solved in 4 N HCl in acetic acid (10 mL) containing 0.1% of anisole. After the mixture was allowed to stand for 10 min, methanol (75 mL) and 10% Pd/C (20 mg) were added. The mixture was hydrogenated for 12 h at atmospheric pressure, and the mixture was filtered free of catalyst. The filtrate was concentrated to 30 mL and ethyl ether (100 mL) was added. The precipitated solid was decantated and washed with ethyl ether and was dried over NaOH. The yield of this chromatographically purified (Sephadex LH-20 in methanol) intermediate **19** (R_f 0.1; *n*-BuOH/AcOH/H_2O, 3:1:1) was 111.2 mg (86%). Oxymorphone (20 mg, 0.075 mmol) was added to a methanol solution (5 mL) of the deprotected peptide **19** and the mixture was heated under reflux for 4 h. The solution was then concentrated to 1 mL and chromatographed on Sephadex LH-20 in methanol. The fractions containing the pure product **7** (R_f 0.18, *n*-BuOH/AcOH/H_2O, 3:1:1) were collected and evaporated to dryness. Yield 71 mg (78%); mp 252 °C dec; $[\alpha]^{23}_D$ −93.1° (*c* 1, DMF). Amino acids analysis: Arg:Ile:Leu:Phe; 1.91:1.02:1.00:0.98. Anal. ($C_{52}H_{78}N_{14}O_{10}\cdot 3HCl\cdot 3H_2O$) C, H, N.

Naltrexone *N*-(Hydrazinocarbonyl)phenylalanylleucylarginylarginylisoleucine Methyl Ester Hydrochloride Derivative (8). Naltrexone hydrochloride (28.3 mg, 0.075 mmol) was dissolved in 5 mL of a methanol solution of compound **19** (0.075 mmol), and the mixture was heated under reflux for 4 h. After concentration of solution to 1 mL, the product was chromatographed on Sephadex LH-20 in methanol. The TLC-pure fraction with R_f 0.22 (*n*-BuOH/AcOH/H_2O, 3:1:1) was collected and the solvent removed. Yield 72 mg (75%); mp 260 °C dec; $[\alpha]^{23}_D$ −110.5° (*c* 1, DMF). Amino acid analysis: Arg:Ile:Leu:Phe, 1.90:0.99:1.00:1.01. Anal. ($C_{55}H_{82}N_{14}O_{10}\cdot 3HCl\cdot 4H_2O$) C, H, N.

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New Cyanomorpholinyl Byproduct of Doxorubicin Reductive Alkylation

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Previously we reported that reductive alkylation of doxorubicin with 2,2′-oxybis[acetaldehyde] and $NaBH_3CN$ to form the 4″-morpholinyl derivative also gave the intensely potent 3″-cyano-4″-morpholinyl as a byproduct, by addition of CN^- to an iminium intermediate in place of hydride. We now find that sugar 4′-OH is a third nucleophile that can add to the iminium intermediate in this reaction. Bridging of the 4′-OH to the morpholine ring at C.5″ formed a novel byproduct with an oxazolidino ring fused to the sugar and morpholine. The new product was minor at neutral pH but predominant at an acidic pH. When tested against tumors in mice it was 4–6 times more potent than doxorubicin. Hence, in comparison with the 3″-cyano-4″-morpholinyl, potency was reduced up to 100-fold by the O bridge. Analytical HPLC showed the presence of three of the four possible diastereoisomers, and two were isolated. The diastereoisomers appeared to differ in stability. In vitro tests suggested that biological potency varied inversely with stability.

The Borch reductive alkylation of daunorubicin and doxorubicin with 2,2′-oxybis[acetaldehyde] (**7**) and $NaBH_3CN$ has given cyanomorpholino byproducts[1] (**2** and **4**, respectively) in addition to the expected morpholino derivatives[1,2] (**1** and **3**) that incorporate the amino N of the daunosamine moiety. The nonbasic cyanomorpholino derivative **4** of doxorubicin showed 100- to 1000-fold increases in antitumor potency, absence of cardiotoxicity, and activity against tumors that are resistant to doxorubicin.[1,3-7] This constitutes one of the strongest leads in the field of anthracycline drug development and analogue synthesis. We now report isolation of the O-bridged oxazolidino compounds **5** and **6** as additional active byproducts from the reductive alkylation.

Synthesis

Generally, the reaction was carried out in aqueous acetonitrile with a neutral solution of **7** in 15-fold excess, which was mixed with 1 molar equiv of $NaBH_3CN$ ($2H^-$ required for the morpholine or 1 for the cyanomorpholine), and finally with doxorubicin hydrochloride. Attempts to improve the yield of **4** through systematic changes in reaction time, stoichiometry, order of mixing, or addition of NaCN were consistently unsuccessful. The usual yields (HPLC analysis) of **3** (42%), **4** (23%), and accompanying 13-dihydro derivatives (21%, 10%) were virtually unaffected. Similarly, small changes in pH made little difference. When, however, acidity was significantly increased by adjusting the dialdehyde solution to pH 2.7 with acetic acid, a new product in the neutral fraction became predominant (38% by HPLC) while the yields of **3** and **4** fell to 19% and 11% and almost none of the 13-dihydro derivatives were formed. When 1 molar equiv of NaCN was added at this pH, the HPLC yield of the new product was raised to 53%, with further decreases in **3** and **4**.

Further chromatographic workup on silica gel with reverse-phase HPLC analysis showed that this product existed in three isomeric forms, with identical mass spectra, and designated **6A**, **6B**, and **6C**. Most chromatographic fractions were mixtures, but small samples enriched in **6B** and **6C** were also obtained. The near identity of the UV–visible spectra with those of doxorubicin or **1**–**4** indicated again there was no change in the chromophore. The identification of the several fractions all as having structure

† SRI International.
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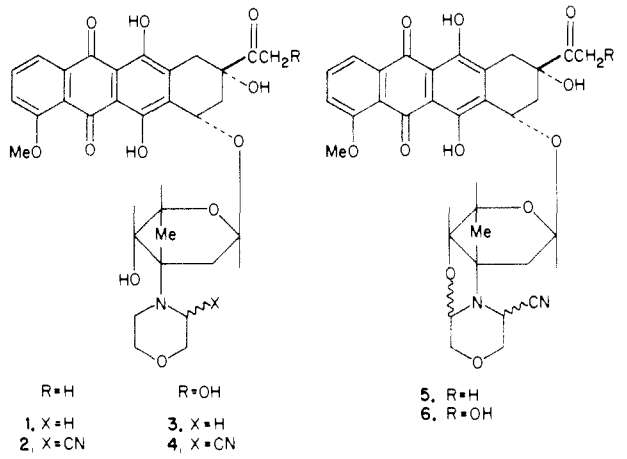


6 was based on their identical mass spectra. Confirming elemental analyses disclosed the presence of one CN as in 4 but with two less H's. As with 4, HCN was formed (m/e 27) upon electron-impact mass spectroscopy (EI-MS) of underivatized samples of 6. Desorption chemical ionization mass spectroscopy (DCI-MS, negative ion) showed both M and M - HCN and sugar fragments X and X - HCN (Chart I). Fragment Y would be equivalent to X by mass spectral evidence and a parent structure containing Y could have been considered as an alternative to 6, but this was excluded by the ^{1}H NMR spectra (see Experimental Section and Tables I and II), which showed the absence of any vinyl protons. The NMR spectra of fractions of 6 were very similar and showed few differences from 4, but all showed absence of the multiplet at δ 2.69, which in 4 had been assigned to NCH_2 at 5″.[8] In a mixture of **6A** and **6B**, new broad singlets observed at δ 4.65 and 4.58 (combined integration = one H) could be assigned to OCHN at 5″ of the two isomers. (In **6C** this proton was assigned to a multiplet at δ 4.07 that overlapped with H-4′ at 4.05 d and with 4-OCH_3 at 4.08 s.) Bridging of the 4′-OH to the morpholinyl ring at C-5″ had been inferred from the empirical formula with two less H's than in 4, and from the mechanism of morpholine ring construction involving, in two stages, iminium ions that are susceptible to nucleophilic addition. Chart II is an elaboration of the scheme used[1] to explain formation of 3 and 4, further showing attack by the 4′-OH on the iminium intermediate **22** (numbered as in ref 1) bearing a cyano substituent. Hence 4′-OH is a third nucleophile that can add to an iminium intermediate in this reaction, in competition with cyanide or hydride, depending upon pH. With these results in mind, reexamination of the products from the usual reductive alkylation at neutral pH disclosed the presence of a few percent of 6, consisting of similar mixtures of the three isomers. That bridging by the 4′-OH became predominant at lower pH suggests a loss in hydride reactivity with acid, even in the short reaction period of 10–15 min,[9] and the near absence of 13-dihydro derivatives among the products encountered is consistent with that. Simultaneous with this work, the reaction of glutaraldehyde and KCN with α,β-amino alcohols in the absence of a reducing agent at pH 3 was reported to give simple

Chart I. Mass Spectral Sugar Fragments

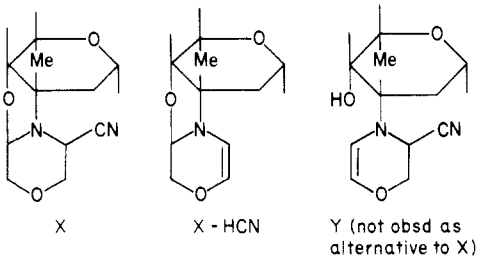
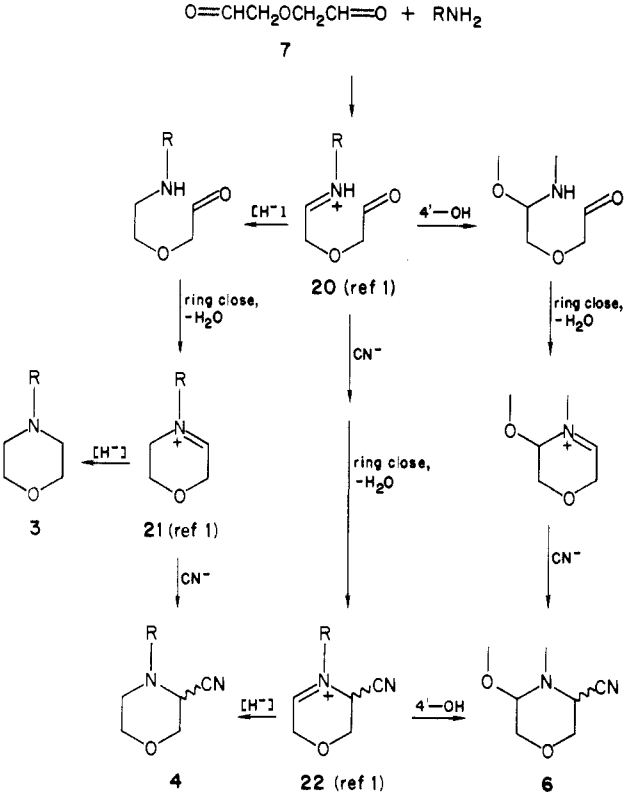


Chart II. Iminium Intermediates[a]



[a] R = doxorubicin (R = daunorubicin → 1, 2, and 5).

oxazolidinopiperidine structures[10] analogous to 6.

The O-bridged oxazolidino byproduct 5 from daunorubicin was also observed in several experiments. In one, 5 equiv of NaCN was added to the solution of 7 (15 equiv) and the pH was adjusted to 6.0; 1 equiv of daunorubicin hydrochloride was then added followed by 3 equiv of $NaBH_3CN$. After 30 min, workup and HPLC analysis showed the presence of 50% of 1, 30% of 2 (accompanied by 3–5% of the 13-dihydros), and 8% of the neutral byproduct that proved to be 5. In another experiment, NaCN was added but $NaBH_3CN$ was omitted, and none of the products (1 and 3) requiring a reductive step was obtained. The product was predominantly 5, as two resolvable diastereoisomers, identified by MS and ^{1}H NMR. Amounts adequate for in vitro screening were obtained. A minor product (2–5%) of this experiment was the previously described[1] 3″,5″-dicyano derivative—the only case in which we have encountered a dicyanomorpholine. It appeared that addition of the 4′-OH to an iminium ion (**20** or **22**) was preferred over CN^-. A similar treatment of doxorubicin hydrochloride with 7 and NaCN, but no $NaBH_3CN$,

(8) Positions in the sugar ring are numbered with primes and in the morpholinyl ring as double prime. The CN is attached at 3″ and the O bridge at 5″.

(9) Although $NaBH_3CN$ is remarkable for its acid stability relative to $NaBH_4$, decomposition of BH_3CN^- is induced by even small amounts of acid unless the solution is cooled to 0 °C. Berchied, J. R.; Purcell, K. F. *Inorg. Chem.* **1970**, *9*, 624.

(10) Guerrier, L.; Royer, J.; Grierson, D.; Husson, H.-P. *J. Am. Chem. Soc.* **1983**, *105*, 7754. Similar reactions of α,γ-amino alcohols over a pH range of 3–7 were cited: Langdale-Smith, R. A. *J. Org. Chem.* **1971**, *36*, 226.

Table I. ^{1}H NMR Chemical Shifts (δ)[a]

proton assignments[b]	compounds: 90 Hz,[c] MRA (3)	400 MHz,[d] MRA-CN (4)[e]	400 MHz,[f] O-MRA-CN (6B)[g]	400 MHz,[f] O-MRA-CN (6C)[h]
6-OH (ex)	13.88 s	14.02 s	13.98 s	13.96 s
11-OH (ex)	13.07 s	13.26 s	13.26 s	13.25 s
9-OH (ex)	4.68 s	4.54 s, 4.42 s	4.68 s	4.83 s
H-1	7.90 d	8.05 d, 8.04 d	8.03 d	8.04 d
H-2	7.72 t	7.80 t, 7.79 t	7.78 t	7.78 t
H-3	7.38 d	7.41 d, 7.40 d	7.39 d	7.39 d
4-OCH_3	4.07 s	4.11 s, 4.10 s	4.08 s	[4.08 s]
H-7	5.20 s (br)	5.34 m, 5.30 m	5.32 dd	5.32 dd
H-8A	[2.65–1.95 m]	2.22 m	2.15 dd	2.13 dd
H-8B	[2.65–1.95 m]	2.38 m	2.44 dt	2.51 dt
H-10A	2.83 d	3.07 d, 3.06 d	3.03 d	3.00 d
H-10B	3.09 d	3.30 d	3.28 dd	[3.26 dd]
2 × H-14	4.75 s	4.79 s, 4.78 s	4.75 s[i]	4.75 s
H-1′	5.51 s (br)	5.61 d, 5.57 d	5.47 t	5.45 dd
H-2′A	} 1.80 m	} 1.84 m	1.84 dt	1.54 ddd
H-2′B			2.09 dt	2.11 ddd
H-3′	3.20–2.80 m	[2.69 m]	3.60 m	[3.25 dt]
H-4′	[3.67 m]	[3.71 m]	3.65 d	[4.05 d]
H-5′	3.98 m	4.05 m	[4.04 m]	3.96 dq
3 × H-6′	1.38 d	1.40 d, 1.39 d	1.37 d	1.30 d
H-2″A, H-2″B } OCH_2	[3.67 m]	[4.03–3.66], 3.58 m	3.63 d [4.04 d][j]	3.76 dd 4.13 d[l]
H-6″A, H-6″B } OCH_2			3.80 dd 4.15 d[k]	3.43 dd 4.15 dd[m]
2 × H-3″ (NCH_2)	[2.65–1.95 m]			
2 × H-5″ (NCH_2)		[2.69 m]		
H-3″ (NCHCN)		[4.03–3.87 m]	[4.07 m][j]	4.02 d[l]
H-5″ (OCHN)			4.64 s (br)[k]	[4.07 m][m]

[a] In ppm from internal Me_4Si, δ = 0.0, in $CDCl_3$. [b] Signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), br (broad), and ex (exchanged with D_2O). Brackets indicate overlapping signals. Integrated signal areas were as predicted from the structures. See ref 8 for position numbering. [c] On a Varian EM 390 spectrometer. Data from ref 1. [d] On a Bruker WH-400 at the University of Alberta, Edmonton. We thank Prof. J. William Lown, Chemistry Department, for arranging to have 4 run by Dr. S. Yen. [e] The data are from ref 1, on a 65:35 mixture of diastereoisomers. [f] On a Varian XL-400 spectrometer at SRI. We thank G. Detre for the data. [g] The sample was 91% pure containing 2% of **6A** and 7% of **6C**. A sample containing 28% of **6A** was run at 300 MHz on a Nicolet Spectrometer at Stanford University and at 500 MHz through the courtesy of Dr. Daniel Lednicer of Adria Laboratories. Side peaks attributable to signals from **6A** were at δ 13.96 s (6-OH), 1.38 d (3 × H-6′), and 4.57 br s (H-5″). Otherwise, the spectra were essentially the same. [h] The sample was 92% pure containing 7% of **6A**. [i] In a DCl-free sample of $CDCl_3$ this was a doublet (J = 4.6 Hz) and a triplet for 14-OH appeared at δ 2.98. There were no other spectral differences from the presence or absence or solvent DCl. [j] Assigned by decoupling at 3.63 ppm. [k] Decoupling at 3.80 ppm. [l] Decoupling at 3.76 ppm. [m] Decoupling at 3.43 ppm.

Table II. ^{1}H NMR Coupling Constants (Hz)[a,b]

assigned couplings (J)	compounds: 400 MHz, O-MRA-CN (6B)	400 MHz, O-MRA-CN (6C)	daunorubicin[c]	*N*,*O*-isopropylidene-daunorubicin[c]
7,8eq	2.3	2.3	2.5	2.5
7,8ax	3.9	3.7	4.1	4.0
8eq,8ax	14.7	14.7	14.9	15.0
10eq,10ax	19.0	18.0	19.0	19.0
1′,2′A	5.8	8.6	4.1(eq,ax)[d]	8.0
1′,2′B	5.8	5.9	~1.0(eq,eq)[d]	6.0
2′A,2′B	15.3	15.9	13.3[d]	15.5
2′A,3		8.6	12.2(ax,ax)[d]	4.0
2′B,3		3.2	5.2(ax,eq)[d]	3.0
4′,5′	2.1	2.1	1.5	2.5*e*
2″A,2″B	10.1	11.6		
2″A,3″		2.7		
5″,6″A	2.4	9.1		
5″,6″B		3.1		
6A″,6″B	13.0	10.1		

[a] Procedural and experimental details as in Table I. [b] The expected (Arcomone, *Tetrahedron Lett.* **1968**, 3349, 3353) $J_{1,2} = J_{2,3}$ = 8.0, $J_{10_A,10_B}$ = 19 Hz, $J_{5',6'}$ = 6.5 Hz were observed in all spectra and are not listed. [c] Data from ref 14. [d] In $CDCl_3$–benzene. [e] In acetone.

gave primarily unstable products which were not recovered after chromatography.

Stereoisomerism

Because there are two new chiral centers in **5** and **6**, each of these compounds can exist in four diastereoisomeric forms. Identifying three isomers of **6** is consistent with this. The fourth isomer may be among the unknowns detected in the initial product by HPLC but lost on workup. Losses and changes in isomeric composition were,

Table III. Biological Screening Data

compound no.	compound abbrev	isomer ratio[f]	inhibn of synth in leukemia L1210 cells: ED_{50},[b] μM DNA	inhibn of synth in leukemia L1210 cells: ED_{50},[b] μM RNA	ΔT_m of isol helical DNA in soln[c] °C	antitumor efficacy at opt dose in mice:[a] % T/C (mg/kg) ip leukemia P388[d] d 1 ip[g]	ip leukemia P388[d] q4d 5, 9, 13 ip[h]	ip B16 melanoma[e] d 1 ip[g]	ip B16 melanoma[e] q4d 1, 5, 9 iv[g]
	doxorubicin		1.6	0.58	12.8	252 (7.5)	160 (8.0)	192 (7.5)	206 (10)
4	MRA-CN[i]	A/B = 60:40	0.0030	0.00053	8.7	262 (0.012)	187 (0.075)	≥138 (0.0063)[j]	inactive
6	O-MRA-CN	A/B/C = 28:69:0	0.55	0.22	12.8	150 (1.25)	135 (2.0)	≥153 (0.625)[j]	141 (2.5)
6	O-MRA-CN	A/B/C = 2:91:7	1.4	0.79	12.2				
6	O-MRA-CN	A/B/C = 7:0:92	25	2.0	2.6				
5	O-MRD-CN	A/B = 4:91	1.95	0.84	8.3				
5	O-MRD-CN	A/B = 76:12	0.42	0.22	5.0				

[a] T/C = average survival time of treated mice/control mice = antitumor efficacy. [b] ED_{50} = drug concentration for 50% inhibition of the incorporation of [^{3}H]thymidine in the DNA or [^{3}H]uridine in the RNA of actively growing L1210 cells in culture. Drugs were initially dissolved in Me_2SO, and the solution was diluted to a final concentration of 1% Me_2SO. [c] ΔT_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} M EDTA and 5% Me_2SO. Values ≤1 indicate insignificant degree of binding to DNA. [d] Injected 10^6 cells on day 0. T/C ≥120 required for activity. [e] Implanted 0.5 mL of brei on day 0. T/C ≥135 required for activity. [f] In order of reverse-phase HPLC elution. Totals <100% when there are unidentified impurities. [g] Results from Adria Laboratories. [h] Results from NCI. [i] All data on 4 are from ref 1. [j] Incomplete; require test at lower doses.

in fact, encountered during chromatographic purification of **6**. Comparing HPLC analyses before and after columning showed that about half the weight of **6** was lost (382 mg → 171 mg, calculated from amounts given in the Experimental Section). (In contrast, chromatographic recoveries of **4** were about 75%.) As the major isomer, **6B** was also decreased by about one-half (calcd 244 mg → 113 mg), but **6A** decreased to 5% of the amount before chromatography (calcd 127 mg → 7 mg), whereas **6C** increased 5-fold (calcd 11 mg → 51 mg). This suggested that decomposition was accompanied by equilibration toward **6C** as the most stable isomer. A similar equilibration upon chromatography has been described[11] for diastereoisomers of an α-cyanopiperidine.

Differences between the isomers in structural geometry and in steric access to the α-CN could be seen with Drieding molecular models. Models could be constructed for all four of the possible isomers. The evident structural differences were not adequate to explain the absence of one isomer or the differences in stability. Several folded or extended conformations were possible for each of the four isomers, even without going beyond the conventional chair conformation of pyranose and morpholine rings or the axial orientation of the α-CN that is preferred in simple piperidines.[11-13] This type of fused tricyclic system seems to be largely unprecedented, and with the evidently required folding into U or Z forms, the byproduct formation of these structures would scarcely have been predicted.

That conventional assumptions about conformation should not be made in this system was suggested by the ^{1}H NMR coupling constants derived at 400 MHz (see Experimental Section and Table II). On the pyranose ring, $J_{1',2'}$ values were not those of the usually preferred $^1C_{4'}$ (L) chair (e.g., 1.0 Hz for eq,eq and 4.0 Hz for eq,ax as listed for daunorubicin[14]). For **6B**, H-1′ (t) showed J = 5.8 Hz for couplings to both 2′-protons. For **6C**, H-1′ (dxd) showed couplings of 5.9 and 8.6 Hz like those (6.0 and 8.0 Hz) assigned[14] to the skew pyranose of *N,O*-isopropylidenedaunorubicin. Other differences between isomers that were encountered in the pyranose ring were the downfield shifts of H-3′ from δ 2.69 (in **4**) to 3.60 (**6B**) and 3.25 (**6C**).

Morpholine proton assignments depended on the initial assignment in **6B** of the lone proton downfield from the others at δ 4.64 (new relative to **4**) to H-5″ (the OCHN at the O-bridge attachment). As a broad singlet, it clearly lacked diaxial splitting with either H-6″ and would conventionally be designated equatorial. A similar singlet (δ 4.58) was discerned for **6A**. For **6C**, H-5″ was assigned to a partially obscured multiplet at δ 4.07 m, and a diaxial splitting was found with 6″A ($J_{5'',6''A}$ = 9.1 Hz). These assignments suggest that **6A** and **6B** may have the same 5″-chirality, with H-5″ equatorial and the O bridge axial. Then **6C** may have the opposite 5″-chirality with the O-bridge equatorial. The near identity of **6B** and **6C** in chemical shifts for H-3″ (δ 4.07 m and 4.02 d) is consistent with identical chiralities of **6B** and **6C** at the α-CN. The data do not lend themselves to further speculation without additional study, but these isomeric structures should be useful for comparisons of molecular geometry and biological potency.

Biological Results

Table III shows a comparison of doxorubicin and the cyanomorpholino derivative **4** with the new oxazolidinocyanomorpholino derivative **6** in several in vivo and in vitro screens. Three samples of **6** were tested in vitro. One, a mixture of **6A** (28%) and **6B** (69%), was also tested in vivo. The others were samples enriched in **6B** (91%) and in **6C** (92%) and were available in small amounts. When tested in L1210 cells for the inhibition of DNA and RNA synthesis,[15] the sample of **6B** (91%) was remarkably identical in potency with doxorubicin. In comparison to **4**,[16] however, there was a 500- to 1500-fold loss in potency with the oxazolidino bridge. The mixture of **6A** and **6B** appeared to be more potent than **6B**, but the difference may be within experimental error. The sample of **6C**, on the other hand, was considerably poorer in potency, especially against DNA synthesis (18-fold; 3-fold poorer vs.

(11) Bonin, M.; Romero, J. R.; Grierson, D. S.; Husson, H.-P. *J. Org. Chem.* **1984**, *49*, 2392.
(12) Essawi, M. Y. H.; Portoghese, P. S. *J. Org. Chem.* **1983**, *48*, 2138.
(13) Earlier assignments of an α-CN to a preferred equatorial orientation may have been based on insufficient NMR data. Gless, R. D.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 1324. Koskinen, A.; Lounasmaa, M. *Heterocycles* **1983**, *20*, 563.
(14) Mondelli, R.; Arnone, A.; Fronza, G.; Cassinelli, G.; Gioia, B.; Vigevani, A. *J. Chem. Res., Synop* **1984**, 180.
(15) Test procedure from Tong et al. (Tong, G. L.; Lee, W. W.; Black, D. R.; Henry, D. W. *J. Med. Chem.* **1976**, *19*, 395) as modified in footnote *b* of Table II.
(16) **4** was the 65:35 mixture of diastereoisomers usually formed by reductive alkylation.

Table IV. Octanol–Buffer Partition Coefficients (Log P)[a]

compound no.	compound abbrev	log P octanol–phosphate, pH 7.4	octanol–phosphate, pH 6.5	octanol–phosphate, pH 6.0	octanol–citrate, pH 4.0
	doxorubicin	0.07			
3	MRA	1.73	1.42	0.96	−1.1
4	MRA-CN	1.91	1.88	1.93	1.81
6	O-MRA-CN	2.41	2.29	2.40	2.24

[a] Log P = logarithm of ratio of partitioned concentration in organic phase/concentration in H_2O phase, measured from UV absorbances at 235.5 nm. Number of determinations was from two to five (one on 3 at pH 4.0), using two or three octanol–buffer ratios varying from 1:10 to 1:50; standard deviations ranged from ±0.02 to ±0.10. Buffer concentrations were 0.1 M. Compound 4 was the 60:40 mixture of diastereoisomers; 6 was a 29:68 mixture of A/B.

RNA synthesis). Clearly, in structure 6, biological activity can vary with diastereoisomerism. We previously[1] cited the imino quinone of 4 as a case where activity did not vary with diastereoisomerism.

The sample of 6C also showed barely significant stabilization of helical DNA toward thermal denaturation (ΔT_m = 2.6°), whereas the other two samples of 6 were indistinguishable from doxorubicin in this test for DNA binding. In fact, the values (12.8°, 12.2°) for those samples are surprisingly high for 6 as another nonbasic analogue. It is usually assumed that a basic amine is required for intercalative binding of anthracyclines with DNA. The nonbasicity of both 4 and 6 was shown (Table IV) by the lack of change in partition coefficient (i.e., log P) with octanol when the aqueous phosphate buffer was varied from pH 7.4 to 4.0, whereas the log P of 3 was dramatically changed over this range. Unlike 3, both 4 and 6 remained in the organic phase.

A series of antitumor tests in mice showed similar potency relationships between doxorubicin, 4, and 6 (as the 28:69 A/B mixture). In tests against leukemia P388 by intraperitoneal (ip) dosing in two schedules, once on day 1 and once each on days 5,9,13,[17] this sample of 6 was much less potent (100 times and 27 times, respectively) than 4 but was 4–6 times more potent than doxorubicin. In both regimens, 6 showed moderate efficacy (T/C = 150% and 135%). In tests against B16 melanoma[17] as a solid tumor model, results after ip dosing again showed the retention of moderate antitumor efficacy in 6, with a 100-fold loss of potency relative to 4 and a 10-fold gain relative to doxorubicin. However, further testing of 4 and 6 at lower levels in this regimen is needed to quantify these results. Of particular interest was the activity of 6 (T/C = 141% at 2.5 mg/kg) against B16 melanoma after intravenous (iv) administration. The iv route is important in clinical use. The usually potent cyanomorpholino compound 4 was inactive in this regimen (although 4 was active[1] iv vs. mouse P388). Doxorubicin showed higher efficacy (T/C = 206%) but was 4 times less potent.

In related studies, these compounds were recently compared for their growth inhibitory potencies against a doxorubicin-resistant subline of leukemia P388.[18] The cyanomorpholino compound 4 was apparently the first analogue that was not cross-resistant[5–7,18] with doxorubicin; it retained potency against resistant tumor lines both in mice and in cell culture. Possible activity against clinically resistant tumors is an important criterion for analogue selection and drug development. Interestingly, 6, though less potent than 4, retained the non-cross-resistance of 4 against the P388 subline in culture,[18] as measured by comparison of resistance indices (ratios of IC_{50} vs. resistant/sensitive sublines of P388). Consequently, 6 as the A/B 28:69 mixture was tested against the doxorubicin-resistant P388 subline in the mouse. The results were not those predicted in cell culture, as 6 was inactive in two ip regimens (days 1, 5, 9 and days 1–5) up to doses of 4 mg/kg. Reasons for this discrepancy are not clear.

The test results with 6 show further the strong structural specificity for activity in the morpholino series. In every test, there is a dramatic loss in potency in going from 4 to the O-bridged structure 6. This may be an effect of increased structural rigidity in the fused tricyclic system. The loss in potency and the retention of cross-resistance in vivo may be somewhat offset by the appearance of activity against B16 melanoma after iv dosing. Of significant interest is that the in vitro potency of 6 varies with its diastereoisomeric forms. The least stable (and presumably most reactive) isomer 6A is the most potent, and the most stable (and least reactive) isomer 6C is the least potent. This is consistent with our suggestions[4,5,18] that the biological activity of 4 may depend on the chemical reactivity of the α-cyano amine as a new functional group in the anthracycline molecule. The nature of the reactivity, perhaps involving dissociation of the CN, will require further study.

Experimental Section

Solutions in organic solvents were dried over anhydrous Na_2SO_4 and filtered through Celite. Evaporations were carried out under reduced pressure (bath ≤ 35 °C) on a rotary evaporator. Residues from CH_2Cl_2 or $CHCl_3$ solutions were generally solvated glasses. Evaporating solutions of these residues in CH_2Cl_2–CH_3OH mixtures (4:1, then 1:2) gave amorphous solids that could be triturated with CH_3OH and dried free of solvation. Thin-layer chromatography (TLC) was carried out on silica gel (0.25 mm GF, Analtech) plates. Preparative TLC was done on 20 × 20 cm plates with 0.5, 1.0, or 2.0 mm of silica gel. Product purity was quantified by reverse-phase analytical HPLC, monitoring at 254 nm, flow rate 2 mL/min. System A was a Waters RCM-100 Radial Compression Separation System using a 10-μm, Radial Pak C-18 column in 0.05 M, pH 4.0, citrate buffer and CH_3CN (ratio of buffer to solvent given for each compound). System B was a Spectra-Physics SP-8100LC using a Waters Z-Module Radial-Pak Nova C-18 5-μm column in 0.1 M NaH_2PO_4 (pH 4.4) and CH_3CN and is now preferred for better resolution and reproducibility. UV–vis spectra were determined on a Perkin-Elmer Model 575 recording spectrometer. Electron-impact mass spectra (EI-MS) were determined on an LKB 9000 GC-MS at 12 eV interfaced with a PDP12 computer, generally on per(trimethylsilyl) derivatives prepared at 80 °C. DCI-MS were determined on a Ribermag R10-10C GC-MS with NH_3 as the reagent gas.

(17) Screening tests at Adria Laboratories, Inc., Columbus, OH, or under the auspices of the National Cancer Institute, Division of Cancer Treatment, Development Therapeutics Program, were done according to NCI protocols described by Geran et al.: Geran, R. I.; Greenberg, H. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1–103. We thank Dr. V. L. Narayanan for the data from NCI.

(18) Streeter, D. G.; Taylor, D. L.; Acton, E. M.; Peters, J. H. *Cancer. Chemother. Pharmacol.* **1984**, *14*, 160. The sample of 6 was the A/B = 28:69 mixture. The other samples of 6 were later shown to be non-cross-resistant also.

^{1}H NMR Spectra. Chemical shift data for **6B** and **6C** are listed in Table I, in comparison with the previous data on **3** and **4**. Signals for **6A** observed in a mixture with **6B** are listed in Table I, footnote *g*. Most of the assignments were made by comparison of **6B** and **6C** with **4**, as the differences, discussed above, were few. Decoupling experiments were used, as noted, for the analysis of **6B** and **6C** but not for the previous spectra[1] of **4** as the mixture of diastereoisomers or of **3** at 90 MHz. Hence coupling constants in the pyranose and morpholine rings of **4** were not available for comparison. In Table II, pyranose couplings for **6B** and **6C** were compared with literature values[14] of daunorubicin and its relevant *N,O*-isopropylidene derivative. Analyses of proton resonance in the morpholine ring began with the assignment in **6B** of the new, lone proton at δ 4.64 to H-5″, at the attachment of the O bridge. Further assignments were from the decouplings. The spectrum we observed for **6C** was identical with a spectrum provided by Dr. Sergio Penco, Farmitalia Carlo Erba, Milan, on a sample independently isolated from the reductive alkylation.

4′,5″-Anhydro-3′-deamino-3′-(3″-cyano-5″-hydroxy-4″-morpholinyl)doxorubicin (6). The procedure[1] for reductive alkylation of doxorubicin with 2,2′-oxybis[acetaldehyde] was repeated, except that the dialdehyde solution at pH 4 was adjusted to pH 2.7 with glacial acetic acid (0.1 mL/1.5 mmol of periodate used) instead of to pH 7, and the solution was then treated with a solution of NaCN (equimolar to the doxorubicin) in 2 N acetic acid. The pH of the red, semiaqueous reaction mixture was not measured. Upon workup of the product from 696 mg of doxorubicin, the combined $CHCl_3$ extracts were simply evaporated, and the residual glass (0.724 g, 95% of theory) was analyzed by HPLC. System A (56:44) showed the presence of 10% of **4** (retention time 3.8 min; no 13-dihydro derivative at 2.3 min), 53% of **6** (8.1 min), and 8% of **3** (10.5 min; 1% 13-dihydro at 5.7 min). The residue was partitioned[1] between $CHCl_3$ and 0.1 N acetic acid to give 0.554 g of neutral products from the $CHCl_3$ layer. System B (63:37) showed the presence of 8% of **4A** (5.3 min), 4% of **4B** (5.5 min), 23% of **6A** (11.5 min), 44% of **6B** (13.4 min), and 2% of **6C** (17.1 min). A solution of this material in 2 mL of CH_2Cl_2 was added to a 1.5 × 37.5 cm column of silica gel, which was eluted with CH_2Cl_2 (100 mL) and then CH_2Cl_2–CH_3OH (99:1), 300 mL; 98:2, 400 mL; 97:3, 200 mL; and 90:10, 200 mL). Eluate fraction I (335 mL) was set aside. Fraction II (35 mL) was evaporated to yield 0.022 g (**6A**/**6C** = 8:92); fraction III (80 mL), 0.047 g (**6A**/**6B**/**6C** = 6:43:51); fraction IV (65 mL), 0.102 g (**6A**/**6B**/**6C** = 2:91:7). Purity of **6** (total yield 0.171 g, 22%) in these samples was 96–98%, with <0.1% of **4**. All samples of **6** were essentially identical by spectral analyses. DCI-MS, *m/e* 637 (M + H), 610 (M − HCN + H), 241 (sugar moiety X + H_2O), 223 (X), 214 (X − HCN + H_2O), 196 (X − HCN); EI-MS at 70 eV showed a base peak at *m/e* 27 assigned to HCN. UV–vis λ_{max} (CH_3OH) 233 nm (ϵ 40 500), 252 (27 200), 289 (9470), 478 (12 900), 495 (12 800), 530 (7010). Anal. ($C_{32}H_{32}N_2O_{12}\cdot 0.5H_2O$) C, H, N.

The nonbasicity of **6** was demonstrated in an experiment where a 550-mg sample containing roughly equal amounts of **3**, **4**, and **6** (ca. 25% each, along with 13-dihydro derivatives of **3** and **4**) was dissolved in 50 mL of $CHCl_3$–CH_3OH (39:1) and extracted repeatedly with 0.1 N acetic acid in 15-mL portions. The $CHCl_3$ solution was evaporated and shown by TLC analysis ($CHCl_3$–acetone–CH_3OH, 9:5:1) of the residue (217 mg) to contain essentially all of the **4** and **6** and a trace of **3**. The acid extracts, upon neutralization, extraction, and evaporation, yielded a residue (209 mg) that contained essentially all of the **3**, a trace of **4**, and no **6**.

4′,5″-Anhydro-3′-deamino-3′-(3″-cyano-5″-hydroxy-4″-morpholinyl)daunorubicin (5). A solution of 0.208 g (2.00 mmol of 1,4-anhydroerythritol in 4 mL of H_2O was oxidized with 0.321 g (1.50 mmol) of sodium metaperiodate at room temperature for 2 h.[1,2] The resultant dialdehyde solution at pH 5 was treated with 0.056 g (0.10 mmol) of daunorubicin hydrochloride. After 30 min, the mixture was diluted with 5 mL of acetonitrile, and a solution of 0.049 g (1.0 mmol) of NaCN in 1.0 mL of H_2O (adjusted to pH 6.3 with acetic acid) was added. The mixture was stirred for 1 h, diluted with 5% aqueous $NaHCO_3$, and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried and evaporated to yield 0.071 g of residue (111% of theory). Preparative TLC in $CHCl_3$–CH_3OH (19:1) gave 28 mg of **5**. Purification by preparative TLC in EtOAc gave two fractions. The first (9 mg) contained 91% of **5B** according to HPLC analysis in system B (54:46), retention time 13.0 min; and 4% of **5A**, HPLC retention time 11.8 min (i.e., **5B** was faster by TLC but slower by reverse-phase HPLC). The second (12 mg) contained 12% of **5B** and 76% of **5A**. Compound **5B** was identified by DCI-MS, *m/e* 621 (M + H), 594 (M − HCN + H); negative ion DCI-MS, *m/e* 620 (M), 593 (M − HCN); and ^{1}H NMR at 100 MHz, which (as for **6**) showed absence of any 5″-H_2 resonance near δ 2.7 and appearance of a new signal at δ 4.67 s for OCHN at 5″. The DCI-MS of **5A** was identical with that of **5B**, and the ^{1}H NMR showed the new singlet for OCHCN at δ 4.60.

Further MS analysis of another fraction from the preparative TLC in $CHCl_3$–CH_3OH (19:1) showed evidence (negative ion DCI, *m/e* 647; EI-MS after trimethylsilylation[1] as described) for presence of the 3″,5″-dicyanomorpholine.

Partition coefficients were measured by modification of a previous technique.[19] For the aqueous phase, distilled water was saturated with reagent grade 1-octanol. For the organic phase, 1-octanol was saturated with distilled water. Samples were dissolved in either phase. Equal volumes of the phases were mixed and inverted 100–150 times in 5 min to achieve equilibrium. The water and the octanol phases were separated by centrifugation at 2000 rpm for 5 min. The concentration of sample in the octanol or aqueous phase was determined with 1-cm cells in a Perkin-Elmer 575 spectrophotometer, and the concentration in the other phase was obtained by difference. All determinations were done in duplicate at 2 or 3 octanol–water ratios.[20]

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