Synthesis and Biological Evaluation of Some 14-O-Acyl Derivatives of Adriamycin

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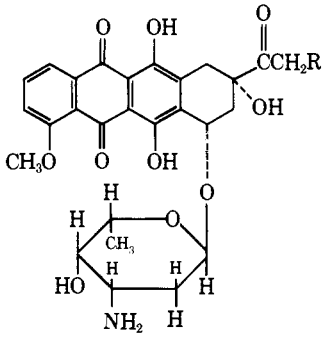
The following 14-O-acyl derivatives of adriamycin were synthesized: acetate, propionate, octanoate, benzoate, phenylacetate, and nicotinate. The compounds were evaluated for cytotoxic, antiviral, and antitumor activities.

The substitution of one hydrogen atom in the daunorubicin (1) acetyl side chain with a hydroxyl gives adriamycin (doxorubicin, 2).[1,2] This compound is a clinically effective agent for therapeutic treatment of leukemias and of a variety of tumors.[3] We now report the synthesis and biological activity data of some 14-O-acyl derivatives of 2, corresponding to structures 3–8. Compounds 3–8 were prepared from 14-bromodaunorubicin[4] by reaction with the sodium or potassium salts of the corresponding acids in acetone. The products were isolated as the hydrochlorides.



1, R = H
2, R = OH
3, R = $OCOCH_3$
4, R = $OCOCH_2CH_3$
5, R = $OCO(CH_2)_6CH_3$
6, R = $OCOC_6H_5$
7, R = $OCOCH_2C_6H_5$
8, R = OCO–(pyridyl)

The compounds were evaluated for cytotoxic, antiviral, and antitumor activity. The results of the *in vitro* tests are reported in Table I. All new derivatives were active on cell proliferation and on Murine Sarcoma virus (Moloney) foci formation. It can be noticed that the observed differences in the *in vitro* activity of compounds 3–8 do not correlate with the partition coefficients (Table II). All compounds induced a comparable or greater increase in survival time or inhibition of tumor growth in tumor-bearing mice when compared with adriamycin. Table III shows results against Sarcoma 180 (solid and ascites), MSV-M induced sarcoma, intravenously transplanted Gross leukemia, and transplanted mammary carcinoma in mice. Adriamycin 14-octanoate (5) resulted in the most active compound in all the experimental tumors tested.

In order to investigate the presence of enzymes able to hydrolize the side-chain ester function to give adriamycin in the blood and in tissues of mice, tritium-labeled 5 was incubated with serum and homogenized tissues. The results are shown in Table IV and indicate a very rapid conversion of 5 to adriamycin in serum and in liver homogenate. Substantial hydrolysis was also evident with kidney, heart, and spleen homogenates. In liver the recovery of 7-deoxyadriamycinone was in agreement with the findings of other authors.[5]

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Table I. Cytotoxic and Antiviral Activity of Compounds 1–8 *in Vitro*[a]

Compound	Cell proliferation Hela[b]	Cell proliferation MEF[c]	MSV-M foci formation on MEF[c]
Daunorubicin (1)	0.53	0.031	0.021
Adriamycin (2)	1.18	0.034	0.020
Adriamycin 14-acetate (3)	1.20	0.056	0.028
Adriamycin 14-propionate (4)	1.57	0.050	0.022
Adriamycin 14-octanoate (5)	1.41	0.063	0.028
Adriamycin 14-benzoate (6)	2.19	0.076	0.027
Adriamycin 14-phenylacetate (7)	1.43	0.050	0.025
Adriamycin 14-nicotinate (8)	2.92	0.048	0.027

[a]Data are expressed as 50% inhibiting dose (n*M*/ml). [b]Treatment for 8 hr. [c]Treatment for 72 hr.

Table II. Partition Coefficients of Daunorubicin, Adriamycin, and Esters of Adriamycin

Compound	1-butanol/buffer (pH 7.0)[a]
Daunorubicin (1)	4.3
Adriamycin (2)	1.6
Adriamycin 14-acetate (3)	5.8
Adriamycin 14-propionate (4)	10.5
Adriamycin 14-octanoate (5)	∞
Adriamycin 14-benzoate (6)	42.5
Adriamycin 14-phenylacetate (7)	38.6
Adriamycin 14-nicotinate (8)	14.1

[a]Phosphate buffer, *M*/15.

The distribution of 5 in mice was also investigated. Table V shows tha pattern of tissues $C \times t$ values as determined by measuring radioactivity of the different organs at different times following the intravenous administration of tritiated 2 and 5. The $C \times t$ values were different for the two drugs, as lower levels of tritium were found for 5 in the heart and in kidneys, while in lungs, spleen, and liver higher and more persistent levels of radioactivity were observed. These findings are important because of the cardiotoxic effects observed in patients submitted to prolonged adriamycin therapy.

Results reported in this paper show that significant levels of antitumor activity are retained among this group of derivatives of adriamycin and that esterification of the side-chain hydroxyl leads to modifications in the distribution characteristics which can be of relevance for the therapeutic activity.

Experimental Section

Chemical Synthesis. Melting points, observed in open capillaries, are uncorrected. Optical rotations were determined at 20 ± 5° in methanol (*c* 0.05) with a Perkin-Elmer Model 141 automatic polarimeter. Where analyses are indicated only by symbols of the

Table III. Activity of Optimal Doses of Adriamycin Derivatives on a Spectrum of Experimental Tumors in Mice

Compound	S 180 solid[a]		MSV-M sarcoma[b]		S 180 ascites[c]			Gross leukemia[d]			Mammary carcinoma[e]	
	Dose[f]	Tumor growth	Dose[f]	Tumor growth	Dose[f]	AST[g]	LTS[h]	Dose[f]	ATS[g]	LTS[h]	Dose[f]	Tumor incidence
Controls		100		100		100	0/10		100	0/10		100
Daunorubicin (1)	3.25	50	3.25	63	0.25	222	6/11	3.25	176	0/10		
Adriamycin (2)	2.5	31	2.25	52	0.5	227	8/11	2	187	1/30	2	50
Adriamycin 14-acetate (3)	2.5	36	1.5	45	2	252	7/10	2.5	168	0/10	2.5	10
Adriamycin 14-propionate (4)	3.25	33			2	232	1/10	4	147	0/10		
Adriamycin 14-octanoate (5)	3.5	27	2.75	10	2	250	5/10	4.1	214	3/30	2.5	20
Adriamycin 14-benzoate (6)	3.25	32			1	287	2/10	2.5	138	0/10		
Adriamycin 14-phenylacetate (7)	3.25	38			4	207	2/10	3.25	156	0/10		
Adriamycin 14-nicotinate (8)	3.25	36			2	250	1/10	4	227	0/10		

[a]Treatment ip on days 1–8. [b]Treatment iv or sc on days 3–5. [c]Treatment ip on day 1. [d]Treatment iv on days 3–5. [e]Treatment iv (q2d) on days 1–11, 17–27. [f]mg/kg/day. [g]Average survival time. [h]Long-term (60 days from tumor transplantation) survivors. [i]Dissolved in 5% aqueous ethanol.

Table IV. Distribution of Radioactivity Present in the Incubation Mixtures in the Different Chromatographic Peaks[a]

Chromatographic peak	Incubation mixtures												
	Buffer			Serum		Liver		Kidney		Heart		Spleen	
	15[c]	30	60	15[c]	30	30[c]	60	30[c]	60	30[c]	60	30[c]	60
Daunosamine	0	0	0	0	7	26	29	2	2	4	7	4	1
Adriamycin	7	8	11	100	84	11	5	32	44	26	31	39	69
Adriamycin octanoate	93	92	89	0	0	3	3	64	51	65	57	55	35
Total aglycones	0	0	0	0	9	60[b]	63[b]	2	3	5	5	2	2

[a]Data are expressed as per cent of total recovered radioactivity. [b]7-Deoxyadriamycinone. [c]In minutes.

Table V. Calculated $C \times t$ Values of Tritium in Tissues of Mice at 8 hr after iv Treatment with Labeled Adriamycin or Adriamycin 14-Octanoate[a]

Tissue	$C \times t$ (nM × hr/g)	
	Adriamycin	Adriamycin 14-octanoate
Heart	60.9	36.0
Lungs	73.3	136.9
Liver	140.0	212.5
Kidney	176.6	145.1
Spleen	59.0	95.0

[a]Radioactivity is expressed as nanomolar equivalents of the administered labeled compound.

elements, the analytical results obtained for those elements were within ±0.4% of theorical values. Tlc on silica gel HF (E. Merck), with the solvent system CH_2Cl_2–MeOH–H_2O (100:20:2 by volume), was used for identification purposes and homogeneity test. The ir (KBr) and pmr spectra of each compound were consistent with the assigned structure.

Adriamycin 14-Acetate (3). A mixture of 14-bromodaunorubicin hydrochloride (3.2 g, 5 mmol) and fused potassium acetate (7.4 g, 75 mmol) in 1500 ml of anhydrous acetone was stirred at refluxing temperature for 45 min, then filtered, and evaporated. The crude solid was dissolved in 300 ml of 0.1 N HCl and extracted with $CHCl_3$ in order to eliminate aglycones and then with n-butyl alcohol. Evaporation of the combined extracts afforded the crystalline compound 3 (1.55 g, 50%), recrystallized from MeOH–n-butyl alcohol: mp 198° dec; $[\alpha]_D$ +250°; $\nu_{C=O}$ ester 1735 cm^{-1}. *Anal.* ($C_{29}H_{31}NO_{12}$·HCl) C, H, Cl.

Adriamycin 14-Propionate (4). 14-Bromodaunorubicin hydrochloride (2 g, 3.1 mmol) was added to dried sodium propionate (6 g, 62.5 mmol) in 1600 ml of anhydrous acetone. The mixture was stirred at refluxing temperature for 2 hr. The compound 4 (1.5 g, 76%), obtained using the same isolation procedure followed for 3, was recrystallized from MeOH–n-butyl alcohol: mp 184–185° dec; $[\alpha]_D$ +230°; $\nu_{C=O}$ ester 1730 cm^{-1}. *Anal.* ($C_{30}H_{33}NO_{12}$·HCl) C, H, Cl.

Adriamycin 14-Octanoate (5). 14-Bromodaunorubicin hydrobromide (3 g, 4.35 mmol) was added to dried sodium octanoate (9 g, 54 mmol) in 1700 ml of anhydrous acetone. The mixture was stirred at refluxing temperature for 1 hr, then filtered, and evaporated. The crude solid was dissolved in MeOH (100 ml) and 0.1 N HCl (300 ml), and the solution extracted with $CHCl_3$. The crystalline mixture of 5 and octanoic acid, obtained by evaporation of the combined extracts, was washed with ethyl acetate and Et_2O to eliminate the acid. The pure 5 (2.5 g, 81%) was recrystallized from MeOH–$CHCl_3$: mp 162–163° dec; $[\alpha]_D$ +222°; $\nu_{C=O}$ ester 1730 cm^{-1}. *Anal.* ($C_{35}H_{43}NO_{12}$·HCl) C, H, Cl.

Adriamycin 14-Benzoate (6). A mixture of 14-bromodaunorubicin hydrochloride (2.5 g, 3.9 mmol) and dried sodium benzoate (7.5 g, 52 mmol) in 1500 ml of anhydrous acetone was stirred at refluxing temperature for 2 hr, then filtered, and evaporated. The crude solid was suspended in 250 ml of 1% aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extracts were evaporated and the residue was dissolved in 50 ml of $CHCl_3$ and 5 ml of MeOH. The pH was adjusted to 3 with 1 N HCl in MeOH and HCl salt crystallized from solution (1.6 g, 60%): mp 182–183° dec; $[\alpha]_D$ +224°; $\nu_{C=O}$ ester 1720 cm^{-1}. *Anal.* ($C_{34}H_{33}NO_{12}$·HCl) C, H, Cl.

Adriamycin 14-Phenylacetate (7). A mixture of 14-bromodaunorubicin hydrochloride (2 g, 3.1 mmol) and dried sodium phenylacetate (6 g, 38 mmol) in 1200 ml of anhydrous acetone was stirred at refluxing temperature for 1 hr. The isolation procedure was the same followed for compound 3. The product (1.5 g, 69%) was recrystallized from MeOH–n-butyl alcohol: mp 171–175° dec; $[\alpha]_D$ +224°; $\nu_{C=O}$ ester 1730 cm^{-1}. *Anal.* ($C_{35}H_{35}NO_{12}$·HCl) C, H, Cl.

Adriamycin 14-Nicotinate (8). A mixture of 14-bromodaunorubicin hydrochloride (2 g, 3.1 mmol) and dried potassium nicotinate (6 g, 37 mmol) in 1200 ml of anhydrous acetone was stirred at refluxing temperature for 2 hr. The isolation procedure was the same followed for compound 6. The product (1.2 g, 56.5%) was crystallized from MeOH–n-butyl alcohol: mp 180–185° dec; $[\alpha]_D$ +212°; $\nu_{C=O}$ ester 1725 cm^{-1}. *Anal.* ($C_{33}H_{32}N_2O_{12}$·HCl) C, H, Cl.

Tritium-Labeled Adriamycin 14-Octanoate. This compound was obtained following the method described for the synthesis of 5 starting from [^{3}H]daunorubicin. Tritiated daunorubicin was obtained by Dr. G. P. Vicario, Istituto Donegani, Novara, Italy, by

means of the Wilzbach technique[6] and purified to constant specific radioactivity (21 μCi/mg). Labeled 5 used in this study showed a specific radioactivity of 4.07 μCi/mg and was chromatographically pure. Distribution of tritium was 71% in the aglycone and 29% in daunosamine residue.

Biological Activity Evaluation. The compounds were evaluated for cytotoxic, antiviral, and antitumor activity, according to methods similar to those used for daunomycin derivatives.[7-9] Drugs were dissolved in Ringer solution, except adriamycin 14-octanoate and adriamycin 14-benzoate, which were administered in aqueous solution containing 1% (v/v) of dimethyl sulfoxide. In one experiment (activity on Gross leukemia), compound 5 was dissolved in 5% aqueous ethyl alcohol.

In vitro tests were carried out on HeLa cells and on secondary mouse embryo fibroblasts (MEF), infected or not with the Murine Sarcoma virus (Moloney) (MSV-M). HeLa cells were treated for 8 hr; then the drugs were removed and replaced by normal medium; cells were counted in an haemocytometer after 48 hr. MEF were plated on 35-mm Falcon plastic dishes, infected with MSV-M and treated for 3 days with compounds under study. The number of foci was evaluated microscopically 5 days after the infection. Uninfected MEF were similarly treated; at the end of the experiment cells were counted in an haemocytometer.

The antitumor activity of derivatives under study was tested on Sarcoma 180 (solid and ascites), MSV-M induced sarcoma, intravenously transplanted Gross leukemia, and transplanted mammary carcinoma. CD 1 mice (Charles River Breeding Laboratories, Calco, Italy) were used in the test on Sarcoma 180 and MSV-M induced sarcoma; C_3H/He Dp mice were used in the experiments on the other transplanted tumors. Each experimental group consisted of at least ten mice. Compounds were administered ip or iv in a volume of 10 ml/kg of body weight, according to different schedules of treatment. Each drug was tested over a wide range of doses. For the sake of brevity, only results obtained for the optimal doses are reported (Table III).

Incubation Experiments. CD 1 male albino mice were sacrificed by decapitation under ether anaesthesia. Hearts, livers, kidneys, and spleens were excised and homogenized in 2 vol of 0.25 *M* sucrose, 10 m*M* $MgCl_2$, and 10 m*M* tris-HCl, pH 7.6, using a glass Teflon homogenizer. The suspension was centrifuged at 3000 g for 10 min and the supernatant was used for the incubation experiments. The incubation mixtures were prepared by adding 0.5 ml of an aqueous solution of labeled 5 to 3.5 ml of each supernatant. Final concentration of 5 was 85 μg/ml. Incubations were carried out at 37° in open 10-ml flasks and samples were taken at the times indicated (Table IV). Similar incubations were performed using blood serum previously diluted with 2 vol of the above-mentioned buffer solution. Aliquots of the samples were freeze-dried, extracted with a mixture of equal volumes of chloroform and methanol, and analyzed by tlc. Recovery of total radioactivity was higher than 90%. Thin-layer chromatography was carried out in silica gel plates. The following systems were used: I, chloroform–methanol–water (13:6:1 by volume); II, methylene chloride–methanol–water (100:20:2 by volume); III, chloroform–ethanol–acetic acid–water (179:4:5:12 by volume); IV, ethyl acetate–benzene–petroleum ether (bp 40–70°) (70:25:5 by volume). When systems I and II were used, plates were buffered at pH 7 with phosphate buffer. The estimation of labeled compounds involved scraping of chromatogram in sections of the same length, extracting each section with 0.5 ml of methanol in a counting vial, and adding 10 ml of the scintillation mixture to the extract. The R_f values of the compounds related to adriamycin 14-octanoate metabolism are reported in Table VI.

Table VI. Chromatographic R_f Values

Compound	Solvent system I	II	III	IV
Daunosamine	0.00	0.00		
Adriamycin	0.25	0.15		
Adriamycin octanoate	0.60	0.25		
Adriamycinone	0.90	0.70	0.35	0.25
7-Deoxyadriamycinone	0.90	0.70	0.35	0.40

Distribution Studies. CD 1 mice were injected iv with equimolar doses of tritiated adriamycin[10] (4.35 mg/kg) and of tritiated adriamycin 14-octanoate (5.31 mg/kg). Animals were killed at different times after treatment and samples of the organs were obtained and processed for liquid scintillation counting by combustion in a Packard samples oxidizer.

Measurement of Radioactivity. A Packard Tri-Carb liquid scintillation spectrometer Model 3375 and counting medium of 100 g of naphthalene, 7 g of PPO, and 0.3 g of POPOP/l. of dioxane were used. The counting efficiency of the samples was determined by the channel ratio method for quenching correction.

References

(1) F. Arcamone, G. Cassinelli, G. Fantini, A. Grein, P. Orezzi, C. Pol, and C. Spalla, *Biotechnol. Bioeng.*, 11, 1101 (1969).
(2) F. Arcamone, G. Franceschi, S. Penco, and A. Selva, *Tetrahedron Lett.*, 1107 (1969).
(3) "International Symposium on Adriamycin," S. K. Carter, A. Di Marco, M. Ghione, J. H. Krakoff, and G. Mathé, Ed., Springer-Verlag, Berlin, 1972.
(4) F. Arcamone, G. Franceschi, and S. Penco, *Belgium Patent* 731,398 (Oct 13, 1969).
(5) F. J. Bullock, R. J. Bruni, and M. A. Asbell, *J. Pharmacol. Exp. Ther.*, 182, 70 (1972).
(6) K. E. Wilzbach, *J. Amer. Chem. Soc.*, 79, 1013 (1957).
(7) A. M. Casazza, A. Di Marco, and G. Di Cuonzo, *Cancer Res.*, 31, 1971 (1971).
(8) A. Di Marco, L. Lenaz, A. M. Casazza, and B. M. Scarpinato, *Cancer Chemother. Rep.*, 56, 153 (1972).
(9) A. Di Marco, A. M. Casazza, T. Dasdia, G. Giuliani, L. Lenaz, A. Necco, and C. Soranzo, *Cancer Chemother, Rep.*, 57, 269 (1973).
(10) F. Arcamone, G. Cassinelli, G. Franceschi, S. Penco, C. Pol, S. Redaelli, and C. Selva, see ref 3, p 9.

Steroidal Imidazole-1-carboxylic Acid Esters

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A variety of steroidal esters of imidazole 1-carboxylic acid and related acids was prepared by reaction of the steroidal alcohol with *N,N'*-carbonyldiimidazole or by displacement of phenol from steroid phenylcarbonates. One compound, **21c** (the imidazole-1-carboxylate of 19-norethisterone), was found to have an interesting separation of progestational from androgenic activity.

In connection with another project, we were interested in the preparation of the bisester of 17α-hydroxyprogesterone (**1a**) and carbonic acid. Treatment of **1a** with *N,N'*-carbonyldiimidazole (CDI) by the procedure of Staab and Mannschreck[1] gave not the desired carbonate but instead the imidazole-1-carboxylic acid ester **1b**.

Prior to our studies, no examples of characterized steroidal esters of imidazole-1-carboxylic acid had been published (although Sondheimer and coworkers had reported[2] an *in situ* preparation of **2a,b**). Since the completion of our work Kuhl and Taubert[3] have characterized two further examples of such esters.

On the other hand, nonsteroidal esters of imidazole-1-carboxylic acid and acids of related heterocyclic bases are well known; they have been prepared by a number of methods,† including the acylation of either the free

†For the excellent review on heterocyclic amides, see ref 4.