THE SYNTHESIS AND ANTITUMOR ACTIVITY OF *N*-GLYCOSYL DERIVATIVES OF DAUNORUBICIN[1]

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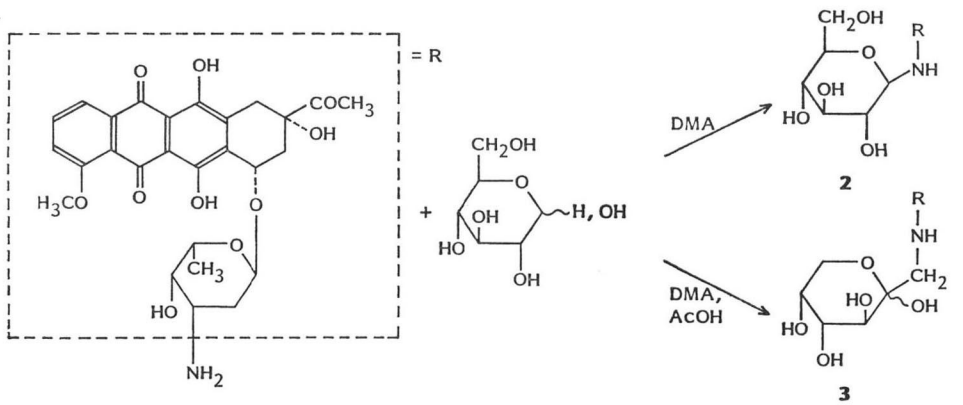
The synthesis and antitumor properties of *N*-glycosyl derivatives of daunorubicin formed in the reaction with D-glucose, D-ribose and maltose is described.

Daunorubicin and its 14-hydroxyl analogue, adriamycin, are broadly applied in therapy of neoplasia diseases[2,3]. However the delirious side effects, especially cardiotoxicity, caused upon treatment with these drugs, limit their full scope of clinical utilization. An extensive search for analogues that might exhibit more favorable therapeutic properties, as compared with the parent drugs, has been conducted in many laboratories[4].

The present paper deals with the syntheses, structures and antitumor properties of derivatives of daunorubicin (**1**) obtained in the reaction of the antibiotic with carbohydrates.

Daunorubicin, in the form of free base, dissolved in *N*,*N*-dimethylformamide (DMF) or *N*,*N*-dimethylacetamide (DMA), treated with molar excess of D-glucose yielded the *N*-glucoside (**2**). Addition to the reaction mixture of acetic acid in slightly molar excess afforded an Amadori rearrangement compound, *N*-(1-deoxyfructos-1-yl)daunorubicin (**3**) (Scheme 1). The course of the reaction was observed by means of TLC in the solvent systems: chloroform - methanol - water, 13: 6: 1 and ethyl acetate - acetic acid - water, 5: 1: 1. In the first one the formation of both products **2** and **3** was observed. However in the second case only the Amadori compound **3** could be detected because of the instability of the *N*-glucoside. The latter easily undergoes hydrolysis and therefore its properties were not studied in detail. Formation of similar Amadori compounds had been observed in the

Scheme 1.

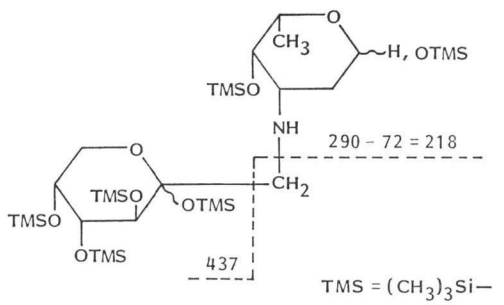


reaction of daunorubicin with D-ribose and maltose.

The electronic absorption spectra of the derivatives were identical with that of the parent compound. The IR spectra as well as the ^{1}H NMR data of the peracetylated derivatives are in accordance with the postulated structures. The structure of **3** was proved in the mass spectrum of the persilylated disaccharide moiety obtained by hydrolysis of the derivative and its silylation with *N*-trimethylsilylimidazole in hexane. The most characteristic features of the spectrum were: the presence of the molecular ions at *m/z* 741, and fragmentation at *m/z* 437, 347 and 218 (Scheme 2) typical for the Amadori compounds[5,6].

The synthesized Amadori compounds of daunorubicin and the parent antibiotic were assayed *in vivo* against L-1210 and P-388 leukemia in mice. The investigations of activity against L-1210 were done in our laboratory, and the screening against P-388 was performed under the auspices of the Natural Products Branch, Division of Cancer Treatment, N.C.I., USA. The results are summarized in Table 1. All the tested derivatives exhibit antileukemic activity similar to daunorubicin however at higher doses.

Scheme 2.



Experimental

Instrumental Analysis

Mass spectra were obtained on a Varian Mat-711 double focusing spectrometer by means of a direct introduction probe. The instrumental conditions were as follows: a) electron impact mode electron energy 70 eV, emission current 0.8 mV, accelerating voltage 8 kV, ion source temperature 250°C, resolution (10% valley definition)=1,000 and 10,000 for exact mass determination; b) field desorption mode-wire heating current 14~18 mA, ion source temperature 70~100°C, accelerating voltage 8 kV.

IR spectra were taken on a UR-10 (Carl Zeiss, Jena) spectrophotometer in KBr pellets. Melting points are uncorrected. ^{1}H NMR spectra were determined in $CDCl_3$ with a Tesla 80 MHz spectrometer.

Synthesis

N-(Glucos-1-yl)daunorubicin (**2**): 0.53 g (1 mM) of daunorubicin free base and 0.3 g (1.6 mM) of D-glucose in 3 ml of DMA were stirred 12 hours at 35°C. The product was precipitated upon addition of 150 ml of ethyl ether, centrifuged, washed with the same solvent and purified by means of Sephadex LH-20 column chromatography with methanol. The fractions containing the pure derivative were concentrated, the product precipitated upon addition of ethyl ether and finally crystallized from ethanol - ethyl ether mixture. Yield 0.5 g, mp 192~195°C (dec). IR (KBr) ν_{max} cm^{-1} 3500~3280 (OH, NH), 1725 (C=O), 1620 and 1580 (chelated quinones). FD-MS *m/z* 689 (M^+).

Anal Calcd for $C_{33}H_{41}NO_{16}$: C 56.06, H 5.86, N 1.98.
Found: C 55.62, H 5.95, N 1.91.

N-(1-Deoxyfructos-1-yl)daunorubicin (**3**): 0.53 g (1 mM) of daunorubicin free base and 0.3 g (1.6 mM) of glucose were dissolved in 10 ml DMA and 0.1 ml (1.5 mM) acetic acid and the mixture stirred for six hours at 35°C. The product was precipitated with 150 ml of ethyl ether, centrifuged, washed two times with the same solvent and purified by means of partition chromatography on silica gel with solvent system chloroform - methanol - water - acetic acid, 10: 5: 1: 0.15. The obtained derivative was dissolved in methanol and treated with a stoichiometric amount of hydrogen chloride in methanol and precipitated upon addition of ether to give the hydrochloride of *N*-(1-deoxyfructos-1-yl)-

Table 1. Antitumor activity of *N*-glycosyl derivatives of daunorubicin.

Compound tested (NSC number)	Antitumor activity				
	L-1210[a]		P-388[b]		
	Dose (mg/kg)	T/C±$\bar{s}$ (%)	Dose (mg/kg)	T/C (%)[c]	
Daunorubicin (**1**)			50	102	
(NSC-359653)			25	127	
	10	78± 5	12.5	112	
	5	111± 8	6.25	112	
	2.5	133± 2	3.13	108	
	1.25	150±15			
	0.62	111± 5			
	0.31	111± 4			
N-(1-Deoxyfructos-1-yl)-			200	108	
daunorubicin (**3**)	80	130±15	100	182	
(NSC-351132)	40	183±25	50	137	122[d]
	20	125±12	25	113	112
	10	133±18	12.5		117
			6.25		100
			3.13		103
N-(1-Deoxyketoarabinos-1-yl)-	40	156±17	50		119
daunorubicin (**4**)	20	156±18	25	105	119
(NSC-351133)	10	111±15	12.5	100	128
	5	111±12	6.25	103	99
			3.13	99	95
N-(1-Deoxy-4-(α-D-glucopyranoside)-			200	99	
fructos-1-yl)daunorubicin (**5**)	80	88± 5	100	134	
(NSC-351134)	40	137±13	50	134	137
	20	150± 7	25	118	126
	10	128± 4	12.5		119
	5	122± 6	6.25		117
	2.5	111± 8	3.13		119

[a] The results of experiments carried out in our laboratory (see Experimental).

[b] Data obtained under auspices of the National Cancer Institute, Division of Cancer Treatment, Drug Research and Development Branch. CDF_1 mice were injected ip with 10^6 P-388 lymphocytic leukemia cells on day 0 and treated ip on days 5, 9 and 13 with the drug-dose specified.

[c] Ratio of median survival time expressed as percent of untreated controls.

[d] Data from second series of tests.

daunorubicin as an amorphous solid. Yield 0.4 g, mp 181~184°C (dec), $[\alpha]_D^{25}$ −71.1° (*c* 0.15, methanol). IR (KBr) ν_{max} cm^{-1} 3500~3250 (very broad, OH, NH), 1720 (C=O), 1620 and 1585 (chelated quinones). FD-MS *m/z*=689 (M^+, 100% relative intensity), 671 (M−18, 25%).

The ^1H NMR spectrum was taken up for the peracetylated derivative obtained after treatment of **3** with pyridine - acetic anhydride, 2: 1, ~2 days, in the dark at room temperature. At this time pyridine and acetic anhydride were evaporated *in vacuo*, and the dry residue purified on silica gel using ethyl acetate as eluting solvent. ^1H NMR δ ppm 1.2 (d, 3H, Me-6′), 1.8~2.2 (br m, 14H, OAc), 2.4 (m, 9H, Me-14, Ph-OAc), 3.8 (s, 3H, OMe).

Anal Calcd for $C_{33}H_{42}NO_{18}Cl$: C 53.31, H 5.59, N 1.88.
Found: C 52.82, H 5.59, N 1.66.

According to the procedure described for **3** the following compounds were synthesized, purified, and isolated as free bases:

N-(1-Deoxyketoarabinos-1-yl)daunorubicin (**4**): Obtained from 0.53 g (1 mM) daunorubicin, 0.45 g (3 mM) ribose and 0.1 ml acetic acid in 10 ml DMA. Yield 0.3 g, mp 170~174°C (dec). IR (KBr)

ν_{max} cm^{-1} 3200~3500, 1726, 1620 and 1585. FD-MS *m/z* 659 (M^+).

The ^1H NMR spectrum was taken up for the peracetylated derivative prepared as for **3**. ^1H NMR δ ppm 1.2 (d, 3H, Me-6'), 1.8~2.2 (br m, 12H, OAc), 2.4 (m, 9H, Me-14, Ph-OAc), 3.8 (s, 3H, OMe).

Anal Calcd for $C_{32}H_{39}NO_{15}$: C 56.75, H 5.24, N 2.08.
Found: C 55.94, H 5.40, N 2.01.

N-(1-Deoxy-4-(α-D-glucopyranoside)-fructos-1-yl)daunorubicin (**5**): Obtained from 0.53 g (1 mM) daunorubicin, 0.72 g (2 mM) maltose and 0.125 ml acetic acid in 10 ml DMA. Yield 0.5 g, mp 187~189°C (dec). IR (KBr) ν_{max} cm^{-1} 3200~3600, 1720, 1620 and 1582. FD-MS *m/z* 852 $(M+H)^+$.

The ^1H NMR spectrum was taken up for the peracetylated derivative prepared as for **3**. ^1H NMR δ ppm 1.2 (d, 3H, Me-6'), 1.8~2.3 (br m, 20H, OAc), 2.4 (m, 9H, Me-14, Ph-OAc), 3.9 (s, 3H, OMe).

Anal Calcd for $C_{39}H_{51}NO_{21}$: C 53.88, H 5.70, N 1.6.
Found: C 53.61, H 5.85, N 1.48.

N-(1-Deoxyfructos-1-yl)-3-amino-2,3,6-trideoxy-L-lyxose (**6**): 0.3 g of **3** in 5 ml of 0.1 N HCl was heated for 0.5 hour at 80~90°C. The insoluble aglycone was filtered off, the filtrate concentrated with the aid of butanol and the amino sugar precipitated upon addition of ethyl ether. The product was purified by means of chromatography on silica gel (Merck 60H) developed with butanol - acetic acid - water, 5: 1: 1, and after isolation as described above it was crystallized from an ethyl ether - propanol mixture. Yield 40 mg, $[\alpha]_D^{20}+7°$ (*c* 0.3, H_2O), mp 161~165° (dec). FD-MS *m/z* 310 $(M+H)^+$.

10 mg of **6** was suspended in 5 ml of *N*-trimethylsilylimidazole - hexane, 1: 10, kept for 12 hours, washed three times with water, concentrated and introduced directly on the mass spectrometer. MS *m/z* (relative intensity %) 43 (100), 58 (50), 73 (34), 103 (6), 117 (8), 147 (12), 217 (9), 218 (40), 219 (6), 302 (9), 322 (1.4), 347 (1.6), 437 (12), 438 (3), 439 (1), 506 (3.6), 507 (1.8), 636 (0.4), 651 (0.3), 726 (0.7), 741 (0.3, M^+).

Antitumor Activity on L-1210 Transplanted Leukemia in Mice

CDF_1 mice were injected ip with 10^5 L-1210 lymphocytic leukemia cells on day 0 and treated daily day 1 through 5. The antibiotics were administered ip using a 5% NaCl solution vehicle (0.1 ml)/10 g of body weight. Experiments were carried out using 18 mice in the control group and 8 mice in the test group; each compound was tested in 3 or more experiments. Experiments were presented as the average ratio of median survival time of treated to control mice (T/C). Standard deviations of average results (*s*) were calculated also.

Acknowledgments

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