H's), 8.2 (1, s, NH)].

Ethyl 3-Hydroxy-7-*tert*-butyl-2-oxoindoline-3-acetate. Ethyl bromoacetate (24.5 ml, 0.22 mol) was added to a stirred suspension of 7-*tert*-butylisatin (15.0 g, 0.074 mol) and granulated zinc (14.0 g, 0.214 g-atom) in C_6H_6 (200 ml). The mixture was heated at reflux for 20 hr and then poured onto cracked ice and 20% aqueous H_2SO_4. The organic phase was washed with H_2O, dried, and evaporated to give a residue which was chromatographed on silica gel. Elution with C_6H_6–EtOAc (7:3) gave the product (15.5 g, 72%) as an oil: NMR δ 1.15 (3, t, J = 7 Hz, CH_2CH_3), 1.35 [9, s, 1.35, $C(CH_3)_3$], 2.93 (2, s, CH_2CO), 4.12 (2, q, J = 7 Hz, CH_2CH_3), 4.65 (1, s, OH), 7.0–7.4 (3, m, aromatic H), 9.4 (1, s, NH).

7-*tert*-Butyltryptophol. Ethyl 3-hydroxy-7-*tert*-butyl-2-oxoindoline-3-acetate (15.5 g) and $LiAlH_4$ (12.0 g) were combined in THF (300 ml) and the mixture was heated at reflux for 2 hr. A conventional work-up procedure afforded the product (11.0 g, 95%) as an oil: NMR δ 1.45 [9, s, $C(CH_3)_3$], 1.95 (1, s, OH), 3.0 (2, t, J = 7.0 Hz, $=CCH_2$), 3.8 (2, t, J = 7.0 Hz, CH_2O), 6.95–7.60 (4, m, aromatic H's), 8.4 (1, s, NH).

2-Cyclopropylphenylhydrazine Hydrochloride. 2-Cyclopropylaniline (26.6 g, 0.2 mol) dissolved in a mixture of concentrated HCl (150 ml) and H_2O (150 ml) was treated at −5° during 40 min with a solution of $NaNO_2$ (14.5 g) in H_2O (28 ml). After 1 hr at 0°, a solution of $SnCl_2 \cdot 2H_2O$ (112.5 g) in concentrated HCl (100 ml) was added during 30 min at −15°. After stirring for 90 min, the resulting precipitate was isolated and stirred with Et_2O and 10% aqueous NaOH. The Et_2O phase was washed with brine, dried, and treated with anhydrous HCl in Et_2O. The precipitate was collected, washed with Et_2O, and dried to give 24.7 g of the product (66.9%): mp 168–169°; NMR δ 0.5–1.1 (4, m, CH_2CH_2), 1.35–1.85 (1, m, CH), 4.13 (3, s, $NHNH_2$), 6.6–7.4 (4, m, aromatic H's).

7-Cyclopropyltryptophol. To a stirred suspension of 2-cyclopropylphenylhydrazine hydrochloride (24.9 g, 0.14 mol) in H_2O (7 ml) and dioxane (110 ml) was added a solution at 2,3-dihydrofuran[12] (16.8 g, 0.28 mol) during 10 min. The mixture was heated at 100° for 6 hr, cooled, and diluted with Et_2O (1.5 l.). The Et_2O phase was decanted from an insoluble residue, dried, and evaporated. The residue was chromatographed on silica gel. Elution with C_6H_6–EtOAc (3.1) gave the product (6.7 g, 24.9%) as an oil: NMR δ 0.8 (4, m, cyclopropane CH_2), 2.0 (1, m, CH), 3.0 (2, t, J = 6.5 Hz, $=CCH_2$), 3.9 (2, t, J = 6.5 Hz, CH_2O), 6.9–7.6 (4, m, aromatic H's), 8.8 (1, broad, NH).

(f) Tryptophols → Tetrahydropyrano[3,4-*b*]indole-1-acetic Acids. 1-Ethyl-8-(2-propyl)-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid (22). A mixture of 7-(2-propyl)tryptophol (1.9 g, 0.0094 mol), ethyl propionylacetate (1.63 g, 0.011 mol), and *p*-toluenesulfonic acid (200 mg) was refluxed in C_6H_6 (50 ml) under a Dean-Stark trap for 5 hr. The C_6H_6 solution was washed with 5% aqueous $NaHCO_3$ and worked up in the conventional manner to give an oil which was eluted from a silica gel column with C_6H_6–Et_2O (4:1) to afford the ethyl ester of the product (1.0 g, 32%). It was dissolved in EtOH (25 ml) and 10% aqueous NaOH (25 ml) was added. The mixture was refluxed for 4 hr and after a conventional work-up procedure the product was obtained as a solid residue. It was crystallized from Et_2O–hexane to give the product **22**. Analytical data and melting points for **22** and other final products prepared by the same method are collected in Table I.

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Adriamycin Analogs. Periodate Oxidation of Adriamycin

George Tong, William W. Lee, Douglas R. Black, and David W. Henry*

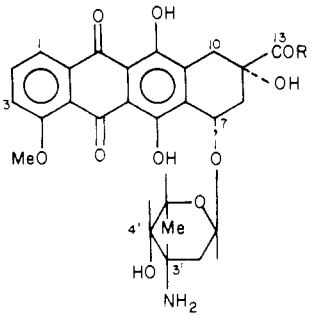
Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025. *Received August 6, 1975*

Adriamycin is selectively cleaved in high yield at the C_{13}–C_{14} bond by 1 equiv of sodium metaperiodate to yield carboxylic acid **3**. The corresponding methyl ester **4** is obtained by Fischer esterification. Spectral studies indicate that **3** and **4** bind to calf thymus DNA in a manner similar to adriamycin and daunomycin but T_m measurements suggest that less stable complexes are formed. Ester **4** inhibits DNA and RNA synthesis in cultured L1210 cells at levels comparable to adriamycin but acid **3** is much less effective. Both new compounds are moderately effective as antitumor agents against P388 lymphocytic leukemia in the mouse.

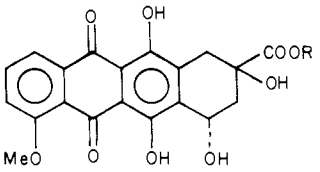
Recent reviews[1,2] have emphasized the importance of adriamycin (1) in the treatment of a wide spectrum of human cancers. However, its administration is accompanied by various undesirable side effects,[1] especially cardiomyopathy,[1,3,4] and less toxic analogs would greatly extend its usefulness. Daunomycin (**2**), a closely related antibiotic that appears restricted primarily to use against leukemia,[2] also suffers from similar defects. We are reporting the synthesis and early biological evaluation of two new analogs, carboxylic acid **3** and the corresponding

methyl ester 4.

Treatment of adriamycin hydrochloride in aqueous methanol at 0° with 1 equiv of sodium metaperiodate effects selective cleavage of the C_{13}–C_{14} bond of the hydroxyacetyl moiety with the formation of carboxylic acid 3 in high yield. Carboxylic acid 3 exists as a zwitterion according to its ir spectrum and precipitates as a red, highly insoluble, gelatinous material. It is converted to the hydrochloride salt for solubilization and ease of handling. Acid 3 was characterized by elemental and spectral analyses and by hydrolysis to aglycone 5 and the amino sugar daunosamine.



1, R = CH_2OH
2, R = CH_3
3, R = OH
4, R = OCH_3



5, R = H
6, R = CH_3

Paper chromatographic comparison with authentic daunosamine established that the latter product survived the oxidation intact. The periodate cleavage is highly specific under our conditions; although the C_9–C_{13} and $C_{3'}$–$C_{4'}$ bonds are also potentially susceptible, we saw no evidence of attack at these positions. Hurwitz et al.[5] recently reported the use of sodium periodate to cleave the $C_{3'}$–$C_{4'}$ bond of both daunomycin and adriamycin to form the corresponding ring-opened dialdehydes. Although these authors did not chemically characterize their products, we believe that their choice of reaction conditions (pH 7.2 buffer) differed sufficiently from ours to alter the selectivity of the reagent. The optimum rate of cleavage of vicinal amino alcohols is reported to occur at pH 7–8, whereas hydroxy ketones are most readily cleaved at pH 3–5.[6] Based on a pK_a of 8.22 for adriamycin hydrochloride,[7] our reaction medium is calculated to be about pH 5 initially and is more acidic at the end of the reaction.

Acid 3 is esterified with unusual ease under acid-catalyzed conditions. In the formation of the HCl salt of 3 in methanol, excess acid must be avoided. The presence of one-third of an equivalent of excess HCl in methanolic solution at room temperature results in good yields of methyl ester 4 after 24 h. Methanolysis of the glycosidic bond also occurs to a significant extent, a 14% yield of esterified aglycone 6 having been isolated in one experiment.

As reviewed recently by Di Marco and Arcamone,[8] the cytotoxic effects of adriamycin and daunomycin are thought to be mediated by a nuclear DNA–drug complex, and it is well established that both antibiotics bind strongly to isolated helical DNA by an intercalative mechanism. It was therefore of interest to compare DNA binding parameters of adriamycin and daunomycin with those of modified analogs 3 and 4. The ultraviolet and visible spectra of 3 and 4 were, as expected, essentially identical with those of the natural products. When titrated with increasing concentrations of sonicated calf thymus DNA, the resulting bathochromic shifts and decreased absorptivity of the visible maxima of adriamycin, daunomycin, 3, and 4 essentially coincided over a concentration ratio range of 1:1–16:1 and matched reported values.[8] Table I presents comparative data.

This result suggests that the DNA binding mechanisms of 3 and 4 do not differ substantially from those of adriamycin or daunomycin, at least insofar as the chromophore is involved. However, we noted a highly significant difference from the parent antibiotics when we compared their effects on stabilization of helical DNA to thermal denaturation. Under the standard conditions employed in our work, adriamycin elevated the melting temperature of calf thymus DNA by 17.8°; corresponding values for acid 3 and ester 4 were 2.6 and 12.6°, respectively (Table I). Clearly, some details of the binding processes of 3 and 4 to DNA differ from those of adriamycin and daunomycin in a manner causing destabilization of the complex. Electrostatic repulsion between the negatively charged DNA and the anionic center of the drug carboxyl could account for the small ΔT_m of 3.

As an inhibitor of nucleic acid synthesis in cultured L1210 cells, acid 3 proved to be nearly inactive; it provided ED_{50} values of >100 and 65 μM for DNA synthesis and RNA synthesis, respectively. These values contrast sharply with the corresponding values for adriamycin and daunomycin, all of which are <1 μM (Table I). Ester 4 was nearly as potent as adriamycin in this test, giving ED_{50} values of 2.0 and 0.9 μM for DNA and RNA inhibition, respectively. The marked difference between 3 and 4 in this in vitro system could be attributed to decreased cellular uptake caused by the highly polar character of 3. Aglycones 5 and 6 did not inhibit DNA and RNA synthesis significantly in this test below 100 μM.

Analogs 3 and 4 were assayed in vivo against transplanted P388 lymphocytic leukemia in the mouse; Table I presents the data. Both compounds increased the survival time of tumor-bearing animals, but substantially higher doses were required than when adriamycin or daunomycin were used. Acid 3 was better than ester 4 and about equivalent to daunomycin in efficacy, but both analogs (and daunomycin) were clearly less effective than adriamycin. The slight in vivo superiority of 3 over 4 contrasts strongly with the results of the in vitro tests, in which 3 was much less active. No explanation for this discrepancy is obvious, but it would not be unexpected that two compounds differing substantially in polarity would be metabolized or transported differently by the host.

Compounds 3 and 4 do not seriously challenge adriamycin as antitumor drugs in terms of efficacy or potency. However, they could be significant in future structure-activity studies if their cardiotoxicity potential were reduced, and this characteristic is being evaluated. Perhaps 3 and 4 are most interesting at present because they are the only in vivo active adriamycin analogs reported to date in which the carbon skeleton of the parent antibiotic has been modified.

Experimental Section

Melting points were taken on a Fisher-Johns hot-stage apparatus and are not corrected. NMR spectra were obtained on Varian A-60 or XL-100 spectrometers. Infrared spectra were obtained on a Beckman IR-4 instrument. Analyses for indicated elements were within ±0.4% unless otherwise indicated.

Uv–visible spectra were obtained on Cary 11 or Cary 14 recording spectrophotometers. In drug–DNA titration experiments, 0.005 M Tris buffer at pH 6 was used. To help avoid precipitation of drug–DNA complex at low ratios of drug to DNA, the calf thymus DNA (Miles Laboratories) stock solution (400 mg/240 ml) was sonicated with ice chilling in 40- to 50-ml portions for

Table I. Comparison of Test Data for Adriamycin and Analogs

Drug	Concn, M × 10^5	Visible spectra,[a] λ max (ε) Without DNA	Visible spectra,[a] λ max (ε) [DNA]/[drug] = 16:1	ΔT_m,[a] °C	Nucleic acid syn inhibn,[a] ED_{50}, μM DNA	Nucleic acid syn inhibn,[a] ED_{50}, μM RNA	Antitumor act. in mice (P-388, qd 1-9)[b] Dose, mg/kg	Antitumor act. in mice (P-388, qd 1-9)[b] T/C[c]
Adriamycin	2.10	479 (10500), 495 (10500), 533 sh (5700)	485 sh (6400), 507 (6900), 542 sh (4500)	17.8	0.8	0.9	4[d]	71
							2	106
							1	212
							0.5	182
							0.25	162
							0.12	156
							0.06	139
Daunomycin	2.00	479 (10500), 495 (10400), 535 sh (5500)	485 sh (6200), 508 (6800), 542 sh (4400)	16.8	0.3	0.3	4[d]	96
							2	150
							1	157
							0.5	150
							0.25	142
							0.12	130
							0.06	112
3	1.54	478 (9500), 496 (9400), 535 sh (5100)	485 sh (5900), 508 (7050), 544 sh (4000)	2.6	>100	65	50[e]	151
							25	163
							12.5	151
							6.25	142
							3.12	107
							1.56	100
4	1.63	478 (9600), 497 (9200), 535 sh (4900)	485 sh (6000), 508 (6500), 543 sh (4000)	12.6	2.0	0.9	75[e]	141
							50	125, 146
							25	126, 115
							12.5	112, 126
							6.25	108, 111
							3.12	103
							1.56	107

[a] For details see Experimental Section. [b] Assays performed by Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute. BDF or CDF mice are injected ip with 10^6 P-388 lymphocytic leukemia cells on day 0 and treated ip on days 1-9 with the specified drug dose. Detailed protocols are described in ref 9. [c] Ratio of average survival time of treated mice to that of untreated controls in percent. The average survival time of untreated controls is approximately 11 days. No significant acute toxic drug deaths (survival less than 5 days after initiation of treatment) were seen at any of the dose levels reported. [d] Eighteen mice per dose level. [e] Six mice per dose level.

7 min per portion with a Biosonik sonicator (low range, intensity setting at 60). The drug–DNA solutions were prepared by pipetting a constant volume of stock drug solution into a volumetric flask, adding the required volume of stock DNA solution, and bringing to constant total volume with buffer. Drug–DNA ratios of 1:1, 1:2, 1:4, 1:8, and 1:16 are run routinely. DNA concentration is based on percent phosphorus content as provided by the supplier.

For determining the DNA melting temperature, 0.010 M phosphate buffer containing 10^{-4} M EDTA at pH 6 was used. Drug and DNA concentrations were 10^{-5} and 10^{-4} M, respectively. The 259-nm DNA peak was monitored in a Beckman DU uv spectrophotometer fitted with a jacketed cell compartment. The cells were heated by circulating ethylene glycol–water or silicone oil from a Lauda K-2/R controlled-temperature bath heated at a constant rate of 10°/h. Absorbance was read at 1° intervals. Cell temperature was monitored by a thermocouple immersed in buffer in an unused cell in the compartment. The T_m was obtained by plotting absorbance against temperature and taking the midpoint of the curve between the high and low temperature constant-absorbance regions. The melting temperature of uncomplexed DNA under these conditions is 63.6°.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-9-carboxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Hydrochloride (3). To a stirred solution of 1.450 g (2.5 mmol) of adriamycin hydrochloride in 150 ml of MeOH–water (1:2) cooled in an ice bath was added dropwise a cold solution of 0.535 g (2.5 mmol) of $NaIO_4$ in 25 ml of water. After stirring at ice-bath temperatures for 2 h, the clear red, viscous solution (pH 2.3) was adjusted to pH 6.0 with 25 ml of 0.2 M $NaHCO_3$ solution added dropwise. The resulting gelatinous precipitate was stirred in the ice bath for 1 h and then collected by centrifugation at 7700g for 20 min (Sorvall SS-34 rotor at 8000 rpm in an SS-3 centrifuge). The gelatinous precipitate was washed with 2 × 50 ml of water by centrifugation (7700g for 20 min) and then transferred to a medium fritted disk funnel and filtered for 2 days in the dark to afford 1.257 g (95%) of the free base of **3**. To a stirred suspension of the free base (1.257 g, 2.37 mmol) in 25 ml of water cooled in an ice bath was added 23.7 ml of 0.1 N HCl. The gelatinous acid dissolved after about 1 h of stirring to yield a clear, slightly viscous solution that was frozen and lyophilized in the dark. A solution of the lyophilized product in 25 ml of MeOH was stirred, and 75 ml of ether was added dropwise. The resulting precipitate was collected, washed with 3 × 15 ml of ether, and dried at room temperature (0.1 mm, 17 h) to give 1.225 g (85%) of **3**·HCl as a red powder: mp 180–182° dec; ir (Nujol) 2.90 (OH, NH), 5.76 (C=O, CO_2H), 6.13, 6.28 μm (C=O, H-bonded quinone); uv–visible λ max (CH_3OH) 234 nm (ε 34700), 252 (26100), 288 (8800), 478 (11800), 495 (12100), 530 (6830); NMR (Me_2SO-d_6) δ 7.78 (m, 2, H-1, 3), 7.56 (m, 1, H-2), 5.31 (br s, 1, H-1'), 4.89 (br s, 1, H-7), 4.18 (d, 1, H-5'), 3.97 (s, 3, OCH_3), 3.61 (br s, 1, H-4'), 3.39 (m, 1, H-3'), 2.92 (br s, 2, H-10), 2.21 (m, 2, H-8), 1.74 (m, 2, H-2'), 1.16 (d, 3, CH_3-6'); $[\alpha]^{20}D$ +110 ± 12° (*c* 0.028, EtOH); TLC on SiHF [$CHCl_3$–CH_3OH–H_2O (40:10:1)] R_f 0.03. Anal. ($C_{26}H_{27}NO_{11}$·HCl·0.75H_2O) C, H, N; Cl: calcd, 6.12; found, 5.46, 5.34.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-9-carboxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-3,12-naphthacenedione Methyl Ester Hydrochloride (4). To a solution of 0.703 g (1.24 mmol) of **3**·HCl in 25 ml of MeOH was added 5.0 ml (0.4 mmol) of 0.08 M methanolic HCl. After being at room temperature in the dark for 24 h, the reaction mixture was cooled and poured into 75 ml of ice water. The acidic aqueous solution was washed with 2 × 25 ml of $CHCl_3$ and then basified with 0.210 g (2.5 mmol) of $NaHCO_3$. The aqueous mixture was extracted with 4 × 25 ml of $CHCl_3$, and the combined extract was washed with 10 ml of water, dried, and evaporated. The residue was dissolved in 13.5 ml (1.08 mmol) of 0.08 M methanolic HCl; the solution was stirred and diluted with 40 ml of ether added dropwise. The resulting precipitate was collected, washed with 3 × 5 ml of ether, and dried at room temperature (0.1 mm, 15 h) to afford 0.481 g (67%) of the ester hydrochloride (**4**·HCl), mp 172.5–174° dec. The ana-

lytical sample of 4·HCl, prepared in another run, had mp 173–174.5° dec: ir (Nujol) 2.92 (OH), 5.75 (C=O, ester), 6.17, 6.32 (C=O, chelated quinone), 7.98 μm (COOR); uv-visible λ max (MeOH) 234 nm (ε 38000), 252 (25100), 288 (8560), 478 (11900), 495 (11900), 530 (6380); NMR (Me_2SO-d_6) δ 7.83 (m, 2, H-1,3), 7.62 (m, 1, H-2), 5.31 (br s, 1, H-1'), 4.89 (br s, 1, H-7), 4.19 (d, 1, H-5'), 3.97 (s, 3, OCH_3), 3.72 (s, 3, CO_2CH_3), 2.96 (br s, 2, H-10), 2.28 (br s, 2, H-8), 1.75 (m, 2, H-2'), 1.16 (d, 3, CH_3-6'); $[\alpha]^{20}D$ 175 ± 8° (c 0.052, EtOH); TLC on SiHF [$CHCl_3$–CH_3OH–H_2O (40:10:1)] R_f 0.21. Anal. ($C_{27}H_{29}NO_{11}$·HCl·1.5H_2O) C, H, N; Cl: calcd, 5.84; found, 5.34.

(7*S*,9*S*)-9-Carboxy-4-methoxy-7,8,9,10-tetrahydro-6,7,9,-11-tetrahydroxy-5,12-naphthacenedione (5). A 0.079-g (0.15 mmol) sample of 3·HCl was dissolved in 5 ml of 0.2 N HCl, and the solution was heated at 90° for 30 min (a precipitate began to form after ~5 min). The mixture was cooled to room temperature, and the precipitate was collected and washed with 4 × 0.5 ml of water to afford a dark-red powder, 0.064 g. A 0.050-g sample of this powder was dissolved in 10 ml of hot $CHCl_3$–MeOH (19:1) and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 5 ml of hot $CHCl_3$–MeOH (9:1), and the hot solution was diluted slowly with 5 ml of C_6H_6 added dropwise. After standing overnight in the dark, the gelatinous precipitate was collected, washed with 4 × 0.25 ml of C_6H_6, and dried at room temperature (0.1 mm, 17 h) to afford a dark-red powder: 0.040 g (80%); mp 171–175° dec; ir (Nujol) 2.85 (OH), 3.75–4.3 (CO_2H), 5.75 (C=O, CO_2H), 6.15, 6.30 μm (H-bonded quinone); uv-visible λ max (MeOH) 233 nm (ε 36800), 252 (26600), 288 (8800), 479 (12000), 495 (12200), 530 (6830); NMR (Me_2SO-d_6) δ 13.84 (s, 1, OH-6,11), 13.11 (br s, 1, OH-6,11), 7.76 (br s, 2, H-1,3), 7.51 (m, 1, H-2), 4.4 → 6.0 (v br, OH-7,9, CO_2H), 4.94 (br s, 1, H-7), 3.95 (s, 3, OCH_3), 2.96 (q, 2, J = 18 Hz, H-10), 2.19 (br s, 2, H-8); $[\alpha]^{20}D$ +14 ± 8° (c 0.042, dioxane); TLC on SiHF [$CHCl_3$–CH_2OH–H_2O (20:10:1)] R_f 0.03; TLC on Avicell [$CHCl_3$–CH_3OH–H_2O (40:10:1)] R_f 0.88. Anal. ($C_{20}H_{16}O_9$·0.5H_2O) C, H.

The acidic filtrate from the hydrolysis was frozen and lyophilized to afford an amino sugar that was identical chromatographically with daunosamine hydrochloride in EtOH–H_2O (7:3), R_f 0.74, in *n*-BuOH–AcOH–H_2O (5:2:3), R_f 0.38, and in MeOH–H_2O–NH_4OH (90:10:1), R_f 0.70, when detected with silver nitrate–sodium hydroxide spray.

(7*S*,9*S*)-4-Methoxy-9-carboxy-7,8,9,10-tetrahydro-6,7,9,-11-tetrahydroxy-5,12-naphthacenedione Methyl Ester (6). To a solution of 1.89 mmol of 3·HCl in 30 ml of MeOH was added 5.0 ml (0.63 mmol) of 0.125 M methanolic HCl. After stirring at room temperature in the dark for 23 h, the reaction mixture was cooled to 0° and poured into 100 ml of ice water. The acidic aqueous solution was extracted with 2 × 35 ml of $CHCl_3$, and the combined extracts were washed with 10 ml each of dilute $NaHCO_3$ and water. After drying, the organic phase was evaporated to a glassy residue, 0.167 g. A 0.150-g sample of this residue was dissolved in 8 ml of hot $CHCl_3$–MeOH (9:1). The hot solution was diluted with 10 ml of $CHCl_3$ and then was heated and concentrated under a gentle stream of nitrogen to about 8 ml to remove the MeOH. After being at room temperature in the dark overnight, the crystalline precipitate was collected, washed with $CHCl_3$, and dried at room temperature (0.1 mm, 16 h) to afford very fine fibrous needles of 6: 0.073 g; mp 227–230° dec. An additional 0.035 g [total 0.108 g (14%)] of 6 was obtained from the mother liquors: mp 225–230° dec; ir (Nujol) 2.84 (OH), 5.75 (C=O, ester), 6.20, 6.31 μm (C=O, quinone); uv-visible λ max (MeOH) 233 nm (ε 37400), 251 (25300), 288 (8700), 478 (11600), 494 (11700), 529 (6400); NMR ($CDCl_3$) δ 14.05 (s, 1, OH-6,11), 13.38 (s, 1, OH-6,11), 8.00 (d, 1, J = 8 Hz, H-1), 7.75 (t, 1, J = 8 Hz, H-2), 7.35 (d, 1, J = 8 Hz, H-3), 5.36 ("m", 1, H-7), 4.07 (s, 3, OCH_3), 3.93 (s, 1, OH), 3.87 (s, 3, CO_2CH_3), 3.80 (s, 1, OH), 3.30 ("d", 1, J = 19 Hz, H-10B), 3.06 ("d", 1, J = 19 Hz, H-10A), 2.48 (m, 1, H-8B), 2.29 (m, 1, H-8A); $[\alpha]^{21}D$ +154 ± 8° (c 0.047, dioxane); TLC on SiHF [$CHCl_3$–CH_3OH (19:1)] R_f 0.40. Anal. ($C_{21}H_{18}O_9$) C, H.

L1210 Nucleic Acid Synthesis Inhibition Assay. L1210 cells were obtained from Dr. Phillip Thayer of Arthur D. Little and were grown in RPMI 1640 medium containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% heat inactivated fetal bovine serum. Cells were stirred in suspension at 34° and were maintained at a cell density of 1–4 × 10^6/ml. The average cell doubling time was less than 24 h and cell viability was greater than 95%.

The nucleic acid synthesis inhibition assay is based in part on the work of Meriwether and Bachur.[10] L1210 cells were adjusted to a cell density of 2 × 10^6/ml and HEPES buffer (pH 7.2) was added to 20 mM. This cell suspension (1 ml) was added to 1 ml of each antitumor drug solution also made up in 1640 medium containing antibiotics, 10% fetal calf serum, and 20 mM HEPES, pH 7.2 buffer. Test compounds were typically prepared as a 200-μM sonicated solution. Drugs were assayed initially at concentrations of 100, 31.6, 10.0, 3.16, 1.00, 0.316, and 0.100 μM. Assays with drug concentrations bracketing the ED_{50} were repeated for confirmation.

L1210 cells were incubated with test compounds in a shaking water bath for 3 h at 37°. Control L1210 cells were also included in each assay as was a daunorubicin internal standard. Cells were then incubated for 1 h with either 0.5 μCi/ml of [^{3}H]thymidine or [^{3}H]uridine. The assay solutions were then made 10% (w/v) with TCA and allowed to stand for at least 1 h at 0°. The resulting precipitates were collected on Whatman GF/A glass fiber filter (prewet with 20% TCA) and washed extensively with 10% TCA. Each filter was transferred to a glass scintillation vial and the precipitate dissolved with 1 ml of NCS tissue solubilizer (Amersham Searle) diluted 1:3 with scintillation solution (42 ml of liquifluor per liter of toluene; New England Nuclear). After 30–60 min, 5 ml of scintillation solution containing 0.1% glacial acetic acid was added and radioactivity was determined by scintillation spectrometry.

The percent synthesis of DNA or RNA in the test samples was calculated relative to synthesis in the controls. The percentage of inhibition of synthesis closest above and below 50% was plotted; by extrapolation the concentration of test compound causing 50% inhibition of either RNA and DNA synthesis was determined.

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