Synthesis of Daunorubicin Analogues with Novel 9-Acyl Substituents[1]

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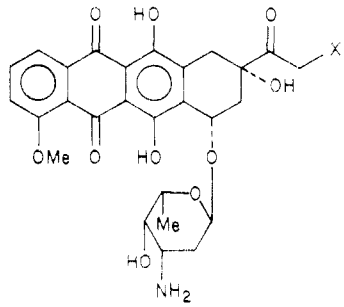
Synthetic approaches to anthracyclines bearing novel 9-acyl substituents were investigated. Reaction of the lithium enolate of *N*-(trifluoroacetyl)daunorubicin (**9**) with methyl iodide in tetrahydrofuran afforded only the 9-propionyl derivative **10** in high yield. Reaction of **10** under identical conditions cleanly afforded the 9-isobutyryl derivative **11**. Extension of this procedure to other alkylating agents (ethyl iodide, benzyl bromide, and heptyl iodide) required hexamethylphosphoramide as cosolvent and afforded mixtures of mono- and dialkylated products as well as recovered **9**. The amino group was deblocked with NaOH in aqueous tetrahydrofuran, except in the case of the dibenzyl derivative **13** which was inert under these conditions. The 9-formyl analogue **23** was prepared via $NaIO_4$ cleavage of 13-dihydroadriamycin (**21**). Antitumor evaluation against P388 leukemia in mice showed **23** to have activity comparable to the parent compounds, while the C-alkylated analogues were less active.

The anthracycline antibiotics daunorubicin[2] (**1**) and



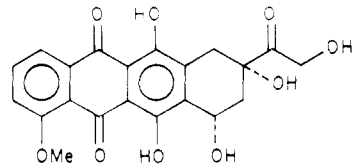
1, X = H
2, X = OH

adriamycin[3] (**2**) are clinically useful antineoplastic agents, with adriamycin having an especially broad spectrum of activity that includes various solid tumors that are normally resistant to most modes of chemotherapy. However, clinical use of these drugs is hampered by a number of undesirable side effects, the most serious being a dose-related and irreversible cardiotoxicity.[4] As part of our efforts to prepare analogues of **1** and **2** having improved therapeutic properties, we now report the synthesis and biological evaluation of a number of anthracyclines bearing novel acyl groups at C-9.

The apparent clinical superiority of adriamycin over daunorubicin, from which it differs only by the presence of a hydroxyl group at C-14, led us to investigate the side chain as a site for structural modification. Recent reports indicate that **1** and **2** can tolerate substantial modification and even removal of the 9-acyl group while retaining antitumor activity. These prior modifications involved either degradation of the side chain,[5] modifying the oxidation level or derivatization of the 13-carbonyl,[6] or the introduction of a novel heterosubstituent at C-14.[7] Our present approach to novel 9-acylanthracyclines involved the generation of new carbon–carbon bonds at C-14, except for the 9-formyl derivative **23** obtained by a degradative sequence.

Chemistry. The methodology developed in our laboratory for the synthesis of [14-^{14}C]daunorubicin and adriamycin[8] seemed appropriate as a general synthesis of 9-acylanthracyclines. In this approach, adriamycinone (**3**) is reacted with the appropriate Grignard reagent, and the resulting glycol **4** is cleaved with $NaIO_4$ to afford the 13-ketone. While this sequence afforded daunomycinone (**5**) in a 56% chemical yield from **3**, it failed in several cases when R ≠ Me. A 10% yield of **6** from **3** was the next best result obtained by this method, with Grignard addition to the quinone carbonyls appearing to be a serious side reaction.

The ready availability of 14-bromodaunomycinone (**7**)[12]



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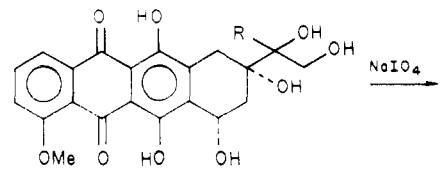

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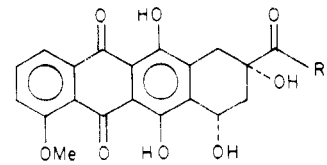

5, R = Me
6, R = Ph
7, R = CH_2Br
8, R = CH_2CH_3

led us to evaluate it as a 9-acylanthracycline synthon. Reaction of **7** with lithiodimethyl cuprate afforded only a low yield of the desired 9-propionyl aglycon **8**, with daunomycinone being the major product. The formation of **5** can be attributed to halogen–metal exchange forming the enolate, which decomposes to **5** on aqueous workup. Treatment of the reaction mixture with excess methyl iodide prior to workup[9] increased the yield of **8** to 49%. Moreover, the absence of any O-methylated products, despite the presence of four hydroxyl groups, indicated that direct alkylation of the 14-enolate may be a feasible route to C-alkylated aglycons such as **8**.

Indeed, reaction of the enolate of **5** (generated with 12 equiv of lithium diisopropylamide (LDA) in THF at −78 °C) with excess methyl iodide afforded **8** in 76% yield. In light of the demonstrated susceptibility of the phenolic hydroxyls to O-methylation (dimethyl sulfate, K_2CO_3),[10] the chemospecificity observed in the C-alkylation procedure is impressive. The absence of O-methylation is probably due to the greater association of the lithium cation to the oxygen atoms, as compared with the larger potassium ion, thus enhancing the extent of C-alkylation.[11]

Pilot experiments showed the glycoside bond to be stable to the C-alkylation conditions, and extension of this procedure to the intact glycosides went smoothly, thus obviating the need for the relatively laborious aglycon–sugar condensation. The amino group of **1** was protected via conversion to the trifluoroacetamide **9**[12] with *S*-ethyl

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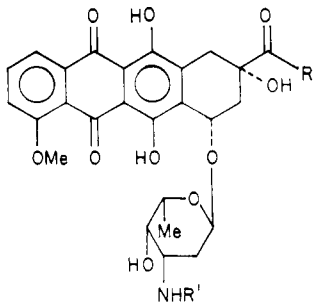


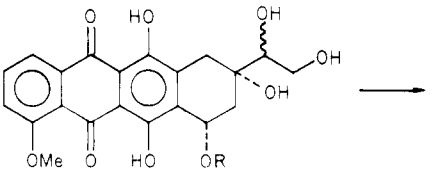
9, R = CH_3; R′ = $COCF_3$
10, R = CH_2CH_3; R′ = $COCF_3$
11, R = $CH(CH_3)_2$; R′ = $COCF_3$
12, R = CH_2CH_2Ph; R′ = $COCF_3$
13, R = $CH(CH_2Ph)_2$; R′ = $COCF_3$
14, R = $(CH_2)_6CH_3$; R′ = $COCF_3$
15, R = $CH(CH_2CH_3)_2$; R′ = $COCF_3$
16, R = $(CH_2)_6CH_3$; R′ = COCF
17, R = $CH(n\text{-}C_6H_{13})_2$; R′ = $COCF_3$
18, R = C_2H_5; R′ = H
19, R = $CH(CH_3)_2$; R′ = H
20, R = CH_2CH_2Ph; R′ = H

trifluorothioacetate and sodium methoxide. Generation of the enolate of **9** with LDA in THF followed by excess methyl iodide afforded the 9-propionyl glycoside **10** in 77% yield. Although **9** could not be dimethylated directly, **10** was smoothly alkylated under the above conditions to afford **11** in high yield.

Table I summarizes the results of our C-alkylation studies. Extension of the scope of the glycoside C-alkylation to permit the use of reagents other than methyl iodide required hexamethylphosphoramide (HMPA) as cosolvent. In the absence of HMPA, ethyl iodide was inert, and only low yields of alkylated products could be obtained with benzyl bromide and heptyl iodide. Although monoalkylation predominated, significant amounts of dialkylated products and unreacted **9** were obtained.

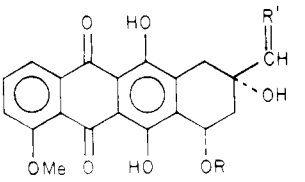
The amino group was deprotected with NaOH in aqueous THF at 0 °C to afford the amino glycosides **18**–**20**, which were isolated as the HCl salts. However, this procedure was unsuccessful with the dibenzyl derivative **13**, which was recovered unchanged after prolonged reaction under the above and more rigorous conditions. An examination of a Drieding model of **13** offers a possible explanation of this result. The second phenyl group can afford appreciable steric hindrance to the approach of base to the trifluoroacetamide moiety. The side chain of **12** has no effect on the reactivity of the trifluoroacetamido moiety, since it can readily assume a conformation minimizing such interaction. However, in **13** one of the phenyl groups is forced into close proximity to the ring A and sugar portions of the molecule.

The 9-formyl analogue **23** was obtained in a manner conceptually similar to the Grignard approach (vide supra). Adriamycin (**2**) was reduced with $NaCNBH_3$, and the resulting alcohol **21**[13] was treated with sodium metaperiodate to afford **23**, which was isolated as the hydrate. Since **23** showed both a weak aldehyde proton signal in its ^1H NMR and weak aldehyde carbonyl absorbance in its IR spectra, additional confirmation of its structure was sought. Acid hydrolysis of **23** afforded an aglycon (**24**) identical (TLC, IR) with that obtained from periodate cleavage of 13-dihydroadriamycinone (**22**). Further characterization of **24** was obtained by its conversion to the 2,4-dinitrophenylhydrazone **25**.



21, R

22, R = H



23, R =

, R = O

24, R = H; R′ = O
25, R = H; R′ = $NNHC_6H_3$-*o*, *p*-$(NO_2)_2$

Results and Discussion

Table II presents a comparison of test data for the parent antibiotics and the 9-acylanthracyclines prepared in this study. All of the compounds bind to DNA to a significant degree, as indicated by their ability to stabilize helical DNA to thermal denaturation (ΔT_m). The ΔT_m value is the difference in degree Celsius between the melting temperature of calf thymus DNA at pH 7 and the same DNA in the presence of drug at a drug–DNA phosphate ratio of 1:10. Each new analogue also displays potent inhibition of DNA and RNA syntheses in cultured L1210 cells. The most complete set of in vivo antitumor data is in the advanced P388 test (QD5, 9, 13). In this test, daunorubicin (**1**) effects only a very modest increase in survival time. Along with daunorubicin, all C-14 alkylated analogues (**18**–**20**) show weak but significant antitumor activity in this test, branched analogue **19** being the most borderline case. Compound **19** was inactive in the P388 QD1-9 test according to NCI criteria. Neither **18** nor **20** was tested in this latter system. Formyl derivative **23** showed efficacy intermediate between those of daunorubicin and adriamycin in both in vivo tests.

The available data indicate that acyl groups on the 9 position larger than acetyl do not interfere substantially with DNA binding or with nucleic acid synthesis inhibition in cultured cells. Similarly, antitumor efficacy in vivo is not grossly affected by the larger and increasingly lipophilic acyl moieties of compounds **18**–**20** in the advanced P388 test when compared with daunorubicin. Potency in this three-compound group is reduced substantially, however, and roughly according to the lipophilicity of the side chain. These data taken together suggest that distribution of the drugs is being adversely affected by these structural modifications. 9-Formyl analogue **23** is the only member of the group with a small acyl group and should be less lipophilic than daunorubicin. It provides equal or somewhat enhanced efficacy relative to daunorubicin but at much larger doses, thus not falling into the lipophilicity–potency correlation seen with the other analogues. The existence of **23** as the *gem*-diol hydrate, with tetrahedral stereochemistry at C-13, might be expected to give it unique status in the group. This is also reflected by its relatively poor ranking in ΔT_m and nucleic acid synthesis inhibition.

Although none of the new anthracycline compounds prepared in this study appear especially interesting as clinical antitumor drug candidates, the new chemistry involved in their synthesis provides a potentially useful

Table I. Summary of Enolate C-Alkylation

substrate	alkylating agent	solvent	products[a] (% yield) monoalkyl	dialkyl	recov substr
5	CH_3I	THF	8 (76)		
9	CH_3I	THF	10 (77)		
10	CH_3I	THF	11 (82)		
9	$C_6H_5CH_2Br$	2:1 THF–HMPA	12 (36)	13 (27)	9 (16)
9	CH_3CH_2I	2:1 THF–HMPA	14 (31)	15 (11)*	9 (5)
9	$CH_3(CH_2)_6I$	2:1 THF–HMPA	16 (43)	17 (12)*	9 (25)

[a] Yields refer to isolated products, homogenous by TLC and having expected IR and NMR spectra. Acceptable elemental analyses were obtained for all new compounds except where, as noted by an asterisk, sufficient material was not available.

Table II. Comparison of Biological Data for Daunorubicin, Adriamycin, and 9-Acyl Analogues Prepared in This Study

compd	ΔT_m,[a] °C	nucleic acid synth inhibn,[a] ED_{50}, μM DNA	RNA	antitumor activity in mice[b] P388, qd 5, 9, 13 opt dose, mg/kg	T/C[c] (n)[d]	P388, qd 1–9 opt dose, mg/kg	T/C (n)
1	11.2	0.66	0.33	8	132 ± 4 (29)	0.78	160 ± 27 (8)
2	13.4	1.5	0.58	8	159 ± 20 (29)	0.78	197 ± 26 (8)
18	11.0	0.67	0.29	12.5	128 ± 8 (2)		
19	10.3	1.0	0.40	37.5	122 ± 6 (2)	3.13	117
20	8.6	1.5	1.6	50	129 ± 6 (2)		
23	6.5	4.1	2.0	50	154	4	169

[a] ΔT_m and ED_{50} values were determined by the method of G. Tong, W. W. Lee, D. R. Black, and D. W. Henry, *J. Med. Chem.*, **19**, 395 (1976), except that the drugs were initially dissolved in a volume of Me_2SO that resulted in a final Me_2SO concentration of 5 and 1%, respectively, in the assay medium. This modification greatly aided solubilization and did not affect assay results according to extensive control experiments. [b] Assays were arranged through the Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute. RDF or CDF mice are injected ip with 10^6 P388 lymphoctic leukemia cells on day 0 and are treated ip on days 1–9 or 5, 9, and 13 with the specified drug dose. For detailed protocols, see R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**(2), 9 (1972). [c] Ratio of average survival times of treated mice to untreated controls in percent. The average survival time of untreated controls is approximately 11 days. Activity is defined as values of T/C ≥ 120 in the qd 5, 9, 13 protocol and T/C ≥ 125 in the qd 1–9 protocol. [d] n = number of tests.

tool for more complex structural manipulations of the 9-acyl group of daunorubicin.

Experimental Section

Solvent extracts of aqueous solutions were dried over anhydrous Na_2SO_4. Solutions were concentrated under reduced pressure using a rotary evaporator. All melting points are uncorrected. HMPA was stored over 13X molecular sieves, and THF was distilled from $LiAlH_4$ immediately prior to use. Measurements of 100-MHz NMR were performed by Mr. L. Cary using a Varian XL-100 spectrometer in $CDCl_3$, unless specified otherwise, with Me_4Si as an internal standard. Elemental microanalyses were provided by the microanalytical laboratory of Stanford University.

Thin-layer chromatograms (TLC) were obtained on silica gel GF 250-μm plates (Analtech). Preparative layer chromatograms (PLC) were obtained on 20 × 20 × 0.2 cm silica gel 60 F-254 plates (E. Merck). Preparative liquid chromatography was performed on Bio-SilA 200–325 mesh or E. Merck prepacked silica gel 60 columns.

***N*-(Trifluoroacetyl)daunorubicin (9).** Daunorubicin hydrochloride (1, 1.10 g, 1.95 mmol) was dissolved in MeOH (10 mL) and cooled to 5 °C. Sodium methoxide (108.0 mg, 1.95 mmol) was added and the solution was stirred at 5 °C for 5 min. *S*-Ethyl trifluorothioacetate (0.80 mL) was added, and the mixture was stirred at 23 °C for 16 h. Additional sodium methoxide (~20 mg) was added and stirring was continued for 24 h (total reaction time 36 h). The solvent was removed and the residue partitioned between $CHCl_3$ (200 mL) and 0.5 M citric acid (20 mL). The organic phase was separated, washed with saturated NaCl (20 mL), dried, and evaporated. The residue was precipitated from $CHCl_3$–petroleum ether to afford 1.15 g (94%) of 9.

(7*S*,9*S*)-7,8,9,10-Tetrahydro-6,9,11-trihydroxy-4-methoxy-9-propionyl-7[[2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione (10). Methyllithium (24.5 mL of 0.90 M solution in ether, 22.0 mmol) was added to diisopropylamine (6.5 mL) in THF (6.50 mL) at 0 °C under N_2. The solution was cooled to −78 °C, *N*-trifluoroacetyldaunorubicin (9, 500 mg, 0.80 mmol) in THF (8.0 mL) was added dropwise with stirring, and the mixture was maintained at −78 °C for 15 min. Methyl iodide (6.5 mL) was added, and the mixture was allowed to warm to 0 °C over 30 min and kept at 0 °C for 2 h. The reaction mixture was poured into saturated NH_4Cl ice (150 mL) and extracted with $CHCl_3$ (4 × 40 mL). The extracts were combined, dried, and evaporated. The residue was chromatographed (E. Merck prepacked silica gel 60 column, size B, 98:2 $CHCl_3$–MeOH) to afford 394.5 mg (77%) of **10**: IR 2.85 (OH, NH), 5.75 (sh), 5.80 (C=O), 6.15, 6.30 μm (H-bonded quinone); NMR δ 1.16 (t, 3, 15-H_3), 1.32 (d, 3, 6′-H_3), 1.85–2.35 (m, 4, 8- and 2′-H_2's), 2.88 (q, 3, 10β-H and 14-H_2), 3.28 (d, 1, J = 17 Hz, 10α-H), 3.68 (br s, 1, 4′-H), 4.11 (s, 3, OMe), 4.57 (m, 2, 3′- and 5′-H's), 5.27 (br s, 1, 7-H), 5.54 (br s, 1, 1′-H), 6.65 (d, 1, NH), 7.41 (dd, 1, J = 8 and 1 Hz, 3-H), 7.79 (t, 1, J = 8 Hz, 2-H), 8.06 (dd, 1, J = 8 and 1 Hz, 1-H), 13.30 (s, 1, 11-OH), 14.02 (s, 1, 6-OH); MS *m/e* 637 M^+ (45), 412 (61), 376 (38), 337 (100), 57 (48); UV visible λ_{max} (MeOH) 233 nm (ε 37 665), 252 (26 976), 289 (8901), 480 (11 961), 496 (12 216), 531 (6617); $[\alpha]_D$ +255° (MeOH) (*c* 0.05); TLC (95:5 $CHCl_3$–MeOH) R_f 0.35. Anal. ($C_{33}H_{34}F_3NO_{11}$·H_2O) C, H, N.

(7*S*,9*S*)-9-Isobutyryl-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-7-[[2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione (11). By the procedure described in the above experiment, **10** (634.0 mg, 0.994 mmol) was converted to 531.3 mg (82%) of **11**: IR 2.85 (OH, NH), 5.81 (sh), 5.85 (C=O), 6.15, 6.30 (H-bonded quinone), 9.55, 10.10 μm; NMR δ 1.22 [d, 6, CH(*Me*)$_2$], 1.32 (d, 3, 6′-H_3), 1.95 (m, 2, 2′-H_2), 2.27 (m, 2, 8-H_2), 2.96 (d, 1, J = 19 Hz, 10β-H), 3.29 (d, 1, J = 19 Hz, 10α-H), 3.59 (m, 2, 4′- and 14-H's), 4.12 (s, 3, OMe), 4.29 (m, 2, 3′- and 5′-H's), 5.35 (br s, 1, 7-H), 5.51 (br s, 1, 1′-H), 6.66 (d, 1, NH), 7.40 (dd, 1, J = 8 and 1 Hz, 3-H), 7.79 (t, 1, J = 8 Hz, 2-H), 8.06 (dd, 1, J = 8 and 1 Hz, 1-H), 13.30 (s, 1, 11-OH), 14.03 (s, 1, 6-OH); MS *m/e* 651 M^+ (16), 426 (27), 390 (13), 337 (100); TLC (95:5 $CHCl_3$–MeOH) R_f 0.41. Anal. ($C_{34}H_{36}F_3NO_{11}$·$0.5H_2O$) C, H, N.

Alkylation of the Lithium Enolate of 9 with Benzyl Bromide. Methyllithium (2.0 mL of 0.9 M solution in ether, 2.76 mmol) was added to a stirred solution of diisopropylamine (1.0

mL) in 2:1 THF–HMPA (15 mL) under N_2. The solution was cooled to −78 °C and **9** (50 mg, 0.080 mmol) in 2:1 THF–HMPA (2.0 mL) was added. After 10 min at −78 °C, benzyl bromide (0.5 mL) was added, and the mixture was allowed to warm to 0 °C over 30 min and kept at 0 °C for 1.75 h. The reaction was quenched with saturated NH_4Cl–ice (20 mL) and extracted with ethyl acetate (20 mL). The extract was washed with saturated $NaHCO_3$ (10 mL), water (4 × 15 mL), and saturated NaCl (10 mL), dried, and evaporated. The residue was precipitated from $CHCl_3$–petroleum ether, collected, and chromatographed (E. Merck prepacked silica gel 60, column-size A-99:1, $CHCl_3$–MeOH) to afford 17.5 mg (27%) of **(7*S*,9*S*)-9-(2-benzyl-3-phenylpropionyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-7-[[2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione (13)**: IR 2.85 (OH, NH), 5.80, 5.82 (C=O), 6.15, 6.30 (H-bonded quinone), 9.85, 10.10 μm; NMR δ 1.17 (d, 3, 6′-H_3), 1.84 (m, 4, 8- and 2′-H_2's), 2.72 (m, 4, 15- and 16-H_2's), 3.08 (m, 2, 10-H_2), 3.62 (d, 1, 4′-H), 4.09 (s and m, 6, OMe, 14-, 3′-, and 5′-H's), 5.13 (br s, 1, 7-H), 5.43 (br s, 1, 1′-H), 6.66 (d, 1, NH), 7.30 (m, 11, 3-H and Ar-H's), 7.76 (t, 1, J = 8 Hz, 2-H), 8.04 (dd, 1, J = 8 and 1 Hz, 1-H), 13.18 (s, 1, 11-OH), 14.03 (s, 1, 6-OH); UV–vis λ_{max} (MeOH) 235 nm (ε 38778), 253 (29006), 289 (9807), 480 (13381), 498 (13467), 531 (7666); $[\alpha]_D$ 245° (MeOH) (*c* 0.05); TLC (95:5 $CHCl_3$–MeOH) R_f 0.42. Anal. ($C_{43}H_{40}F_3NO_{11}$·0.5H_2O) C, H, N.

Continued elution afforded 20.5 mg (36%) of **(7*S*,9*S*)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-9-(3-phenylpropionyl)-7-[[2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]-5,12-naphthacenedione (12)**: IR 2.85 (OH, NH), 5.80, 5.80 (C=O), 6.15, 6.30 (H-bonded quinone), 9.85, 10.10 μm; NMR δ 1.27 (d, 3, 6′-H_3), 1.87 (m, 2, 2′-H_2), 2.18 (m, 2, 8-H_2), 2.8–3.4 (m, 6, 10-, 14- and 15-H_2's), 3.66 [d (br s on D_2O exchange), 1, 4′-H], 4.12 (s, 3, OMe), 4.03 (m, 2, 3′-, and 5′-H's), 5.25 (br s, 1, 7-H), 5.51 (br s, 1, 1′-H), 6.63 (d, 1, NH), 7.30 (s, 5, Ar-H's), 7.38 (dd, 1, J = 8 and 1 Hz, 3-H), 7.78 (t, 1, J = 8 Hz, 2-H), 8.04 (dd, 1, J = 8 and 1 Hz, 1-H), 13.29 (s, 1, 11-OH), 14.02 (s, 1, 6-OH), UV–vis λ_{max} (MeOH) 234 nm (ε 37035), 252 (27082), 289 (9067), 479 (12478), 495 (12552), 530 (6911); $[\alpha]_D$ 216° (MeOH) (*c* 0.05); TLC (95:5 $CHCl_3$–MeOH) R_f 0.38. Anal. ($C_{36}H_{34}F_3NO_{11}$) C, H, N. Further elution afforded 7.5 mg (16%) of **9**.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-9-propionyl-5,12-naphthacenedione Hydrochloride (18). To a solution of **10** (325 mg, 0.510 mmol) in THF (36 mL) at 0 °C was added 0.1 N NaOH (36 mL). The solution was kept at 5 °C for 16 h, quenched with 0.5 M citric acid (~10 mL), neutralized with $NaHCO_3$, and extracted with 9:1 $CHCl_3$–MeOH (4 × 40 mL). The extracts were combined, washed with saturated NaCl, dried, and evaporated. The residue was dissolved in MeOH (2 mL) and cooled to 0 °C. Cold methanolic HCl (2 mL of 0.276 M solution) was added followed by ether (100 mL). The precipitate was collected to afford 247.2 mg (84%) of **18**: IR 2.90 (OH, NH), 5.81 (C=O), 6.15, 6.30 μm (H-bonded quinone); NMR (free base) δ 1.17 (t, 3, 15-H_3), 1.36 (d, 3, 6′-H_3), 1.73 (m, 2, 8-H_2), 2.25 (m, 2, 8-H_2), 2.75–3.40 (m, 5, 3′-H, 10- and 14-H_2's), 3.50 (br s, 1, 4′-H), 4.12 (s, 4, OMe and 5′-H), 5.30 (br s, 1, 7-H), 5.52 (br s, 1, 1′-H), 7.39 (dd, 1, J = 8 and 1 Hz, 3-H), 7.78 (t, 1, J = 8 Hz, 2-H), 8.03 (dd, 1, J = 8 and 1 Hz, 1-H); UV–vis λ_{max} (MeOH), 234 nm (ε 33864), 252 (23333), 290 (7949), 478 (10513), 495 (10513), 530 (5641); $[\alpha]_D$ 234° (MeOH) (*c* 0.06); TLC (30:10:1, $CHCl_3$–MeOH–H_2O) R_f 0.36. Anal ($C_{28}H_{31}NO_{10}$·HCl·1.7H_2O) C, H, N.

13-Dihydroadriamycin Hydrochloride (21). To a solution of **2** (1.97 g, 3.39 mmol) in water (90 mL) was added $NaCNBH_3$ (0.64 g, 10.1 mmol) in water (45 mL). The pH was adjusted and maintained at 3.52 with 1 N HOAc while the solution was stirred at 23 °C for 6 h. The reaction mixture was basified to pH 9.52 with 1 M NaOH and lyophilized. The residue was dissolved in water (200 mL) and extracted with 6:4 $CHCl_3$–MeOH (10 × 100 mL). The extracts were combined and evaporated. The residue was dissolved in $CHCl_3$ (200 mL) and washed with H_2O (50 mL). The aqueous phase was reextracted with $CHCl_3$ (5 × 50 mL), and the organic solutions were combined, dried, and evaporated. The residue was dissolved in 4:1 $CHCl_3$–MeOH (75 mL), filtered, and cooled to ~5 °C. HCl (8.25 mL of 0.40 M solution in ether, 3.30 mmol) was added and the HCl salt precipitated with ether (175 mL) to afford 1.79 g (91%) of **21**.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-9-formyl-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Hydrochloride (23). To a solution of **21** (58 mg, 0.095 mmol) in water (3 mL) was added $NaIO_4$ (26 mg, 0.120 mmol) in water (2 mL). The solution was stirred at 0 °C for 5.5 h, basified to pH 9.4 with 1 M NaOH, and extracted with 6:4 $CHCl_3$–MeOH (4 × 25 mL). The extracts were combined and washed with water (35 mL), and the aqueous phase was extracted with $CHCl_3$ (3 × 10 mL). The organic solutions were combined, dried, and evaporated. The residue was dissolved in 6:1 $CHCl_3$–MeOH (5 mL), filtered, and cooled to ~5 °C. HCl (4.57 mL of 0.02 M solution in ether, 0.092 mmol) was added followed by ether (40 mL). The precipitate was collected to afford 36 mg (64%) of **23** essentially homogenous by TLC. An analytical sample was prepared via chromatography (E. Merck silica gel 60 prepacked column, size A, 30:10:1 $CHCl_3$–MeOH–H_2O), which afforded 17 mg of analytically pure **23**: IR 3.0 (OH, NH), 5.80 (wk, C=O), 6.20, 6.35 (H-bonded quinone), 7.80, 8.25, 8.95, 10.10 μm; NMR (Me_2SO-d_6) δ 1.20 (d, 3, 6′-H_3), 1.5–2.3 (m, 4, 8-, and 2′-H_2's), 2.7–3.1 (m, 2, 10-H_2), 4.00 (s, 3, OMe), 5.00 (br s, 1, 7-H), 5.30 (br s, 1, 1′-H), 7.5–8.0 (m, 3, 1-, 2-, and 3-H's), 9.6 (s, CHO); TLC (30:10:1 $CHCl_3$–MeOH–H_2O) R_f 0.35. Anal. ($C_{26}H_{27}NO_{10}$·HCl·1.6H_2O) C, H, N.

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Development of Ethyl 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate, a New Prototype with Oral Antiallergy Activity[1]

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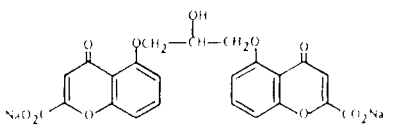
Structural modification of 3,4-dihydro-4-oxoquinazoline-2-carboxylic acid leading to ethyl 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate, a new prototype with oral antiallergy activity of the disodium cromoglycate type, is described. This prototype is 10 times more potent than disodium cromoglycate in the rat passive cutaneous anaphylaxis test. Structure activity studies indicate that a carboxylic acid moiety directly attached to the 2 position of the pyrimidine ring is most favorable for intravenous activity while esters of this acid are preferred for oral activity. The oral activity of ethyl 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate (ED_{50} = 3 mg/kg) places this ester among the more potent orally active antiallergy agents reported to date.

The discovery about a decade ago that inhalation of disodium cromoglycate (1, DSCG) provides protection



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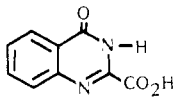
against antigen-induced seasonal allergic asthma[2] has stimulated considerable research seeking agents acting by a similar pharmacological mechanism.

Since DSCG is not absorbed orally this effort has focused on finding orally active agents. In the past few years a number of different chemical series have been reported to possess oral activity in the rat passive cutaneous anaphylaxis (PCA) procedure, a test capable of identifying antiallergy agents pharmacologically related to DSCG. Examples of these orally active series include xanthone-2-carboxylic acids,[3] 2-nitroindan-1,3-diones,[4] 3-(5-tetrazolyl)thioxanthone 10,10-dioxides,[5] 8-azapurinones,[6] 1,4,6,9-tetrahydro-4,6-dioxopyrido[3,2-*g*]quinoline-2,8-dicarboxylic acids,[7] cinnoline-3-propionic acids,[8] 2- and 3-substituted chromones,[9-12] and aryl oxamates.[13]

We wish to report the development of a novel prototype, ethyl 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylate, which exhibits oral DSCG-type antiallergy activity. This compound was developed by successive molecular modification of the carbocyclic ring of 3,4-dihydro-4-oxoquinazoline-2-carboxylic acid (3), leading to



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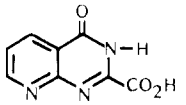

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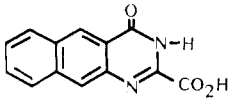

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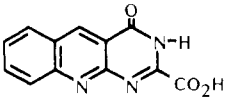

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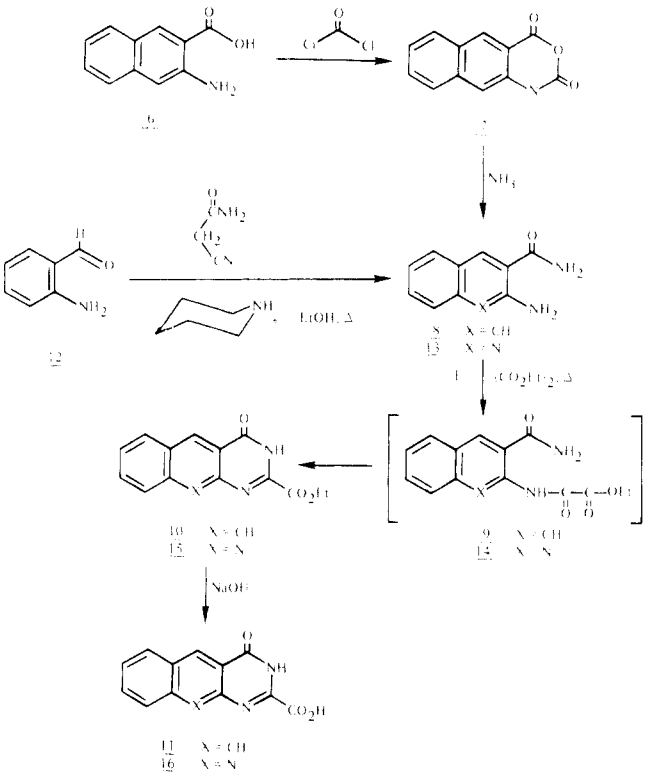
acids **5**, **11**, and then **16**. Initial attempts to increase potency by substitution of the carbocyclic ring of **3** were unsuccessful. However, either incorporation of a nitrogen atom into this ring or fusion of another ring onto **3**, affording **5** and **11**, respectively, did increase potency. A further, marked potency enhancement was achieved with compound **16** which incorporates both of these changes. More significantly, the ethyl ester of **16** displayed potent oral activity.

Scheme I. Synthesis of 3,4-Dihydro-4-oxobenzo[*g*]quinazoline-2-carboxylic Acids (X = CH) and 3,4-Dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic Acids (X = N)



Chemistry. Although a few 3,4-dihydro-4-oxoquinazoline-2-carboxylic acids (**3**) have previously been reported in the literature,[14] this communication reports the first synthesis of 3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidine-2-carboxylic acids (**5**), 3,4-dihydro-4-oxobenzo[*g*]quinazoline-2-carboxylic acids (**11**), and 3,4-dihydro-4-oxopyrimido[4,5-*b*]quinoline-2-carboxylic acids (**16**). Esters of these four series of 3,4-dihydro-4-oxopyrimi-

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