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The Synthesis of New Derivatives of 1-β-D-Arabinofuranosylcytosine

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In order to obtain 1-β-D-arabinofuranosylcytosine derivatives with better antitumor effect, 12 kinds of saturated fatty acyl groups were introduced at the N^4-position of 1-β-D-arabinofuranosylcytosine. The presence of a great excess of water and about two-fold equivalents of carboxylic anhydride was found to be most desirable for selective N^4-acylation. This simple method of one-step N^4-acylation should be generally applicable to cytosine nucleosides and a variety of carboxylic anhydrides.

Keywords——N^4-acyl-ara-C; lipophilicity; antitumor activity; one-step N^4-acylation; straight-chained aliphatic acid

Although 1-β-D-arabinofuranosylcytosine (ara-C) is one of the most important antitumor agents, it requires a very complex and precise dosage schedule to obtain its full therapeutic effect. In order to make this troublesome dosage schedule better, we synthesized N^4-acyl derivatives of ara-C with saturated and straight-chained aliphatic acyl groups. We reported in separate papers that these derivatives showed a better antitumor activity than the parent compound.[2] In this paper, we describe the synthesis of N^4-acyl-ara-C.

It seems that the following properties of ara-C could be made better. First, it is easily metabolized by the enzyme cytidine deaminase into 1-β-D-arabinofuranosyluracil which has no antitumor activity.[3] Second, it disappears quickly from the blood.[3b] One of the best steps against the former is to mask the 4-amino group, and that against the latter is an addition of high lipophilicity to this molecule.[4] These two chief steps can be taken at the same

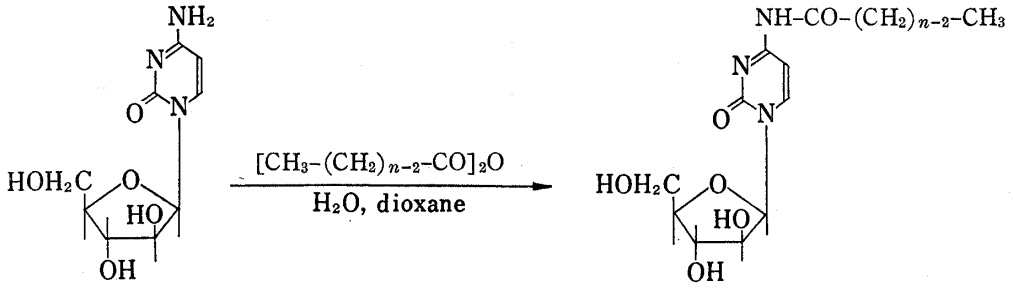


Chart 1

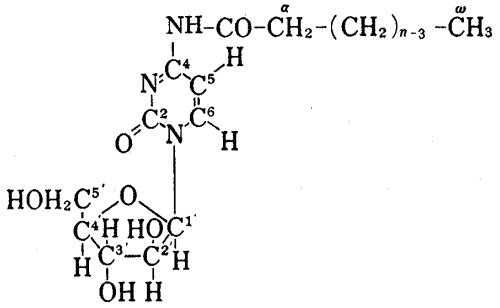
1) Location: *2-1, Samejima, Fuji, Shizuoka.*
2) M. Aoshima, S. Tsukagoshi, Y. Sakurai, J. Oh-ishi, T. Ishida, and H. Kobayashi, *Cancer Res.*, **36**, 2726 (1976); M. Aoshima, S. Tsukagoshi, Y. Sakurai, J. Oh-ishi, T. Ishida, and H. Kobayashi, *Cancer Res.*, **37**, 2481 (1977).
3) *a*) G.W. Camiener and C.G. Smith, *Biochem. Pharmacol.*, **14**, 1405 (1965); *b*) W.A. Creasey, R.J. Papac, M.E. Markiw, P. Calabresi, and A.D. Welch, *Biochem. Pharmacol.*, **15**, 1417 (1966).
4) T. Okabayashi, S. Mihara, D.B. Repke, and J.G. Moffatt, *Cancer Res.*, **37**, 619 (1977); D.H.W. Ho and G.L. Neil, *Cancer Res.*, **37**, 1640 (1977).

TABLE I. N^4-Acyl-1-β-D-arabinofuranosylcytosine

Compd. Acyl group	mp (°C) Recryst. solvent	Yield (%)	Formula	Analysis (%) Found (Calcd.) C	H	N	$[\alpha]_D$ (Temp.) (c=1.0, THF[a])	UV (ε) $\lambda_{max}^{iso\text{-}PrOH}$	IR ν_{max}^{KBr} cm^{-1} CH_2	CH_3	–CO–	–C=N–	NHCO
Butyryl (n=4)	108—110 (33% EtOH)	93	$C_{13}H_{19}N_3O_6$	49.95 (49.83)	6.16 (6.12)	13.42 (13.41)	+114° (23°)	216 (14900) 248 (13800) 303 (7500)	2920	2865	1720	1635	1635 1570
Valeryl (n=5)	131—133 (CH_3CN)	90	$C_{14}H_{21}N_3O_6$	51.55 (51.37)	6.42 (6.47)	12.95 (12.84)	+113° (23°)	216 (22300) 248 (13400) 303 (7300)	2950	2900	1700	1640	1640 1580
Caproyl (n=6)	130—133 (CH_3CN)	91	$C_{15}H_{23}N_3O_6$	52.73 (52.77)	6.72 (6.79)	12.35 (12.31)	+112° (23°)	216 (16700) 248 (15000) 303 (8200)	2950	2860	1700	1650	1650 1560
Heptyryl (n=7)	137—140 (CH_3CN)	91	$C_{16}H_{25}N_3O_6$	54.01 (54.07)	7.02 (7.09)	11.89 (11.83)	+111° (23°)	216 (16800) 248 (15100) 303 (8100)	2930	2850	1700	1650	1650 1560
Caprylyl (n=8)	151—153 (50% EtOH)	98	$C_{17}H_{27}N_3O_6$	55.21 (55.26)	7.22 (7.38)	11.20 (11.38)	+109° (23°)	216 (16900) 248 (15200) 303 (8300)	2910	2845	1710	1635	1635 1550
Capryl (n=10)	144—145 (Acetone)	92	$C_{19}H_{31}N_3O_6$	57.39 (57.41)	7.89 (7.86)	10.51 (10.57)	+99° (23°)	216 (22200) 248 (14700) 303 (7900)	2920	2850	1690	1640	1640 1550
Lauroyl (n=12)	145—149 (Acetone)	92	$C_{21}H_{35}N_3O_6$	59.26 (59.27)	8.25 (8.29)	9.91 (9.88)	+89° (23°)	216 (17000) 248 (14500) 303 (8200)	2930	2860	1700	1650	1650 1550
Myristoyl (n=14)	143—148 (Acetone)	91	$C_{23}H_{39}N_3O_6$	60.92 (60.90)	8.66 (8.67)	9.25 (9.27)	+84° (24°)	216 (14900) 248 (14500) 303 (8000)	2920	2850	1690	1640	1640 1550
Palmitoyl (n=16)	139—144 (CCl_4–DMSO)	92	$C_{25}H_{43}N_3O_6$	62.39 (62.34)	9.02 (9.00)	8.71 (8.73)	+79° (24°)	216 (14800) 248 (14100) 203 (7600)	2930	2860	1700	1650	1650 1550
Stearoyl (n=18)	147—151 (AcOEt)	90	$C_{27}H_{47}N_3O_6$	63.67 (63.61)	9.31 (9.31)	8.14 (8.24)	+75° (24°)	216 (13700) 248 (13700) 303 (7700)	2915	2845	1705	1635	1635 1540
Arachidoyl (n=20)	142—145 (DMSO)	92	$C_{29}H_{51}N_3O_6$	64.75 (64.77)	9.57 (9.56)	7.89 (7.82)	+71° (24°)	216 (15500) 248 (14600) 303 (8000)	2920	2850	1700	1650	1650 1550
Behenoyl (n=22)	141—142 (DMSO)	92	$C_{31}H_{55}N_3O_6$	65.79 (65.81)	9.82 (9.80)	7.41 (7.43)	+70° (22°)	216 (16400) 248 (15200) 303 (8200)	2910	2840	1690	1640	1640 1550

a) THF=tetrahydrofuran.

TABLE II. ^{1}H NMR Spectra of N^4-Acyl-1-β-D-arabinofuranosylcytosines in Pyridine-d_5 Solution (δ in ppm, J in Hz)



Compd.	ω-CH_3	$(CH_2)_{n-3}$	α-CH_2	5′-CH_2	2′-CH 3′-CH 4′-CH	1′-CH	5-CH	6-CH
Butyryl (n=4)	0.90 t, 3H J=7.0	1.72 m, 2H	2.58 t, 2H J=7.0	4.36 d, 2H J=4.0	4.5—5.2 m, 3H	6.98 d, 1H J=4.0	7.68 d, 1H J=8.0	8.68 d, 1H J=8.0
Valeryl (n=5)	0.80 t, 3H J=7.0	1.0—2.0 4H	2.60 t, 2H J=7.0	4.38 d, 2H J=4.0	4.5—5.2 m, 3H	7.04 d, 1H J=4.0	7.70 d, 1H J=8.0	8.72 d, 1H J=8.0
Caproyl (n=6)	0.80 t, 3H J=7.0	1.0—2.0 6H	2.62 t, 2H J=7.0	4.38 d, 2H J=4.0	4.5—5.3 m, 3H	7.06 d, 1H J=4.0	7.74 d, 1H J=8.0	8.76 d, 1H J=8.0
Heptyryl (n=7)	0.80 t, 3H J=7.0	1.0—2.0 8H	2.66 t, 2H J=7.0	4.38 d, 2H J=4.0	4.5—5.2 m, 3H	7.05 d, 1H J=4.2	7.74 d, 1H J=8.0	8.74 d, 1H J=8.0
Caprylyl (n=8)	0.80 t, 3H J=7.0	1.0—2.0 10H	2.64 t, 2H J=7.0	4.38 d, 2H J=4.0	4.5—5.3 m, 3H	7.04 d, 1H J=3.9	7.74 d, 1H J=7.9	8.74 d, 1H J=7.9
Capryl (n=10)	0.85 t, 3H J=7.0	1.0—2.0 14H	2.66 t, 2H J=7.0	4.36 d, 2H J=4.0	4.5—5.3 m, 3H	6.98 d, 1H J=4.0	7.72 d, 1H J=7.9	8.70 d, 1H J=7.9
Lauroyl (n=12)	0.88 t, 3H J=7.0	1.0—2.0 18H	2.66 t, 2H J=7.0	4.34 d, 2H J=4.0	4.5—5.3 m, 3H	7.00 d, 1H J=3.9	7.72 d, 1H J=8.0	8.70 d, 1H J=8.0
Myristoyl (n=14)	0.88 t, 3H J=7.0	1.0—2.0 22H	2.68 t, 2H J=7.0	4.36 d, 2H J=4.0	4.5—5.2 m, 3H	7.00 d, 1H J=3.8	7.74 d, 1H J=7.9	8.70 d, 1H J=7.9
Palmitoyl (n=16)	0.88 t, 3H J=7.0	1.0—2.0 26H	2.64 t, 2H J=7.0	4.34 d, 2H J=4.0	4.5—5.2 m, 3H	7.02 d, 1H J=4.0	7.72 d, 1H J=8.0	8.70 d, 1H J=8.0
Stearoyl (n=18)	0.86 t, 3H J=7.0	1.0—2.0 30H	2.61 t, 2H J=7.0	4.26 d, 2H J=4.0	4.4—5.1 m, 3H	6.90 d, 1H J=4.0	7.60 d, 1H J=8.0	8.62 d, 1H J=8.0
Arachidoyl (n=20)	0.86 t, 3H J=7.0	1.0—2.0 34H	2.58 t, 2H J=7.0	4.26 d, 2H J=4.0	4.4—5.2 m, 3H	6.90 d, 1H J=4.0	7.62 d, 1H J=8.0	8.60 d, 1H J=8.0
Behenoyl (n=22)	0.90 t, 3H J=7.0	1.0—2.0 38H	2.65 t, 2H J=7.0	4.28 d, 2H J=4.0	4.4—5.2 m, 3H	6.85 d, 1H J=4.0	7.60 d, 1H J=8.0	8.58 d, 1H J=8.0

time by the introduction of a high lipophilic group at the 4-amino position. Thus, saturated and straight-chained aliphatic acyl groups were selected as high lipophilic groups.

The 4-amino position of ara-C was selectively acylated by the use of about two-fold equivalents of carboxylic anhydride in the presence of a great excess of water and of awater-miscible organic solvent. We used dioxane preferably among water-miscible organic solvents such as acetone, acetonitrile, dimethylformamide, dimethyl sulfoxide, dioxane, tetrahydrofuran, and other non-hydroxylic solvents. In this selective acylation, molar ratios of acid anhydride and water to ara-C are decisively important. This suggests that water plays a dual

role of dissolving ara-C and keeping the hydroxyl groups of the sugar moiety away from acylation.

The reaction products were identified as N^4-acyl-ara-C by means of elemental analysis, ultraviolet (UV), infrared (IR), and nuclear magnetic resonance (NMR) spectra (Table I and II). In the case of N^4-stearoyl-ara-C, for example, that one stearoyl group was introduced into one molecule was indicated by both elemental analyses and integrated intensity in its NMR spectra, that the stearoyl group was introduced into the cytosine moiety was suggested by the great difference in UV spectra between the product and ara-C, and that the position into which the stearoyl group was introduced was not hydroxyl groups of the arabinose moiety but the 4-amino position of the cytosine moiety was implied by the absence of absorption due to ester bond and the presence of absorption of amide bond in its IR spectrum. Based on these data, the product was identified as N^4-stearoyl-ara-C. Identifications of other derivatives were made by the same way as this.

Among the reported methods of selective N^4-acylation of cytosine nucleoside, the following two should be recommended for simplicity and high yield. One is the N^4-acylation of cytidine or ara-C in methanol with anhydrides[5] and the other is the N^4-acylation of cytidine in the presence of pyridine with anhydrides.[6] However, our method is not less effective than these for simplicity and high yield, and seems to find a wide application for the N^4-acylation of cytosine nucleosides with anhydrides of a variety of carboxylic acids such as aromatic acids, dicarboxylic acids, and so on.

Experimental

All melting points are uncorrected. Optical rotations were recorded on a JASCO DIP-4 automatic polarimeter. UV spectra were recorded on a Hitachi 124 spectrophotometer; IR spectra, with a Hitachi EPI-G3 spectrophotometer; NMR spectra, with a JEOL JNM-MH-100 spectrometer using tetramethylsilane as an internal standard. All physical data are listed in Tables I and II.

N^4-Butyryl-1-β-D-arabinofuranosylcytosine——To a solution of 1-β-D-arabinofuranosylcytosine (300 mg, 1.23 mmol) in H_2O (1.6 ml) were added dioxane (5 ml) and butyric anhydride (415 mg, 2.63 mmol) with stirring at room temperature. After the mixture was stirred for 48 hr at room temperature, it was evaporated to dryness *in vacuo* at 60° and the residue was recrystallized from H_2O–EtOH (2: 1) to colorless needles. Yield, 357 mg (1.14 mmol, 92.7%).

N^4-Caprylyl-1-β-D-arabinofuranosylcytosine——To a solution of 1-β-D-arabinofuranosylcytosine (300 mg, 1.23 mmol) in H_2O (2 ml) were added dioxane (12 ml) and caprylic anhydride (667 mg, 2.47 mmol) with stirring at room temperature. After the mixture was stirred for 48 hr at room temperature, it was evaporated to dryness *in vacuo* at 60°. After the residue was washed with hexane in order to remove free caprylic acid, it was recrystallized from H_2O–EtOH (1: 1). Yield, 443 mg (1.20 mmol, 97.5%).

N^4-Stearoyl-1-β-D-arabinofuranosylcytosine——To a solution of 1-β-D-arabinofuranosylcytosine (300 mg, 1.23 mmol) in H_2O (2 ml) were added dioxane (30 ml) and stearic anhydride (1.36 g, 2.47 mmol) with stirring at room temperature. After the mixture was stirred for 5 hr at 80°, it was evaporated to dryness *in vacuo* at 60°. After the residue was washed with benzene in order to remove free stearic acid, it was recrystallized from AcOEt. Yield, 566 mg (1.11 mmol, 90.3%).

5) K.A. Watanabe and J.J. Fox, *Angew. Chem.*, **78**, 589 (1966); I. Wempen, N. Miller, E.A. Falco, and J.J. Fox, *J. Med. Chem.*, **11**, 144 (1968).
6) T. Saoaki and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **15**, 894 (1967).