Communications to the Editor

A NEW ANTHRACYCLINE METABOLITE D788-1 (10-CARBOXY-13-DEOXOCARMINOMYCIN) IN DAUNORUBICIN BEER

Sir:

We found a new anthracycline metabolite in the culture broth of daunorubicin or baumycin (4′-substituted daunorubicin)[1]-producing *Streptomyces* strains. The metabolite accumulated much more in the culture fluid rather than in the mycelial cake in contrast to a cellular accumulation of most anthracycline products. Since it contained a carboxyl group and was slightly soluble in organic solvents such as $CHCl_3$ and EtOAc, it was not extracted by solvent from the culture broth. We could find it by the use of adsorption resin on the culture broth.

This paper deals with the isolation and purification of this anthracycline metabolite, designated as D788-1 (**I**), from the culture broth of a blocked mutant of *Streptomyces* sp. D788, which was newly isolated from soil, and its chemical characterization. During this work, we also found that a novel type of anthracycline antibiotic akrobomycin[2], isolated from the culture broth of a carminomycin producer, could be derived from **I** by chemical alteration.

The microbial strain Gl-1 used was 4′-substitution-less mutant which was obtained from baumycin-producing *Streptomyces* sp. D788, and was blocked to accumulate daunorubicin with a high co-production of **I**. The fermentation medium used was soluble starch 7.5%, dry yeast 5%, yeast extract 0.3%, NaCl 0.2% and $CaCO_3$ 0.3%, pH 8.2. The fermentation was carried out in 250-ml Erlenmeyer flasks each containing 20 ml of the medium at 28°C for 6 days on a rotary shaker (220 rpm). Assay of **I** in the fermentation broth or at the isolation and purification steps was performed by HPLC on a Hitachi 655 liquid chromatographic apparatus with a reverse phase using an analytical Hitachi gel No. 3056 column (4×15 mm). The mobile phase was acetonitrile - 0.025 M camphoresulfonic acid (pH 3.8) (45:55) and the product detection was monitored by UV absorption at 254 nm. **I** showed a single peak at a retention time of 3.4 minutes, while that of the coproduced daunorubicin was 6.1 minutes. The yield of **I** and daunorubicin in the final fermentation broth was about 4:6.

The fermentation broth (3 liters) was harvested and adjusted to pH 1.8 with H_2SO_4 followed by stirring for 30 minutes at room temperature. The broth was filtered and the mycelial cake was then extracted with one liter of acetone (pH 1.7). The acetone extract was evaporated to about 300 ml *in vacuo* and was combined to the filtrate, adjusting to pH 2.3 with 4 N NaOH and passed through a column of Diaion HP-20 (150 ml) to adsorb the anthracycline products. The column was washed with pH 2.3 water (dil HCl) and then eluted with 50% acetone (pH 2.3). Red-pigmented fractions were pooled, evaporated *in vacuo* and adjusted to pH 7.5 with solid $NaHCO_3$. After removal of daunorubicin by $CHCl_3$ extraction, the aqueous layer was again adjusted to pH 2.5 with HCl, extracted with $CHCl_3$ and then BuOH. The BuOH extract was evaporated to dryness *in vacuo* to yield the crude reddish powder (1.08 g). Further purification was carried out by a silica gel column chromatography (Wako gel C-200, 80 g) using as eluent $CHCl_3$ - MeOH - H_2O - AcOH (40: 10: 1: 0.1). The pure fractions containing **I** were pooled and evaporated to dryness *in vacuo*. The residue was dissolved in 10% $NaHCO_3$ aqueous solution followed by washing with $CHCl_3$. The aqueous layer was additionally washed with $CHCl_3$ after adjusting to pH 2.5 with HCl and then extracted with BuOH. The BuOH extract was evaporated to dryness *in vacuo*. The red pigment residue thus obtained was crystallized in MeOH - toluene to yield 316 mg as a fine red crystalline powder.

Physico-chemical properties are: mp 161~163°C (dec); $[\alpha]_D^{25}$ +200° (*c* 0.113, MeOH); IR (KBr) cm^{-1} 1710 (carboxyl), 1600 (quinone), 1380, 1190 (phenolic OH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 495 (222), 530 (157), 575 (24).

^1H NMR of **I** (400 MHz, CD_3OD) showed the signals assigned to the aglycone moiety: δ 7.76 (1H, d, $J_{1,2}$=8.0 Hz, 1-H), 7.69 (1H, t, $J_{2,1}$=8.0 Hz, $J_{2,3}$=8.0 Hz, 2-H), 7.26 (1H, d, $J_{3,2}$=8.0 Hz, 3-H), 5.11 (1H, d, $J_{7,8a}$=5.0 Hz, 7-H), 4.21

(1H, s, 10-H), 2.26 (1H, dd, $J_{8a,8b}$=15.0 Hz, $J_{8a,7}$=5.0 Hz, 8a-H), 2.34 (1H, d, $J_{8a,8b}$=15.0 Hz, 8b-H), 1.92 (1H, m, $J_{13b,14}$=7.0 Hz, 13b-H), 1.63 (1H, m, $J_{13a,14}$=7.0 Hz, 13a-H), 1.15 (3H, t, $J_{14,13a}$=$J_{14,13b}$=7.0 Hz, 14-CH_3), and to daunosamine residue linked to C-7 of the aglycone: δ 5.49 (1H, d, $J_{1',2'b}$=4.0 Hz, 1'-H), 4.28 (1H, q, $J_{5',6'}$=7.0 Hz, 5'-H), 3.66 (1H, br s, 4'-H), 3.60 (1H, m, $J_{3',2'b}$=12.0 Hz, $J_{3',2'a}$=5.0 Hz, $J_{3',4'}$=3.0 Hz, 3'-H), 2.07 (1H, m, $J_{2'b,2'a}$=12.0 Hz, $J_{2'b,3'}$=12.0 Hz, $J_{2'b,1'}$=4.0 Hz, 2'b-H), 1.96 (1H, m, $J_{2'a,2'b}$=12.0 Hz, $J_{2'a,3'}$=5.0 Hz, 2'a-H), 1.29 (3H, d, $J_{6',5'}$=7.0 Hz, 6'-CH_3).

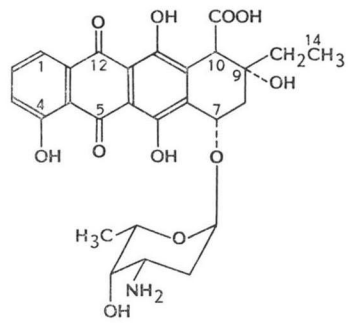
The chemical shifts and assignments of **I** in ^{13}C NMR spectrum are shown in Table 1 in comparison of those of β-rhodomycinone. The aglycone moiety of **I** was identical with β-rhodomycinone in the signals of their corresponding carbons, except for an additional signal 173.6 in **I** due to a carboxyl group (C-15). The FD-MS spectrum of **I** did not show a molecular ion peak m/z 543 ($C_{27}H_{29}NO_{11}$), but an intensive fragment peak m/z 499 (M−44) with the loss of CO_2.

Table 1. ^{13}C NMR chemical shifts of **I** and β-rhodomycinone.

Carbon	**I** (ppm in CD_3OD)	β-Rhodomycinone (ppm in $CDCl_3$-CD_3OD)	Remark
1	120.5	119.8	Aglycone moiety
2	138.3	137.5	
3	125.8	124.9	
4	163.5	162.6	
4a	116.9	116.8	
5	191.8	191.1	
5a	112.5	112.1	
6	157.6	156.5	
6a	137.0	137.4	
7	72.6	65.9	
8	(35.3)	33.8	
9	71.5	73.0	
10	53.2	62.2	
10a	137.2	138.3	
11	157.9	157.5	
11a	112.2	111.8	
12	187.2	186.5	
12a	134.4	133.8	
13	(33.7)	30.6	
14	7.3	6.3	
15	173.6	—	
1'	101.6		Daunosamine moiety
2'	29.5		
3'	48.6		
4'	67.9		
5'	68.0		
6'	17.0		

TMS was used as internal standard (100 MHz). Similar values in parenthesis may be interchanged.

Fig. 1. The structure of D788-1 (**I**).



Acid hydrolysis (0.1 N HCl, 85°C, 30 minutes) of **I** afforded a red aglycone and an aminosugar, the latter being identified to daunosamine by a qualitative TLC analysis[3] compared with an authentic sample obtained by same acid hydrolysis of daunorubicin. Chemical treatment of the red aglycone with CH_2N_2 gave a methylated compound which was identical to ε-rhodomycinone by ^1H NMR study. It was thus proved that **I** had a stereochemical configuration of 7(*S*), 9(*R*) and 10(*R*). From these results, we identified **I** as 10-carboxy-13-deoxocarminomycin as shown in Fig. 1.

During the isolation and purification of **I**, we found that **I** was chemically unstable and was decomposed to a new violet compound. This chemical alteration occurred much more readily when **I** was present in acetone, especially at alkaline pH. The spectral analysis of this compound proved that it was identical to akrobomycin which was isolated from the culture broth of *Actinomadura roseoviolacea* 1029-AVI[2]. Thus, it seems that akrobomycin was not a natural product but a chemically degraded compound of **I**.

I was as active as daunorubicin against the cell growth of L1210 cell culture under continuous exposure (IC_{50} value: 0.027 μg/ml for **I** and 0.02 μg/ml for daunorubicin), but the inhibition of nucleic acid synthesis by **I** in the cell culture (IC_{50} value: over 5 μg/ml for both DNA and RNA syntheses) was quite weak as compared

with that of daunorubicin (IC_{50} value: 0.42 and 0.16 μg/ml for DNA and RNA syntheses, respectively). *In vivo* antitumor activity of **I** against L1210 leukemia in mice was about 130% (T/C) at an optimum dose (20 mg/kg) (ip-ip, day 1 to 9, daily) and was less active than daunorubicin.

SHIZUKA FUJII
KATSURO KUBO
OSAMU JOHDO
AKIHIRO YOSHIMOTO
TOMOYUKI ISHIKURA
HIROSHI NAGANAWA†
TSUTOMU SAWA†
TOMIO TAKEUCHI†
HAMAO UMEZAWA†

Central Research Laboratories
Sanraku Incorporated
Johnan, Fujisawa-shi, Kanagawa
251, Japan
†Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo
141, Japan

(Received October 24, 1985)

References

1) TAKAHASHI, Y.; H. NAGANAWA, T. TAKEUCHI, H. UMEZAWA, T. KOMIYAMA, T. OKI & T. INUI: The structure of baumycins A1, A2, B1, B2, C1 and C2. J. Antibiotics 30: 622~624, 1977
2) IMAMURA, K.; A. ODAGAWA, K. TANABE, Y. HAYAKAWA & N. ŌTAKE: Akrobomycin, a new anthracycline antibiotic. J. Antibiotics 37: 83~84, 1984
3) OKI, T.; A. YOSHIMOTO, Y. MATSUZAWA, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of anthracycline antibiotics by *Streptomyces galilaeus*. I. Glycosidation of various anthracyclinones by an aclacinomycin-negative mutant and biosynthesis of aclacinomycins from aklavinone. J. Antibiotics 33: 1331~1340, 1980