dispersion of sodium hydride and 300 mL of dry dimethyl sulfoxide was stirred at 50–70 °C, under nitrogen until gas was no longer evolved. The resulting grayish-green solution was diluted with 150 mL of dry tetrahydrofuran and cooled in an ice bath, and a solution of 58.4 g of methyl 2-[(dimethylamino)sulfonyl]-benzoate (16) in 100 mL of tetrahydrofuran was added over a period of 10 min. The mixture was stirred at room temperature for 45 min, poured into 2.0 L of ice–water which had previously been acidified to pH 2–3 by the addition of HCl, and *quickly* extracted with chloroform. Washing the chloroform solution three times with cold water (to remove all traces of acid), drying over $MgSO_4$, and evaporation of the solvent gave 57.9 g of crude ketosulfoxide as a pale brown syrup which was found to crystallize on long standing. The crude sulfoxide was immediately reduced as follows: it was dissolved in 2500 mL of 10% aqueous tetrahydrofuran and treated with aluminum amalgam prepared from 65 g of aluminum as described by Corey and Chaykovsky;[13] the reaction temperature was maintained at 10–20 °C, and the reaction time was 20 min. The reaction mixture was filtered, the filtrate was evaporated to remove the tetrahydrofuran, and the residue was dissolved in ether. The ether solution was washed successively with cold 1 N sodium hydroxide and water, dried, and evaporated. Recrystallization of the residue from diisopropyl ether gave 23.1 g of 17, mp 92.5–93.5 °C. Anal. ($C_{10}H_{13}NO_3S$) C, H, N, S.

3-[2-[(Dimethylamino)sulfonyl]phenyl]-*N*-(5-methyl-3-isoxazolyl)-3-oxopropanamide (20). A mixture of 6.8 g (0.03 mol) of 2-acetyl-*N*,*N*-dimethylbenzenesulfonamide (17), 0.09 mol of sodium hydride, and 50 mL of dimethyl carbonate was heated at reflux for 1.5 h. Methanol was added to destroy the excess sodium hydride, and the mixture was poured into ice–water and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated to a syrup. This crude β-keto ester (19) was dissolved in 300 mL of xylene and was heated at reflux with 3.3 g (0.033 mol) of 5-methyl-3-isoxazolamine for 18 h using a Soxhlet apparatus, the thimble of which contained Linde 4A molecular sieves. The reaction mixture was extracted with cold 1 N sodium hydroxide, and the aqueous layer was acidified and extracted with dichloromethane. The dichloromethane solution was evaporated to a residue, which was crystallized from methanol to give 7.1 g of 20, mp 133–136 °C. Recrystallization from ethyl acetate gave 5.0 g (47.5%) of an analytical sample: IR (Nujol) ν_{max} 3300, 1712, 1673, 1618, 1163 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.35 (s, 3, CH_3), 2.65 [s, 6, $N(CH_3)_2$], 4.05 (s, 2, CH_2, D_2O exchangeable), 6.60 (s, 1, $C_{4'}$ H), 7.70 (m, 4, aromatic), 10.85 (br, 1, NH, D_2O exchangeable).

Acknowledgment. The authors thank A. D. Lewis and his associates of the Department of Analytical–Physical Chemistry for spectral determinations. Microanalyses were done by Mrs. U. Zeek and her associates.

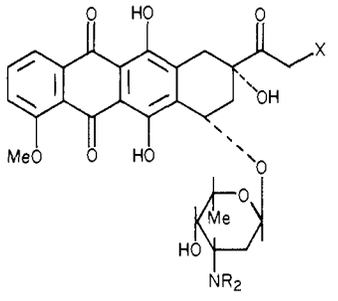
Enhanced Antitumor Properties of 3′-(4-Morpholinyl) and 3′-(4-Methoxy-1-piperidinyl) Derivatives of 3′-Deaminodaunorubicin

Carol W. Mosher,* Helen Y. Wu, Allan N. Fujiwara, and Edward M. Acton

Bio-Organic Chemistry Laboratory, Life Sciences Division, SRI International, Menlo Park, California 94025.
Received June 8, 1981

Reductive N,N-dialkylation of daunorubicin with 2,2′-oxydiacetaldehyde and $NaBH_3CN$ occurred in two steps without interruption and with cyclization to form 3′-(4-morpholinyl)-3′-deaminodaunorubicin. This derivative retained the antitumor efficacy of doxorubicin against mouse leukemia P388 but at one-fortieth the dose; hence, it is the most potent anthracycline analogue synthesized so far. The 4-methoxy-1-piperidinyl derivative, similarly prepared with 3-methoxyglutaraldehyde, showed improved efficacy against P388, though at normal doses. Results with a series of analogues indicate that incorporation of the N in the new ring and the presence of an ether O at the 4-position are critical for enhanced activity.

New analogues of doxorubicin (adriamycin, 1) and



1, X = OH; R = H
2, X = H; R = H
3, X = H; R = $CH_2C_6H_5$

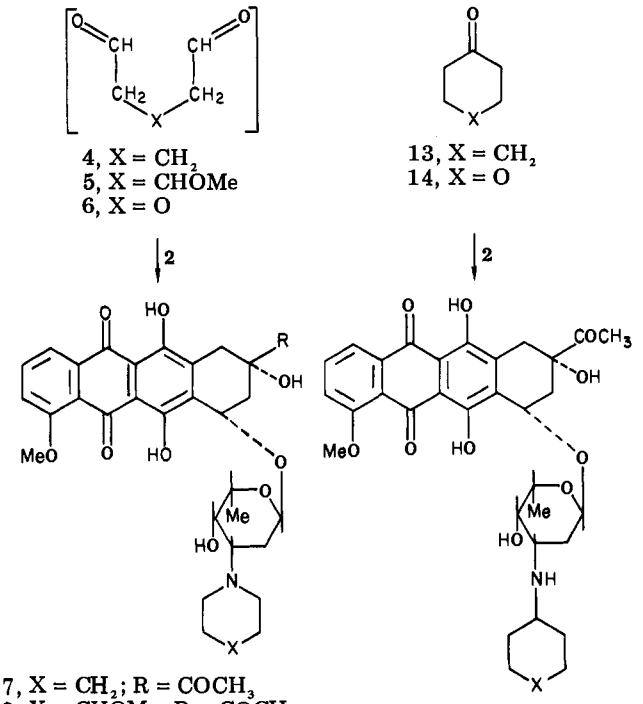
daunorubicin (2) that might be more active or less toxic as cancer drugs are of considerable importance.[1] Doxorubicin appears to be the most active single agent against cancer. It is a principal agent in the treatment of an unusually wide number of solid tumors, and along with daunorubicin it is used in the treatment of leukemias. Despite this, many patients with these presumably treatable tumors fail to respond, and there are other types of tumors (colon cancer, melanoma) where essentially no patients respond. All patients treated with these drugs, however, encounter some risk of cardiotoxicity. Over 500 derivatives and analogues have been screened for antitumor properties just by the National Cancer Institute.[2] Many were active but could not be extensively evaluated. We have been exploring reductive alkylation[3] of the amino function with aldehydes and ketones in the presence of $NaBH_3CN$, as a useful one-step method for the semisynthesis of active analogues with altered patterns of biological effects.[4,5] *N*,*N*-Dibenzyldaunorubicin (3) is one example with markedly superior activity against mouse leukemia P388, even though it required higher doses and did not show the expected interactions with DNA. Because of superior activity also against murine colon and mammary tumors, 3 is currently under preclinical development at the NCI.

The series of active *N*-alkyl derivatives included the *N*,*N*-pentamethylene derivative of 1, with the amino N

(1) S. K. Carter, *Cancer Chemother. Pharmacol.*, 4, 5 (1980).
(2) M. C. Lowe and J. I. Smallwood, *Cancer Chemother. Pharmacol.*, 5, 61 (1980).
(3) R. J. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 93, 2987 (1971).
(4) G. L. Tong, H. Y. Wu, T. H. Smith, and D. W. Henry, *J. Med. Chem.*, 22, 912 (1979).
(5) Reviewed by E. M. Acton, in "Anthracyclines: Current Status and New Developments", S. T. Crooke and S. D. Reich, Eds., Academic Press, New York, 1980, pp 15–25.

0022-2623/82/1825-0018$01.25/0

Scheme I



4, X = CH_2
5, X = CHOMe
6, X = O

13, X = CH_2
14, X = O

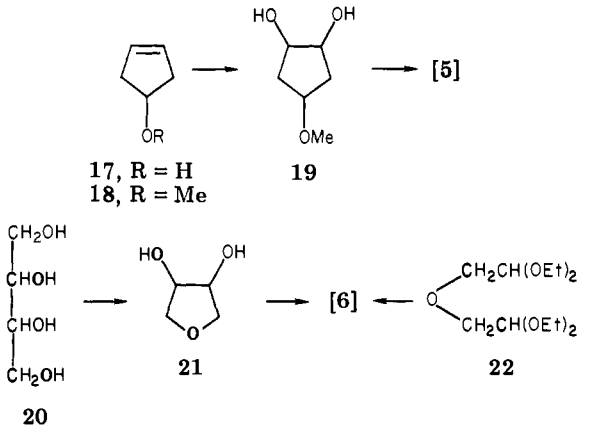
7, X = CH_2; R = $COCH_3$
8, X = CHOMe; R = $COCH_3$
9, X = O; R = $COCH_3$
10, X = CH_2; R = $CHOHCH_3$
11, X = CHOMe; R = $CHOHCH_3$
12, X = O; R = $CHOHCH_3$

15, X = CH_2
16, X = O

incorporated in a new piperidino ring by dialkylation with 1 mol of glutaraldehyde (**4**). Subsequent preparation of the corresponding *N,N*-pentamethylene derivative **7** of daunorubicin (**2**) is shown in Scheme I. (The 13,13-dihydro derivatives, e.g., **10**, were obtained as the usual byproducts.) These ring-added analogues were of interest because of the apparent preference for inhibition of RNA synthesis (i.e., lower ED_{50}) compared to DNA synthesis in cultured lymphoid leukemia L1210 cells.[4,5] It was recently proposed[6] that more potent inhibition of RNA synthesis denotes a separate class of anthracyclines with altered biological properties. This report describes enhanced antitumor properties in related derivatives of **2** with 4-methoxy-1-piperidinyl[7] and 4-morpholinyl rings[7,8] (**8** and **9**, respectively). These derivatives are compared to the inactive or weakly active *N*-cyclohexyl and *N*-tetrahydropyran-4-yl analogues (**15** and **16**).

Chemistry. Reductive N-alkylation (Scheme I) of daunorubicin (**2**) with 3-methoxyglutaraldehyde (**5**) and with 2,2′-oxydiacetaldehyde (**6**) generated the 4-methoxy-1-piperidinyl and 4-morpholinyl derivatives **8** and **9**. The dialdehydes were best synthesized by periodate cleavage of the corresponding cyclic 1,2-diols (Scheme II). 4-Methoxycyclopentane-1,2-diol (**19**), the precursor to **5**, was prepared from 4-hydroxycyclopentene[9] (**17**), which was converted to the methyl ether [10] (**18**) and hydroxylated

Scheme II



17, R = H
18, R = Me

19

20

21

22

using trimethylamine *N*-oxide and osmium tetroxide[11] in the presence of pyridine. 2,2′-Oxydiacetaldehyde (**6**) was similarly obtained by cleavage[12,13] of 1,4-anhydroerythritol (**21**) but was alternatively prepared by acid hydrolysis of the tetraethyl diacetal[14] **22**. The dialdehydes were used without isolation from the aqueous solutions. The presence of unconsumed periodate was avoided by the used of less than the theoretical amount required for glycol cleavage. Both **5** and **6** appeared to be mixtures of several forms, including the hydrated and intramolecularly cyclized ones. This conclusion was based on complex ^1H NMR spectra, which were observed on the small fraction of product that was extracted from the aqueous reaction mixture or directly on reactions in D_2O solution. The spectra were similar to those reported for preparations of glutaraldehyde[15,16] and 2,2′-oxydiacetaldehyde.[13]

The amounts of dialdehyde available for reaction were based on the amount of periodate used for diol cleavage, assuming quantitative conversion. The aqueous solutions of **5** and **6** were neutralized and treated with **2** (as the hydrochloride) and sodium cyanoborohydride, with acetonitrile added to maintain solution. The reactions of **4**–**6** were complete within 1–4 h. The products were separated by extraction with chloroform or dichloromethane and analyzed by reverse-phase high-performance liquid chromatography (HPLC). In the preparation of **9**, there was considerable formation of nonbasic, anthracycline-derived byproducts; excess **6** was used successfully to suppress formation of the 13,13-dihydro derivative **12** but did not suppress formation of the unidentified byproducts. Extensive chromatographic purifications were required, involving flash chromatography[17] and thin-layer techniques, and the yields of **7**–**12** were low (Table I). In the experiments (i.e., with **6** in small excess) that produced appreciable amounts of **12** along with **9**, these products were separated by HPLC on a preparative reverse-phase column, with monitoring by reverse-phase analytical HPLC.

(6) S. T. Crooke, V. H. Duvernay, L. Galvan, and W. W. Prestayko, *Mol. Pharmacol.*, **14**, 290 (1978).
(7) Presented in part; see E. M. Acton and C. W. Mosher, *Proc. Am. Assoc. Cancer Res.*, **22**, 225 (1981).
(8) Presented in part; see C. W. Mosher and E. M. Acton in "Abstracts of Papers", 181st National Meeting of the American Chemical Society, Atlanta, GA, 1981, American Chemical Society, Washington, DC, 1981, Abstr CARB 31.
(9) J. J. Eisch, J. H. Merkley, and J. E. Galle, *J. Org. Chem.*, **44**, 587 (1979).
(10) I. Fleming and E. J. Thomas, *Tetrahedron*, **28**, 4989 (1972).
(11) R. Ray and D. S. Matteson, *Tetrahedron Lett.*, **21**, 449 (1980).
(12) V. C. Barry, J. E. McCormick, and R. S. McElhinney, *Carbohydr. Res*, **7**, 299 (1968).
(13) H. R. Greenberg and A. S. Perlin, *Carbohydr. Res.*, **35**, 195 (1974).
(14) G. T. J. Field and P. R. Huddleston, Belgian Patent 655 436 (1965); *Chem. Abstr.*, **64**, 18775 (1966); C. L. Zirkle, F. R. Gerns, A. M. Pavloff, and A. Burger, *J. Org. Chem.*, **26**, 395 (1961).
(15) P. M. Hardy, A. C. Nicholls, and H. N. Rydon, *J. Chem. Soc. Perkin Trans. 2*, 2270 (1972).
(16) J. Foos, F. Steel, S. Q. A. Rizvi, and G. Fraenkel, *J. Org. Chem.*, **44**, 2522 (1979).
(17) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).

Table I. Reductive N-Alkylation of Daunorubicin (2)

dialdehyde or cyclic ketone reagent	stoichiometry: dialdehyde or cyclic ketone–2·HCl–$NaBH_3CN$	product·HX: 13-keto	product·HX: 13,13-dihydro	yield,[a] %	TLC R_f (system; see Exptl Sect)	formula	anal.
4	1:1:2	7		17[b,c]	0.08 (A) 0.12 (B) 0.6 (C)	$C_{32}H_{37}NO_{10}\cdot 0.9HCl\cdot 1.25H_2O$	C, H, N, Cl
			10	4[b]	0.4 (C)	$C_{32}H_{39}NO_{10}\cdot HCl\cdot H_2O$	C, H, N, Cl
5	1.5:1:2	8		17[d]	0.11 (A) 0.20 (B)	$C_{33}H_{39}NO_{11}\cdot HCl\cdot 2H_2O$	C, H, N, Cl
			11	9[d]	0.03 (A) 0.09 (B)	$C_{33}H_{41}NO_{11}\cdot HCl\cdot 2H_2O$	C, N, Cl; H[g]
6	18:1:2	9		16[e]	0.53 (A) 0.59 (B)	$C_{31}H_{35}NO_{11}\cdot 0.9HBr\cdot 0.3H_2O$	C, H, N, Br
			12	5[e]	0.21 (A) 0.30 (B)	$C_{31}H_{37}NO_{11}\cdot HCl\cdot 2H_2O$	C, N, Cl; H[h]
13	20:1:3	15		59[f]	0.82 (A) 0.75 (B)	$C_{33}H_{39}NO_{10}\cdot HCl\cdot H_2O$	C, H, N
14	20:1:3	16		51[f]	0.06 (A) 0.18 (B)	$C_{32}H_{37}NO_{11}\cdot 0.8HCl\cdot 3H_2O$	C, N, Cl; H[i]

[a] Yields of isolated products, homogeneous by TLC, exhibiting expected UV and ^1H NMR spectra, are based on amount of 2·HCl used in each case. [b] The aqueous 25% solution (Aldrich) of 4 was added to a solution (40 mg/mL) of 2·HCl in CH_3CN–H_2O (3:1); the mixture was stirred for 30 min and then added dropwise to a CH_3CN solution of $NaBH_3CN$ (14 mg/mL). After this mixture was stirred 30 min, it was diluted with H_2O and extracted with CH_2Cl_2. The crude product, from the dried CH_2Cl_2 layer, was purified by preparative TLC (C). [c] Final purification on a Lichroprep column with CH_3CN–0.05 M citrate buffer, pH 4 (45:55). [d] An aqueous solution (10 mg/mL) of 5 (based on 19) was diluted 7-fold with CH_3CN, treated with 2·HCl, stirred for 45 min, and treated with $NaBH_3CN$ in CH_3CN (25 mg/mL). The mixture was stirred 2 h, diluted 2-fold with H_2O, and extracted with $CHCl_3$. The extract was purified by flash chromatography[17] (B) or by thick-layer chromatography ($CHCl_3$–CH_3OH, 10:1). [e] See Experimental Section. Several reaction mixtures were combined and worked up. When the molar ratio of reactants was 1.5:1:16, the crude reaction mixture contained 9 and 12 in a ratio of 1:2, when the ratio of reactants was 1.5:1:2, the ratio of 9 and 12 was 3:2 (analyzed by reverse phase HPLC). [f] A solution of $NaBH_3CN$ in CH_3CN–H_2O (10:1) was added to a reaction solution of the ketone and 2·HCl in CH_3CN–H_2O (3:1) and stirred for 18 h. A $CHCl_3$ extract of the mixture was purified by preparative TLC($CHCl_3$–CH_3OH, 4:1). [g] H: calcd, 6.62; found, 5.97. [h] H: calcd, 6.30; found, 5.80. [i] H: calcd, 6.35; found, 5.69.

Table II. UV-Visible Spectral Data

	$\epsilon \times 10^{-3}$					
compd·HX[a]	λ_{max}: 233 nm	251 nm	287–289 nm	476–479 nm	493–496 nm	525–532 nm (sh)
2	37.5	26.1	8.60	12.1	12.3	6.30
7	38.2	25.6	8.73	12.3	12.2	6.29
10	34.7	25.4	8.10	11.6	11.7	6.40
8	39.7	27.1	9.19	12.8	12.8	6.87
11	39.0	26.8	9.00	12.7	12.7	6.83
9	36.3	25.6	8.39	11.8	11.7	6.29
12	37.4	27.9	8.79	12.5	12.7	7.18
15	39.5	27.9	9.36	12.8	13.0	7.24
16	39.2	26.9	9.13	12.8	12.8	6.81

[a] CH_3OH solution.

The occurrence of **12** as a diastereoisomeric mixture was indicated by two signals for the 6-OH, the 11-OH, and the 14-H_3 protons in the 360-MHz NMR spectrum. Since for each pair the differences between the two signals were very small (0.007, 0.010, and 0.018 ppm, respectively), they were detected only at 360 MHz. Each product (**7–12**) was purified as the free base and converted to the hydrohalide salt. Control of pH in this step is critical. The best technique was to treat a stirred aqueous suspension of the amorphous anthracycline with dilute acid until pH 4.7–4.9 was attained and lyophilize the resultant solution. Avoidance of excess acid was critical. A sample of **9** that analyzed for 1.3HBr underwent considerable glycoside cleavage within a few days at 5 °C, whereas a sample with 1.0HBr could be stored at room temperature indefinitely.

To explore the attachment of rings that do not incorporate the amino N, reductive alkylations were conducted with cyclohexanone and tetrahydropyran-4-one to give **15** and **16**, respectively. These reactions gave better yields (Table I). The expected 13,13-dihydro derivatives of **15** and **16** were observed in the reaction mixtures but were not isolated because the biological activity of **15** and **16** proved to be poor.

The products were characterized by ^1H NMR and UV–visible spectra. The usual resonances were observed that are diagnostic[4,18] for derivatives of **2** (aryl H's, 1'-H, 7-H, OCH_3, $COCH_3$, and 6'-CH_3), as described for **9** and **12**. Spectra of the free bases were better resolved and more readily analyzed than those of the hydrohalide salts. Chemical-shift assignments for 3'-H and 4'-H (3.4–3.9 ppm) and 2'-H_2, 8-H_2, and 10-H_2 (1.5–3.4 ppm) were often ambiguous due to overlapping or partial obscuring. Particular difficulty was encountered in attempts to analyze and assign protons from the rings attached at the 3'-position. The $-CH_2NCH_2-$ shifts were assigned to broad multiplets at 2.3–2.7 ppm, $-CH_2OCH_2-$ to multiplets at 3.6–3.8 ppm, and the remaining piperidino and tetrahydropyranyl ring protons to the 1.5–3.4 region by comparing with NMR spectra of simpler compounds (*N*-methylmorpholine, *N*-methylpiperidine, and tetrahydropyran) and by integration of these regions. The failure to observe sharply resolved signals may be due, in part, to a high degree of conformational mobility in these rings.

The extinctions from the UV–visible spectra (Table II) were generally within 10% of the values for **2**.

Biological Results

Table III presents the initial antitumor screening results against lymphocytic leukemia P388 in mice,[19] in a dose schedule that allows this implanted tumor to progress for 4 days before treatment. Also presented are data from in vitro tests relevant to proposed mechanisms of anthracycline action. The observed increase in the thermal denaturation temperatures (T_m) of isolated helical DNA in solution after addition of the anthracycline is a measure[20] of DNA binding. Inhibition of DNA and RNA synthesis in lymphoid leukemia L1210 cells is measured[20] by the dose (ED_{50}) that inhibits by 50% the incorporation of [^3H]-thymidine and [^3H]uridine. The capacity for anthracyclines to undergo bioreductive activation at the quinone and to produce oxygen-derived radicals in a cyclic process can be measured by the stimulation of O_2 consumption in the presence of microsomes and NADPH, upon adding the compound.[21]

Compounds **7–12**, all those with the amino N incorporated in the added six-membered ring, were superior to **1** and **2** in antitumor activity. The piperidino compounds **7** and **10** offered only a small improvement, but the 4-methoxypiperidinyl analogues **8** and **11** both showed an important increase in antitumor efficacy (T/C = 199%). As standard of comparison, the antitumor efficacy of **1** in this test regimen is T/C = 160%. For all anthracyclines, the highest values recorded by the NCI (as averages from more than one test) appear to be T/C = 209%, observed for **3** and for one other analogue. Few analogues have shown the doubling (T/C = 199%) in survival time produced by **8** and **11**, in the deliberately stringent q4d 5, 9, 13 test regimen, judging from results by the NCI on the approximately 150 analogues submitted by SRI and from results on eight analogues selected for consideration by the NCI's Decision Network Committee.[22] Even more striking is the increase in potency (or reduction in optimum dose) observed with the 4-morpholinyl compound **9**. The optimum dose level, 0.2 mg/kg, was one-fortieth of that required by **1**. Compound **9** did not provide a significant improvement in antitumor efficacy (T/C = 166%, compared to 160% for **1**), but the increase in potency is the greatest observed with any anthracycline analogue so far and could be important. The retention of activity at such low doses suggests the possibility for loss of undesired side effects in cancer treatment with **9**. The 13,13-dihydro analogue **12** similarly showed a 20-fold increase in potency. Such increases have also been observed in continued testing against B16 melanoma (qd 1), where **9** was active at 0.25 mg/kg (T/C = 135%) compared to 4 mg/kg for **1** (T/C = 262%) or to 6.25 mg/kg for **7** (T/C = 127%), even though the efficacy against this tumor was modest.

It was unexpected, then, that *N*-cyclohexyldaunorubicin (**15**), a homologue of **7**, should be completely inactive, even at a dose level of 200 mg/kg. Activity at high doses was restored by insertion of a ring O at the 4-position in the tetrahydropyran-4-yl analogue **16** (T/C = 136% at 100 mg/kg). The efficacy was comparable to that of daunorubicin (**2**), but the potency was 12 times poorer—or 500 times poorer than that of **9**. It appears the enhanced activity we have seen in the series **7–12** requires the N to be incorporated in the added ring, with no linking atom between the new ring and the sugar. Perhaps the degree of structural rigidity this imposes is critical at a receptor site. The presence of the ether O also appears to be critical, as seen by comparing **8** and **9** to **7**, or **16** to **15**. Perhaps this provides an interactive site not previously identified among derivatives of **1** and **2**.

All the compounds that were active in vivo gave ΔT_m values indicative of DNA binding. This test was predictive in that the inactive **15** showed no ΔT_m, and the weakly active **16** gave only a borderline value. It is unclear why the very potent morpholinyl compounds **9** and **12** gave lower ΔT_m values than **1** and **2** or why **7** gave a higher value. The inactive *N*-cyclohexyl compound **15** was also

(18) F. Arcamone, G. Cassinelli, G. Franceschi, P. Orezzi, and R. Mondelli, *Tetrahedron Lett.*, 3353 (1968).

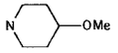
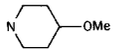
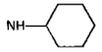
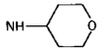
(19) Screening for antitumor properties was done under the auspices of the National Cancer Institute, Division of Cancer Treatment, Developmental Therapeutics Program, according to its protocols; R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3(2), 1–103 (1972). We thank Dr. V. L. Narayanan for providing the data.

(20) G. L. Tong, W. W. Lee, D. R. Black, and D. W. Henry, *J. Med. Chem.*, 19, 395 (1976).

(21) N. R. Bachur, S. L. Gordon, M. V. Gee, and H. Kon, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 954 (1979).

(22) Decision Network Committee, Division of Cancer Treatment, NCI; open meeting, June 12, 1980. For example, Aclacinomycin A, an important analogue in clinical trial, was indistinguishable (T/C = 150%) from **1** in this test.

Table III. Comparison of Biological Test Data

3′-substituent	compd·HX	antitumor efficacy[a] at opt dose vs. leukemia P388 in mice treated q4d 5, 9, 13: % T/C, mg/kg	ΔT_m[d,e] of isolated helical DNA in solution, °C	inhibn of synth[e,f] in leukemia L1210 cells: ED_{50}, μM		augmentation of microsomal O_2 consumption,[g] rel to consumption by doxorubicin, %
				DNA	RNA	
NH_2	1	160[b] (8.0)	13.4	1.5	0.58	100
NH_2	2	130[c] (8.0)	11.2	0.66	0.33	82, 109
	7	177 (6.25)	16.8	0.50	0.05	8
	10	162 (5.0)	12.9	0.62	0.04	
	8	199 (6.25)	13.3	0.63	0.12	82
	11	199 (12.5)	9.5	0.58	0.08	64
	9	166 (0.2)	6.1	0.76,[h] 0.34	0.10,[h] 0.014	25
	12	153 (0.4)	4.1	2.2	0.53	29
	15	inact (≤200)	0.7	64	16	57
	16	136 (100)	4.0	2.0	0.61	68

[a] Ratio of average survival time of treated mice to untreated controls × 100. Values of T/C > 120 signify activity. The reported values are averages of two test results on all analogues, except for the inactive 15, which was tested only once. Assays were conducted under the auspices of the NCI; see ref 19. Mice injected ip with P338 cells on day 0 were treated on days 5, 9, and 13 with a series of doses. We thank Dr. Ven L. Narayanan for the results. [b] T/C = 160 ± 20%, based on an average of 60 values received from the NCI, 1976–1981. [c] T/C = 130 ± 8%, based on an average of 50 values received from the NCI, 1976–1980. [d] $\Delta T_m = T_m$ of DNA–drug complex – T_m of DNA (calf thymus). Concentration of drug, 5.2×10^{-6} M. Concentration of DNA (P), 5.2×10^{-5} M in 0.01 M phosphate buffer (pH 7) containing 10^{-5} EDTA and 5% Me_2SO. Values <1 indicate insignificant degree of binding to DNA. [e] Determined as in ref 20, except that the drugs were initially dissolved in a volume of Me_2SO that gave a final concentration of Me_2SO of 5 and 1% in the T_m and synthetic inhibition assays, respectively. We thank Dorris L. Taylor for these studies. [f] ED_{50} is the concentration of drug necessary to reduce by 50% the incorporation of tritiated thymidine in the DNA or tritiated uridine in the RNA of actively growing L1210 cells. [g] The rate of O_2 consumption by liver microsomes and NADPH in the presence of the analogue, compared to the rate in presence of doxorubicin. Endogenous O_2 consumption is generally stimulated by the addition of drug. We thank Dr. John H. Peters and G. Ross Gordon for these assays. [h] Two values obtained on two preparations of 95–97% purity, the impurities not accounting for the variations in these values.

a poor inhibitor of DNA/RNA synthesis in L1210 cells. Otherwise, this test did not distinguish clearly between the active compounds. The weakly active **16** was not significantly different from **1**. The piperidino compounds (**7** and **10**) showed significant inhibitor selectivity toward RNA synthesis [DNA/RNA (ED_{50}) ratios of 10:1 and 15:1], but such elevated ratios were not observed for **8** and **11** or consistently for **9** and **12**. In the present series of compounds, the test results for drug-stimulated consumption of O_2 did not correlate with antitumor properties in vivo or with DNA interaction in vitro.[7,23] The inactive *N*-cyclohexyl compound **15**, for example, gave about half the enhancement of **1**, but the very potent morpholino compound **9** gave only one-fourth the effect. These comparisons so far are with only single concentrations of drug in the presence of microsomes and NADPH; however, if one assumes that the concentrations are appropriately chosen, the results may accurately reflect the relative O_2-cycling properties in vivo. A lessened apparent capacity for **9** to generate O_2-derived radicals was independently observed in a test for nicking of PM-2 closed circular DNA, which involves exposure of the DNA to chemically prereduced drug that presumably autoxidizes with the generation of DNA-damaging radicals. The extent of single-strand scission was only half that observed with **1**. The separation of antitumor effects from radical-generating effects in **9** is intriguing and suggests further studies of possibly reduced cardiotoxicity with this very potent analogue.

Experimental Section

Solutions in organic solvents were dried over anhydrous Na_2SO_4. Solutions were concentrated under reduced pressure on a rotary evaporator. NMR spectra were determined on a Varian EM 390 or XL-100-15 spectrometer equipped with a Fourier transform or on a Bruker HXS-360, located at the Stanford Magnetic Resonance Laboratory, Stanford University, on solutions as noted with Me_4Si (δ 0.0) internal reference and signals described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad); integrated signal areas were as predicted from the structures. UV–vis spectra were determined on a Perkin-Elmer Model 575 recording spectrometer, and MS were determined on a LKB 9000 GC–MS interfaced with a PDP12 computer. Thin-layer chromatography (TLC) was accomplished on silica gel (0.25 mm GF, Analtech) plates with solvent systems (A) CH_2Cl_2–*i*-PrOH (9:1), (B) $CHCl_3$–MeOH (8:1), and (C) $CHCl_3$–MeOH–H_2O (40:10:1). Preparative TLC was accomplished on 2-mm silica gel F-254 (E. Merck) plates, and flash chromatography was accomplished on a silica gel 60 (230–400 mesh, E. Merck) column (2.5 × 15 cm). Analytical reverse-phase HPLC was carried out using a Waters RP-18 Radialpak column with solvent system CH_3CN–0.05 M sodium citrate buffer (pH 4) (60:40), monitoring at 254 nm, flow rate 2 mL/min. Preparative liquid chromatography was performed on a Waters Prep LC/500 system.

3'-Deamino-3'-morpholinodaunorubicin Hydrobromide (9·HBr). A solution of 31.5 g (0.30 mol) of 1,4-anhydroerythritol[24] (**21**) in 400 mL of H_2O was stirred and cooled in an ice–water bath while 53.0 g (0.25 mol) of $NaIO_4$ was added in small portions. Stirring was continued for 1 h at room temperature, a small amount of $NaIO_3$ separating. After the mixture had stood overnight, the pH was adjusted to 7.3 with a few drops of saturated $NaHCO_3$ solution, and 50 mL of water was added to give complete solution.

A solution of 9.2 g (16.3 mmol) of **2**·HCl in 50 mL of CH_3CN–H_2O (1:1) was added to the dialdehyde solution and stirring continued at room temperature. The mixture became cloudy and small amounts of solid separated after 45 min; addition of 75 mL of CH_3CN effected only partial solution. A solution of 2.02 g (32.6 mmol) of $NaBH_3CN$ in 6 mL of H_2O was added. The mixture was stirred for 1 h, diluted with water, and extracted with $CHCl_3$ (5 × 200 mL). Solid (0.91 g) that dissolved in neither phase was removed by filtration.

The $CHCl_3$ solution consisting of 32% **9**, 7% **12**, and 67% byproducts, as indicated by reverse-phase HPLC, was concentrated to about half its volume and extracted with aqueous HCl (10 × 100 mL of pH 2) until little color appeared in the aqueous phase. Separation of the phases was aided by centrifugation. Concentration of the dried $CHCl_3$ solution left 4.24 g of solid residue. Reverse-phase HPLC and TLC (A) showed this to be a mixture of products, including daunomycinone and unidentified materials with retention times similar to that of daunomycinone.

The acidic aqueous solution was made alkaline (pH 8) with $NaHCO_3$ and extracted 6 times with 100-mL portions of $CHCl_3$. The dried organic layer was concentrated, yielding 5.60 g of **9**, about 80% pure. This was combined with 0.45 g of similar material from a previous preparation and purified by flash chromatography,[17] using solvent system A. Like fractions were combined, after examination by TLC (A) and/or HPLC (reverse phase), and repurified several times by flash chromatography to give 1.55 g of **9** of 97% purity. An additional 0.69 g of similar purity was obtained by purification of less pure fractions on thick-layer chromatographic plates; total yield of free base **9**, based on **2**, was 18%: NMR (360 MHz; $CDCl_3$) δ 13.98 (s, 6-OH), 13.27 (s, 11-OH), 8.02 (d, 1-H, $J_{1,2}$ = 8 Hz), 7.79 (t, 2-H), 7.39 (d, 3-H, $J_{2,3}$ = 8 Hz), 5.56 (d, 1'-H, J = 1 Hz), 5.27 (br s, 7-H), 4.09 (s, 4-OMe), 4.0–4.1 (m, 5'-H), 3.68 [m, morpholino $(CH_2)_2$-O, 3'-H, 4'-H], 3.22 (d, 10-H_B, J_{gem} = 19 Hz), 2.95 (d, 10-H_A, J_{gem} = 19 Hz, OH), 2.35–2.65 m [morpholino $(CH_2)_2$N, 8-H_B), 2.42 (s, 14-H_3), 2.12 (q, 8-H_A, J_{gem} = 1 Hz, $J_{7,8A}$ = 19 Hz), 1.77 (m, 2'-H_2), 1.65 (br s, OH), 1.38 (d, 6'-H_3, J = 7.56 Hz).

A portion of free base **9**, 217 mg, was converted to the hydrobromide by adding 40 mL of H_2O to the insoluble **9** and then adding 0.3 N HBr solution dropwise, slowly, swirling the mixture, and monitoring with a pH electrode until complete solution had occurred; addition was halted at pH 4.7. This solution was lyophilized, and the residual salt was triturated with ether, collected on a filter, and dried (0.1 mm, room temperature, 48 h), yielding 216 mg (88%). This salt, like others prepared similarly, was hygroscopic. A similar sample (with 1.0HBr) was stored as the solid at room temperature for 1 year with no measureable decomposition. However, a small excess of HBr is deleterious: a sample with 1.3HBr, stored for one week at 5 °C, underwent 6% glycosidic cleavage; after 1 week at room temperature, it underwent 21% cleavage: TLC (A) R_f 0.53, (B) 0.59. Reverse-phase HPLC retiontion time 14.4 min (compared to daunomycinone, 3.6 min; **2**, 7.8 min). MS [as the $(Me_3Si)_4$ derivative], m/e 855 (M), 870 (M – Me), 855 (M – Me_2), 796 (M – $OSiMe_3$), 142 [OCH_2CH_2-CH-*c*-N$(CH_2CH_2)_2$O], 113 [CH_2CH-*c*-N$(CH_2CH_2)_2$O]. Anal. ($C_{31}H_{35}NO_{11}$·0.9HBr·0.3H_2O) C, H, N, Br.

3'-Deamino-3'-morpholino-13,13-dihydrodaunorubicin Hydrochloride (12·HCl). The crude solids from several small runs, containing a mixture of hydrochlorides of **9** and **12** and minor impurities, were combined. (These had been obtained by extracting the reductive alkylation reaction mixture with $CHCl_3$; the $CHCl_3$ solution was extracted with aqueous HCl (pH 2) and the aqueous phase was lyophilized.) After the solids were triturated and extracted several times with $CHCl_3$, they were dissolved in water and the solution made basic (pH 8) and extracted with $CHCl_3$; the composition, analyzed by reverse-phase HPLC, was 73% **12**, 21% **9**, in addition to several impurities. Partial purification by direct phase chromatography (silica gel, Waters Prep LC 500, 10% *i*-PrOH in CH_2Cl_2) yielded, in order of elution, **9** and **12**. The combined later fractions containing 87% **12** and 9% **9** were further purified on a prepacked reverse-phase column (Lichroprep RP-8, 40–63 μM, size B, 310 × 25 cm, using 0.05 M citrate buffer, pH 4 in CH_3CN, 65–20%), eluting **12** in early fractions, followed by **9**. $CHCl_3$ extracts of the basified fractions were analyzed by reverse-phase HPLC. The combined $CHCl_3$ extracts of fractions containing **12** of 97% purity were washed with water, dried, concentrated and converted to the hydrochloride salt by adding 0.2 N HCl to an aqueous mixture until solution was complete (pH 5.0), followed by lyophilization: TLC (A) 0.21,

(23) Presented in part; see G. R. Gordon, J. H. Peters, and E. M. Acton, in "Abstracts of Papers", 2nd Chemical Congress of the North Americal Continent, San Francisco, CA, Aug 24–29, 1980, American Chemical Society, Washington, DC, 1980, Abstracts, BIOL 136.

(24) F. H. Otey and C. L. Mehltretter, *J. Org. Chem.*, **26**, 1673 (1961).

(B) 0.30; reverse-phase HPLC retention time 10.0 min (compared to **2**, 7.8 min; daunomycinone, 3.6 min; **9**, 14.4 min).

A small sample was converted back to free base **12** by dissolving it in H_2O, making the solution basic with $NaHCO_3$ solution, and then extracting it with $CHCl_3$. The $CHCl_3$ solution was dried, concentrated, and used to obtain an NMR spectrum: NMR (360 MHz; $CDCl_3$) δ 13.975 and 13.968 (2 s, 6-OH), 13.319 and 13.309 (2 s, 11-OH), 8.021 (d, 1-H, $J_{1,2}$ = 7.55 Hz), 7.777 (t, 2-H), 7.385 (d, 3-H, $J_{2,3}$ = 8.45 Hz), 5.561 (d, 1'-H, J = 2.99 Hz), 5.29 (br s, 7-H), 4.081 (s, 4-OMe), 4.0–4.1 (m, 5'-H), 3.67 [m, morpholino $(CH_2)_2O$, 3'-H, 4'-H), 3.20 (d, 10-H_B, J_{gem} = 20 Hz), 2.97 (br s, OH), 2.25–2.75 [m, morpholino $(CH_2)_2N$, 13-H, 10-H_A, (OH, OH)], 1.65–1.95 (m, 2'-H_2, 8-H_2), 1.39 (d, 6'-H_3, J = 6.53 Hz), 1.336 and 1.292 (2 d, 14-H_3, J = 6.4 Hz).

1,2-Dihydroxy-4-methoxycyclopentane (19). 4-Methoxycyclopentene[10] (18) was converted[11] to **19**. The light yellow syrup, obtained in 47% yield by repeated ether extraction of the reaction mixture diluted with $NaHSO_3$ (20% solution) and saturated with NaCl, showed a trace impurity on TLC but was suitable for use in the next step: TLC (B) 0.4; NMR ($CDCl_3$) δ 3.8–4.3 (m, 1-H, 2-H, 4-H), 3.60 (s, 1-OH, 2-OH), 3.28 (s, OMe), 1.8–2.0 (m, 3-H_2, 4-H_2).

Acknowledgment. This work was supported by the National Cancer Institute (NIH), Cancer Research Emphasis Grant CA 25711. We acknowledge use of the Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305, supported by NSF Grant GP 23633 and NIH Grant RR00711. We thank Lee Garver for NMR spectral data and Dr. David W. Thomas for MS data and analysis. We thank the National Cancer Institute and Rhône-Poulenc-Santé for gifts of daunorubicin hydrochloride. Elemental analyses were performed by E. Meier, Department of Chemistry, Stanford University, Stanford, CA 94305.

Adriamycin Analogues. Preparation and Antitumor Evaluation of 7-*O*-(β-D-Glucosaminyl)daunomycinone and 7-*O*-(β-D-Glucosaminyl)adriamycinone and Their *N*-Trifluoroacetyl Derivatives

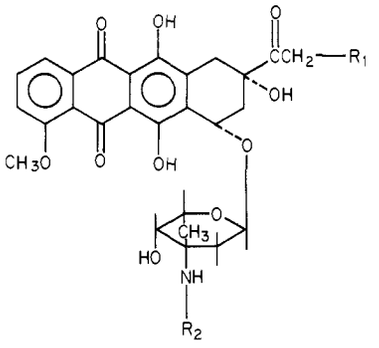
Mervyn Israel* and Robert J. Murray

Division of Pharmacology, Sidney Farber Cancer Institute, Boston, Massachusetts 02115. *Received March 10, 1981*

The title compounds were prepared by Koenigs–Knorr condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-α-D-glucopyranosyl bromide with daunomycinone or a side-chain protected adriamycinone, followed by selective hydrolysis of blocking groups. Despite poor complexation with DNA and weak growth-inhibitory properties in vitro, the glucosaminyl analogues of the antitumor antibiotics daunorubicin and adriamycin, at their optimal (highest nontoxic) doses, exhibited antileukemic activity equivalent to that of adriamycin against a usually drug-refractory mouse leukemia model system (L1210) in vivo. These findings, together with other data from these laboratories, continue to support the hypothesis that the mechanism of action of adriamycin and related agents cannot be due exclusively to DNA binding, as has earlier been believed.

The antitumor antibiotics daunorubicin (**1a**) and adriamycin (**1b**) are clinically important cancer chemotherapeutic agents. Adriamycin occupies a special place in cancer medicine because of its effectiveness against a range of solid tumors normally refractory to drug treatment.[1-3] These agents are known to bind strongly to DNA, and this biological property has generally been accepted as their mechanism of antitumor action.[3,4]

In connection with a major program on the chemistry, biology, and pharmacology of anthracyclines, these laboratories have been responsible for the synthesis, preclinical development, and clinical introduction of the promising adriamycin analogue *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32, **1c**).[5,6] In contrast to the natural occurring agents **1a** and **1b**, the semisynthetic derivative **1c** does not bind to double-helical calf thymus DNA.[7,8] However, **1c**



1a (daunorubicin), $R_1 = R_2 = H$
b (adriamycin), $R_1 = OH$; $R_2 = H$
c (AD 32), $R_1 = OCO(CH_2)_3CH_3$; $R_2 = COCF_3$

exhibits very significant in vivo antitumor activity: therapeutic superiority to adriamycin has been seen across a spectrum of experimental murine tumor systems, including early and advanced leukemias, solid tumors, and a leukemia subline selected for adriamycin resistance.[5,9,10] Clinical antitumor activity, and low toxicity relative to

(1) A. DiMarco and L. Lenaz, in "Cancer Medicine", J. F. Holland and E. Frei III, Eds., Lea and Febiger, Philadelphia, 1973, p 826.
(2) R. H. Blum and S. K. Carter, *Ann. Intern. Med.*, **80**, 249 (1974).
(3) T. Skovsgaard and N. I. Nissen, *Dan. Med. Bull.*, **22**, 62 (1975).
(4) A. DiMarco and F. Arcamone, *Arzneim.*-Forsch., **25**, 368 (1975), and earlier references cited therein.
(5) M. Israel, E. J. Modest, and E. Frei III, *Cancer Res.*, **35**, 1365 (1975).
(6) M. Israel and E. J. Modest (assignors to Sidney Farber Cancer Institute, Inc.), U.S. Patent 4 035 566, July 12, 1977.
(7) S. K. Sengupta, R. Seshadri, E. J. Modest, and M. Israel, *Proc. Am. Assoc. Cancer Res.*, **17**, 109 (1976).
(8) L. F. Chuang, R. T. Kawahata, and R. Y. Chuang, *FEBS Lett.*, 117, 247 (1980).
(9) L. M. Parker, M. Hirst, and M. Israel, *Cancer Treat. Rep.*, **62**, 119 (1978).
(10) A. Vecchi, M. Cairo, A. Mantovani, M. Sironi, and F. Spreafico, *Cancer Treat. Rep.*, **62**, 111 (1978).

0022-2623/82/1825-0024$01.25/0