.20	—	—	—
12b	156.00	—	—	—
13	24.03	3.01	3.02	3.02
14	80.06	—	—	—
15	53.39	3.82		
16	42.28	2.76		
17	62.92	4.56, 4.35		
1′	96.95	5.27		
2′	27.10	1.96, 1.64		
3′	65.36	2.58		
4′	70.57	3.11		
5′	74.11	3.45		
6′	18.10	1.37		
$N(CH_3)_2$	40.23	2.34		
11-OH	—	12.82	13.3	13.0
17-OH	—	3.90		
4′-OH	—	4.18		

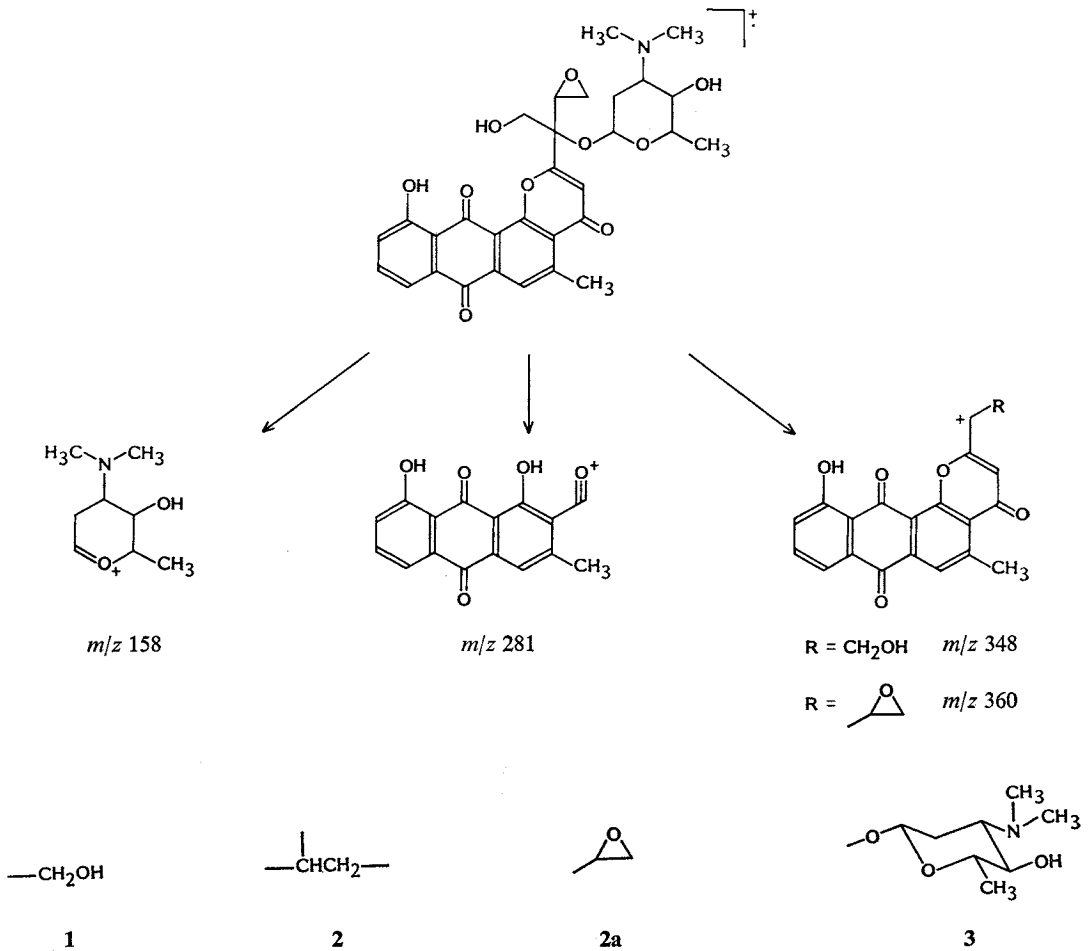
molecular weight of 565. A high resolution mass spectrum afforded the molecular formula of $C_{30}H_{81}NO_{10}$. The UV spectrum in EtOH has maxima at 326, 244 and 209 nm.

Two resonances (δ 187.34 and 181.54) in the ^{13}C NMR spectrum are characteristic of quinones. The ^{1}H NMR spectrum of A51493A was compared to the published data for a number of quinones derived from natural products and in particular rubiflavinones C-1 and C-2[1]. Based on these data, it was concluded that A51493A and rubiflavinones C-1 and C-2 posses the same nucleus (Fig. 1 and Table 1).

It is apparent that the difference from rubiflavinone resides in the side chain at C-2. ^{1}H NMR decoupling has shown the presence of the partial structures **1**, **2** and **3**. The ^{13}C NMR assignments of the nucleus, **1**, **2** and **3** were made through distortionless enhancement by polarization transfer (DEPT), one bond 2D heteronuclear correlation and fully coupled 2D heteronuclear correlation[4] experiments. The carbon chemical shifts for the resonances of **2** have shifts of δ 53.39 for the methine and δ 42.28 for the methylene. The one bond ^{13}C-^{1}H coupling (measured from the fully coupled 2D heteronuclear correlation) at these two carbons is approximately 177 Hz. This value and the chemical shifts are consistent with values found in epoxides[5,6]. Therefore, **2** can be amended to become an epoxide, **2a**. The stereochemistry of the various positions of **3** was determined through the magnitude of the vicinal couplings. The couplings of 3′-H to 4′-H, 4′-H to 5′-H and 1′-H to 2′-H_{ax} are of sufficient magnitude (*ca.* 8 Hz) to require that these protons be axial in stereochemistry.

A comparison of the molecular formula of

Fig. 2. The EI-MS fragmentation for A51493A.



A51493A and the sum of the formulas of the partial structures shows that there is only one carbon that is unaccounted. This carbon is observed at δ 80.06 in the ^{13}C NMR spectrum and has long range proton couplings (as shown in a fully coupled ^{13}C-^{1}H correlation) to the anomeric proton of **3**, the methylene of **1**, the methine of the **2a** and to 3-H. It is clear from this that all the partial structures are interconnected through this quaternary carbon and establishes the structure for A51493A.

The EI-MS of A51493A has prominent peaks at *m/z* 158 ($C_8H_{16}NO_2$), 281 ($C_{16}H_9O_5$), 348 ($C_{20}H_{12}O_6$) and 360 ($C_{21}H_{12}O_6$). The presence of these peaks is in accord with the structure proposed, and their formation is outlined in Fig. 2.

References

1) NADIG, H. & U. SEQUIN: Isolation and structure elucidation of some components of the antitumor antibiotic mixture 'rubiflavin'. Helv. Chim. Acta 70: 1217~1228, 1987
2) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. 22: 265~394, 1972
3) SHIRLING, E. B. & D. GOTTLIEB: Methods for the characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
4) BAX, A. (*Ed.*): Two-dimensional Nuclear Magnetic Resonance in Liquids. p. 52, Reidel, London, 1986
5) SHAPIRO, M.: Conformational analysis of 1-substituted 2,3-epoxypropanes. A carbon-13 nuclear magnetic resonance study. J. Org. Chem. 42: 1434~1436, 1977
6) ELIEL, E. L. & K. M. PIETRUSIEWICZ: ^{13}C NMR of nonaromatic heterocyclic compounds. *In* Topics in Carbon-13 NMR Spectroscopy. *Ed.*, G. C. LEVY, pp. 174~176, John Wiley & Sons, New York, 1979