A NEW CLASS OF BIOSYNTHETIC ANTHRACYCLINES: ANTHRACYCLINONE GLUCURONIDES

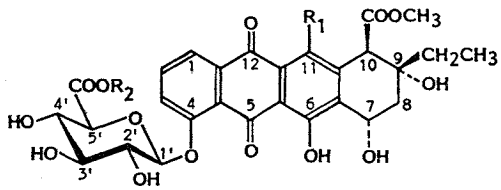
Sir:

Our previous search for new anthracyclines produced by mutants of *Streptomyces peucetius*[1], the daunorubicin[2] producing microorganism, led to the discovery of doxorubicin (adriamycin)[3] and other new anthracyclines[4~8]. By further mutagenic treatment of *Streptomyces peucetius* var. *aureus*, a mutant of *Streptomyces peucetius* producing 4-*O*-demethyl-11-deoxydoxorubicin and analogues[7], we have recently isolated other new mutant strains. Among them, two mutants designed *Streptomyces peucetius* var. *vinaceus* (NRRL 15344) and *Streptomyces peucetius* var. *castaneus* (NRRL 15345), were found to produce respectively 4-*O*-(β-D-glucopyranuronosyl)-ε-rhodomycinone (**1**)[9] and 4-*O*-(β′-D-glucopyranuronosyl)aklavinone (**3**), representatives of a new class of anthracyclines, shown in Fig. 1. Here we wish to report the isolation and structure elucidation of these new metabolites and preliminary biological activity data of **1**.

Streptomyces peucetius var. *vinaceus* was grown in a fermentation medium containing (g/liter); glucose 60, brewer dry yeast 30, NaCl 2, KH_2PO_4 1, $CaCO_3$ 2.5, $MgSO_4 \cdot 7H_2O$ 0.1, $FeSO_4 \cdot 7H_2O$ 0.01, $ZnSO_4 \cdot 7H_2O$ 0.01, $CuSO_4 \cdot 5H_2O$ 0.01, and was found to produce **1** in noticeable amounts (maximum concentration on the 7th day of fermentation at 28°C). The culture broth (5 liters) was filtered using diatomaceus earth as filter aid. The wet mycelium was extracted with MeOH and the concentrated extract was combined with the filtered broth, brought to pH 4 and extracted with methylisobutylketone. The organic phase was re-extracted with 3% aq $NaHCO_3$ and the resulting aqueous phase, salted with 5% NaCl, was adsorbed on a column of Amberlite ER 180 (Rhom & Haas Co.). After washing with water, elution was carried out with 10% propanol in water. Selected fractions were concentrated and extracted with methylisobutylketone. Concentration and recrystallization from the same solvent gave crystalline **1** as free acid (0.4 g). Physicochemical properties of **1** were as follows; molecular formula $C_{28}H_{28}O_{15}$, field desorption mass spectrum (FD-MS) (*m/z* 604), mp 168~169°C (dec), λ_{max}^{MeOH} nm (ε) 233 (29,600), 252 (29,900), 474 (11,100), 493 (11,100), 525 (6,000). Acid hydrolysis of **1** (1% aq H_2SO_4, 2 hours, 100°C) gave ε-rhodomycinone[10] and D-glucuronic acid which were also obtained upon incubation of **1** with β-D-glucuronidase (bacterial type VII Sigma) in phosphate buffer 50 mM, pH 7 for 1 hour at 37°C. ^1H NMR of **1** (200 MHz, $CDCl_3$ with one drop of DMSO-d_6) showed (see Table 1) all the signals due to the aglycone, including two free phenolic hydroxyl protons[11], and the glucuronic acid moiety, whose anomeric configuration was determined to be β on the basis of the large value of the coupling constant ($J_{1',2'}$ = 7.1 Hz). The corresponding methyl ester **2** was prepared from **1** (0.1 N methanolic HCl, 2 hours, 20°C) to confirm the assigned structure. As expected, its ^1H NMR spectrum (200 MHz, $CDCl_3$) showed a marked downfield shift of 5′-H ($\Delta\delta$ = +0.4 ppm) due to esterification of the neighboring carboxyl group. ^{13}C NMR chemical shift data (see Table 2) are in good agreement with recent data reported in the literature for 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone[12]. ^{13}C NMR analyses of **1** showed the site of attachment of the glucuronic acid to be C-4 on the aromatic D ring of ε-rhodomycinone; in fact the resonance of C-4 (δ 158.36) is shifted to higher field with respect to ε-rhodomycinone (δ 162.6)[12], corresponding to a change from an OH substitution to a *O*-alkyl substitution in the anthracyclinone[12] and anthraquinone systems[13]. Furthermore no significant changes were observed for the other possible glycosidation sites, namely C-7[14], C-6 and C-11.

Streptomyces peucetius var. *castaneus*, grown on the same culture medium as reported above, gave, following the same procedure applied for **1**, the 11-deoxy analogue **3** as free acid. Physicochemical properties of **3** were as follows; mole-

Fig. 1. Structures of **1**, **2** and **3**.



1 R_1 = OH R_2 = H
2 R_1 = OH R_2 = CH_3
3 R_1 = H R_2 = H

Table 1. ^{1}H NMR chemical shift data ($CDCl_3$, δ) (coupling constants (Hz) in parenthesis).

	1	2	3
Aglycone			
1-H	7.65 (4.4)	7.2~7.4	7.7~8.0
2-H	8.00		
3-H	7.65 (4.4)		
10-H	4.14	4.20	4.01
8-H	1.9~2.3	1.9~2.3	8_{eq} 2.15 (1.5, 14.9) 8_{ax} 2.38 (4.5, 14.9)
7-H	5.14	5.19	5.25 (1.5, 4.5)
13-H	1.40, 1.66	1.50, 1.76	1.30, 1.60
14-H	0.99 (7.3)	1.12 (7.2)	0.99 (7.2)
6-OH[a]	13.30	13.21	13.25
11-OH[a]	13.60	13.47	—
11-H	—	—	7.56
10-$COOCH_3$	3.56	3.69	3.59
Sugar			
1'-H	4.85 (7.1)	5.06	4.88 (7.5)
2'-H			3.81 (7.5, 8.8)
3'-H	3.5~3.8	3.7~4.0	3.5~3.9
4'-H			
5'-H	3.94 (9.0)	4.28 (8.0)	3.97 (9.2)
5'-$COOCH_3$	—	3.82	—

[a] Resonances of the phenolic hydroxyl protons are assigned according to ref 11.

Table 2. ^{13}C NMR chemical shifts of **1**[a].

Carbon	δ	Carbon	δ
C-1	122.03	C-11	156.24
C-2	135.81	C-11a	111.00
C-3	124.44	C-12	186.07
C-4	158.36	C-13	32.38
C-4a	122.03	C-14	6.08
C-5	187.13	10-$COOCH_3$	171.52
C-5a	111.00		51.89
C-6	155.47	C-1'	101.50
C-6a	134.92	C-2'	72.89
C-7	61.64	C-3'	75.14
C-8	34.42	C-4'	71.22
C-9	71.56	C-5'	75.14
C-10	51.52	5'-COOH	170.61
C-10a	138.16		

[a] $CDCl_3$ - CD_3OD (50 : 50) as solvent.

cular formula $C_{28}H_{28}O_{14}$, FD-MS (*m/z* 588), mp 153~154°C (dec), λ_{max}^{MeOH} nm (ε) 228 (27,200), 258 (25,800), 412 (8,100). Acid and enzymatic hydrolysis, under the same conditions reported above for compound **1**, gave aklavinone and D-glucuronic acid. ^{1}H NMR spectrum of **3** (200 MHz, $CDCl_3$ with one drop of DMSO-d_6) was essentially identical to that of compound **1**, except for the additional singlet in the aromatic region due to 11-H and the increased shielding of 10-H equatorial (δ 4.01) due to the lack of the *peri* aromatic hydroxyl group. The glucuronic moiety showed a β configuration at the anomeric center ($J_{1',2'}$=7.5 Hz) (see Table 1).

The ε-rhodomycinone glucuronide **1** was found to be cytotoxic toward HeLa cells in a viability test (ID_{50} 40 ng/ml, compared with 16 ng/ml for doxorubicin). Studies on blocked mutants of a daunorubicin producer and bioconversion of precursors have demonstrated that daunorubicin is biosynthesized by glycosidation of ε-rhodomycinone[15] which was also isolated from fermentation broths of *S. peucetius*[16]. Furthermore aklavinone was suggested to be the biosynthetic precursor of daunomycinone[17]. However up to now all the new anthracyclines we have isolated from mutants of *S. peucetius*[3~8] are characterized by the presence of the same amino sugar, daunosamine (3-amino-2,3,6-trideoxy-L-lyxohexose)[18] glycosidically linked to the 7-benzylic hydroxyl group of different aglycones modified at 4, 11, 13 and 14 positions. From the structural point of view, compounds **1** and **3** can be considered representative of a new class of anthracyclines because of two unusual features; the presence of the D-glucuronic acid moiety and the 4-*O*-glycosidation site. Only recently a related metabolite, 4-*O*-(β-D-glucopyranosyl)-ε-rhodo-

mycinone has been obtained by microbial glycosidation of ε-rhodomycinone using a blocked mutant of *Actinomadura roseoviolacea*[12]. On the other hand, it has to be pointed out that 4-*O*-glucuronides of 4-*O*-demethyl-7-deoxy-13-dihydro-daunomycinone and -adriamycinone have been detected in bile, plasma or urine as human metabolites of daunorubicin[19] and doxorubicin[20] respectively. Finally compounds **1** and **3** can be added to the rare examples of microbial products containing the D-glucuronic acid moiety, such as a glucuronyl diglyceride[21], the nucleoside antibiotic pentopyranic acid[22], the sesquiterpenoid deoxypentalenylglucuron[23] and the monobactams formadicins A and B[24].

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