4-*O*-(β-D-GLUCOPYRANOSYL)-ε-RHODOMYCINONE, A NEW MICROBIAL TRANSFORMATION PRODUCT OF RHODOMYCINONE

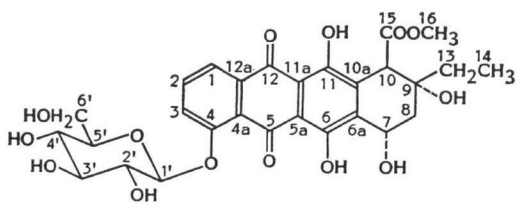
Sir:

Although the biosynthetic pathway of anthracycline antibiotics has been extensively investigated with daunorubicin[1] and aclarubicin[2], details about mechanisms of the glycosidation and formation processes of unusual carbohydrate moieties remain to be revealed. During our biosynthetic studies on anthracycline antibiotics, we found that ε-rhodomycinone (**2**) was converted to 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone (**1**) (Fig. 1) by the blocked mutant, *Actinomadura roseoviolacea* MuWl[3]. We report herein the isolation and structure determination of **1**.

For the microbial conversion of **2**, a medium of the following composition was used; yeast extract 0.4%, meat extract 2%, malt extract 1% and glucose 0.4%. The pH of the medium was adjusted to 7.0 before sterilization. Seed culture (2 ml) and 1 ml of dimethyl sulfoxide solution of **2** (3 mg/ml) were simultaneously added into 100 ml of the medium contained in a 500-ml Erlenmeyer flask. The conversion was carried out by shaking culture on a rotary shaker at 37°C for 2 days.

The culture filtrate (500 ml) was adjusted to pH 2.0 and applied to a column of Diaion HP-20 which was washed successively with water and 50% MeOH, and then **1** was eluted with 100% MeOH. The eluate was concentrated to a small volume *in vacuo* and the metabolite was extracted with EtOAc. The solvent fraction was evaporated to dryness *in vacuo* and the residue was subjected to silica gel column chromatography. The column was washed with $CHCl_3$ and **1** was eluted with $CHCl_3$ - MeOH (10: 1). Further purification was achieved by Sephadex LH-20 column chromatography with MeOH to give 3 mg of **1**.

Fig. 1. The structure of **1**.



Physico-chemical properties of **1** are as follows: $C_{28}H_{30}O_{14}$, FD-MS *m/z* 613 $(M+Na)^+$, mp 182~183°C, λ_{max} (in MeOH) nm (ε) 234 (46,600), 254 (42,900), 475 (11,800), 494 (12,000) and 526 (6,600), λ_{max} (in MeOH+0.1 N NaOH) nm 210 (ε) (88,300), 250 (45,400), 560 (12,000) and 590 (11,000). These UV spectral data of **1** are identical with those of daunorubicin[4]. Acid hydrolysis of **1** with 0.5 N HCl at 100°C for 30 minutes yielded **2** and D-glucose.

The 400 MHz ^1H NMR spectrum of **1** (Fig. 2)

Table 1. ^{13}C NMR spectral data.

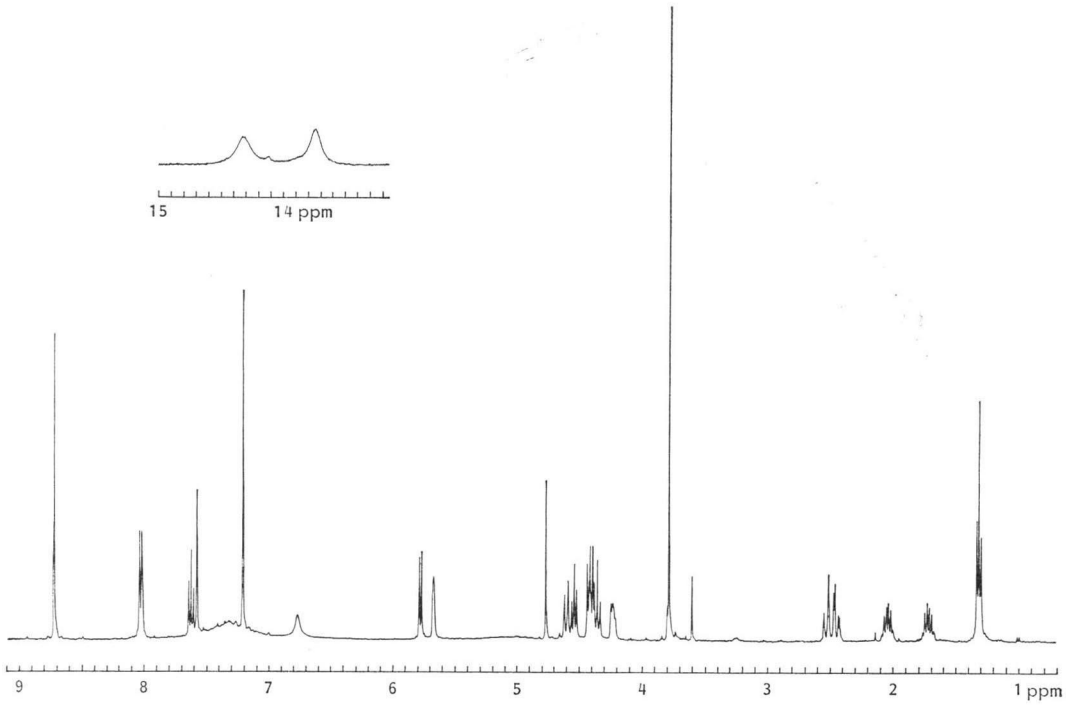
Carbon	1	2	Methyl β-D-glucopyranoside[5]
5	187.0	190.5	
12	186.5	186.0	
15	171.7	171.6	
4	159.6	162.6	
11	(157.0)	(157.3)	
6	(156.1)	(157.2)	
10a	139.8	139.8	
2	135.9	137.3	
12a	135.4*	**	
6a	133.7	133.8	
3	123.8	124.9	
4a	122.1	119.6	
1	121.2	116.6	
5a	(112.8)	(111.8)	
11a	(111.8)	(111.6)	
1′	103.4		105.5
5′	(79.7)		78.3
3′	(78.5)		78.3
2′	75.2		74.9
9	72.4	72.4	
4′	71.5		71.6
6′	62.9		62.7
7	62.4	62.3	
10	53.1	53.2	
16	52.7	52.7	
8	36.5	36.3	
13	34.0	34.0	
14	8.0	8.0	

In ppm (δ); obtained from C_5D_5N solutions containing TMS as internal reference. Similar values in parentheses may be interchanged.

* This value was obtained by suppressing the overlapping solvent peak by ^{13}C spin-echo modulation through ^{13}C, ^2H spin-spin coupling (see ref 6).

** This value could not be obtained due to the overlapping of the solvent peak.

Fig. 2. ^1H NMR spectrum of **1** (400 MHz in C_5D_5N).



showed signals due to the glucose moiety at δ_H 5.80 (H-1′, d, J=7.8 Hz), 4.56 (H-2′, dd, J=7.8, 7.8 Hz), 4.43 (H-3′, dd, J=7.8, 8.5 Hz), 4.38 (H-4′, dd, J=8.5, 8.5 Hz), 4.25 (H-5′, m), 4.62 (H-6′, dd, J=12.2, 2.0 Hz) and 4.44 (H-6′, dd, J=12.2, 5.9 Hz). The anomeric configuration was determined to be β by the large coupling constant between H-1′ and H-2′.

The 100 MHz ^{13}C NMR spectra of **1** and **2** are summarized in Table 1. The signals of **2** were assigned by reference to those of daunorubicin[5]. Since the chemical shift of C-7 of **1** (δ_c 62.4) is similar to that of **2**, the C-7 position must be free. On the other hand, one resonance due to the quinone carbonyl carbon of **1** (δ_c 187.0) is shifted to higher field as compared with the corresponding signal of **2** (δ_c 190.5). These data imply that the hydrogen bonding of this quinone carbonyl (C-5 or C-12) is disrupted by the glycosidation of a phenolic hydroxyl function in **1**. Thus the glucose must be connected to the oxygen at either 4, 6 or 11. Nuclear Overhauser effect (NOE) and long range coupling observed between the anomeric proton at δ_H 5.80 and the aromatic protons at δ_H 8.03 (2H, due to an overlap of H-1 and H-3) gave conclusive evidence for the final structure of **1**. Since H-1 is located far away from the anomeric proton wherever the sugar is attached, the observed NOE and long range coupling were concluded to be caused by interaction between H-3 and the anomeric proton. Therefore the carbohydrate residue must be connected to C-4 through a glycosidic linkage to establish the structure of **1** as 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone.

As far as we know, **1** is the first 4-*O*-glycosylanthracyclinone derivative to be isolated in nature. It is interesting to note that **1** was produced only when **2** was added at lag phase; under this condition carminomycins and akrobomycin were not produced.

4-*O*-(β-D-Glucopyranosyl)-ε-rhodomycinone was not active against Gram-positive and Gram-negative bacteria at concentration as high as 1,000 μg/ml, and did not show any cytotoxicity (100 μg/ml).

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