Communications to the Editor

THE STRUCTURE OF A NEW ANTHRACYCLINE, CINERUBIN X PRODUCED BY A BLOCKED MUTANT OF *STREPTOMYCES VIOLACEOCHROMOGENES*

Sir:

As part of our biosynthetic studies on anthracycline antibiotics, we attempted to develop a cloning system using a cinerubin-producing organism isolated in our laboratory *Streptomyces violaceochromogenes* as a host. This strain was found to be transformed by the plasmid, pIJ702[1]. As a first step to clone the structural gene controlling the cinerubin biosynthesis, we isolated cinerubin-negative mutants of *S. violaceochromogenes*. Among these mutants the strain C73 was found to produce a new anthracycline named cinerubin X. In this paper we wish to report the isolation and structure of cinerubin X.

The strain C73 was cultivated at 27°C for 4 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of starch 2.5%, soybean meal 1.5%, yeast extract 0.2% and $CaCO_3$ 0.4%. After centrifuging the culture broth (1 liter), the mycelial cake was extracted with acetone. The acetone extract was concentrated to a small volume *in vacuo* and was then extracted with EtOAc. The solvent layer was concentrated and subjected to silica gel column chromatography. The column was washed with $CHCl_3$ and cinerubin X was then eluted with $CHCl_3$ - MeOH (20:1). Further purification was achieved by LH-20 column chromatography with $CHCl_3$ - MeOH (1:1) to give cinerubin X (20 mg).

Physico-chemical properties of cinerubin X are as follows: $C_{40}H_{48}O_{16}$, FAB-MS (negative) *m/z* 783 $(M-H)^-$, mp 151~152°C, $[\alpha]_D^{25}$ +80° (*c* 0.1, $CHCl_3$), UV λ_{max}^{MeOH} nm (ε) 258 (23,300), 290 (7,860), 493 (14,200), 505 (11,000) and 525 (9,100).

The ^1H NMR spectrum of cinerubin X was similar to that of cinerubin A[2] except for disappearance of the *N*-dimethyl signal. In agreement with this, the molecular weight of cinerubin X was smaller than that of cinerubin A by 43 which corresponded to the substitution of *N*-dimethyl group with a hydrogen atom. Analyses of the 2D-COSY and C-H correlation spectra revealed that cinerubin X contained ε-pyrromycinone and three types of sugars, *i.e.* rhodinose, 2-deoxyfucose and cinerulose A.

On acid hydrolysis with 0.1 N HCl at 85°C for 30 minutes, cinerubin X yielded ε-pyrromycinone, L-rhodinose, L-2-deoxyfucose and L-cinerulose A. The ^{13}C-chemical shift assignments of the cinerubin X are shown in Table 1. The anomeric configurations of three sugars were determined to be all α by the coupling constants of the anomeric protons of rhodinose (J=3.0, <1 Hz), 2-deoxyfucose (J=3.2, <1 Hz) and cinerulose A (J=6.0, 6.0 Hz); the coupling constant of cinerulose A being identical with that of aclarubicin A[3].

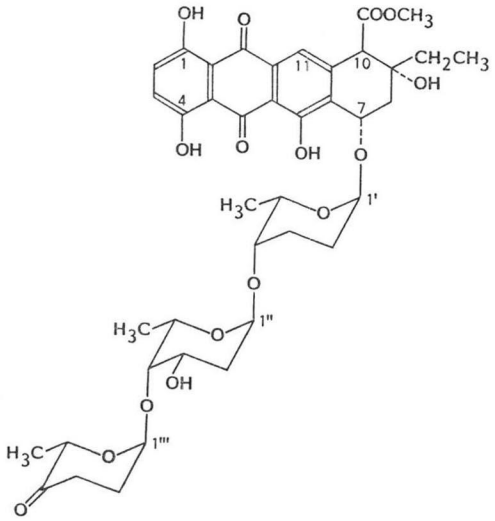
A nuclear Overhauser effect observed between 7-H and the anomeric proton of rhodinose proved that rhodinose was combined to C-7 of

Table 1. ^{13}C NMR spectral data of cinerubin X.

Carbon	Chemical shift	Carbon	Chemical shift
5	190.0	1′	101.2
12	185.2	2′	24.6
15	170.8	3′	24.6
6	161.8	4′	74.9
1	157.9*	5′	66.9
4	157.4*	6′	17.4
10a	142.2	1″	100.0
6a	132.4	2″	34.5
11a	131.3	3″	65.4
2	130.0#	4″	82.3
3	129.4#	5″	67.4
11	120.2	6″	17.3
5a	114.5	1‴	100.0
12a	112.3+	2‴	27.8
4a	112.1+	3‴	34.0
9	71.8	4‴	209.2
7	70.6	5‴	71.6
10	57.3	6‴	15.0
16	52.6		
8	33.7		
13	32.3		
14	7.0		

In ppm (δ); obtained from $CDCl_3$ solutions.
*, #, +: Assignments may be exchanged.

Fig. 1. The structure of cinerubin X.



the aglycone. The low field chemical shift of C-4 (δ_c 82.3) of 2-deoxyfucose was explained in terms of the glycosidation by cinerulose A. Therefore, the structure of cinerubin X was determined to be L-cinerulosyl-L-2-deoxyfucosyl-L-rhodinosyl-ε-pyrromycinone (Fig. 1).

The accumulation of cinerubin X implies that the strain C73 has lost the enzyme catalyzing the transamination of hypothetical 3′-deamino-3′-oxocinerubin X which would be converted to cinerubin X. The mutant C73 may be a very good host for cloning of the transaminase gene of cinerubin. Tested so far, cinerubin X was active only against *Bacillus subtilis* at 50 μg/ml.

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