DCC, 684 mg (3.28 mmol), in 10 mL of THF. After the solution was stirred for 3 h and ice-bath cooled, it was allowed to stand for 3 days at room temperature, concentrated, and worked up as in method A to secure 165 mg (27%) of **13**, mp 214–216 °C.

4-Methyl-1,9-bis[carbonyl-L-threonyl-D-valyl-L-propylsarcosyl-L-*N*-methylvaline-(*threonine hydroxyl*) Lactone] Phenazine (2). All glassware was carefully dried and reactions were protected against moisture. A solution of 90 mg (0.16 mmol) of blocked pentapeptide **13** in 0.5 mL of trifluoroacetic acid was allowed to stand for 30 min and then concentrated, leaving a pale amber residue which was dissolved in 0.2 mL of dry DMF and 75 μL (0.55 mmol) of triethylamine.

The acid chloride of **5** was prepared by mixing 14.6 mg (0.052 mmol) of **5** (dried iv) with 0.3 mL of dry DMF and 12 μL (0.17 mmol) of $SOCl_2$, freshly distilled. After the mixture had been stirred for 5 min, solution occurred, followed immediately by separation of a yellow solid. The mixture was heated at 40 °C for 30 min and concentrated, leaving a yellow-green solid residue, which was slurried in 0.2 mL of dry DMF and added to the DMF solution of peptide and triethylamine.

The mixture was stirred at room temperature and the course of reaction followed by TLC [on Whatman reversed phase KC_{18} plates (solvent system A)]. After 5 h, the mixture showed two yellow spots, one at R_f 0.9, corresponding to **5**, and one at R_f 0.4, which was desired product **2**. After 6 days, some **5** was still seen on TLC. Precipitated $Et_3N{\cdot}HCl$ was removed by filtration and washed with a small volume of DMF; addition of H_2O to the filtrate caused separation of a small amount of **5**, which was removed by filtration. Concentration of the filtrate left 182 mg of yellow oil: TLC R_f (solvent system F) 0.5, 0.1, R_f (solvent system G) 0.9, 0.6; white fluorescence at origin in both systems. This was purified on the Sephadex column (86 cm). After 216 mL of colorless eluate had been collected, 2-mL fractions were collected and TLC of yellow fractions examined. Fractions 6–23 showed a major yellow spot, R_f 0.5 (solvent system F), with a minor brown spot at the origin. From fractions 40–46 was obtained 33 mg (yellow oil mixed with whitish solid), showing two spots on TLC (solvent system G), R_f 0.9, 0.6, with streaking; fractions 104–128 yielded 2 mg of yellow solid, identical on reversed phase TLC with **5**.

Concentration of fractions 6–23 yielded 34 mg (54% crude yield) of yellow solid, which was partially purified by several recrystallizations from EtOAc and from acetone, and finally by thick-layer chromatography using EtOAc–acetone (2:1); the yellow band at R_f 0.5 was cut out and extracted with acetone, yielding 19 mg (30%) of **2**: bright yellow crystalline solid; mp 243.5–245 °C; TLC R_f (solvent system F) 0.5, R_f (solvent system A) 0.38; UV λ_{max} (0.01 M phosphate buffer, pH 7) 197 nm (ε 64300), 254 (57000), 350 (sh)(7330), 365 (11300). Anal. ($C_{61}H_{84}N_{12}O_{14}{\cdot}H_2O$) C, H, N.

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7-(Aminoethyl) Ether and Thioether of Daunomycinone[1]

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One-step treatment of daunomycinone with excess 2-aminoethanethiol and 2-aminoethanol in trifluoroacetic acid afforded at C-7 the thioether (77% yield) and ether (30% after recycling), respectively. Stereoselectivity for the natural 7*S* over the 7*R* configuration was greater for the ether (97:3) than for the thioether (2.5:1). Esterification of daunomycin at C-7 with β-alanine was accomplished through the mixed anhydride of Z(OMe)-β-alanine. Preliminary biological tests suggest that the antitumor and DNA interactive properties of the anthracyclines can be retained in such structures.

The chemically labile glycosidic bond at the 7 position of daunorubicin (**1**) and adriamycin (**2**) is cleaved rapidly in the metabolism of these useful anticancer drugs. Both hydrolytic and reductive glycosidases deactivate **1** and **2**

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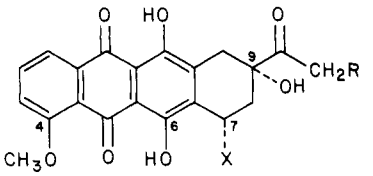

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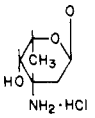
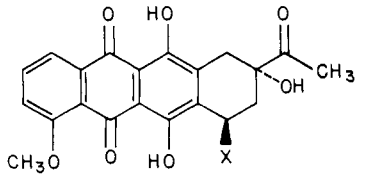
1	H	
2	OH	
3	H	OH
4	OH	OH
5	H	H
6	OH	H
7	H	$OCOCH_2CH_2NHZ(OMe)$
8	H	$OCOCH_2CH_2NH_2 \cdot HCl$
9	H	$SCH_2CH_2NH_2 \cdot HCl$
10	H	$OCH_2CH_2NH_2 \cdot HCl$



11, X = OH
12, X = $SCH_2CH_2NH_2 \cdot HCl$

with formation of the aglycones **3** and **4** and their 7-deoxy analogues **5** and **6**.[3] The reductive cleavage, which is the major pathway in human metabolism, is unprecedented, and the mechanism of this process is of considerable interest. There is experimental evidence that the 6-OH of **1** and **2** (and of the related anthracycline, steffimycin) plays an essential role in the reductive cleavage.[4,5] Because NADPH is required as cofactor, a suggested mechanism is nucleophilic attack of hydride ion from NADPH at carbon-7.[4] Several authors have proposed an *o*-quinonemethide (involving a carbonyl at C-6) as intermediate structure.[4-6] It was also proposed that a more favored quinonemethide, involving extended conjugation from C-7 to C-5, could be formed following bioreduction of the quinone ring as an activation step.[6] The $Na_2S_2O_4$ reduction of **1** to give[7] 7-deoxydaunomycinone (**5**) was cited as an example of this in a purely chemical system, because it no doubt involves initial reduction of the quinone. Thus, **1** and **2** might fit the concept of bioreductive alkylation agents[6,8] through the reactivity developed at C-7. The possible covalent binding between C-7 and various biological nucleophiles was suggested[4] as a basis for the toxic side effects of **1** and **2**. These biological nucleophiles might even include DNA, since it is recognized[9] that the familiar noncovalent DNA-binding properties of **1** and **2** are insufficient to explain the various effects that occur with DNA. Exploration of these ideas by the synthesis and evaluation of analogues with various leaving groups at C-7 was suggested.[6]

Changes in the 7-*O*-glycosyl group are also of interest for simplified structures which might retain the activity of **1** and **2**, be more accessible to total synthesis, and even show reduced side effects. The basic amino substituent of the sugar moiety is important[10] for the potent activity of **1** and **2**, but considerable simplication in the sugar structure might be permitted so long as the free amino group is retained. This is illustrated by the moderate antitumor activity in vivo that we previously reported[10] for the β-alanine ester **8** of daunomycinone (**3**). Updated values are given in Table I. The ester **8** was synthesized by coupling **3** with *N*-[[(*p*-methoxybenzyl)oxy]carbonyl]-β-alanine[11] by the mixed anhydride method.[12] Choice of the methoxy-substituted blocking group was dictated by the need for mild deblocking of intermediate **7** with anhydrous hydrogen chloride in cold dioxane. Chromatographic purification of **8** was required, and the overall yield was 6–7%. For an alternative, improved synthesis of additional analogues, it seemed that the potential alkylating activity at C-7 might be employed for the direct attachment of various 7 substituents, if the proper conditions could be devised. This report describes the first examples of the synthesis of anthracycline analogues by alkylation with daunomycinone at C-7.

Chemistry. More stable than the ester link of **8** would be an ether or thioether bond at C-7. Initial experiments were toward synthesis of a thioether, since sulfur is a better nucleophile than oxygen. Treatment of **1** (as free base) with sodium benzylmercaptide in dimethylformamide (DMF) afforded a 15–20% yield of the 7-(benzylthio) compound (X = SCH_2Ph; of undetermined stereochemistry at C-7), as indicated by mass spectral analysis for the expected molecular ion and by ^1H NMR for the expected phenyl signal at δ 7.27. About 10% of the 7-deoxy compound **5** was formed as well. The ejection of the sugar and subsequent coupling may have occurred through a quinonemethide intermediate. However, the reaction could not be extended. Attempted coupling with aminoethyl mercaptides gave complex mixtures containing considerable amounts of **5**.

Acid-catalyzed coupling was an alternative, based on the acidic methanolysis of nongalamycin or nogalarol to give 7-*O*-methylnogalarol.[13] Furthermore, the acid-catalyzed epimerization that occurs in favor of the natural 7,9-cis configuration[14] of **3** suggested that acidic coupling might also favor the natural configuration at C-7.

Successful coupling was accomplished when **3** was treated with 5 equiv of 2-aminoethanethiol in trifluoroacetic acid at room temperature. The crude yield of thioether was 77%, isolated as the hydrochloride. The product proved to be a mixture of isomers **9** and **12** in a ratio of 2.5:1. The major isomer **9** was purified by recrystallization. A fraction enriched in the minor isomer **12** was obtained by chromatography. By spectral analysis, it differed little from **9**, except in the ^1H NMR signal for H-7, and **9** and **12** were identified as 7-epimers according to a previous method of assignment[7,14-16] based on the peak widths ($\nu_{1/2}$) for H-7. Thus, the major isomer showed $\nu_{1/2}$ = 7 Hz (which approximates $J_{eq,eq} + J_{eq,ax}$) for H-7 with an equatorial orientation in the natural 7*S* configuration of **9**, while the minor isomer **12** showed $\nu_{1/2}$ = 13 Hz for the axial H-7 of the 7*R* configuration. The related values for **3** and 7-*epi*-daunomycinone (**11**) were 7 and 17 Hz, respectively.[16] The coupling reaction to give **9** and **12** was less favored if only 1 equiv of thiol was used (20% yield) or if daunorubicin (**1**) was used in place of **3** (5% yield with 1 equiv of thiol).

The coupling reaction was also successful in the formation at C-7 of an ether bond with 2-aminoethanol. The yield was lower, but the stereoselectivity in favor of the natural 7*S* configuration was greater. Heating of **3** with a 30-fold excess of 2-aminoethanol in trifluoroacetic acid was required. The reaction was interrupted after 4 h, when only 11% of the amino ether **10** had been formed but when decomposition of unreacted **3** was still negligible. During this time, the unreacted **3** had undergone considerable epimerization to **11** but little aromatization to dianhydrodaunomycinone, and the mixture of **3** and **11** could be

Table I. Comparison of Biological Test Data

no.	leuk P388 in mice: antitumor efficacy at opt dose[a] qd 1–9: % T/C (mg/kg)	leuk P388 in mice: antitumor efficacy at opt dose[a] q4d 5, 9, 13: % T/C (mg/kg)	isolated helical DNA in soln:[b] ΔT_m, °C	leuk L1210 cells in cult: inhibn of synth;[c] ED_{50}, μM DNA	leuk L1210 cells in cult: inhibn of synth;[c] ED_{50}, μM RNA
1	160 ± 27 (0.78)	133 (2)[d]	11.2	0.4	0.3
2	197 ± 26 (0.78)	130 (1)[d]	13.6	2	0.7
8	169, 126 (6.25)	116 (50)[d]	1.0	8	6
9		109 (200)	6.3	6	4
10		124,[d] 112 (50)	8.2	2	1

[a] Reference 17. Mice injected ip with leukemia cells on day 0 were treated on days 1–9 or days 5, 9, and 13 with the specified dose. Increase in survival time of (treated mice)/(control mice) = T/C or antitumor efficacy and must be ≥125% for an active result. Data are from single tests, except for 1 and 2, qd 1–9, where averages of eight tests are given. [b] The thermal denaturation temperature was determined as in ref 18, except that the 0.010 M phosphate buffer was at pH 7.0 and contained EDTA 10^{-5} M plus 5% Me_2SO to solubilize the compounds. [c] Procedure as in ref 18 for 8 but with addition of 1% Me_2SO for 1, 2, 9, and 10. [d] Simultaneous single test result.

separated from the product 10 and retreated. After three recyclings, the yield of 10 was 30%. A trace contaminant was presumably the 7-epimer and was removed by crystallization. The 7S configuration of 10 was assigned from the peak width of the signal for an equatorial H-7, $\nu_{1/2}$ = 9 Hz.

One experiment was done to test the ease of reductive cleavage at C-7 of the new analogues compared to 1. The thioether 9 in aqueous methanol–tetrahydrofuran solution was treated[16] with 4 equiv of $Na_2S_2O_4$ and excess $NaHCO_3$. After 15 min there was a high yield of a product that was chromatographically identical with 5, suggesting that 9 is a substrate for reductive activation and cleavage.

Biology. Compounds 8–10, compared with 1 and 2, were screened for antitumor properties using lymphocytic leukemia P388 in mice.[17] Test results are presented in Table I. A schedule of nine daily doses (qd 1–9) was initially used on 1, 2, and 8, but the NCI's currently preferred schedule (q4d 5, 9, 13) allows the implanted leukemia to progress for 4 days before the first dose is administered, as a more stringent test. Thus, the β-alanine ester 8 was active when tested qd 1–9 but was inactive against the advanced leukemia. The thioether 9 and ether 10 were tested only q4d 5, 9, 13. Compound 9 was inactive, even at a very high dose (200 mg/kg).

The ether 10 showed borderline activity (T/C = 124%) alongside 1 and 2 (133 and 130%) in one simultaneous test, but this result was not repeated when 10 was retested alone. It was surprising that prototypal structures such as 8 or 10 would show any activity at all, after the amino sugar moiety of 1 and 2 was so grossly simplified, the linkage at C-7 was changed, and (in 10) the distance to the NH_2 was shortened. The detection of even marginal activity with 10 suggests further elaboration of the amino ether side chain to optimize its structure.

Interest in such structures was further suggested when the DNA interactive properties[18] of the compounds were measured. Compounds 9 and 10 retained an appreciable effect (ΔT_m) on the thermal denaturation temperature of isolated helical DNA, though not as strong as 1 and 2, but 8 showed no significant effect. Compound 8 was also the least inhibitory to DNA and RNA synthesis in L1210 cells, as measured by the doses (ED_{50}) required for inhibition of incorporation of [^{3}H]thymidine and [^{3}H]uridine. On the other hand, the ether 10 was nearly as inhibitory as 1 and 2.

Experimental Section

Solutions in organic solvents were dried over Na_2SO_4 and filtered. Evaporations were carried out in vacuo on a spin evaporator. Melting points are uncorrected. Spectra were determined as follows: UV–visible, Cary 11 or Cary 14 recording spectrometers; IR in Nujol mull, Perkin-Elmer 137; ^{1}H NMR in Me_2SO-d_6 solutions (Me_4Si internal reference, δ 0.0), Varian XL-100 (signals are designated s, singlet; d, doublet; t, triplet; or m, multiplet); mass spectra, LKB Model 9000 GC–MS at 12 eV, interfaced with PDP12 computer. The R_f values are for thin-layer chromatography (TLC) on silica gel plates. Analytical high-pressure liquid chromatography (LC) was done on a Spectra Physics Model 3500 liquid chromatograph using a Spherisorb Silica ODS 10 column (0.4 × 30 cm) eluted with MeOH–0.1 M NaH_2PO_4 (2:1) at a flow of 2.0 mL/min and with UV detection at 254 nm. Optical rotations were determined on a Jasco ORD/UV5 spectropolarimeter at 589 nm.

7-*O*-[*N*-[[(4-Methoxybenzyl)oxy]carbonyl]-β-alanyl]-daunomycinone (7). An anhydrous solution of 94 mg (0.37 mmol) of Z(OMe)-β-alanine[11] (predried at 0.1 mm over P_2O_5) in 1.5 mL of pyridine (stored over Linde 4Å molecular sieve) was stirred in an ice bath and treated with 0.10 mL (0.86 mmol) of freshly distilled benzenesulfonyl chloride. After 10 min, 199 mg (0.50 mmol) of predried 3 was added, and most of the solid dissolved. After 9 h, another equal portion of separately prepared mixed anhydride solution was added, and stirring was continued for an additional 11 h, when no 3 was detectable by TLC (CH_2Cl_2–MeOH, 10:1). The red solution was poured into 10 mL of H_2O and was extracted with CH_2Cl_2 (3 × 25 mL). The extracts were washed with H_2O, dried, and concentrated to 422 mg of solid. A solution in a few milliliters of warm CH_2Cl_2 almost immediately deposited 117 mg (37%) of a red-orange precipitate: mp 198–204 °C dec; TLC R_f (CH_2Cl_2–MeOH, 25:1) 0.8 vs. R_f 0.5 for 3; IR 2.87, 2.93 (OH, NH), 5.75, 5.83 μm (C=O). An analytical sample from another run had mp 205–207 °C dec. Anal. ($C_{33}H_{31}NO_{12}$·0.5H_2O) C, H, N. At eight times the scale, the yield was about 17%.

7-*O*-(β-Alanyl)daunomycinone Hydrochloride (8). The blocked ester 7 (962 mg, 1.52 mmol) was finely ground, predried in vacuo, and suspended in 80 mL of 2 N anhydrous HCl in dioxane. The reaction occurred in suspension with stirring for 4 h. The solvent was evaporated in vacuo and the remaining HCl was removed by coevaporating with CH_2Cl_2. The residue was stirred with 20 mL of MeOH, and the insolubles were removed by filtration through Celite (diatomaceous earth filter-aid). The product in the filtrate was adsorbed (with evaporation of the MeOH) on 20 g of silica gel (Bio-Sil A, 200–325 mesh) for subsequent chromatography on a column (50 × 3 cm) of 150 g of silica gel, which was eluted with CH_2Cl_2–MeOH (5:1). Fractions containing small amounts of unreacted 7 mixed with 3 were followed by impure 8 and finally by fractions containing 220 mg of pure 8. The Celite from the filter was extracted with hot $CHCl_3$ for recovery of unreacted 7, which was converted to an additional 184 mg of pure 8. The combined product was dissolved in 95% EtOH, precipitated with Et_2O, and added to 45 mg from rechromatography to yield 335 mg (43%): TLC R_f (CH_2Cl_2–MeOH, 5:1) 0.2; ^{1}H NMR (Me_2SO-d_6) δ 7.9 (m, H-1, H-3), 7.65 (m, H-2), 6.2 (br s, H-7, $\nu_{1/2}$ = 7 Hz), 4.02 (s, OCH_3), 3.6–2.5 (10-H_2 and NCH_2CH_2C=O), 2.36 (s, CH_3CO), 2.2 ppm (m, H-8). Anal. ($C_{24}H_{23}NO_9$·HCl·1.75H_2O) C, H, Cl, N.

The free base was formed by dissolving 8 in a buffer at pH 10 and extracting repeatedly with $CHCl_3$–MeOH (10:1). It was

treated with acetic anhydride to yield the **acetamide**, which was recrystallized from EtOH: mp 131–134 °C with previous softening; TLC R_f (MeOH–$CHCl_3$–H_2O, 40:10:1) 0.9; ^1H NMR (Me_2SO-d_6) δ 13.76 (s, 6-OH), 13.12 (s, 11-OH), 6.12 (br s, H-7, $\nu_{1/2}$ = 6 Hz), 5.54 (s, 9-OH), 4.00 (s, OCH_3), 2.36 (s, CH_3CO), 1.86 ppm (s, CH_3CON). Anal. ($C_{26}H_{25}NO_{10}$) C, H, N.

(7*S*,9*S*)-9-Acetyl-7-[(2-aminoethyl)thio]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Hydrochloride (9). A solution of 0.797 g (2.00 mmol) of **3** and 1.14 g (10.0 mmol) of 2-aminoethanethiol hydrochloride in 30 mL of trifluoroacetic acid was stirred at room temperature for 18 h and evaporated. Excess acid was removed by coevaporation of the residue with C_6H_6–$CHCl_3$–MeOH (5:4:1). The residue was partitioned between 75 mL of $CHCl_3$–MeOH (9:1) and 75 mL of water. The acidic aqueous layer was basified with solid $NaHCO_3$ and extracted with $CHCl_3$–MeOH (9:1; 5 × 100 mL). The combined extracts were washed with water, dried, and evaporated. The residual free base was dissolved in 20 mL of $CHCl_3$ and converted with 20 mL of 0.09 M anhydrous HCl in MeOH to the hydrochloride, which was precipitated by adding 40 mL of ether. The dark red powder (0.765 g, 77%) was collected and dissolved in 8 mL of $CHCl_3$–MeOH (4:1), and the solution was added to a column (2.4 × 37 cm) of silica gel (61 g of Mallinkrodt SilicAR CC-7, 200–325 mesh; in $CHCl_3$–MeOH, 9:1). The column was eluted with $CHCl_3$–MeOH (90:10, 1000 mL; 85:15, 400 mL; 80:20, 500 mL) and $CHCl_3$–MeOH–H_2O (20:10:1, 500 mL), while 10-mL fractions were collected. Fractions 86–130 were combined and evaporated to afford 0.639 g of a mixture of hydrochloride and free base. Conversion to the hydrochloride was completed by acidifying an aqueous solution to pH 4 with 0.1 N HCl, washing the solution with $CHCl_3$ (2 × 50 mL), and freeze-drying. The residue (0.547 g) was crystallized from 12 mL of $CHCl_3$–0.09 M HCl in MeOH (4:1) to yield 0.267 g (26%): mp 193–196 °C dec; UV-vis (MeOH) λ_{max} 233 nm ($\epsilon \times 10^{-3}$ 32.2), 256 (26.1), 286 sh, 480 (12.9), 498 (12.6), 532 (6.68); ^1H NMR (at 55 °C) δ 14.16 (br s, 6-OH), 13.23 (br s, 11-OH), 8.15 (br s, NH_3^+), 7.86 (m, H-1, H-3), 7.63 (m, H-2), 4.37 (m, H-7, $\nu_{1/2}$ = 7 Hz), 4.01 (s, OCH_3), 3.11 (br s, SCH_2CH_2N), 2.93 (s, 10-H_2), 2.31 ppm (s, $COCH_3$); MS [as $(Me_3Si)_4$ derivative] *m/e* 745; $[\alpha]^{21}_D$ +238° (*c* 0.06, MeOH). The product was homogeneous on TLC, R_f ($CHCl_3$–MeOH–H_2O, 40:10:1) 0.21, and on analytical high pressure LC (retention time 9.0 min). Anal. ($C_{23}H_{23}NO_7S$·HCl·1.5H_2O) C, H, Cl, N.

The combined mother liquors from crystallization of **9** were evaporated, and the residue was dissolved in 20 mL of H_2O. The solution was washed with CH_2Cl_2 and freeze-dried to afford 0.305 g, which was shown by analytical high-pressure LC to be mainly the 7*R* epimer **12** (75%; retention time 6.1 min) mixed with **9** (23%; 9.0 min) and an unidentified contaminant (2%; 24 min). Further purification of the epimer **12** was accomplished by semipreparative high-pressure LC on a reverse-phase column (0.78 × 61 cm) of Bondapak C-18/Porasil B (37–75 μm) eluted with MeOH–0.1 M NH_4OAc (1:1, followed by a 4:1 mixture), 4.0 mL/min, and with UV detection at 308 nm. Six runs (50 mg in 2 mL of MeOH for each injection) were made. Aqueous fractions enriched in the epimer were combined, acidified to pH 3 with dilute HOAc, extracted with CH_2Cl_2 to remove neutral contaminants, and basified with $NaHCO_3$. The free base was recovered by extraction with CH_2Cl_2 and evaporation. The residue was dissolved in CH_2Cl_2–MeOH (4:1) and converted with 0.17 N anhydrous methanolic HCl to the HCl salt, which was precipitated with ether to give 0.044 g of the 7*R* epimer **12**, 87% pure containing 13% of **9** by high-pressure LC. Spectral data were nearly identical with those for **9** except for the ^1H NMR signal for H-7: 4.47 ppm (m, $\nu_{1/2}$ = 13 Hz); MS [as $(Me_3Si)_4$ derivative] *m/e* 745. Anal. ($C_{23}H_{23}NO_7S$·HCl·0.75H_2O) C, H, Cl, N.

7-*O*-(2-Aminoethyl)daunomycinone Hydrochloride (10). A solution of 1.20 g (3.00 mmol) of **3** and 5.50 g (90 mmol) of 2-aminoethanol in 50 mL of trifluoroacetic acid was stirred and heated at 55–60 °C for 4 h and then evaporated. After coevaporation with C_6H_6–CH_2Cl_2–MeOH (5:4:1) to remove excess acid, the residue was partitioned between 100 mL of CH_2Cl_2–MeOH (4:1) and 100 mL of H_2O. The organic phase was washed with water, dried, and evaporated to yield 0.923 g, which was shown by TLC in $CHCl_3$–MeOH (39:1) to be largely a mixture of **3** (R_f 0.27) and its 7-epimer **11** (R_f 0.20), contaminated with a little dianhydrodaunomycinone[19] (R_f 0.60) and minor impurities. The combined acidic aqueous extracts were clarified by filtration, basified with $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were washed with H_2O, dried, and evaporated to yield 0.179 g of free base as a violet residue. It was suspended in 15 mL of H_2O and dissolved by acidification to pH 3.5 with 0.1 N HCl. The solution was washed with CH_2Cl_2 and freeze-dried to yield 0.164 g (11%) of **10**. Analytical high-pressure LC showed that **10** (retention time = 9.7 min) contained 3% of an unidentified contaminant (retention time = 6.4 min), presumably the 7-epimer. The recovered daunomycinone fraction (**3** and **11**) was recycled three times to give a total yield of 30%. A 0.437-g sample of the amorphous hydrochloride was crystallized by solution in 9 mL of $CHCl_3$–0.17 M HCl in MeOH (4:1) and slow evaporation in the dark to dryness. All attempts at crystallization directly from solution gave a gelatinous precipitate. The crystalline residue was triturated with 6 mL of $CHCl_3$–0.17 N HCl in MeOH (9:1), and the precipitate was collected and washed with a little fresh solvent to afford 0.240 g of homogeneous **10**. An additional 0.057 g was recovered from the mother liquor: mp 183–185 °C dec; TLC R_f ($CHCl_3$–MeOH–H_2O, 40:10:1) 0.18; UV-vis (MeOH) λ_{max} 233 nm ($\epsilon \times 10^{-3}$ 40.1), 252 (25.1), 288 (8.74), 476 (12.3), 494 (12.0), 5.28 sh (6.23); ^1H NMR (at 55 °C) δ 13.84 (br s, 6-OH), 13.10 (br s, 11-OH), 7.87 (m, H-1, H-3), 7.63 (m, H-2), 4.82 (m, H-7, $\nu_{1/2}$ = 9 Hz), 3.99 (s, OCH_3), 3.92 (OCH_2), 3.1–2.6 (CH_2N + 10-H_2), 2.36 (s, $COCH_3$, H-8B obscured), 2.02 (d × d, H-8A, $J_{7,8A}$ = 5 Hz, J_{gem} = 15 Hz); $[\alpha]^{21}_D$ +223° (*c* 0.05, MeOH). Anal. ($C_{23}H_{23}NO_8$·HCl·2H_2O) C, H, Cl, N.

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Synthesis and Biological Activities of Arginine-vasopressin Analogues Designed from a Conformation–Activity Approach[1]

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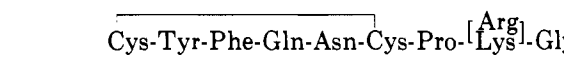
Using the proposed conformation of vasopressin thought to be preferred when the mammalian antidiuretic hormone is bound to its renal receptor, an analogue, [1-β-mercaptopropionic acid,2-phenylalanine,7-(3,4-dehydroproline)]arginine-vasopressin, was designed that contained three synthetic modifications. It posessed a rat antidiuretic potency of 13 000 ± 1250 units/mg, no measurable rat pressor activity, an in vitro rat uterotonic potency of 6.0 ± 0.6 units/mg, and an avian vasodepressor potency of 2.9 ± 0.5 units/mg. The strong dissociation of an apparently very high antidiuretic activity of this analogue from its other biological activities prompted the synthesis of the singly and doubly modified analogues of this series of 3,4-dehydroproline-containing derivatives. [1-β-Mercaptopropionic acid,7-(3,4-dehydroproline)]arginine-vasopressin, [2-phenylalanine,7-(3,4-dehydroproline)]arginine-vasopressin, and [7-(3,4-dehydroproline)]arginine-vasopressin were found to have the following specific activities (units/mg), respectively: rat antidiuretic, 4134 ± 306, 1541 ± 336, 1260 ± 126; rat pressor, 240 ± 4.5, 63.8 ± 3.9, 255 ± 23; rat uterotonic, 69 ± 5, 0.80 ± 0.11, 40.6 + 4.7; avian vasodepressor, 345 ± 19, 4.2 ± 0.9, 76.9 ± 48. Using the same synthetic procedure, arginine-vasopressin was prepared in an overall yield of 51% with rat antidiuretic and pressor potencies of 511 ± 61 and 519 ± 13, respectively. Renal clearance studies revealed that arginine-vasopressin and [1-β-mercaptopropionic acid,2-phenylalanine,7-(3,4-dehydroproline)]arginine-vasopressin affect water and electrolyte handling by the kidney through a similar mechanism, but that the decrease in urine flow seen for the synthetic analogue is augmented by a decrease in glomerular filtration rate.

The conformation of a peptide hormone can serve as a model to design analogues in which a particular biological activity is maintained or enhanced while, at the same time, other biological activities of the parent hormone are diminished or even abolished.[2] Synthetic modifications that increase the capacity of "binding elements"[3] present in the hormone would be one way to accomplish this goal. Another way to enhance productive hormone–receptor interactions would be by modifications that increase the time the peptide spends in the "biologically active" conformation. One could postulate that a structural change reduces the inherent flexibility of the peptide backbone[4,5] or enhances compatibility beween the side chains of the analogue containing the binding elements and/or "active elements"[3] and the complementary receptor moieties.

For the vasopressins,



we proposed a model of the biologically active conformation thought to be favored for the elicitation of their antidiuretic activity.[6] In this model, amino acid side chains located at the corner positions of the two β turns (residues in positions 3, 4, 7, and 8) contain the binding elements (for schematic representation see Figure 1). The basic moiety of the position 8 residue and the carboxamide moiety of the asparagine residue in position 5 are the active elements. Using this model, we recently designed an analogue of vasopressin, [1-β-mercaptopropionic acid,2-phenylalanine,7-(3,4-dehydroproline)]arginine-vasopressin, ([β-Mpr^1,Phe^2,Δ^3-Pro^7]AVP) that contained three synthetic modifications and that possessed a remarkably high antidiuretic potency[4] (Table I). In this analogue the proline residue in position 7 of vasopressin was substituted by 3,4-dehydroproline, which has a double bond. The introduction of a deformable electron cloud was expected to enhance receptor binding of the resultant analogue, provided the steric fit at the receptor was correct.[7] The biologically active model of vasopressin predicts that the hydroxyl moiety on the tyrosine residue in position 2 is not an active element; hence, its deletion should not diminish the antidiuretic potency of the resultant Phe^2 analogue. The N-terminal amino group was replaced by a hydrogen atom. This deamination, which results in a tightening of the peptide backbone,[8] gives rise to vasopressin analogues that exhibit increased stability to enzymatic inactivation both in vitro and in vivo[9,10] (for a review see ref 11).

The enhancement of antidiuretic potency and specificity achieved with [β-Mpr^1,Phe^2,Δ^3-Pro^7]AVP prompted us to extend our biological testing of this analogue, as well as to synthesize and examine biologically the singly and doubly modified analogues of this series, which contain 3,4-dehydroproline in position 7. Accordingly, we synthesized [1-β-mercaptopropionic acid,7-(3,4-dehydroproline)]arginine-vasopressin ([β-Mpr^1,Δ^3-Pro^7]AVP),[12] [2-phenylalanine,7-(3,4-dehydroproline)]arginine-vasopressin ([Phe^2,Δ^3-Pro^7]AVP), and [7-(3,4-dehydroproline)]arginine-vasopressin ([Δ^3-Pro^7]AVP).[12]

The required protected peptide intermediates were prepared by the solid-phase technique,[13] using a chloromethylated polystyrene copolymer (1%)–divinylbenzene support to which glycine had been esterified, and a scheme of deprotection, neutralization, and coupling already described.[14] In general, the *tert*-butyloxycarbonyl (Boc) group was used for the temporary protection of the N$^\alpha$ group and was removed by treatment with 50% trifluoroacetic acid (TFA) in CH_2Cl_2. Coupling was affected by dicyclohexylcarbodiimide (DCC) or DCC mediated with 1-hydroxybenzotriazole (HBT),[15] except when indicated. The benzyl group was used for the protection of the

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