Total Chemical Synthesis and Antitumor Evaluation of the 9-Aza Analogue of *N*-(Trifluoroacetyl)-4-demethoxydaunomycin

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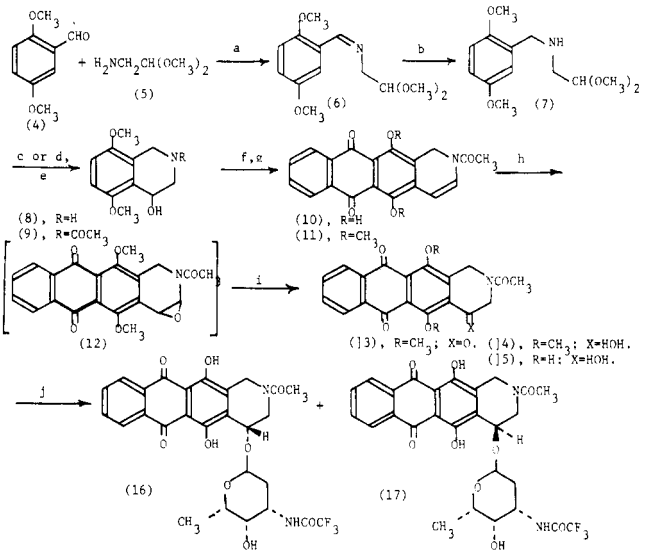
The 9-aza analogue of *N*-(trifluoroacetyl)-4-demethoxydaunomycin has been synthesized from 2,5-dimethoxybenzaldehyde. Pomeranz–Fritsch condensation followed by borohydride reduction and acid-catalyzed cyclization led smoothly to 4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline. Selective N-acetylation and subsequent Friedel–Crafts acylation with phthalic anhydride produced 2-acetyl-5,12-dihydroxy-1,2-dihydro-2-azanaphthacene-6,11-dione, which was protected as its dimethyl ether and epoxidized to an acylated aza Brigl's anhydride. This was converted to (±)-2-acetyl-4-hydroxy-5,12-dimethoxy-1,2,3,4-tetrahydro-2-azanaphthacene-6,11-dione by dehydration to the 4-keto analogue followed by cyanoborohydride reduction either stepwise or in situ. The protecting groups were removed with boron trichloride and the resulting aglycone glycosidated with optically active *N,O*-bis(trifluoroacetyl)daunosamine bromide and silver trifluoromethanesulfonate. The resulting diastereoisomers were separated by column chromatography and their structures established by CD and NMR spectroscopy. Unexpectedly it was not possible to remove the *N*-trifluoroacetyl blocking group without aromatization to the azanaphthaquinone. Both (*R*)- and (*S*)-acetyl-4-*O*-[*N*-(trifluoroacetyl)daunosaminyl]-5,12-dihydroxy-2-azanaphthacene-6,11-dione were inactive ip in mice carrying the P388 tumor. Drugs were given at various concentrations on days 0, 5, and 9.

Despite the enormous effort that has gone into synthesis and evaluation of anthracyclines, comparatively little work has involved transformation of the alicyclic A ring of the glycosides.[1,2] However, it is known that removal of the C-9 hydroxyl is compatible with antitumor activity in mice, although requiring higher doses.[3] Furthermore, removal of the C-4 methoxy moiety is associated with enhanced potency.[4-6] Considering that these two molecular features might produce an additive result, this set the stage for the design of the previously unknown A-ring bioisosteres.

Medicinal chemists often use the operational strategy of bioisosterism.[7] When the atomic interchange is made away from the pharmacophore, retention of biological activity is the expected outcome. When the change is made in the portion of the molecule in contact with the receptor, then a rather more profound effect either on the nature or the intensity of the activity can be expected. This makes this strategem a rather interesting probe for these features in a molecule.

Consideration of the chemical structure of the clinically useful antitumor anthracycline antibiotics doxorubicin (**1**) and daunomycin (**2**) shows that bioisosteric replacements for carbon in the alicyclic A ring can only be realistically performed at C-9. The C-9 acetyl moiety is known to be important but not obligatory for activity.[8] Thus, the minimum allowable valence for a replacement atom is three and a nitrogen substitution for carbon could be envisioned even though this would require sacrifice of the C-9 hydroxyl function. If successful, we envisioned that several advantages could accrue. The N atom would be present as a neutral amide rather than a basic amine and might not result in unusual tissue distribution patterns compared to the naturally occurring analogues. The acyl group would be easily varied so as to allow facile preparation of a series of homologues permitting definition of the optimal congener should the target molecule prove to be interestingly active. Further, the only chiral center in the aglycon portion would be at C-7 so that the isomer problem often encountered at late stages in anthracycline synthetic schemes would be minimized.[9] Despite putative lack of a dominant influence on in vivo activity, relatively recent X-ray studies and other considerations suggest a significant hydrogen-bonding role between the C-9 OH group and DNA when these drugs intercalate in vitro.[10,11]

Scheme I



In advance of experiment, the energy sacrificed by leaving out this function would not seem sufficient to abolish intercalation should this be crucial for in vivo activity. The change would also not appear to interfere significantly with

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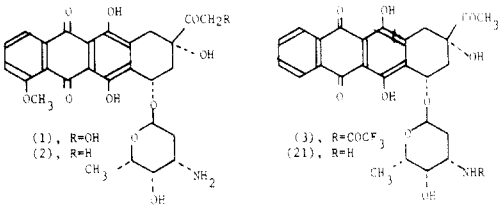
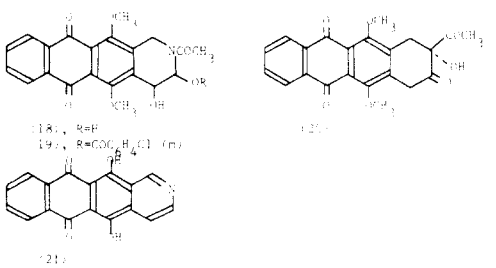
bioreductive activation toward electrophilic reactions, a mechanism that has attracted considerable experimental attention recently.[12] One could also expect that the chemistry required to accomplish this bioisosteric replacement would have features of intrinsic interest as well. We report herein our experiences with the 9-aza analogue (16) of *N*-(trifluoroacetyl)-4-demethoxydaunomycin (3).



The successful synthesis starts with the modified Pomeranz–Fritsch condensation of 2,5-dimethoxybenzaldehyde (4) and 2-aminoacetaldehyde dimethyl acetal (5) to produce the corresponding benzylideneamino acetal (6) (Scheme I). Reduction with sodium borohydride leads to the known (dimethoxybenzyl)amino acetal 7.[13] Acid-catalyzed cyclodehydration gave the previously undescribed 4-hydroxytetrahydroisoquinoline derivative 8 in 95% yield. Selective N-acetylation to 9, by either pentafluorophenyl acetate[14] (80% yield) or, more conveniently and more conventionally, with acetic anhydride–sodium acetate at reduced temperature (86% yield), set the stage for assembly of the tetracyclic system.

Friedel–Crafts acylation of 9 with phthalic anhydride gave a 45% yield of the desired dihydroazanaphthacenequinone (10). The benzylic hydroxyl group was lost in this process and had to be reintroduced. This proved not to be difficult. First, the B-ring phenolic hydroxyls were protected as their methyl ethers (11, 90% yield), and the olefinic linkage was epoxidized with *m*-chloroperbenzoic acid. As expected, the intermediate acylated aza Brigl's anhydride (12) was unstable[15] and readily isomerized during acidic treatment to the desired 4-keto analogue (13) in 80% overall yield. This could be reduced selectively in 69% yield with sodium cyanoborohydride to the desired benzyl alcohol (14). Alternatively, the process could be compressed into a single step by not isolating the product of epoxidation but by performing the reduction in situ. This succeeded in 78% yield. An attractive feature of this sequence is that almost all of the intermediate species one can envision to result from the epoxidation (12, 13, 18) will give the desired product.



The protecting methyl ether groups were selectively removed in quantitative yield without dehydration through use of boron trichloride.

Glycosidation was carried out with use of optically active *N,O*-bis(trifluoroacetyl)duanosaminyl bromide and silver trifluoromethanesulfonate.[16] Both C-1′ β mono-*N*-trifluoroacetyl protected diastereoisomers (16, 17) were produced in good yield and were readily separated by silica gel column chromatography. The C-1′ α analogues were apparently minor products and were not isolated. Assignment of stereochemistry was accomplished both from the NMR coupling pattern (both isomers had a half-heighth width of 6–7 cps for H-1′ characteristic of the β-anomers[17]) and by comparison of the circular dichroism spectra with that of daunomycin. Compound 16 showed a strongly positive peak at 282.5 nm while compound 17 had a strongly negative trough at 282 nm. Daunomycin and its analogues are known to have a negative extremum at that wavelength.[18]

Unexpectedly, it proved impossible under a wide variety of conditions (see Experimental Section) to remove the *N*-trifluoroacetyl moiety of 17 without undesired aromatization to 21. It is well-known that the *N*-trifluoroacetyl moiety is compatible with antitumor activity in mice with certain anthracycline analogues even though increased doses are required as compared with the free amines.[19] There are examples, however, in which *N*-TFA derivatives lack the antitumor activity displayed by the corresponding amines.[20,21] Thus it was felt that there was a realistic chance of detecting in vivo activity of 17, especially bearing in mind the well-known potency enhancement that removal of the C-4 methoxyl group brings.

Biological Results

Both glycosides (16 and 17) were tested intraperitoneally against P388 acute lymphocytic leukemia in mice with use of conditions under which 4-demethoxydaunomycin gave a T/C value of 160 (at a dosage of 0.75 mg/kg given on days 1, 5, and 9 after tumor implantation). The new 9-aza glycosides were administered at doses of 100, 50, 25, 12.5, 6.25, 3.72, and 1.56 mg/kg ip. Even at 100 mg/kg, the T/C values obtained were 105 for the 7*R* analogue and 98 for the 7*S* analogue. These compounds are inactive.

This finding is perplexing. Two of the major theories of the molecular modes of action of the anthracyclines involve intercalation and bioreductive activation prior to alkylation of essential biopolymers.[20] Neither mechanism depends in a major way upon the presence of a C-9 OH group, and analogues are known that lack this feature and yet possess significant antitumor activity. The problem may lay simply in the nature of the protecting group employed.[21] Evaluation of this feature will require resynthesis with a different sugar component. Taken at face value, it would appear from the findings in this study that the side-chain keto moiety cannot be replaced by an aliphatic amide group.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus or on a Fisher-Johns hot stage and are uncorrected. IR spectra

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were recorded with a Beckman 33 or a Perkin-Elmer 727 infrared spectrophotometer. UV spectra were obtained with a Cary 219 spectrophotometer. ^{1}H NMR spectra were obtained in $CDCl_3$ with a Varian EM360 or FT-80A spectrometer using tetramethylsilane as internal standard. Mass spectral analyses were conducted on a Riber 10-10 or a MAT CH_5 mass spectrometer at an ionizing voltage of 70 eV and temperatures varying with the substrate. Microanalyses were performed on a Hewlett-Packard 185B instrument, and the results are within ±0.4% of the theoretical values where indicated by symbols for the elements. Analytical thin-layer chromatography was conducted with E. Merck silica gel 60 F-254 precoated plates or with Brinkmann Instruments Sil G/UV-254 precoated plastic sheets. For preparative chromatography on silica gel impregnated with 4% KH_2PO_4, the gel (flash chromatography grade) was conditioned by preparation of a thick slurry in a beaker containing 20.0 g of KH_2PO_4 in 500 mL of distilled water. The slurry was aged at room temperature for 30 min and filtered under suction (water pump) until nearly dry. The impregnated gel was transferred into a big crystallizing dish and was kept in a drying oven at 110 °C for about 1 h and cooled. The dry gel was kept in a dessicator before use.

[*N*-(2,5-Dimethoxybenzylidene)amino]acetaldehyde Dimethyl Acetal (6). To a solution of 2,5-dimethoxybenzaldehyde (16.6 g, 0.1 mmol) in toluene (55 mL) was added aminoacetaldehyde dimethyl acetal (10.51 g, 0.1 mmol). The mixture was refluxed for 4 h with a Dean–Stark trap to remove water. Distillation of solvent under vacuum gave the Schiff's base **6** (25.30 g, 100% yield): ^{1}H NMR ($CDCl_3$) δ 3.35 (s, 6 H, $CN(OMe)_2$), 3.53–3.93 (m, 8 H, Ar $(OMe)_2$, NCH_2), 4.6 (t, J = 6.0 Hz, 1 H, $CH(OMe)_2$), 6.78–6.93 (m, 2 H, Ar H), 7.53 (d, J = 2.7 Hz, 1 H, Ar H), 8.7 (br s, 1 H, CH=N); IR (neat) 2945, 2848, 1640, 1497, 1463, 1365, 1305, 1282, 1263, 1225, 1165, 1127, 1045, 965, 923, 882, 820, 720 cm^{-1}; EIMS, m/z (relative intensity) 253 (M^+, 9.7), 222 (10.2), 192 (8.0), 190 (11.4), 178 (15.9), 177 (20.5), 160 (20.5), 151 (29.0), 148 (33.0), 121 (43.2), 92 (22.7), 91 (25.0), 75 (100.0); HRMS, m/z 253.13113 ($C_{13}H_{19}NO_4$ requires 253.13129).

[*N*-(2,5-Dimethoxybenzyl)amino]acetaldehyde Dimethyl Acetal (7). [*N*-(2,5-dimethoxybenzylidene)amino]acetaldehyde dimethyl acetal (**6**) (15.0 g, 0.059 mmol) was dissolved in ethanol (80 mL) and sodium borohydride (9.0 g, 0.238 mmol) was added in small installments over 10 min at room temperature. The reaction was monitored by TLC (Si gel, EtOAc–hexane = 1:1). After stirring for 20 h under argon, the reaction mixture was poured into cold water (300 mL), extracted with ether (4 × 100 mL), dried (Na_2SO_4), and evaporated to give **7** as an oil (15.10 g, 99.9%): ^{1}H NMR ($CDCl_3$) δ 1.67 (br s, 1 H, NH), 2.68 (d, J = 5.0 Hz, 2 H, Ar CH_2NCH_2), 3.28 (s, 6 H, $CH(OMe)_2$), 3.63–3.78 (m, 8 H, Ar $(OMe)_2$), Ar CH_2), 4.43 (t, J = 5.0 Hz, 1 H, $CH(OMe)_2$), 6.57–6.67 (m, 2 H, Ar H), 6.73–6.83 (m, 1 H, Ar H); IR (neat) 3370, 2950, 2850, 1595, 1508, 1466, 1362, 1278, 1220, 1130, 1046, 960, 910, 855, 795, 725, 700 cm^{-1}; EIMS, m/z 255 (M^+, 4.2), 224 (0.7), 223 (0.7), 192 (5.3), 180 (32.7), 151 (100.0), 121 (22.4), 91 (9.2), 75 (22.5); HRMS, m/z 255.14647 ($C_{13}H_{21}NO_4$ requires 255.14693).

4-Hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8). [*N*-(2,5-dimethoxybenzyl)amino]acetaldehyde dimethyl acetal (**7**) (5.0 g, 19.58 mmol) was added dropwise to cold (0–5 °C) hydrochloric acid (6 N, 120 mL) under an argon atmosphere. After addition, the cooling bath was replaced by a heating oil bath and heated to 40–42 °C. Progress of the reaction was followed by TLC (silica gel, MeOH–$CHCl_3$ = 1:20). The reaction was stopped after 30 h, concentrated to 30 mL under vacuum, and cooled to 0–5 °C. The cold reaction mixture was basified by dropwise addition of 10% NaOH and extracted with chloroform (4 × 100 mL). The chloroform extract was dried (Na_2SO_4) and evaporated to give an oil, which solidified on trituration with ether. The solid was recrystallized from methylene chloride/ether to yield 4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (3.9 g, 95%): mp 122 °C; ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 2 H, NH, OH, exchange with D_2O), 2.75–3.35 (ABX, J = 16, 3.8 Hz, 2 H, Ar $CHOHCH_2$), 3.6–4.2 (m, 2 H, Ar CH_2NH), 3.70 (s, 3 H, Ar OMe), 3.78 (s, 3 H, Ar OMe), 4.75 (t, J = 3.8 Hz, 1 H, Ar CHOH), 6.68 (s, 2 H, Ar H); IR ($CHCl_3$) ν_{max} 3600, 3332, 3000, 2950, 2850, 1605, 1485, 1463, 1445, 1386, 1340, 1303, 1270, 1128, 1097, 1030, 1000, 970, 950, 903, 895, 770, 663 cm^{-1}; EIMS, m/z 209 (M^+, 29.2), 192 (17.5), 180 (100.0), 165 (74.5), 150 (15.3), 134 (37.5), 121 (19.8), 120 (21.5), 107 (17.3), 105 (12.9), 91 (31.3), 79 (20.6), 77 (38.0), 65 (17.3), 51 (18.2); HRMS, m/z 209.10422 ($C_{11}H_{15}NO_3$ requires 209.1051). Anal. ($C_{11}H_{15}NO_3$) C, H, N.

***N*-Acetyl-4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (9). (a) With Pentafluorophenyl Acetate.** A solution of 4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**8**) (0.60 g, 2.87 mmol) in DMF (7.0 mL) was cooled to 0–5 °C, and pentafluorophenyl acetate (1.96 g, 8.67 mmol) was added dropwise over 5 min. Progress of the reaction was followed by TLC (silica gel, EtOAc–PhH = 1:1). The reaction mixture was stirred at room temperature for 16 h and ether (20.0 mL) was added to precipitate the product. The solid obtained was recrystallized from methylene chloride and ether to give *N*-acetyl-4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**9**) (0.58 g, 80.5%): mp 169 °C; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, $NCOCH_3$), 2.85 (br s, OH), 3.25–4.10 (ABX, J = 14.4, 3.8 Hz, 2 H, H-3), 3.78 (s, OMe), 3.83 (s, OMe), 3.90–5.38 (m, 2 H, H-1), 4.95 (br t, J = 4.0 Hz, 1 H, Ar CHOH), 6.7 (br s, 2 H, Ar H). The number of peaks and their wide apparent coupling (J = 22 Hz) suggest two slowly interconverting conformations. This broad dispersion is seen in several subsequent spectra including those of **9**, **14–17**. IR ($CHCl_3$) 3600, 3010, 2850, 1635, 1610, 1490, 1465, 1442, 1347, 1268, 1128, 1097, 1080, 1060, 1010, 958, 900, 820 cm^{-1}; EIMS, m/z 251 (M^+, 0.9), 233 (56.6), 190 (100.0), 176 (12.8), 165 (14.0), 160 (18.6), 134 (5.6), 121 (6.1), 91 (10.0), 77 (13.9), 51 (4.7), 43 (34.9); HRMS, m/z 251.11473 ($C_{13}H_{17}NO_4$ requires 251.11565).

(b) With Acetic Anhydride. 4-Hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**8**) (0.50 g, 2.39 mmol) was mixed with anhydrous sodium acetate (0.39 g, 4.75 mmol) and cooled to 0–5 °C (ice bath). To the cold mixture was added acetic anhydride (1.96 g, 19.2 mmol) dropwise in 5 min. The reaction was allowed to attain room temperature and was stirred for 2 h at this temperature. TLC (silica gel, MeOH–$CHCl_3$ = 1:20) of the reaction after 2 h showed complete consumption of starting material. The reaction was poured into water (100 mL) and extracted with methylene chloride (3 × 50 mL). The methylene chloride extract waas washed with water (3 × 100 mL), dried (Na_2SO_4), and evaporated to give *N*-acetyl-4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.518 g, 86.3%).

2-Acetyl-5,12-dihydroxy-1,2-dihydro-2-azanaphthacene-6,11-dione (10). Aluminum chloride (4.98 g, 37.3 mmol), sodium chloride (0.995 g, 17.03 mmol), phthalic anhydride (0.498 g, 3.36 mmol), and *N*-acetyl-4-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**9**) (0.40 g, 1.59 mmol) were mixed intimately in a glovebox under an atmosphere of argon. The mixture was transferred to a preheated flask at 180–182 °C and was stirred with a glass rod for 5 min. The reaction was cooled to 0–5 °C and a saturated solution of oxalic acid (100 mL) was added dropwise over 15 min. The reaction was stirred with oxalic acid for 4 h at room temperature and was extracted with chloroform (4 × 100 mL). The chloroform extract was dried (Na_2SO_4) and evaporated and crude product (0.377 g) was separated by flash column chromatography (silica gel impregnated with 4% KH_2PO_4, EtOAc–PhH = 1:1) to yield **10** (0.237 g, 44.5%): mp 224–225 °C (recrystallized from PhH and petroleum ether, bp 40–60 °C); ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3 H, $NCOCH_3$), 4.95 (s, 2 H, Ar CH_2), 6.13 (d, J = 8.0 Hz, 1 H, AcNCH=CH Ar), 6.83 (d, J = 8.0 Hz, 1 H, Ar CH=CHNAc), 7.75 (m, 2 H, Ar $H_{8,3}$), 8.23 (m, 2 H, Ar $H_{7,10}$), 13.10 (s, 2 H, Ar OH); IR (KBr) 1680, 1610 (s), 1590, 1390, 1350, 1274, 1070, 1026, 965, 923, 970, 800, 746 cm^{-1}; EIMS, m/z 335 (M^+, 34), 292 (100), 276 (6.8), 190 (7.1), 163 (6.8), 152 (12.6), 77 (14.5), 43 (88.4); HRMS, m/z 335.07928 ($C_{19}H_{13}NO_5$ requires 335.07928). Anal. ($C_{19}H_{13}NO_5$) C, H, N.

2-Acetyl-5,12-dimethoxy-1,2-dihydro-2-azanaphthacene-6,11-dione (11). To a solution of 2-acetyl-5,12-dihydroxy-1,2-dihydro-2-azanaphthacene-6,11-dione (**10**) (0.10 g, 0.298 mmol) in acetone (40 mL) were added potassium carbonate (0.272 g, 1.97 mmol) and dimethyl sulfate (0.133 g, 1.05 mmol). The mixture was refluxed under argon for 12 h and reaction progress was monitored by TLC (silica gel impregnated with 4% KH_2PO_4, EtOAc–PhH = 1:1). The reaction was cooled to room temperature and salts were filtered off under suction. The filtrate was evaporated and the solid obtained was separated by flash column chromatography (silica gel impregnated with 4% KH_2PO_4, EtOAc–PhH = 1:1) to yield **11** (0.097 g, 89.6%): mp 218–218.5 °C (from methylene chloride and petroleum ether, bp 40–60 °C); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H, $NCOCH_3$), 3.83 (s, 3 H, OMe), 3.85

(s, 3 H, OMe), 4.9 (s, 2 H, Ar CH_2), 6.03 (d, J = 8.0 Hz, 1 H, AcNC*H*=CH Ar), 6.75 (d, J = 8.0 Hz, 1 H, Ar C*H*=CHNAc), 7.6 (m, 2 H Ar $H_{8,9}$), 8.03 (m, 2 H, Ar $H_{7,10}$); IR ($CHCl_3$) 3050, 2995, 1670, 1622, 1560, 1463, 1390, 1360, 1340, 1290, 1270, 1175, 1138, 1120, 1063, 1045, 1004, 980, 963, 888, 860 cm^{-1}; EIMS, m/z 363 (M^+, 39), 320 (54.8), 306 (34.8), 290 (27.8), 276 (7.9), 263 (7.4), 234 (7.2), 206 (3.7), 190 (4.5), 178 (6.3), 163 (6.9), 151 (8.3), 83 (11.1), 43 (100.0); HRMS, m/z 363.10967 ($C_{21}H_{17}NO_5$ requires 363.11056). Anal. ($C_{21}H_{17}NO_5$) C, H, N.

2-Acetyl-5,12-dimethoxy-3*H*-1,2-dihydro-2-azanaphthacene-4,6,11-trione (13). A solution of 2-acetyl-5,12-dimethoxy-1,2-dihydro-2-azanaphthacene-6,11-dione (11) (0.50 g, 1.38 mmol) in methylene chloride (50 mL) was cooled to −10 °C, and *m*-chloroperoxybenzoic acid (80–85%, 0.386 g, 1.79 mmol) was added over 15 min. The reaction was allowed to attain room temperature and was stirred at this temperature for 6 h. The reaction was washed with sodium bisulfite (2%, 20 mL), sodium bicarbonate (5%, 25 mL), and water (3 × 75 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The yellow solid obtained was dried under vacuum for 20 min and was dissolved in chloroform (100 mL). To the solution was added *p*-toluenesulfonic acid (0.03 g), and this was refluxed for 30 min with use of a Dean–Stark trap whose side arm was filled with molecular sieves (4 Å). The reaction was cooled to room temperature, washed with sodium bicarbonate (5%, 20 mL), dried (Na_2SO_4), and evaporated. The reddish oily product turned into a yellow solid on scratching with ether. The solid was purified by flash column chromatography (silica gel impregnated with 4% KH_2PO_4) to yield **13** (0.42 g, 80.5%): mp 170–171 °C (from methylene chloride and hexane); ^1H NMR ($CDCl_3$) δ 2.18 (s, 3 H, $NCOCH_3$), 4.0 (s, 3 H, OMe), 4.03 (s, 3 H, OMe), 4.30 (s, 1 H, $COCH_2NAc$), 4.45 (s, 1 H, $COCH_2NAc$), 4.95 (br d, 2 H, $ArCH_2NAc$), 7.75 (m, 2 H, Ar $H_{8,9}$), 8.15 (m, 2 H, Ar $H_{7,10}$); IR ($CHCl_3$) 3025, 2975, 1718, 1680, 1603, 1560, 1440, 1402, 1330, 1290, 1260, 1173, 1124, 1085, 1060, 1000, 950, 890 cm^{-1}; EIMS, m/z 379 (M^+, 45.1), 364 (4.0), 336 (32.6), 322 (28.4), 306 (9.7), 293 (10.1), 279 (5.5), 265 (5.5), 250 (9.7), 194 (8.1), 165 (8.6), 152 (11.7), 138 (11.8), 43 (100.0); HRMS, m/z 379.10511 ($C_{21}H_{17}NO_6$ requires 379.105578).

(±)-2-Acetyl-4-hydroxy-5,12-dimethoxy-1,2,3,4-tetrahydro-2-azanaphthacene-6,11-dione (14). (a) Reduction of 2-Acetyl-5,12-dimethoxy-3*H*-2,3-dihydro-2-azanaphthacene-4,6,11-trione (13). Ketone **13** (0.42 g, 1.11 mmol) was dissolved in tetrahydrofuran (40 mL) and methanol (13 mL) and a few crystals of methyl orange were added. Sodium cyanoborohydride (5.60 g) was added in 200-mg portions every 30 min to the yellow solution. Each addition of sodium cyanoborohydride was followed by dropwise addition of 2 N methanolic hydrogen chloride until the red color in the solution was restored. The reaction progress was monitored by TLC (silica gel impregnated with 4% KH_2PO_4, Me_2CO–CH_2Cl_2 = 1.1). The reaction was stopped after 3 h, poured into water (400 mL), and extracted with chloroform (3 × 100 mL). The organic extract was dried (Na_2SO_4) and evaporated to give a reddish oil, which turned into a brown solid on scratching with ether. The solid was purified by flask column chromatography (silica gel impregnated with 4% KH_2PO_4, Me_2CO–CH_2Cl_2 = 1:1) to yield **14** (0.29 g, 68.5%).

(b) From 2-Acetyl-5,12-dimethoxy-1,2-dihydro-2-azanaphthacene-6,11-dione (11) via Epoxidation and Subsequent Reduction. Compound **11** (0.4 g, 1.10 mmol) was treated with *m*-chloroperoxybenzoic acid (0.31 g, 1.44 mmol) in methylene chloride (45 mL) as described earlier (see preparation of **13**). The product was dissolved in THF (100 mL) and sodium cyanoborohydride (3.4 g) was added. A small amount of methyl orange was added as an indicator. Hydrochloric acid (6 N, 3.50 mL) was added dropwise over 40 min and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured into cold water (200 mL), extracted with chloroform (4 × 50 mL), dried (Na_2SO_4), and evaporated. The crude solid product was separated by flash column chromatography (silica gel impregnated with 4% KH_2PO_4; Me_2CO–CH_2Cl_2 = 1:1 and MeOH–$CHCl_3$ = 1:20) to give **14** (0.326 g, 77.7%): mp 231–232 °C; ^1H NMR ($CDCl_3$) δ 2.22 (s, $NCOCH_3$), 3.25–4.25 (m, 2 H, ABX, H-3), 3.95 (s, OMe), 4.00 (s, OMe), 4.25–5.65 (m, 2 H, H-1), 5.10 (m, 1 H, J = 5 Hz, H-4), 7.55–7.85 (m Ar $H_{8,9}$), 8.0–8.3 (m, Ar $H_{7,10}$); IR (KBr) 3350, 2975, 2890, 1680, 1620, 1565, 1490, 1460, 1338, 1265, 1230, 1108, 1003, 1000, 925, 880, 860 cm^{-1}; EIMS, m/z 381 (M^+, 0.9), 363 (36.5), 320 (41.8), 306 (17.7), 2.90 (7.5), 276 (3.9), 181 (3.2), 165 (7.7), 152 (8.7), 139 (6.0), 43 (100%); HRMS, m/z 381.12128 ($C_{21}H_{19}NO_6$ requires 381.121227). Anal. ($C_{21}H_{19}NO_6$) C, H, N.

(±)-2-Acetyl-4,5,12-trihydroxy-1,2,3,4-tetrahydro-2-azanaphthacene-6,11-dione (15). A solution of dimethyl ether **14** (0.1 g, 0.262 mmol) in methylene chloride (100 mL) was cooled to −76 °C and boron trichloride (0.586 g, 5.0 mmol) was added dropwise over 10 min. The reaction mixture was allowed to attain room temperature and was stirred for 1 h at room temperature. Methanol (10.0 mL) was added dropwise to cold (0 °C) reaction and the yellow solution was poured into cold water (150 mL). The mixture was extracted with chloroform (3 × 50 mL), dried (Na_2SO_4), and evaporated. The product was purified by flash column chromatography (silica gel impregnated with 4% KH_2PO_4, MeOH–$CHCl_3$ = 1:20) to yield reddish orange **15** (0.0924 g, 99.9%): mp 213–214 °C; ^1H NMR ($CDCl_3$) δ 2.15 (s, $NCOCH_3$), 3.25–4.15 (ABX, 2 H, H-3), 3.75–5.5 (m, 2 H, H-1), 4.9 (br t, 1 H, $W_{1/2}$ = 7 Hz), 7.75–8.05 (m, 2 H, Ar $H_{8,9}$), 8.05–8.3 (m, 2 H, Ar $H_{7,10}$), 13.05 (br s, 1 H, Ar OH); IR (KBr) 3400, 3325, 1650 (s), 1630, 1590, 1410, 1372, 1340, 1220, 1120, 1060, 1000, 950, 880, 800 cm^{-1}; EIMS, m/z 353 (M^+, 0.9), 335 (21.8), 292 (51.4), 254 (23.3), 239 (7.0), 152 (10.7), 139 (6.1), 43 (100); HRMS, m/z 353.09136 ($C_{19}H_{15}NO_6$ requires 353.08983).

(*R*)- and (*S*)-2-Acetyl-4-*O*-[*N*-(trifluoroacetyl)daunosaminyl]-5,12-dihydroxy-2-azanaphthacene-6,11-dione (16 + 17). To a solution of racemic 2-acetyl-4,5,12-trihydroxy-1,2,3,4-tetrahydro-2-azanaphthacene-6,11-dione (**15**) (0.0926 g, 0.262 mmol) in methylene chloride (40 mL) was added powdered 4-Å molecular sieves (0.70 g) and a solution of *N,N*-bis(trifluoroacetyl)daunosaminyl bromide (0.187 g, 0.465 mmol) in methylene chloride (40 mL). The mixture was cooled to −10 °C and silver trifluoromethanesulfonate (0.184 g, 0.716 mmol) in ether (8 mL) was added dropwise over 10 min. The reaction mixture was allowed to attain room temperature in 76 min and was poured into sodium bicarbonate solution (10%, 200 mL). The solution was filtered under suction and the aqueous layer was extracted with methylene chloride (3 × 60 mL). The organic layer was dried (Na_2SO_4) and evaporated and the reddish solid was refluxed with methanol (60 mL) for 20 min. Methanol was removed under vacuum and the product was separated by flash column chromatography (silica gel impregnated with 4% KH_2PO_4; MeOH–$CHCl_3$ = 1:20) to yield (*R*)-2-acetyl-4-*O*-[*N*-(trifluoroacetyl)daunosaminyl]-5,12-dihydroxy-2-azanaphthacene-6,11-dione (**16**) (66.8 mg, 44.0%): mp 164–165 °C; ^1H NMR ($CDCl_3$) δ 1.25 (d, J = 6.5 Hz, 3 H, 6′-Me), 1.6–1.93 (m, 2 H, H-2′), 2.2 (s, 3 H, $NCOCH_3$), 2.5–2.78 (m, 1 H), 3.38–3.65 (m, 1 H), 4.1–4.65 (m, 4 H), 4.78–5.25 (m, 3 H), 5.3 (br s, 1 H, $W_{1/2}$ = 7.0 Hz, H-1′), 6.65 (br d, J = 8.0 Hz, 1 H, 3′-NH), 7.65–7.98 (m, 2 H, Ar $H_{8,9}$), 8.15–8.50 (m, 2 H, Ar $H_{7,10}$), 13.08 (s, 1 H, Ar OH), 13.18 (s, 1 H, Ar OH); IR (KBr) 3519, 3466, 3422, 3412, 3337, 3316, 3266, 2978, 2938, 1721, 1628, 1590, 1541, 1456, 1420, 1377, 1345, 1316, 1285, 1236, 1206, 1183, 1167, 1121, 1042, 1013, 986, 791; EIMS, m/z 353 ($M^+ - C_8F_3H_{10}NO_3$, 0.9), 335 (28.2), 292 (30.5), 254 (6.8), 226 (4.6), 192 (4.0), 180 (6.3), 169 (11.4), 155 (27.8), 140 (31.3), 114 (14.2), 86 (100), 69 (46.0), 57 (79.0), 43 (70.5); UV–vis λ_{max} (CH_3OH) 225 nm (ϵ 24 920), 251 (40 950e, 283 (10 375), 460 (10 130), 480 (10 455), 514 (6035); CD (CH_3OH) $[\theta]_{282.5}$ 2.49 × 10^4.

(*S*)-2-Acetyl-4-*O*-[*N*-(trifluoroacetyl)daunosaminyl]-5,12-dihydroxy-2-azanaphthacene-6,11-dione (**17**) (56.5 mg, 37.22%): mp 153–154 °C; ^1H NMR ($CDCl_3$) δ 1.20 (d, J = 6.0 Hz, 3 H, 6′-Me), 1.6–2.1 (dd, J = 15.5, 2 H, H-2′), 2.28 (s, 3 H, $NCOCH_3$), 2.68–3.70 (m, 2 H, H-3), 3.60 (m, 1 H, H-4′), 4.0–4.6 (m, 4 H, H-3′ and H-5′), 4.2–5.1 (m, 2 H, H-1), 4.95 (br t, partly obscured, H-4), 5.38 (br t, 1 H, $W_{1/2}$ = 6.0 Hz, H-1′), 6.65 (br d, J = 8.0 Hz, 1 H, NH), 7.60–7.93 (m, 2 H, Ar $H_{8,9}$), 8.10–8.4 (m, 2 H, Ar $H_{7,10}$), 13.15 (br s, 2 H, Ar OH); IR (KBr) 3465, 3420, 3322, 3290, 2978, 2938, 1723, 1628, 1590, 1551, 1416, 1377, 1343, 1316, 1283, 1236, 1208, 1167, 1123, 1042, 1015, 986, 791; EIMS, m/z 353 ($M^+ - C_8F_3H_{10}NO_3$, 1.5), 335 (15.3), 292 (21.4), 254 (4.6), 226 (5.5), 192 (11.4), 180 (6.8), 169 (11.4), 155 (26.7), 140 (35.2), 114 (19.6), 86 (95.7), 69 (61.9), 57 (100.0), 43 (81.6); UV–vis λ_{max} (CH_3OH) 224.5 nm (ϵ 23 060), 251 (40 610), 283 (9265), 460 (9980), 481 (10 100), 513 (5790); CD (CH_3OH) $[\theta]_{282}$ −2.05 × 10^4.

5,12-Dihydroxy-2-azanaphthacene-6,11-dione (21). Deblocking of *N*-trifluoro glycosides **16** and **17** was attempted under

a variety of conditions. Under mildly acidic conditions (0.1 N HCl) at various concentrations or temperatures in various aqueous methanolic solutions either no reaction was observed or the aglycon was formed. With a variety of concentrations of K_2CO_3 or NaOH in aqueous alcohols or DMF at a variety of times and temperatures, aromatization was the only reaction. In a typical experiment, the glycoside (**16**, 2 mg) was dissolved in 5.0 mL of THF and this was cooled to 0 °C in an ice bath before ice cold 0.1 N NaOH was added dropwise over a 15-min interval whereupon the initially yellow solution turned blue. After 5 h the reaction was complete as judged by TLC examination and 0.5 M citric acid (1.38 mL) was added dropwise to achieve a reddish solution of pH about 6. This solution was adjusted to pH 8 with 10% sodium bicarbonate solution with use of a syringe and then extracted with chloroform–MeOH (9:1). The organic layer was dried with sodium sulfate and evaporated to dryness to produce **21** as a brownish crystalline powder identical with a sample synthesized by Friedel–Crafts condensation between 5,8-dimethoxyisoquinoline and phthalic anhydride with $AlCl_3$–NaCl: HRMS, m/z 291.05226 ($C_{17}H_9NO_4$ requires 291.05309). This was the base peak, and only very small fragment peaks were otherwise observed. The product was too insoluble in normal solvents for ^1H NMR examination, so it was acetylated with acetic anhydride and pyridine in the usual fashion for further characterization. The product was a yellow powder: mp 240 °C; MS, m/Z 375 (M^+ – CH_2CO), 333 (M^+ – $2CH_2CO$), 291 (100); ^1H NMR ($CDCl_3$) δ 2.66 (s, 6 H, $COCH_3$), 7.5–8.38 (m, 4 H, Ar H), 8.63–9.75 (m, 3 H, PyH).

Testing in Mice against the P388 Lymphocytic Leukemia Model. 4-Demethoxydaunomycin (**20**), (7*S*)-9-aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin (**17**), and (7*R*)-9-aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin (**16**) were dissolved in Cremophor/saline to the final concentrations given below. Female CDF_1 and DBA_2 mice (Harlan Laboratory, Indianapolis, IN) housed in gang cages were fed Purine Laboratory Chow and water ad libitum and adapted to this regime for at least 1 week before use. The P388 tumor was maintained by continuous passage in DBA_2 mice. On day 0, ascitic fluid was removed and diluted with Hank's balanced salt solution, cells were counted, and 10^6 tumor cells were implanted ip in a total volume of 0.2 mL. Twenty-four hours later, mice were randomly segregated into treatment groups and drug was given ip to groups of nine mice for each dilution. Dosing was repeated on days 5 and 9 also. The mice were observed for 30 days and T/C (percent) values were determined from the survival rate as compared to the controls.[22]

4-Demethoxydaunorubicin: 3 mg/kg (T/C 82); 1.5 (97); 0.75 (160); 0.375 (121).

(7*S*)-9-Aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin: 100 mg/kg (T/C 98); 50 (102); 25 (97); 12.5 (97); 6.25 (100); 3.72 (97); 1.56 (95).

(7*R*)-9-Aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin: 100 mg/kg (T/C 105); 50 (96); 25 (90); 12.5 (98); 6.25 (97); 3.72 (97); 1.56 (95).

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Notes

Synthesis and Biological Activity of Substance P C-Terminal Hexapeptide Analogues: Structure–Activity Studies

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A series of analogues of the C-terminal hexapeptide of substance P, modified at the glutaminyl residue, was synthesized and their relative activities as spasmogens were determined in the guinea pig ileum and rat colon muscularis mucosae preparations in vitro. In general, when compared to SP_{6-11}, the loss of the carboxamide group has little effect on activity in the colon and reduces activity on the ileum. The exception to this is the Orn^6 analogue which retains activity on both preparations and is proposed as a useful tool for structure–activity studies. It is concluded that the hydrogen-bonding potential of the position 6 substituent may be an important determinant of biological activity.

In most pharmacological test systems which respond to the undecapeptide tachykinin, substance P (SP, **1**) biological activity is retained in C-terminal fragments as small as the hexapeptide Gln-Phe-Phe-Gly-Leu-Met-NH_2 (SP_{6-11}, **2**). This applies to the gastrointestinal smooth muscle[1,2] and salivary secretion[3] and to the hypotensive effects of this group of peptides.[4] In neuronal preparations (rat spinal cord and rat superior cervical ganglion, in vitro), the C-terminal hepta- and hexapeptides are somewhat more active than the parent undecapeptide.[5,6] However, the N-terminal residues should not be considered redundant since they may well contribute to the potency of the compound in some test systems, while in others, there is an absolute requirement for the N-terminal basic residues.[7,8] In view of the importance of the C-terminal hexapeptide for biological activity, this sequence should provide a basis for examining some aspects of the structure-activity relationship for tachykinin agonists.

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