INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED REVERSE TRANSCRIPTASE BY 14-*O*-ACYLADRIAMYCINS†

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(Received for publication October 18, 1988)

It was reported that doxorubicin inhibited virus reverse transcriptase. We have synthesized various new 14-*O*-acyladriamycins. The derivatives having a 14-*O*-benzoyl group with hydrophobic aliphatic side chains showed stronger inhibitory activity on human immunodeficiency virus reverse transcriptase and lower cytotoxicity on mouse leukemia cells.

Human immunodeficiency virus (HIV), a causative agent of the acquired immunodeficiency syndrome (AIDS), is a newly recognized retrovirus.[1~4] Inhibitors of reverse transcriptase, an enzyme whose activity is essential for the replication of retroviruses, might be candidates for the chemotherapy of HIV infection. 3′-Azido-3′-deoxythymidine was shown to confer a clinical benefit[5,6] to patients with advanced disease, being metabolized to become a potent inhibitor of HIV reverse transcriptase.[7]

It has been reported that doxorubicin, an antitumor antibiotic, inhibits Rauscher leukemia virus reverse transcriptase,[8] although it shows marked cytotoxicity. Therefore, we synthesized various derivatives of doxorubicin and screened them for derivatives being more effective in HIV reverse transcriptase inhibition and having less cytotoxicity.

Results

Among the anthracycline antibiotics, doxorubicin showed the strongest inhibitory effect on HIV reverse transcriptase as shown in Table 1. Inhibition of HIV reverse transcriptase by known derivatives of doxorubicin substituted at the 14-OH or 3′-NH_2 is shown in Table 2. In our assay system, doxorubicin at 100 μg/ml completely inhibited HIV reverse transcriptase. At the same dose, 3′-*N*-salicylideneadriamycin (**1**)[9] inhibited HIV reverse transcriptase less than doxorubicin, and no inhibitory activity was detected with AD32 (**2**)[10] in which both 14-OH and 3′-NH_2 positions were modified. While, 14-*O*-benzoyladriamycin (**3**)[11] retained full inhibitory activity.

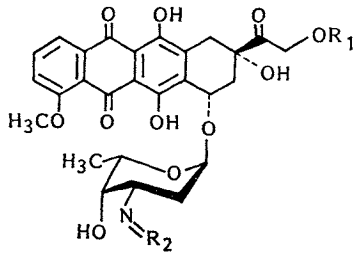
Table 1. Inhibitory effect of anthracyclines on HIV reverse transcriptase.

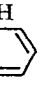
Anthracycline	IC_{50} (μg/ml)
Doxorubicin	55
Daunorubicin	96
Aclarubicin	>100
Pirarubicin	100
Betaclamycin A	>100
Ditrisarubicin B	>100
Oxaunomycin	>100

Therefore, we synthesized two 14-*O*-*n*-alkanoyladriamycins (**4** and **5**) and fifteen 14-*O*-benzoyladriamycin derivatives (**6**~**20**) starting

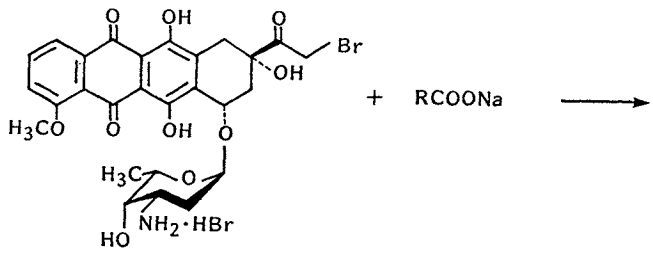
† A part of this work was reported at the 60th Annual Meeting of the Japanese Biochemical Society in Kanazawa on Oct. 12, 1987. "Adriamycin" and "daunomycin" are used for nomenclature of derivatives of doxorubicin and daunorubicin, respectively.

Table 2. Inhibition of HIV reverse transcriptase by known derivatives of doxorubicin.

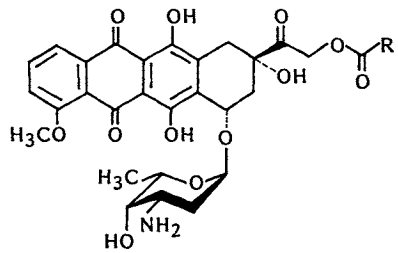


Compound	R_1	R_2	Inhibition (%) at 100 μg/ml
Doxorubicin	H	H, H	98.3
1	H		52.1
2 (AD32)	$CO(CH_2)_3CH_3$	H, $COCF_3$	−27.1
3		H, H	95.7

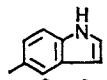
Scheme 1.

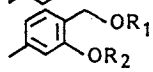


14-Bromodaunomycin hydrobromide

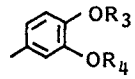


4 R=C_4H_9
5 R=C_9H_{19}

6 R= 

7~14 R= 

7 $R_1=R_2=H$
8 $R_1=R_2=CH_3$
9 $R_1=R_2=C_6H_{13}$
10 $R_1=R_2=C_{11}H_{23}$
11 $R_1=H$, $R_2=C_{11}H_{23}$
12 $R_1=R_2=C_{18}H_{37}$
13 $R_1=H$, $R_2=C_{18}H_{37}$
14 $R_1+R_2=C(CH_3)_2$

15~20 R= 

15 $R_3=R_4=CH_3$
16 $R_3=R_4=C_6H_{13}$
17 $R_3=R_4=C_{11}H_{23}$
18 $R_3=R_4=C_{18}H_{37}$
19 $R_3=R_4=COC_4H_9$
20 $R_3+R_4=CH_2$

from 14-bromodaunomycin[12] hydrobromide by coupling with salts of the corresponding acids (Scheme 1). Compound **7** had a unique substituent at 14-OH, which was a metabolic product[13] of an enzyme inhibitor, forphenicinol.[14] Their inhibitory activities (IC_{50}) of HIV reverse transcriptase and of P388 cell growth are shown in Table 3. For compounds **3**, **4**, **5** and **7**, the IC_{50} values against HIV reverse transcriptase were similar, but the cytotoxicity of **7** was lower than that of the others. Compound **9**, containing hydrophobic side chains, showed higher inhibitory activity and lower cytotoxicity. The ratio of IC_{50} against HIV reverse transcriptase to IC_{50} against P388 cell growth (A/B) was 35 for **9** in contrast to 370 for **7** or 1,500 for doxorubicin. The cytotoxicity was suppressed by elongation of hydrophobic side chains, while inhibitory activity of HIV reverse transcriptase was similar among compounds containing more than 6 carbon chains except for **18**. Compound **12**, having the longest aliphatic chains, had the lowest cytotoxicity, having an A/B value of 6.9.

Table 3. Inhibition of HIV reverse transcriptase and cytotoxicity of 14-*O*-acyladriamycins.

Compound	IC_{50} (μg/ml) HIV RTase (A)	Cytotoxicity (P388) (B)	A/B
Doxorubicin	55	0.037	1,500
3	56	0.084	670
4	56	0.062	900
5	45	0.023	2,000
6	>100	0.21	>480
7	55	0.15	370
8	30	0.29	100
9	17	0.48	35
10[a]	19	0.49	37
11	13	0.50	25
12[a]	22	3.2	6.9
13	40	1.9	21
14	23	0.19	120
15	19	0.33	58
16	20	2.6	7.8
17[a]	16	1.8	8.9
18[a]	100	1.9	53
19[a]	11	0.22	50
20	37	0.16	240

[a] Hydrochloride.

Discussion

For the development of anti-AIDS agents, the enzyme inhibitory activity should be high and the general cytotoxicity should be low. We synthesized derivatives of doxorubicin with 14-*O*-benzoyl group having aliphatic side chains and obtained derivatives (**12**, **16** and **17**) whose enzyme inhibition-cytotoxicity ratio (A/B) was about 1/200 of that of doxorubicin. Insolubility was increased with elongation of side chains, therefore, we solubilized **10**, **12** and **17** as the hydrochloride. However, **18** was hardly soluble even in its hydrochloride form at more than 10 μg/ml and its weak inhibitory activity against the enzyme might reflect this insolubility.

Doxorubicin was reported to inhibit HIV infection of CD4-positive cells in cell culture.[15] Our new derivatives of doxorubicin are more effective in inhibition of HIV reverse transcriptase and have markedly less cytotoxicity than doxorubicin. Inhibitory activity of these derivatives against the cytopathic effect of HIV is now being studied.

Experimental

General Methods

MP's were determined with a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass spectra were obtained on a Hitachi M-80H mass spectrometer. ^{1}H NMR spectra (90 MHz) were measured with a Varian EM-390 NMR spectrometer. TLC were performed on Silica gel (Kieselgel 60 F_{254}, Merck) developed with a mixture of $CHCl_3$ - MeOH - 28% NH_4OH (90 : 20 : 1) and Rf values were calculated.

Materials

β-Globin mRNA was purchased form Bethesda Research Laboratories; [α-^{32}P]dATP (800 Ci/

mmol) from Amersham; ribonuclease inhibitor from Takara Shuzo Co., Ltd.; and $p(dT)_{17}$ from Pharmacia. Doxorubicin, daunorubicin, aclarubicin and pirarubicin[16] were commercially obtained. Betaclamycin A,[17,18] ditrisarubicin B[19] and oxaunomycin[20] were prepared by our institute. 14-Bromodaunomycin[12] were supplied by Meiji Seika Kaisha, Ltd. 3-Hydroxy-4-hydroxymethylbenzoic acid[13] was supplied by Banyu Pharmaceutical Co., Ltd. 3′-*N*-Salicylideneadriamycin (**1**)[9], AD32 (**2**)[10] and 14-*O*-benzoyladriamycin (**3**)[11] were synthesized by us. Other reagents were commercially (guaranteed grades) obtained.

HIV Reverse Transcriptase Assay

Reverse transcriptase assay was carried out by using rabbit β-globin mRNA and HIV lysate and the method will be reported elsewhere by S. ATSUMI *et al.* The reverse transcriptase reaction was started by the addition of 1 μl of rabbit β-globin mRNA (50 ng) to 10 μl of reaction mixture consisting of Tris-HCl (pH 8.3) 50 mM, KCl 70 mM, $MgCl_2$ 10 mM, 2-mercaptoethanol 30 mM, dNTPs 0.09 mM, oligo dT 3.3 μg/ml, human placenta ribonuclease inhibitor 8.4 mU/ml, [α-^{32}P]dATP 0.14 mCi/ml, and HIV lysate.[21] After incubation at 37°C, the reaction was stopped by the addition of an equal volume of 10% TCA, and then the acid-insoluble extracts were collected on Millipore membrane (type AA, pore size 0.8 μm). The membrane was washed 4 times with 5% TCA for 10 minutes each time and rinsed in ethanol. The radioactivity of [α-^{32}P]dATP incorporated into DNA on the membrane was determined with a liquid scintillation counter.

Cytotoxicity Assay

Anthracycline derivatives were added to 5×10^3 mouse P388 leukemia cells in 0.1 ml of RPMI 1640 medium supplemented with 10% FBS. After 48 hours, the number of viable cells was assessed by the MTT method.[22,23]

3,4-Dihexyloxybenzoic Acid (Method A)

To a mixture of protocatechuic acid (3,4-dihydroxybenzoic acid, 200 mg, 1.3 mmol) and 60% oily sodium hydride (208 mg, 5.2 mmol) in 20 ml of anhydrous DMF, hexyl bromide (0.91 ml, 6.5 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was poured into 200 ml of 5% $KHSO_4$ and extracted with 200 ml of $CHCl_3$. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified on silica gel column chromatography (hexane - $CHCl_3$, 3 : 2) to afford hexyl 3,4-dihexyloxybenzoate (441 mg) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (9H, t, $3\times CH_2CH_3$), 4.05 (4H, t, $2\times ArOCH_2$), 4.29 (2H, t, $COOCH_2$), 6.87 (1H, d, 5-H), 7.6 (1H, d, 2-H), 7.63 (1H, dd, 6-H). A solution of the oil in 37 ml of THF - MeOH - 10% NaOH (10 : 5 : 2) was kept at room temperature for 2 days and concentrated to give a residue which was extracted with 100 ml of $CHCl_3$. The organic layer was washed with 5% $KHSO_4$ and H_2O, dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified on silica gel column chromatography ($CHCl_3$ - EtOH, 30 : 1) to afford colorless plates of 3,4-dihexyloxybenzoic acid (271 mg, 65% for two steps): MP 124~126°C; field desorption (FD)-MS *m/z* 322 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.91 (6H, t, $2\times CH_2CH_3$), 4.07 (4H, t, $2\times ArOCH_2$), 6.90 (1H, d, 5-H), 7.62 (1H, d, 2-H), 7.76 (1H, dd, 6-H).

3-Methoxy-4-methoxymethylbenzoic Acid

This compound was synthesized by Method A in 64% yield.

MP 160~163°C; electron impact (EI)-MS *m/z* 196 (M^+); ^{1}H NMR ($CDCl_3$ - CD_3OD, 1 : 1) δ 3.42 (3H, s, $ArCH_2OCH_3$), 3.90 (3H, s, $ArOCH_3$), 4.56 (2H, s, $ArCH_2O$), 7.42 (1H, d, 5-H), 7.60 (1H, br s, 2-H), 7.70 (1H, br d, 6-H).

3-Hexyloxy-4-hexyloxymethylbenzoic Acid

This compound was synthesized by Method A in 45% yield.

Colorless syrup; EI-MS *m/z* 336 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.9 (6H, $2\times CH_2CH_3$), 3.57 (2H, t, $ArCH_2OCH_2$), 4.06 (2H, t, $ArOCH_2$), 4.62 (2H, s, $ArCH_2O$), 7.53 (1H, d, 5-H), 7.59 (1H, s, 2-H), 7.77 (1H, d, 6-H).

3-Undecyloxy-4-undecyloxymethylbenzoic Acid and 4-Hydroxymethyl-3-undecyloxybenzoic Acid

These compounds were synthesized by Method A in yields of 19% and 27%, respectively.

The former: MP 61~63°C; EI-MS *m/z* 476 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.89 (6H, t, 2×CH_2CH_3), 3.57 (2H, t, $ArCH_2OCH_2$), 4.06 (2H, t, $ArOCH_2$), 4.61 (2H, s, $ArCH_2O$), 7.55 (1H, d, 5-H), 7.59 (1H, s, 2-H), 7.78 (1H, d, 6-H).

The latter: MP 82~83°C; EI-MS *m/z* 322 (M^+); ^{1}H NMR ($CDCl_3$ - CD_3OD, 5:1) δ 0.88 (3H, t, CH_2CH_3), 4.07 (2H, t, $ArOCH_2$), 4.75 (2H, s, $ArCH_2O$), 7.45 (1H, d, 5-H), 7.54 (1H, br s, 2-H), 7.70 (1H, br d, 6-H).

3-Octadecyloxy-4-octadecyloxymethylbenzoic Acid and 4-Hydroxymethyl-3-octadecyloxybenzoic Acid

These compounds were synthesized by Method A in yields of 22% and 27%, respectively.

The former: MP 87~88°C; EI-MS *m/z* 672 (M^+); ^{1}H NMR ($CDCl_3$ - CD_3OD, 20:1) δ 0.89 (6H, t, 2×CH_2CH_3), 3.55 (2H, t, $ArCH_2OCH_2$), 4.04 (2H, t, $ArOCH_2$), 4.60 (2H, s, $ArCH_2O$), 7.49 (1H, d, 5-H), 7.55 (1H, s, 2-H), 7.72 (1H, d, 6-H).

The latter: MP 105~107°C; FD-MS *m/z* 420 (M^+); ^{1}H NMR ($CDCl_3$ - CD_3OD, 5:1) δ 0.88 (3H, t, CH_2CH_3), 4.07 (2H, t, $ArOCH_2$), 4.74 (2H, s, $ArCH_2O$), 7.5 (1H, 2-H), 7.69 (1H, br d, 6-H).

O-Isopropylidene 3-Hydroxy-4-hydroxymethylbenzoic Acid

To a solution of 3-hydroxy-4-hydroxymethylbenzoic acid (100 mg, 0.60 mmol) and pyridinium *p*-toluenesulfonate (15 mg, 0.06 mmol) in 2 ml of $(CH_3)_2CO$, 2-methoxypropene (0.068 ml, 0.71 mmol) was added, and the mixture was stirred at room temperature overnight. The mixture was poured into 10 ml of satd $NaHCO_3$, which was concentrated to remove $(CH_3)_2CO$. A resulting aqueous solution was washed with $CHCl_3$ and adjusted pH 2 with 5% $KHSO_4$ and extracted twice with 10 ml of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified on preparative silica gel TLC ($CHCl_3$ - MeOH, 10:1) to afford the isopropylidene derivative (107 mg, 86%) as a colorless solid: MP 153~156°C; EI-MS *m/z* 208 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.58 (6H, s, $C(CH_3)_2$), 4.93 (2H, s, $ArCH_2O$), 7.09 (1H, d, 5-H), 7.64 (1H, s, 2-H), 7.69 (1H, d, 6-H).

3,4-Diundecyloxybenzoic Acid (Method B)

A solution of protocatechuic acid (50 mg, 0.32 mmol) and diphenyldiazomethane (89 mg, 0.46 mmol) in 2.5 ml of EtOAc was kept at room temperature for 4 hours. The solution was concentrated to give a residue which was purified on silica gel column chromatography ($CHCl_3$ → $CHCl_3$ - MeOH, 30:1) to afford protocatechuic acid benzhydryl ester (52 mg, 50%) as a colorless syrup: FD-MS *m/z* 320 (M^+); ^{1}H NMR ($CDCl_3$) δ 6.90 (1H, d, 5-H), 7.07 (1H, s, $CHPh_2$), 7.7 (2H, 2-H, 6-H). To this benzhydryl ester (49 mg, 0.15 mmol) and 60% oily sodium hydride (14 mg, 0.34 mmol) in 5 ml of anhydrous DMF, undecyl iodide (0.078 ml, 0.34 mmol) was added, and the mixture was stirred at room temperature for 2 hours. The mixture was poured into 50 ml of 5% $KHSO_4$ and extracted with 50 ml of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to remove $CHCl_3$. To the concentrate was added TFA (5 ml), and it was kept at room temperature for 30 minutes. The mixture was concentrated to give a residue which was extracted with 50 ml of $CHCl_3$. The organic layer was washed with 5% $KHSO_4$ and H_2O, dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified on preparative silica gel TLC ($CHCl_3$ - MeOH, 10:1) to afford a colorless solid of 3,4-diundecyloxybenzoic acid (49 mg, 69%): MP 98~101°C; FD-MS *m/z* 462 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, 2×CH_2CH_3), 4.05 (4H, t, 2×$ArOCH_2$), 6.89 (1H, d, 5-H).

3,4-Dioctadecyloxybenzoic Acid

This compound was synthesized by Method B in 45% yield from protocatechuic acid benzhydryl ester.

MP 94~117°C; FD-MS *m/z* 659 (MH^+); ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, 2×CH_2CH_3), 4.08 (4H, t, 2×$ArOCH_2$), 6.92 (1H, d, 5-H), 7.62 (1H, d, 2-H), 7.75 (1H, dd, 6-H).

3,4-Divaleryloxybenzoic Acid

This compound was synthesized by Method B in 37% yield from protocatechuic acid benzhydryl

ester.

MP 97~103°C; FD-MS *m/z* 322 (M^+); ^{1}H NMR ($CDCl_3$ - CD_3OD, 20:1) δ 0.95 (6H, t, 2×CH_2CH_3), 2.55 (4H, t, 2×$OCOCH_2$), 7.28 (1H, d, 5-H), 7.92 (1H, s, 2-H), 7.98 (1H, d, 6-H).

14-*O*-(3-Hexyloxy-4-hexyloxymethylbenzoyl)adriamycin (**9**)

A mixture of 14-bromodaunomycin hydrobromide (57 mg, 0.083 mmol) and sodium 3-hexyloxy-4-hexyloxymethylbenzoate (prepared from the acid, 56 mg, 0.17 mmol and 1 N NaOH) in 2.8 ml of anhydrous MeOH was stirred at room temperature overnight. Purification on preparative silica gel TLC ($CHCl_3$ - MeOH - 28% NH_4OH, 90:20:1) gave a red solid of **9** (24 mg, 33%): MP 110~142°C, $[\alpha]_D^{26}$ +180° (*c* 0.01, MeOH); FD-MS *m/z* 862 (MH^+); Rf 0.67; ^{1}H NMR (pyridine-d_5) δ 0.87 (6H, t, 2×CH_2CH_3), 1.63 (3H, d, 6'-H), 3.96 (3H, s, OCH_3), 4.7 (1H, 5'-H), 4.74 (2H, s, ArCH_2O), 5.46 (1H, 7-H), 5.78 (1H, 1'-H), 6.00 (2H, ABq, 14-H).

14-*O*-(3,4-Dihexyloxybenzoyl)adriamycin (**16**)

A mixture of 14-bromodaunomycin hydrobromide (51 mg, 0.074 mmol) and sodium 3,4-dihexyloxybenzoate (prepared from the acid, 48 mg, 0.15 mmol and 1 N NaOH) in 3.9 ml of $CHCl_3$ - anhydrous MeOH (1:2) was stirred at room temperature overnight. Purification on preparative silica gel TLC ($CHCl_3$ - MeOH - 28% NH_4OH, 90:20:1) gave a red solid of **16** (23 mg, 37%): MP 167~170°C; $[\alpha]_D^{25}$ +220° (*c* 0.01, $CHCl_3$); FD-MS *m/z* 847 (M^+); Rf 0.67; ^{1}H NMR (pyridine-d_5) δ 0.85 (6H, t, 2×CH_2CH_3), 1.64 (3H, d, 6'-H), 3.95 (3H, s, OCH_3), 4.70 (1H, q, 5'-H), 5.79 (1H, 1'-H), 6.00 (2H, ABq, 14-H).

14-*O*-Valeryladriamycin (**4**)

In a similar procedure as preparing **9**, **4** was synthesized from sodium valerate.

MP 192~196°C; $[\alpha]_D^{16}$ +190° (*c* 0.01, MeOH); FD-MS *m/z* 627 (M^+); Rf 0.59; ^{1}H NMR (pyridine-d_5) δ 1.59 (3H, d, 6'-H), 3.95 (3H, s, OCH_3), 4.74 (1H, q, 5'-H), 5.45 (1H, 7-H), 5.75 (2H, ABq, 14-H), 5.8 (1H, 1'-H).

14-*O*-Decanoyladriamycin (**5**)

In a similar procedure as preparing **9**, **5** was synthesized from sodium decanoate.

No definite mp; $[\alpha]_D^{18}$ +210° (*c* 0.01, $CHCl_3$ - MeOH, 1:1); FD-MS *m/z* 697 (M^+); Rf 0.62; ^{1}H NMR (pyridine-d_5) δ 0.87 (3H, t, CH_2CH_3), 1.59 (3H, d, 6'-H), 3.94 (3H, s, OCH_3), 4.65 (1H, q, 5'-H), 5.42 (1H, 7-H), 5.73 (2H, ABq, 14-H), 5.8 (1H, 1'-H).

14-*O*-(Indole-5-carbonyl)adriamycin (**6**)

A mixture of 14-bromodaunomycin hydrobromide (60 mg, 0.087 mmol), sodium iodide (262 mg, 1.75 mmol) and sodium indole-5-carboxylate (32 mg, 0.18 mmol) in 6.0 ml of anhydrous DMF was sonicated for 1 minute. The mixture was concentrated to give a residue which was purified on preparative silica gel TLC ($CHCl_3$ - MeOH - 28% NH_4OH, 90:20:1) to afford the crude powder. Column chromatography using Sephadex LH-20 gave a red solid of **6** (26 mg, 43%): MP 220~221°C; $[\alpha]_D^{19}$ +260° (*c* 0.01, MeOH); FD-MS *m/z* 687 (MH^+); Rf 0.46; ^{1}H NMR (pyridine-d_5) δ 1.66 (3H, d, 6'-H), 3.95 (3H, s, OCH_3), 4.71 (1H, q, 5'-H), 5.46 (1H, 7-H), 5.80 (1H, 1'-H), 6.01 (2H, ABq, 14-H), 8.29 (1H, dd, 6''-H), 8.91 (1H, s, 4''-H).

14-*O*-(3-Hydroxy-4-hydroxymethylbenzoyl)adriamycin (**7**)

In a similar procedure as preparing **9**, **7** was synthesized from 3-hydroxy-4-hydroxymethylbenzoic acid.

No definite mp; $[\alpha]_D^{27}$ +150° (*c* 0.01, MeOH); FD-MS *m/z* 693 (M^+); Rf 0.32; ^{1}H NMR (pyridine-d_5) δ 1.53 (3H, d, 6'-H), 3.94 (3H, s, OCH_3), 4.83 (1H, q, 5'-H), 5.29 (2H, s, ArCH_2O), 5.39 (1H, 7-H), 5.80 (1H, 1'-H), 5.91 (2H, ABq, 14-H).

14-*O*-(3-Methoxy-4-methoxymethylbenzoyl)adriamycin (**8**)

In a similar procedure as preparing **9**, **8** was synthesized from 3-methoxy-4-methoxymethylbenzoic acid.

MP 143~163°C; $[\alpha]_D^{27}$ +260° (*c* 0.01, MeOH); FD-MS *m/z* 722 (MH^+); Rf 0.61; ^{1}H NMR

(pyridine-d_5) δ 1.64 (3H, d, 6′-H), 3.37 (3H, s, $ArCH_2OCH_3$), 3.70 (3H, s, 3″-OCH_3), 3.94 (3H, s, 4-OCH_3), 4.57 (2H, s, $ArCH_2OCH_3$), 5.49 (1H, 7-H), 5.79 (1H, 1′-H), 5.99 (2H, ABq, 14-H).

14-*O*-(3-Undecyloxy-4-undecyloxymethylbenzoyl)adriamycin (**10**)

In a similar procedure as preparing **16**, **10** was synthesized from 3-undecyloxy-4-undecyloxymethylbenzoic acid.

MP 160~169°C (hydrochloride); $[\alpha]_D^{28}$ +170° (*c* 0.01, $CHCl_3$) (hydrochloride); FD-MS *m/z* 1,002 (MH^+); Rf 0.72; ^1H NMR (pyridine-d_5) δ 0.88 (6H, t, 2×CH_2CH_3), 1.62 (3H, d, 6′-H), 3.94 (3H, s, OCH_3), 4.64 (1H, q, 5′-H), 4.76 (2H, s, $ArCH_2O$), 5.42 (1H, 7-H), 5.75 (1H, 1′-H), 5.98 (2H, ABq, 14-H).

14-*O*-(4-Hydroxymethyl-3-undecyloxybenzoyl)adriamycin (**11**)

In a similar procedure as preparing **9**, **11** was synthesized from 4-hydroxymethyl-3-undecyloxybenzoic acid.

MP 110~126°C; $[\alpha]_D^{27}$ +190° (*c* 0.01, MeOH); FD-MS *m/z* 848 (MH^+); Rf 0.61; ^1H NMR (pyridine-d_5) δ 0.86 (3H, t, CH_2CH_3), 1.64 (3H, d, 6′-H), 3.94 (3H, s, OCH_3), 4.69 (1H, q, 5′-H), 5.20 (2H, s, $ArCH_2O$), 5.48 (1H, 7-H), 5.79 (1H, 1′-H), 6.01 (2H, ABq, 14-H).

14-*O*-(3-Octadecyloxy-4-octadecyloxymethylbenzoyl)adriamycin (**12**)

In a similar procedure as preparing **16**, **12** was synthesized using 3-octadecyloxy-4-octadecyloxymethylbenzoic acid and sodium iodide.

MP 167~174°C (hydrochloride); $[\alpha]_D^{26}$ +230° (*c* 0.01, $CHCl_3$) (hydrochloride); FD-MS *m/z* 1,198 (MH^+); Rf 0.78; ^1H NMR (pyridine-d_5) δ 0.88 (6H, t, 2×CH_2CH_3), 1.67 (3H, d, 6′-H), 3.94 (3H, s, OCH_3), 4.73 (1H, q, 5′-H), 4.78 (2H, s, $ArCH_2O$), 5.48 (1H, 7-H), 5.80 (1H, 1′-H), 6.04 (2H, ABq, 14-H).

14-*O*-(4-Hydroxymethyl-3-octadecyloxybenzoyl)adriamycin (**13**)

In a similar procedure as preparing **16**, **13** was synthesized from 4-hydroxymethyl-3-octadecyloxybenzoic acid.

MP 122~128°C; $[\alpha]_D^{22}$ +170° (*c* 0.01, $CHCl_3$ - MeOH, 1 : 1); FD-MS *m/z* 946 (MH^+); Rf 0.64; ^1H NMR (pyridine-d_5) δ 0.87 (3H, t, CH_2CH_3), 1.65 (3H, d, 6′-H), 3.95 (3H, s, OCH_3), 4.69 (1H, q, 5′-H), 5.20 (2H, s, $ArCH_2O$), 5.49 (1H, 7-H), 5.79 (1H, 1′-H), 6.00 (2H, ABq, 14-H).

14-*O*-(*O*-Isopropylidene 3-Hydroxy-4-hydroxymethylbenzoyl)adriamycin (**14**)

In a similar procedure as preparing **9**, **14** was synthesized from *O*-isopropylidene 3-hydroxy-4-hydroxymethylbenzoic acid.

MP 195~202°C; $[\alpha]_D^{27}$ +230° (*c* 0.01, MeOH); FD-MS *m/z* 734 (MH^+); Rf 0.61; ^1H NMR (pyridine-d_5) δ 1.50 (6H, s, $C(CH_3)_2$), 1.63 (3H, d, 6′-H), 3.95 (3H, s, OCH_3), 4.69 (1H, q, 5′-H), 4.87 (2H, s, $ArCH_2O$), 5.41 (1H, 7-H), 5.79 (1H, 1′-H), 5.96 (2H, ABq, 14-H).

14-*O*-(3,4-Dimethoxybenzoyl)adriamycin (**15**)

In a similar procedure as preparing **9**, **15** was synthesized from 3,4-dimethoxybenzoic acid.

MP 188~189°C; $[\alpha]_D^{26}$ +320° (*c* 0.01, MeOH); FD-MS *m/z* 707 (M^+); Rf 0.61; ^1H NMR (pyridine-d_5) δ 1.62 (3H, d, 6′-H), 3.73, 3.75 (6H, 2×s, 3″-OCH_3, 4″-OCH_3), 3.94 (3H, s, 4-OCH_3), 4.71 (1H, q, 5′-H), 5.47 (1H, 7-H), 5.80 (1H, 1′-H), 5.98 (2H, ABq, 14-H).

14-*O*-(3,4-Diundecyloxybenzoyl)adriamycin (**17**)

In a similar procedure as preparing **16**, **17** was synthesized from 3,4-diundecyloxybenzoic acid through the tetramethylammonium salt.

MP 185~189°C (hydrochloride); $[\alpha]_D^{24}$ +170° (*c* 0.01, $CHCl_3$ - MeOH, 1 : 1) (hydrochloride); FD-MS *m/z* 988 (MH^+); Rf 0.72; ^1H NMR (pyridine-d_5) δ 0.89 (6H, t, 2×CH_2CH_3), 1.66 (3H, d, 6′-H), 3.94 (3H, s, OCH_3), 4.76 (1H, q, 5′-H), 5.48 (1H, 7-H), 5.82 (1H, 1′-H), 6.03 (2H, ABq, 14-H).

14-*O*-(3,4-Dioctadecyloxybenzoyl)adriamycin (**18**)

In a similar procedure as preparing **16**, **18** was synthesized using 3,4-dioctadecyloxybenzoic acid and sodium iodide.

MP 154°C (hydrochloride); $[\alpha]_D^{23}$ +150° (*c* 0.01, $CHCl_3$) (hydrochloride); FD-MS *m/z* 1,184 (MH^+); Rf 0.78; ^1H NMR (pyridine-d_5) δ 0.88 (6H, t, $2\times CH_2CH_3$), 1.66 (3H, d, 6'-H), 3.94 (3H, s, OCH_3), 4.77 (1H, q, 5'-H), 5.48 (1H, 7-H), 5.81 (1H, 1'-H), 6.03 (2H, ABq, 14-H).

14-*O*-(3,4-Divaleryloxybenzoyl)adriamycin (**19**)

In a similar procedure as preparing **16**, **19** was synthesized from 3,4-divaleryloxybenzoic acid.

MP 176~178°C (hydrochloride); $[\alpha]_D^{23}$ +190° (*c* 0.01, MeOH) (hydrochloride); secondary ion (SI)-MS *m/z* 848 (MH^+); Rf 0.65; ^1H NMR ($CDCl_3$ - CD_3OD, 5:1) δ 0.99 (6H, t, $2\times CH_2CH_3$), 1.37 (3H, d, 6'-H), 4.08 (3H, s, OCH_3), 5.29 (1H, 7-H), 5.48 (2H, ABq, 14-H), 5.5 (1H, 1'-H).

14-*O*-Piperonyloyladriamycin (**20**)

In a similar procedure as preparing **9**, **20** was synthesized from piperonylic acid.

MP 202~204°C; $[\alpha]_D^{19}$ +240° (*c* 0.01, MeOH); FD-MS *m/z* 692 (MH^+); Rf 0.60; ^1H NMR (pyridine-d_5) δ 1.63 (3H, d, 6'-H), 3.95 (3H, s, OCH_3), 4.74 (1H, q, 5'-H), 5.48 (1H, 7-H), 5.82 (1H, 1'-H), 5.95 (2H, ABq, 14-H), 6.01 (2H, s, OCH_2O).

Acknowledgments

This work was supported in part by a grant from the Ministry of Education, Science and Culture.

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