SYNTHESIS AND BIOLOGICAL ACTIVITY OF (7*S*)-*O*-EPOXYALKYL DERIVATIVES OF DAUNOMYCINONE

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Synthesis and antibacterial activity of a number of 7-*O*-epoxyalkyl derivatives of daunomycinone prepared from 7-*O*-alkenyl derivatives of daunomycinone are described along with their inhibitory effect on leukemia P 388 cells.

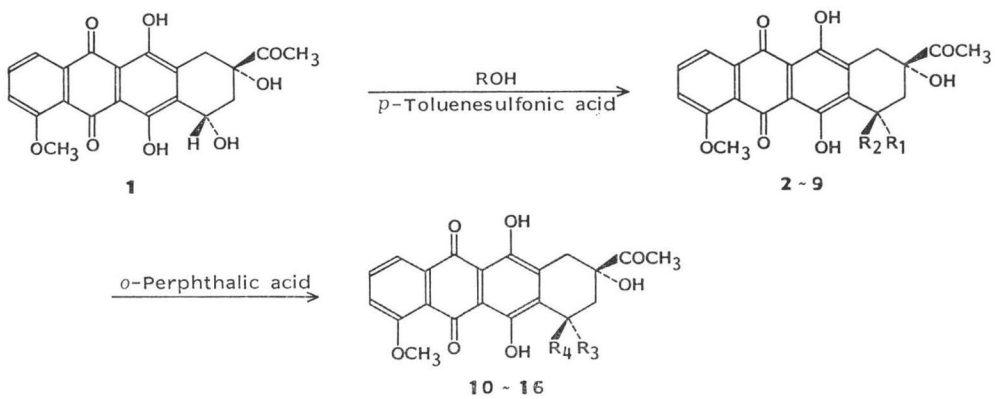
In an effort to increase the biological activity of anthracycline antitumor agents, derivatives have been prepared by modifying both the aglycone and the sugar moiety[1,2]. A number of 7-*O*-derivatives of daunomycinone were prepared in this laboratory, including 7-*O*-alkyl, 7-*O*-alkenyl and 7-*O*-alkynyl derivatives[3,4], 7-*O*-hydroxyalkyl derivatives[5] and their glycosides[6]. Some of them exhibited antibacterial activity.

This study describes the preparation of 7-*O*-epoxyalkyl derivatives of daunomycinone from 7-*O*-alkenyl derivatives of daunomycinone and evaluation of their biological activity.

Chemistry

Synthesis of 7-*O*-epoxyalkyl derivatives of daunomycinone is shown in Chart 1. After daunorubicin hydrolysis, daunomycinone (**1**) is isolated and converted to 7-*O*-alkenyl derivatives by reactions

Chart 1.



1 **2 ~ 9** **10 ~ 16**

R=$-CH_2CH=CH_2$
$-CH_2CH_2CH=CH_2$
$-CH_2CH=CHCH_3$
$-CH_2CH_2CH_2CH=CH_2$

R_1, R_2, R_3, R_4 see Tables 1 and 2.

Table 1. 7-*O*-Alkenyl derivatives of daunomycinone.

Compound	R_1	R_2
2	$-OCH_2CH=CH_2$	H
3	H	$-OCH_2CH=CH_2$
4	$-OCH_2CH_2CH=CH_2$	H
5	H	$-OCH_2CH_2CH=CH_2$
6	$-OCH_2CH=CHCH_3$	H
7	H	$-OCH_2CH=CHCH_3$
8	$-OCH_2CH_2CH_2CH=CH_2$	H
9	H	$-OCH_2CH_2CH_2CH=CH_2$

Table 2. 7-*O*-Epoxyalkyl derivatives of daunomycinone.

Compound	R_3	R_4
10		H
11		H
12	H	
13		H
14	H	
15		H
16	H	

with appropriate unsaturated alcohols in absolute benzene and xylene in the presence of *p*-toluenesulfonic acid. The reactions give rise to 7-*O*-alkenyl derivatives of both the *S* and the *R* series[4] which are listed in Table 1. 7-*O*-Alkenyl derivatives of daunomycinone **2**~**9** are transformed to their corresponding epoxides by *o*-perphthalic acid in absolute chloroform. The resulting 7-*O*-epoxyalkyl derivatives of daunomycinone **10**~**16** are given in Table 2.

Antimicrobial Activity

Compounds **10**~**16** were tested against *Bacillus subtilis*, *Saccharomyces cerevisiae* K and *Escherichia coli* C 600 (Institute of Microbiology). *Brevibacterium flavum* (ATCC 14067) by the plate diffusion method and compared with daunorubicin. None of them including daunorubicin was active against *E. coli*. Against other test microorganisms the (7*R*)-*O*-epoxyalkyl derivatives of daunomycinone (**12, 14** and **16**) were also inactive.

The activity of (7*S*)-*O*-epoxyalkyl derivatives against *B. subtilis* decreases with increasing length of the side chain at position 7, the highest activity being exhibited by compound **10**. Inhibition of growth of *B. subtilis* at a concentration of 0.001 mg/ml by compound **10** was equivalent to the inhibition caused by 0.7 mg/ml daunorubicin. Compound **10** is also the only one to inhibit *S. cerevisiae*.

Inhibition zones of (7*S*)-*O*-epoxyalkyl derivatives of daunomycinone are given in Table 3 for compounds **11**, **13**, **15** and daunorubicin as a control, and in Table 4 for compound **10** and daunorubicin.

Inhibition of Nucleic Acid and Protein Synthesis of P 388 Cells

In an *in vitro* experiment some 7-*O*-epoxyalkyl derivatives of daunomycinone inhibited the nucleic acid and protein synthesis of P 388 cells. The inhibitory effect was estimated as the decrease in incorporation of suitable precursors into the P 388 cells relative to control. Table 5 shows the inhibition of incorporation of adenine, L-valine, thymidine and uridine into the P 388 cells by compounds **10**, **11**, **13**~**15**, daunorubicin and daunomycinone.

Experimental

Melting points were measured on the Kofler stage, optical rotation on an automatic polarimeter Bendix Ericsson, and mass spectra on a Varian MAT-311 spectrometer. ^{1}H NMR spectra were measured on a Jeol FX-60 spectrometer (FD mode, 59.797 MHz) at 25°C in deuteriochloroform, with tetramethylsilane as internal standard. Chemical shifts were calculated from digitized address differences (±0.002 ppm) and are given in the δ scale.

Table 3. Comparative *in vitro* activity of compounds **11**, **13**, **15**[a].

Compound[b]	Organism[c]	
	B. subtilis	*Brevibacterium*
11	17	18
13	12	23
15	11	22
Daunorubicin	19	28

[a] Inhibition zones in mm. [b] Preparation of samples; 1 mg compound dissolved in 1 ml methanol, 0.1 ml sample placed on plate. [c] Preparation of inoculum; the particular test-organism was cultivated under standard static conditions in a 2-liter Roux-bottle for 20 days. The surface of culture was splashed by 50 ml of sterile water and 400 ml of 3% agar medium was inoculated by 0.1 ml of this suspension.

Inhibition of Nucleic Acid and Protein Synthesis

Leukemia P 388 cells obtained from the abdominal cavity 7 days after implantation were freed of the serum, resuspended in a buffer (concentration 3×10^6/ml) and dispensed to test tubes in 1 ml aliquots. After preincubation (1 hour at 37°C) they were supplied with the tested compounds (100 μg/ml in 10 μg DMSO) together

Table 4. Comparative *in vitro* activity of compound **10**[a].

Compound[b]	Organism		
	B. subtilis	*Brevibacterium*	*S. cerevisiae*
10	26	31	17
Daunorubicin	15	24	0

[a] Inhibition zones in mm. [b] Sample preparation; 0.1 mg compound dissolved in 1 ml methanol for testing *B. subtilis* and *Brevibacterium*, 1 mg/ml for testing *S. cerevisiae*, 0.1 ml solution applied per plate.

Table 5. Inhibition of incorporation of precursors into P 388 cells (%).

Compound	[*U*-^{14}C]Adenine	L-[*U*-^{14}C]Valine	[*U*-^{14}C]Thymidine	[*U*-^{14}C]Uridine
10	65.3	80.3	83.3	95.4
11	45.7	29.0	45.5	73.2
13	54.9	26.0	57.8	85.3
14	34.9	1.3	48.0	64.9
15	46.2	41.9	56.7	76.6
Daunorubicin	65.9	49.1	83.1	92.9
Daunomycinone	31.4	+9.8	24.2	38.8

with radioactive substrates; [*U*-^{14}C]adenine (0.76 μg, 13 kBq/μg), L-[*U*-^{14}C]valine (1.12 μg, 5.9 kBq/μg), [*U*-^{14}C]uridine (0.26 μg, 22.5 kBq/μg) and [*U*-^{14}C]thymidine (1.12 μg, 7.14 kBq/μg). Control test tubes contained the cell suspension, labelled precursor and 10 μliter DMSO. The suspension was incubated for 1 hour at 37°C and incorporation of precursors was terminated by adding 1 ml 5% trichloroacetic acid. Inhibitory effect of 7-*O*-epoxyalkyl derivatives of daunomycinone was determined from differences in the utilization of the precursors[7].

Preparation of 7-*O*-Alkenyl Derivatives of Daunomycinone (**2**~**9**)

Daunomycinone (150 mg) was dissolved in the mixture of benzene (6 ml) and xylene (6 ml). Then it was added 2 ml of corresponding unsaturated alcohol (2-propen-1-ol, 3-buten-1-ol, *trans*-2-buten-1-ol respectively 4-penten-1-ol) and *p*-toluenesulfonic acid (50 mg). The reaction was carried out 5~20 hours at 115~120°C. The reaction mixture was diluted with water and the products were extracted with chloroform. Solvent was removed and the residue subjected to column chromatography on silica gel (Lachema, Czechoslovakia); the eluent was benzene. Resulting mixture of corresponding (7*S*) and (7*R*) derivatives was separated by preparative thin-layer chromatography on Silufol 20 in the system benzene - $CHCl_3$ - EtOAc - MeOH, 7: 7: 3: 1.

Yield of reactions was 86~91% and the ratio of (7*S*) and (7*R*) derivatives (calculation based on isolated products) was for reaction with 2-propen-1-ol 6: 5, for 3-buten-1-ol 3: 5, for *trans*-2-buten-1-ol 2: 8 and for 4-penten-1-ol 4: 4.

Absolute configuration on C(7) for compounds **2**~**9** was estimated from ^{1}H NMR spectrum[4].

Preparation of 7-*O*-Epoxyalkyl Derivatives of Daunomycinone (**10**~**16**)

All 7-*O*-epoxyalkyl derivatives (**10**~**16**) were prepared as follows: A sample of 100 mg 7-*O*-alkenyl derivative of daunomycinone (**2**~**9**) was dissolved in 100 ml absolute chloroform and a solution of *o*-perphthalic acid in diethyl ether (41 mg *o*-perphthalic acid in 1 ml) was added. The reaction mixture was left to stand at 20~25°C for 60 hours. The precipitated *o*-phthalic acid was filtered off and the reaction mixture was extracted with water. The chloroform solution was evaporated to dryness and the residue was separated by preparative thin-layer chromatography on silica gel (Silufol 20) in the system $CHCl_3$ - benzene - EtOAc - MeOH, 7: 7: 3: 1. Reaction yields are included in the characteristics of the individual substances **10**~**16**. The (7*R*)-*O*-(2,3-epoxypropyl) derivative of daunomycinone, which was formed in small amounts, was the only compound that was not isolated.

(7*S*)-9-Acetyl-4-methoxy-7-*O*-(2,3-epoxypropyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**10**): Yield 34%; mp 66~68°C; $[\alpha]_D^{20}$ +115° (*c* 0.11, $CHCl_3$); MS 454 (M^+); ^{1}H NMR 1.81 (1H, dd, *J*=3.7, 14.7 Hz), 2.43 (3H, s), 2.63 (1H, dd, *J*=3.4, 14.7 Hz), 2.74 (2H, mt), 2.83, 3.40 (2H, AB system, J_{AB}=19.4 Hz), 3.71~3.98 (3H, mt), 4.08 (3H, s), 4.90 (1H, s, OH), 5.14 (1H, mt, W=7 Hz), 7.39 (1H, dd, *J*=2.4, 7.3 Hz), 7.78 (1H, t, *J*=7.3 Hz), 8.06 (1H, dd, *J*=2.4, 7.3 Hz), 13.29 (1H, s), 13.99 (1H, s).

Anal Calcd for $C_{24}H_{22}O_9$: C 63.43, H 4.88.
Found: C 63.49, H 4.91.

(7*S*)-9-Acetyl-4-methoxy-7-*O*-(3,4-epoxybutyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**11**): Yield 35%; mp 70~72°C; $[\alpha]_D^{20}$ +202° (*c* 0.09, $CHCl_3$); MS 468 (M^+); ^{1}H NMR 1.99 (1H, dd, *J*=3.6, 14.7 Hz), 2.42 (1H, dd, *J*=3.1, 14.7 Hz), 2.43 (3H, s), 2.95, 3.26 (2H, AB system, J_{AB}=19.5 Hz), 3.63~4.01 (3H, mt), 4.09 (3H, s), 5.10 (1H, mt, W=6.7 Hz), 7.38 (1H, dd, *J*=1.8, 7.3 Hz), 7.77 (1H, t, *J*=7.3 Hz), 8.05 (1H, dd, *J*=1.8, 7.3 Hz), 13.29 (1H, s), 13.96 (1H, s).

Anal Calcd for $C_{25}H_{24}O_9$: C 64.09, H 5.18.
Found: C 64.15, H 5.21.

(7*R*)-9-Acetyl-4-methoxy-7-*O*-(3,4-epoxybutyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**12**): Yield 26%; mp 101~104°C; $[\alpha]_D^{20}$ −74° (*c* 0.11, $CHCl_3$); MS 468 (M^+); ^{1}H NMR 1.77 (2H, mt), 2.10 (1H, dd, *J*=4.9, 14.6 Hz), 2.38 (2H, s), 2.49 (2H, mt), 2.62 (1H, dd, *J*=4.9, 14.6 Hz), 3.01 (1H, mt), 2.99, 3.29 (2H, AB system, J_{AB}=17.1 Hz), 3.76 (2H, t, *J*=6.1 Hz), 3.94 (1H, s), 4.09 (3H, s), 5.11 (1H, t, *J*=4.9 Hz), 7.37 (1H, dd, *J*=1.8, 7.9 Hz), 7.77 (1H, t, *J*=7.9 Hz), 8.05 (1H, dd, *J*=1.8, 7.9 Hz), 13.31 (1H, s), 13.87 (1H, s).

Anal Calcd for $C_{25}H_{24}O_9$: C 64.09, H 5.18.
Found: C 64.11, H 5.12.

(7*S*)-9-Acetyl-4-methoxy-7-*O*-(2,3-epoxybutyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**13**): Yield 45%; mp 76~77°C; $[\alpha]_D^{20}$ +173° (*c* 0.15, $CHCl_3$); MS 468 (M^+); ^1H NMR 1.31 (3H, d, *J*=6.8 Hz), 1.98 (1H, dd, *J*=4.0, 14.2 Hz), 2.41 (1H, dd, *J*=2.0, 14.2 Hz), 2.43 (3H, s), 2.97 (2H, mt), 2.90, 3.20 (2H, AB system, J_{AB}=19.0 Hz), 3.96 (2H, d, *J*=3.5 Hz), 4.08 (3H, s), 5.09 (1H, mt, W=8.8 Hz), 7.36 (1H, dd, *J*=1.5, 7.3 Hz), 7.75 (1H, t, *J*=7.3 Hz), 8.02 (1H, dd, *J*=1.5, 7.3 Hz), 13.24 (1H, s), 13.93 (1H, s).

Anal Calcd for $C_{25}H_{24}O_9$: C 64.09, H 5.18.
Found: C 64.12, H 5.15.

(7*R*)-9-Acetyl-4-methoxy-7-*O*-(2,3-epoxybutyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**14**): Yield 43%; mp 110~113°C; $[\alpha]_D^{20}$ −86° (*c* 0.13, $CHCl_3$); MS 468 (M^+); ^1H NMR 1.30 (3H, d, *J*=6.1 Hz), 2.11 (1H, dd, *J*=4.9, 14.8 Hz), 2.39 (3H, s), 2.54 (1H, dd, *J*=0.2, 14.8 Hz), 2.89 (2H, mt), 2.96, 3.29 (2H, AB system, J_{AB}=18.9 Hz), 3.70 (2H, mt), 4.07 (3H, s), 5.11 (1H, dd, *J*=4.9, 9.2 Hz), 7.39 (1H, dd, *J*=1.8, 7.9 Hz), 7.75 (1H, t, *J*=7.9 Hz), 8.02 (1H, dd, *J*=1.8, 7.9 Hz), 13.27 (1H, s), 13.87 (1H, s).

Anal Calcd for $C_{25}H_{24}O_9$: C 64.09, H 5.18.
Found: C 64.05, H 5.22.

(7*S*)-9-Acetyl-4-methoxy-7-*O*-(4,5-epoxypentyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**15**): Yield 21%; mp 65~66°C; $[\alpha]_D^{20}$ +187° (*c* 0.13, $CHCl_3$); MS 482 (M^+); ^1H NMR 2.43 (3H, s), 1.88 (1H, dd, *J*=3.9, 14.7 Hz), 2.93, 3.23 (2H, AB system, J_{AB}=19.3 Hz), 3.93 (3H, mt), 4.10 (3H, s), 5.02 (1H, mt, W=6.3 Hz), 5.15 (1H s), 7.36 (1H, dd, *J*=1.5, 8.3 Hz), 7.76 (1H, t, *J*=8.3 Hz), 7.76 (1H, dd, *J*=1.5, 8.3 Hz), 13.30 (1H, s), 13.94 (1H, s).

Anal Calcd for $C_{26}H_{26}O_9$: C 64.72, H 5.43.
Found: C 64.80, H 5.48.

(7*R*)-9-Acetyl-4-methoxy-7-*O*-(4,5-epoxypentyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (**16**): Yield 20%; mp 145~147°C; $[\alpha]_D^{20}$ −81° (*c* 0.12, $CHCl_3$); MS M^+ was not observed; ^1H NMR 2.41 (3H, s), 3.60~3.90 (mt, OCH protons), 4.10 (3H, s), 4.37 (1H, mt), 5.39 (1H, t, *J*=8.3 Hz), 7.23~8.13 (3H, mt), 13.27 (1H, s), 14.35 (1H, s).

Anal Calcd for $C_{26}H_{26}O_9$: C 64.72, H 5.43.
Found: C 64.65, H 5.40.

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