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Synthesis and Antileukemic Activity of 5-Substituted 2,3-Dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine Diesters

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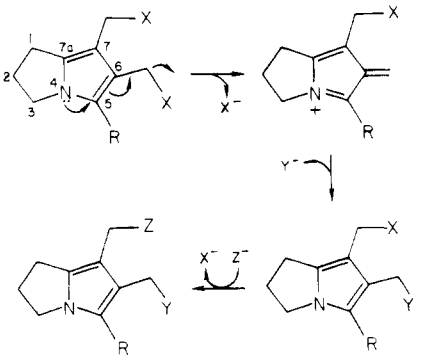
Treatment of *N*-acylproline derivatives, **2**, with acetic anhydride–dimethyl acetylenedicarboxylate (DMAD) gave 5-substituted derivatives of dimethyl 2,3-dihydro-1*H*-pyrrolizine-6,7-dicarboxylate (**5**). The reaction proceeds via a 1,3-dipolar addition of DMAD with the mesoionic oxazalone intermediate **3**, generated in situ, with concomitant elimination of carbon dioxide. Reduction of **5** gave the diols **6** which upon subsequent acylation gave **1**. The bis(*N*-methylcarbamate) **1d** and the diacetate **1i** show a modest level of in vivo antileukemic activity in the L1210 assay. A majority of the diesters, **1**, showed significant antileukemic activity in the in vivo P-388 assay. The bis(carbamate) **1d** afforded "cures" at dose levels as low as 12.5 mg/kg; **1q** showed potent activity at doses as low as 0.78 mg/kg. Several other compounds showed potent activity against P-388 over a greater than fourfold dose range with no acute toxicity. Half-lives for several diacetate derivatives of **1** were determined for aqueous Me_2SO solutions. The preparation of **7** and **8** shows that **1** may react by *O*-alkyl ester cleavage.

A large number of structurally diverse naturally occurring tumor inhibitory compounds have been isolated and identified over the past several years and a major proportion of these compounds contains at least one, often two or three, reactive electrophilic centers in the molecule in addition to several nonelectrophilic moieties.[1] The polyfunctionality is significant because it is the complex interrelationship of these functional groups that contributes to the antitumor activity, cell specificity, and toxicity which these compounds exhibit overall.[2]

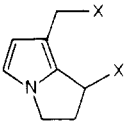
Structure–activity relationship studies with many of these natural products are often limited by the small quantities of material available and by the relatively limited number of modifications which actually can be performed on these complex molecules. Insight into the relationships between structure and activity can, in many instances, only be gained through studies with simpler molecules. The design of these simpler molecules uses the natural product as the base template.[3-5]

During the course of our continuing effort to prepare simple polyfunctional compounds for antitumor evaluation, we synthesized a series of substituted 2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine diesters (**1**). Compounds of this type were chosen for study on the basis of certain similarities with the tumor inhibitory mitomycins[6] and pyrrolizidine alkaloids.[7] The pyrrole metabolites of various pyrrolizidine alkaloids can act as alkylating agents but are too reactive and too toxic for drug use. Mitomycin, on the other hand, possesses similar reactive electrophilic centers but is sufficiently stable to reach the cell nucleus where it can react with DNA. Both the mitomycins and

Scheme I[12]



the pyrrolizidine alkaloid pyrrole metabolites possess the general partial structure



Since the pyrrole metabolites of the pyrrolizidine alkaloids appear to be too reactive to give useful cancer chemotherapeutic activity, it should be possible to modulate this reactivity downward toward that of the mitomycins. The potential electrophilic reactivity of the allylic esters in **1** (via *O*-alkyl cleavage[8]) will be enhanced by participation of the ring nitrogen (Scheme I) similar to the mitomycins and pyrrolizidine alkaloid pyrrole metabolites. Fur-

Table I

Compd (% yield)[a]	R	R'	Recrystn solvent	Mp, °C	Formula[b]
1a (90)	CF_3	CH_3	Et_2O-petr ether[c]	46-47	$C_{14}H_{16}NO_4F_3$
1b (95)	CF_3	$NHCH_3$	*i*-PrOH	182-183 dec[d]	$C_{14}H_{18}N_3O_4F_3$
1c (76)	3,4-Dichlorophenyl	CH_3	$(i\text{-Pr})_2O$	108-109	$C_{19}H_{19}NO_4Cl_2$
1d (91)	3,4-Dichlorophenyl	$NHCH_3$	EtOAc-$(i\text{-Pr})_2O$	152-154 dec[d]	$C_{19}H_{21}N_3O_4Cl_2$
1e (86)	4-Chlorophenyl	CH_3	Et_2O[e]	90.5-91.5 dec	$C_{19}H_{20}NO_4Cl$
1f (79)	4-Chlorophenyl	$NHCH_3$	EtOAc	133-134 dec[d]	$C_{19}H_{22}N_3O_4Cl$
1g (89)	Phenyl	CH_3	Et_2O-petr ether	80-81 dec	$C_{19}H_{21}NO_4$
1h (85)	Phenyl	$NHCH_3$	EtOAc-petr ether	159-160 dec[d]	$C_{19}H_{23}N_3O_4$
1i (62)	4-Methoxyphenyl	CH_3	$(i\text{-Pr})_2O$	114.5-115.2	$C_{20}H_{23}NO_5$
1j (74)	4-Methoxyphenyl	$NHCH_3$	Acetone-EtOAc	179-181 dec[d]	$C_{20}H_{25}N_3O_5$
1k (76)	CH_3	CH_3	Et_2O-petr ether	58-60	$C_{14}H_{19}NO_4$
1m (90)	3,4-Dichlorophenyl	NHC_2H_5	EtOAc-$(i\text{-Pr})_2O$	148-150 dec[d]	$C_{21}H_{25}N_3O_4Cl_2$
1n (83)	3,4-Dichlorophenyl	NH-*n*-C_4H_9	EtOAc-$(i\text{-Pr})_2O$	138-140 dec[d]	$C_{25}H_{33}N_3O_4Cl_2$
1p (88)	3,4-Dichlorophenyl	NH-cyclohexyl	EtOAc-$(i\text{-Pr})_2O$	145-147 dec[d]	$C_{29}H_{37}N_3O_4Cl_2$
1q (69)	4-Fluorophenyl	$NHCH_3$	Acetone-EtOAc	193-196 dec[d]	$C_{19}H_{22}N_3O_4F$
2b (81)	3,4-Dichlorophenyl		EtOAc-petr ether	103-106.5	$C_{12}H_{11}NO_3Cl_2$
2c (88)	4-Chlorophenyl		EtOAc	124-126	$C_{12}H_{12}NO_3Cl$
2d (83)	Phenyl		EtOAc-petr ether	154-156.5[i]	$C_{12}H_{13}NO_3$
2e (85)	4-Methoxyphenyl		EtOAc-petr ether	106-110	$C_{13}H_{15}NO_4$
2g (86)	4-Fluorophenyl		$CHCl_3$	169.5-170.5	$C_{12}H_{12}NO_3F$
5a (78)	CF_3		MeOH-H_2O (4:1)	126-126.8 dec	$C_{12}H_{12}NO_4F_3$
5b (91)	3,4-Dichlorophenyl		MeOH	128-129	$C_{17}H_{15}NO_4Cl_2$
5c (90)	4-Chlorophenyl		MeOH	157.5-158.5	$C_{17}H_{16}NO_4Cl$
5d (87)	Phenyl		MeOH	156-157	$C_{17}H_{17}NO_4$
5e (82)	4-Methoxyphenyl		MeOH	142-143	$C_{18}H_{19}NO_5$
5g (99)	4-Fluorophenyl		MeOH	154-156[h]	$C_{17}H_{16}NO_4F$
6a (92)	CF_3		CH_2Cl_2-petr ether	108.5-109	$C_{10}H_{12}NO_2F_3$
6b (89)	3,4-Dichlorophenyl		CH_2Cl_2-petr ether	142-143 dec[d]	$C_{15}H_{15}NO_2Cl_2$
6c (95)	4-Chlorophenyl		THF-petr ether[f]	156-157 dec[d]	$C_{15}H_{16}NO_2Cl$
6d (94)	Phenyl		THF-petr ether[f]	133-135 dec[d]	$C_{15}H_{17}NO_2$
6e (92)	4-Methoxyphenyl		THF-petr ether[f]	144-145 dec[d]	$C_{16}H_{19}NO_3$
6f (91)	CH_3[g]		Benzene[e]	90-91 dec[d]	$C_{10}H_{15}NO_2$
6g (85)	4-Fluorophenyl		CH_2Cl_2-petr ether	131-132 dec[d]	$C_{15}H_{16}NO_2F$

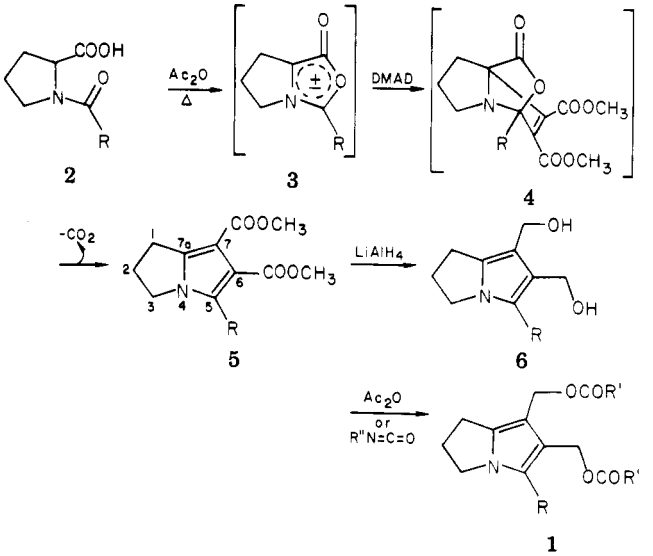
[a] No attempts were made to optimize yields. [b] All of the compounds in this table were analyzed for C, H, and N and the observed values were within ±0.4% of the theoretical values. [c] This compound was purified by sublimation after one crystallization. [d] These melting points were determined in a preheated oil bath in which the compound melted, with decomposition, within 5 s. [e] Purified by cellulose dry column chromatography (petroleum ether) prior to crystallization. [f] CH_2Cl_2-petroleum ether could also be used. [g] Prepared from the known diester, 5f (R = CH_3), ref 10. [h] Solidifies and then remelts at 160-161 °C. [i] Lit.[11] mp 156 °C.

thermore, the electrophilic reactivity of 1 can be controlled by the electronic influence of the C-5 substituent, R.

Chemistry. The synthesis of the 5-substituted 2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine derivatives is outlined in Scheme II. The amide **2**, prepared by treatment of proline with the appropriate acid chloride, was heated in the presence of acetic anhydride and dimethyl acetylenedicarboxylate (DMAD) to provide the mesoionic oxazalone intermediate, **3**. 1,3-Dipolar addition[9] of DMAD with **3** gave **4** which spontaneously eliminated CO_2 to give **5**. The diesters **5a** and **5f**[10] were synthesized directly from proline by treatment with DMAD and trifluoroacetic anhydride or acetic anhydride, respectively. Lithium aluminum hydride reduction of **5** yielded **6** and treatment of **6** with either acetic anhydride or an alkyl isocyanate afforded **1**. The yields, melting points, recrystallization solvents, and elemental analyses of **1**, **2**, **5**, and **6** are summarized in Table I. NMR spectral data for **1**, **5**, and **6** are given in Table II.

The IR spectra of the diesters, **5**, contain two carbonyl absorption bands which show a relationship to the electronic properties of the C-5 substituent. Thus, **5a** has the highest frequency absorptions in the series, 1743 and 1710 cm^{-1} in KBr; **5b–f** have absorptions at 1728/1706, 1727/1703, 1725/1695, 1717/1692, and 1715/1697, respectively. The *p*-fluoro compound, **5g**, has carbonyl absorptions at 1729 and 1700 cm^{-1}. The dicarbamate and diacetate derivatives, **1**, show only one broad carbonyl absorption band in the ranges 1676–1695 and 1720–1742 cm^{-1}, respectively. No obvious correlation could be made between the position of the carbonyl absorption band in **1** and the properties of the C-5 substituent.

Scheme II



The nature of the C-5 substituent also influences the stability of the diesters, **1**. The decomposition was followed by NMR in aqueous Me_2SO at 60 °C; the half-lives of the diacetates were **1i**, 8.5 h; **1g**, 18 h; **6g** diacetate, 26.3 h; **1e**, 40.3 h; and **1c**, 127.5 h. The C-5 methyl compound, **1k**, had a half-life of ca. 3–3.5 h while the C-5 trifluoromethyl

Table II. NMR Spectral Data for 1, 5, and 6[a]

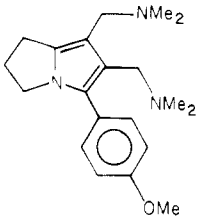
Compd	C-1	C-2	C-3	Aromatic	C-6′ and C-7′	Other
1a	2.42-3.05 (m)		4.10 (t, 7)		5.05 (s), 5.17 (s)	2.07 (s)[b]
1b	2.33-2.97 (m)		4.07 (t, 7)		4.95 (s), 5.00 (s)	2.57 (s),[c] 2.63 (s),[c] 6.87 (br s)[d]
1c	2.93 (t, 7)	2.57 (q, 7)	3.98 (t, 7)	7.18-7.62 (m)	5.07 (s), 5.12 (s)	2.07 (s),[b] 2.12 (s)[b]
1d	2.33-3.00 (m)		4.02 (t, 7)	7.37-7.80 (m)	4.93 (s), 5.00 (s)	2.57 (s),[c] 2.65 (s),[c] 6.87 (br s)[d]
1e	2.90 (t, 7)	2.53 (q, 7)	3.95 (t, 7)	7.40 (s)	5.07 (s), 5.12 (s)	2.08 (s)[b]
1f	2.30-2.97 (m)		3.90 (t, 7)	7.53 (s)	4.92 (s), 5.00 (s)	2.57 (s),[c] 2.65 (s),[c] 6.80 (br s)[d]
1g	2.97 (t, 7)	2.50 (q, 7)	3.95 (t, 7)	7.42 (s)	5.10 (s)	2.07 (s)[b]
1h	2.27-3.00 (m)		3.97 (t, 7)	7.48 (s)	4.93 (s), 5.00 (s)	2.60 (s),[c] 2.67 (s),[c] 6.83 (br s)[d]
1i	2.97 (t, 7)	2.50 (q, 7)	3.97 (t, 7)	6.90-7.42 (m)	5.07 (s), 5.12 (s)	2.08 (s),[b] 3.85 (s)[e]
1j	2.17-2.97 (m)		3.92 (t, 7)	6.97-7.50 (m)	4.87 (s), 4.98 (s)	2.57 (s),[c] 2.65 (s),[c] 3.83 (s),[e] 6.80 (br s)[d]
1k	2.00-3.07 (m)		3.90 (t, 7)		5.17 (s)	2.27 (s),[h] 2.10 (s)[b]
1m	2.77-3.47 (m)[i]	2.53 (br q, 7)	3.93 (t, 7)	7.07-7.60 (m)	5.00 (s), 5.05 (s)	1.13 (t, 7),[j] 4.70-5.33 (br s)[d]
1n	2.72-3.48 (m)[k]	2.52 (br q, 7)	3.92 (t, 7)	7.02-7.52 (m)	4.98 (s), 5.04 (s)	0.68-1.95 (m),[l] 4.82-5.45 (br s)[d]
1p	2.72-3.15 (m)	2.52 (br q, 7)	3.93 (t, 7)	7.05-7.65 (m)	5.00 (s), 5.05 (s)	0.72-2.22 (m),[m] 3.22-3.72 (br s),[m] 4.63 (br s),[d] 4.77 (br s)[d]
1q	2.30-3.07 (m)		3.90 (t, 7)	7.03-7.65 (m)	4.85 (s), 4.95 (s)	2.57 (s),[c] 2.62 (s),[c] 6.76 (br s)[d]
5a	3.17 (t, 7)	2.70 (q, 7)	4.22 (t, 7)			3.90 (s),[f] 4.01 (s)[f]
5b	3.13 (t, 7)	2.58 (q, 7)	3.98 (t, 7)	7.18-7.67 (m)		3.80 (s),[f] 3.83 (s)[f]
5c	3.18 (t, 7)	2.62 (q, 7)	4.03 (t, 7)	7.57 (s)		3.85 (s),[f] 3.90 (s)[f]
5d	3.20 (t, 7)	2.58 (q, 7)	4.05 (t, 7)	7.58 (s)		3.83 (s),[f] 3.90 (s)[f]
5e	3.10 (t, 7)	2.52 (q, 7)	3.92 (t, 7)	6.88-7.48 (m)		3.77 (s),[f] 3.83 (s)[e,f]
5g	3.12 (t, 7)	2.53 (q, 7)	3.93 (t, 7)	6.92-7.62 (m)		3.75 (s),[f] 3.82 (s)[f]
6a	2.35-3.10 (m)		4.10 (t, 7)		4.57 (s), 4.72 (s)	3.73 (br s)[g]
6b	2.92 (t, 7)	2.53 (q, 7)	3.95 (t, 7)	7.13-7.53 (m)	4.55 (s), 4.60 (s)	3.17 (br s)[g]
6c	2.90 (t, 7)	2.50 (q, 7)	4.03 (t, 7)	7.70 (s)	4.40-4.73 (m)[g]	
6d	2.93 (t, 7)	2.52 (q, 7)	4.00 (t, 7)	7.57 (s)	4.68 (s)	3.58 (br s)[g]
6e	2.90 (t, 7)	2.48 (q, 7)	3.90 (t, 7)	6.90-7.45 (m)	4.60 (br s)	3.27 (br s),[g] 3.87 (s)[e]
6f	2.20-2.93 (m)		3.78 (t, 7)		4.37 (s)	2.08 (s),[h] 4.33 (s)[g]
6g	2.88 (t, 7)	2.50 (q, 7)	3.88 (t, 7)	6.90-7.50 (m)	4.52 (s), 4.59 (s)	3.36 (br s)[g]

[a] Chemical shifts are expressed as δ with parenthetic notations for multiplicity and coupling constants (Hz). The carbamates 1b,d,f,h,i,q and 6f were dissolved in Me_2SO-d_6; all other compounds were dissolved in $CDCl_3$ (ca. 1% Me_4Si was used as an internal standard). The heading "other" refers to [b]-$OCOCH_3$; [c]-$NHCH_3$; [d]-OCONH-; [e]-OCH_3; [f]-$COOCH_3$; [g]-OH; [h]-C-5-CH_3; [i]-CH_2CH_3 (overlap with signals for C-1); [j]-CH_2CH_3; [k] overlaps with four protons of *n*-butyl group; [l] 14 protons of *n*-butyl groups; [m]-cyclohexyl protons.

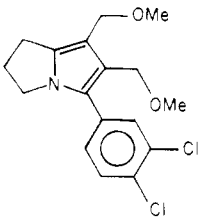
derivative, **1a**, failed to show any signs of decomposition after 200 h. It should also be noted that for any given C-5 substituent, the diacetate was less stable than the dicarbamate.

The calculated Hammett ρ constant for the decomposition of the C-5 phenyl substituted diacetates is −1.493. The negative ρ suggests that some positive charge is developed during the reaction; however, more detailed studies will be necessary in order to evaluate the specific reaction mechanism involved.

Treatment of the diacetate **1i** with dimethylamine gave the diamine **7**. In this reaction a complex between **1i** and dimethylamine precipitated from solution in copious quantity; however, after about 2 weeks the precipitate had completely redissolved and no starting **1i** remained. The structure of **7** was confirmed by IR, NMR, and chemical ionization mass spectrometry. The isolation of **7** confirmed that the bifunctional electrophile **1i** can indeed react via *O*-alkyl cleavage (cf. Scheme I). Dimethylacetamide, the expected product of *O*-acyl cleavage, was not found in the reaction mixture. In a related experiment, the bis(*N*-methylcarbamate), **1d**, was treated with sodium methoxide in methanol. Once again the product of *O*-alkyl cleavage, **8**, was isolated. The structure of **8** was confirmed by an independent synthesis from the diol, **6b**.



7



8

Biological Results and Discussion. The preliminary data for in vivo antileukemic and in vitro cytotoxicity assays are summarized in Table III. Two compounds, **1d** and **1i**, showed a modest level of activity in the L1210 assay; several others afforded some increase in mean survival times but failed to approach the significance level in this assay. A majority of the compounds tested showed significant activity in the P-388 assay.

In the P-388 assay the noteworthy compounds were the bis(carbamate) derivatives of the diols **6b** and **6g**. All of these compounds were quite potent, a feature particularly evident for **1q** which showed significant activity at the lowest dose tested, 0.78 mg/kg; one derivative, **1d**, afforded "cures" at dose levels as low as 12.5 mg/kg. Since these data do not represent minimum effective doses, it is difficult to evaluate the relative activity and toxicity of these compounds; however, it is important to note that activity, with no acute toxicity (as evaluated by toxicity day survivors), is maintained over a several-fold dose range. Two compounds, **1m** and **1p**, show high activity over at least a fourfold dose range (with no acute toxicity) and

have been selected for further evaluation against a wider panel of in vivo antitumor assays.

It would be premature to draw any conclusions regarding structure–activity relationships in this series; however, comparison of the data in Table III does permit some tentative suggestions. The vinylogous carbinolamine moiety appears to be critical for activity (as judged by the inactivity of **5b**) and, although the diol **6b** shows some weak activity, acylation of the two primary hydroxyl groups also seems to be necessary for high activity. The bis(*N*-methylcarbamate) derivative appears to be superior to the more reactive diacetate derivative although this difference may be due to the more facile serum esterase hydrolysis of the latter derivatives. Finally, with regard to C-5 substitution the more reactive *p*-methoxyphenyl compound **1j** appears to be inferior when compared to the less reactive 3,4-dichlorophenyl compound **1d**. This is particularly true if one compares the weight loss of the treated animals.

Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems. Factors such as esterase lability, water solubility, and chemical reactivity will be examined in more detail in an effort to correlate structural parameters with antileukemic activity and host toxicity.

Experimental Section

Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover Unimelt apparatus. IR spectra were determined for KBr wafers (unless otherwise specified) with a Perkin-Elmer 237 spectrophotometer. NMR spectra were determined for $CDCl_3$ solutions (unless otherwise specified) containing ca. 1% Me_4Si as internal standard with a Varian T-60 spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Typical experimental procedures are presented below.

Dimethyl 2,3-Dihydro-5-trifluoromethyl-1*H*-pyrrolizine-6,7-dicarboxylate (5a). L-Proline (23.0 g, 0.2 mol) was dissolved in ice-cold trifluoroacetic anhydride (200 mL); dimethyl acetylenedicarboxylate (100 mL) was added and the mixture was heated under reflux (60 °C bath temperature) for 8 h. The mixture was concentrated to dryness in vacuo and the orange syrup, which solidified on cooling, was crystallized from methanol–water (4:1, 400 mL) to give **5a** (45.65 g, 78%). Two recrystallizations gave the analytical sample of **5a** as large clear rectangular prisms.

***N*-(3,4-Dichlorobenzoyl)proline (2b).** A solution of L-proline (30 g, 0.26 mol), thymolphthalein (5 mg), and Na_2CO_3 (100 g) in water (500 mL) was treated at room temperature with concentrated aqueous NaOH solution until the solution was blue to the indicator. The stirred solution was cooled to ca. 10 °C and treated portionwise with a solution of 3,4-dichlorobenzoyl chloride (58.65 g, 0.28 mol) in ether (100 mL) with periodic addition of concentrated aqueous NaOH solution as necessary to maintain the blue color. The reaction mixture was stirred for an additional 10 min after the addition was completed and then extracted with ether (2 × 150 mL). The aqueous phase was acidified to pH 1 with concentrated HCl and extracted with ethyl acetate (4 × 250 mL). The combined ethyl acetate solution was washed with brine (150 mL), dried (Na_2SO_4), and concentrated in vacuo. The solid residue was crystallized from hot ethyl acetate–petroleum ether (2:1, 300 mL) to give 60.5 g (81%) of **2b** as fine white prisms. Recrystallization from the same solvents afforded the analytical sample of **2b**.

Dimethyl 2,3-Dihydro-5-(3′,4′-dichlorophenyl)-1*H*-pyrrolizine-6,7-dicarboxylate (5b). A solution of **2b** (28.813 g, 0.1 mol) in acetic anhydride (100 mL) and dimethyl acetylenedicarboxylate (50 mL) was stirred in a flask equipped with a reflux condenser and a gas bubbler to monitor CO_2 evolution during the reaction. The mixture was heated to 120 °C over a 15-min period, during which time CO_2 evolution occurred at an increasingly rapid rate; the temperatures were maintained for 1 h *after* the rate of gas evolution had substantially decreased. The reaction mixture was concentrated in vacuo and the residue, which solidified on cooling, was twice crystallized from hot methanol (500 mL) to yield 33.35 g (91%) of **5b** as analytically pure fine white needles.

2,3-Dihydro-5-(3′,4′-dichlorophenyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (6b). A solution of **5b** (57.8 g, 0.157 mol) in dry dichloromethane (250 mL) was added dropwise, over a 30-min period, to a mechanically stirred mixture of lithium aluminum hydride (14.04 g, 2.35 equiv) in anhydrous ether (400 mL) heated under reflux. The stirred mixture was heated at reflux for 1 h after the addition was completed and cooled in an ice bath. The excess hydride was decomposed with wet ether and then with water until the salts were white. The mixture was filtered (through sintered glass) and the inorganic residue was washed with several portions (ca. 100 mL) of hot dichloromethane until the total filtrate volume was 1.2 L. The filtrate was concentrated in vacuo to a volume of 500 mL, warmed to boiling, and diluted with slow addition of 350 mL of petroleum ether. Compound **6b** precipitated as clear, white, chunky prisms (33.8 g); the concentrated mother liquor, after similar treatment, gave an additional 9.71 g of **6b** for a total yield of 89%.

2,3-Dihydro-5-(3′,4′-dichlorophenyl)-6,7-bis(acetoxymethyl)-1*H*-pyrrolizine (1c). A magnetically stirred solution of **6b** (6.244 g, 0.02 mol) in anhydrous pyridine (25 mL) was treated with acetic anhydride (15 mL) for 18 h at room temperature. The volatile reaction components were removed in vacuo, using a toluene azeotrope to remove traces of pyridine, and the brown residue was exhaustively extracted with hot anhydrous ether. The combined triturates were freed of solvent in vacuo, dissolved in ethyl acetate (50 mL), treated with decolorizing charcoal, filtered, and concentrated to dryness in vacuo. The residue was twice crystallized from hot isopropyl ether to give **1c** (5.446 g) as analytically pure chunky prisms. The concentrated mother liquor yielded an additional 1.60 g of **1c** for a total yield of 76%.

2,3-Dihydro-5-(3′,4′-dichlorophenyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine Bis(methylcarbamate) (1d). A solution of **6b** (6.244 g, 0.02 mol) and triethylamine (0.5 mL) in dichloromethane (45 mL) was treated with methyl isocyanate (7 mL, ca. 10 equiv) and refluxed for 1.5 h. The mixture was concentrated to dryness in vacuo and the off-white residue was crystallized from ethyl acetate–isopropyl ether to give **1d** (7.08 g) as white "doughnut"-shaped rosettes. The concentrated mother liquor afforded an additional 0.71 g of **1d** for a total yield of 91%.

Reaction of 1i with Dimethylamine. A solution of 6,7-bis(acetoxymethyl)-2,3-dihydro-5-(*p*-methoxyphenyl)-1*H*-pyrrolizine (**1i**, 0.928 g, 0.0026 mol) and dimethylamine (10 mL, 60 equiv) in dry methylene chloride (40 mL) was stirred at room temperature in a tightly stoppered flask. Within 3 h a fluffy white crystalline precipitate began to form and the mixture became thicker over the first 24 h so that stirring became difficult. Gradually, over ca. 8 days, the precipitate redissolved; the mixture was stirred for a total of 28 days to ensure completion. The solution was concentrated in vacuo until the product began to precipitate and then added to an ice-cold saturated $NaHCO_3$ solution (250 mL) and rapidly extracted with dichloromethane (5 × 50 mL). The combined extracts were washed with brine (50 mL) and dried by passage through a short column of anhydrous sodium sulfate. The solvent was removed in vacuo and the foamy hygroscopic residue was dried under high vacuum over phosphorus peroxide for 24 h. The crude product was twice crystallized from absolute ethanol–isopropyl ether to yield **7** as slightly brownish prisms containing ethanol of crystallization: IR 2987, 2955, 2883, 2840, 1498, 1244, 1179, 1030, and 831 cm^{-1}; NMR δ 1.23 (t, J = 7 Hz, CH_3CH_2OH), 2.33–3.17 (m, 4 H), 2.62 (s, 6 H), 2.83 (s, 6 H), 3.78 (q, J = 7 Hz, CH_3CH_2OH), 3.83–4.17 (m, 2 H), 3.92 (s, 2 H), 3.97 (s, 3 H, OCH_3), 4.03 (s, 2 H), 7.18 (d, $|J_{AB}|$ = 9 Hz, 2 H), and 7.39 (d, $|J_{AB}|$ = 9 Hz, 2 H) [center of AB quartet at 7.28 ($\Delta\gamma_{AB}$ = 13 Hz)], and 8.32 (br s, 1 H, CH_3CH_2OH) (note: the crude product, with no ethanol of crystallization, shows all of the NMR peaks shifted ca. 5–10 Hz lower field); mass spectrum, isobutane chemical ionization, m/e (rel abundance) 329 (26), 328 (M^+ + 1, 100), 327 (22.8), 326 (12.9), 284 (10.8), 283 (63.2), 282 (80.9), 240 (4.7), and 226 (10.8).

Reaction of 1d with Methoxide. A solution of 2,3-dihydro-6,7-bis(hydroxymethyl)-5-(3′,4′-dichlorophenyl)-1*H*-pyrrolizine bis(*N*-methylcarbamate) (**1d**, 0.213 g, 0.0005 mol), sodium methoxide (0.05 g, 2.2 equiv), and methyl iodide (1.0 mL,

Table III. Antileukemic and Cytotoxic Activity of Substituted 2,3-Dihydro-1*H*-pyrrolizines[a]

	L1210[b]				P-388[c]				
Compd	Dose,[e] mg/kg	Toxicity day survivors[f]	Animal wt diff, T − C	% T/C	Dose,[e] mg/kg	Toxicity day survivors[f]	Animal wt diff, T − C	% T/C (cures)[g]	9-KB,[d] ED_{50}, μg/mL
5b	200	6/6	1.1	98	400[h]	6/6	−0.2	88	
	100	6/6	3.4	88	200[h]	6/6	0.2	89	
	50	6/6	2.8	91	100[h]	6/6	0.2	87	
6a	200	0/3	−2.8		200	3/6	1.1	108	25
	100	3/3	−1.1	104	100	5/6	0.6	104	
	50	3/3	−0.3	107	50	6/6	2.1	104	
6b					400[h]	6/6	−1.0	127	
					200[h]	6/6	−0.9	116	
					100[h]	6/6	+0.5	116	
					54[h]	6/6	−0.6	109	
					27[h]	6/6	−0.3	109	
					13.5[h]	6/6	0	107	
1a	200	3/3	−0.8	98	200	6/6	1.1	104	2.9
	100	3/3	−0.6	100	100	6/6	1.8	93	2.4
	50	3/3	−1.0	107	50	6/6	2.1	90	3.0
1b	200	3/3	−0.2	98	200	5/6	−1.5	82	2.5
	100	3/3	−0.8	100	100	6/6	−0.4	116	6.4
	50	3/3	−0.1	104	50	6/6	1.4	117	
1c	200	3/3	−3.3	100	200	6/6	−3.0	127	>100
	100	3/3	−2.2	104	100	6/6	−1.0	122	
	50	3/3	−1.1	100	50	6/6	−0.7	141	
					25[k]	6/6	1.2	125	
					12.5[k]	6/6	1.2	114	
1d	200[i]	3/3	−4.8	63	100[i]	5/6	−1.9	188 (2)	2.7
	100[i]	3/3	−4.3	74	50[i]	6/6	−2.5	155	
	50	3/3	−3.0	124	25[i]	6/6	−0.4	160	>10
	25	3/3	−1.3	109	12.5[i]	6/6	1.6	153 (1)	
	12.5	3/3	−1.5	106					
1e	200	2/3	−3.8	64	200	0/6	0.5		2.8
	100	3/3	−3.8	76	100	6/6	−3.3	66	>10
	50	3/3	−1.8	117	50	6/6	−1.9	118	
	25	3/3	0.6	102	25[k]	6/6	−0.2	123	
	12.5	3/3	0.7	95	12.5[k]	6/6	−0.6	119	
1f	200	0/3	−2.8		200	0/6	0.5		2.6
	100	3/3	−3.8	107	100	1/6	−0.9		2.3
	50	2/3	−2.6	112	50	6/6	−2.5	104	5.2
					25[k]	6/6	−0.8	135	
					12.5[k]	6/6	−0.2	129	
					6.25	6/6	0.2	150	
					3.13	6/6	−0.6	127	
1g	200	0/3	−2.8		200	0/6	0.5		>100
	100	2/3	−2.3	107	100	3/6	−2.8	49	
	50	3/3	−2.6	114	50	6/6	−2.5	79	
					50[i]	6/6	−6.1	87	
					25[k]	6/6	−2.0	133	
					25[i]	6/6	−1.2	142	
					12.5[k]	6/6	−1.2	126	
					12.5[i]	6/6	−0.2	110	
					6.25[k]	5/6	−0.4	107	
					6.25[i]	5/6	−0.3	125	
1h	200	3/3	−3.1	110	200	6/6	−3.5	59	22
	100	3/3	−1.5	104	100	6/6	−1.8	124	
	50	3/3	−2.6	104	50	6/6	−2.1	124	
1i	400[j]	0/6	−0.1						2.5
	200[j]	2/6	−2.5	65					4.0
	200[i]	3/3	−5.2	130					<3.1
	100[j]	4/6	−2.5	65					
	100[i]	3/3	−7.2	78					
	50[j]	6/6	−1.0	77					
	50[i]	3/3	−2.9	113					
1j	200	3/3	−5.0	98	200	5/6	−3.6	55	21
	100	2/3	−4.8	117	100	6/6	−3.7	129	
	50	3/3	−2.3	109	50	6/6	−4.0	131	
					25	6/6	−3.3	132	
					12.5	6/6	−3.2	132	
1m					400[h]	0/6	−1.4		
					200[h]	0/6	−1.4		
					100[h]	2/6	−3.0		
					50[h]	3/6	−4.4		
					50[h,i]	3/6	−8.0		
					25[h]	6/6	−2.4	103	
					25[h,i]	6/6	−4.4	165	
					12.5[h]	6/6	−1.8	170	

Table III (*Continued*)

Compd	L1210[b] Dose,[e] mg/kg	Toxicity day survivors[f]	Animal wt diff, T − C	% T/C	P-388[c] Dose,[e] mg/kg	Toxicity day survivors[f]	Animal wt diff, T − C	% T/C (cures)[g]	9-KB,[d] ED_{50}, μg/mL
1m					12.5[h,i]	6/6	−2.5	168	
					6.25[h]	6/6	−1.8	154	
					6.25[h,i]	6/6	−2.1	168	
1n					400[h]	5/6	−3.6	84	
					200[h]	6/6	−3.2	163	
					100[h]	6/6	−2.0	181	
					100[h,i]	6/6	−0.4	160	
					50[h]	6/6	−1.9	171	
					50[h,i]	6/6	−1.1	137	
					25[h]	6/6	−1.2	159	
					25[h,i]	6/6	−1.9	138	
1p					400[h,i]	6/6	−4.0	79	
					200[h,i]	6/6	−2.8	91	
					100[h,i]	6/6	−1.2	187 (2)	
					100[h]	6/6	−2.5	212	
					50[h,i]	6/6	−0.6	166	
					50[h]	6/6	−2.0	174	
					25[h,i]	6/6	−0.6	150	
					25[h]	6/6	−1.1	168	
1q	12.5[j]	5/6	0.1	118	200[i]	0/6	−1.5		0.89
	6.25[j]	6/6	0.1	107	100[i]	0/6	−1.5		0.22
	3.13[j]	6/6	1.1	115	50[i]	4/6	−3.4	48	
					25[i]	2/6	−2.0		
					12.5	6/6	−3.3	153	
					6.25	6/6	−1.0	145	
					3.13	6/6	−0.3	171	
					1.56[k]	6/6	0	122	
					0.78[k]	6/6	0.9	146	

[a] Antileukemic and cytotoxic activities were determined under the auspices of the National Cancer Institute, National Institutes of Health. For general screening procedures and data interpretation, see R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3 (2), 1 (1972). [b] Ascitic fluid containing ca. 5×10^5 cells was inoculated into female BDF_1 mice (ip route); in this assay mean survival times of % T/C ≥ 125 are considered significant. [c] Ascitic fluid containing ca. 6×10^6 cells was inoculated into female CDF_1 mice (ip route); in this assay median survival times of % T/C ≥ 125 are considered significant. [d] Cytotoxic activity was determined for ethanol solutions of the compound; ED_{50} values of ≤4 μg/mL are considered significant. [e] The compound was administered by the ip route in a Klucel (hydroxypropylcellulose) suspension. A total of nine daily doses was given starting 24 h after tumor inoculation. [f] Recorded on the fifth day. [g] A "cure" in this assay represents 30-day survival. [h] Saline with Tween-80 was used as the vehicle in this assay. [i] Male CDF_1 mice were used in this assay. [j] Female CDF_1 mice were used in this assay. [k] Female BDF_1 mice were used in this assay.

ca. 30 equiv) (8 was also obtained when ethyl iodide was used) in dry methanol (5 mL) was stirred at room temperature in a sealed vial for 18 h (at 100 °C the reaction was complete in 5 min). The reaction mixture was filtered and concentrated in vacuo. The residue was purified by dry column chromatography (silica gel–ethyl acetate); the mobile band was collected and freed of solvent in vacuo. The residue was twice crystallized from isopropyl ether–petroleum ether to give 8 as slightly yellowish prisms: mp 94.6–95.6 °C; IR 2880, 1514, 1473, 1305, 1076, 936, and 827 cm^{-1}; NMR δ 2.27–3.13 (m, 4 H), 3.43 (s, 3 H), 3.45 (s, 3 H), 3.98 (t, J = 7 Hz, 2 H), 4.30 (s, 2 H), 4.45 (s, 2 H), and 7.20–7.80 (m, 3 H).

Anal. Calcd for $C_{17}H_{19}NO_2Cl_2$: C, 60.01; H, 5.63; N, 4.12. Found: C, 60.08; H, 5.67; N, 4.10.

The bis(methyl ether), 8, was also synthesized from the diol **6b**. Thus, **6b** (1.56 g, 0.005 mol) was treated with sodium methoxide (0.6 g, 2.2 equiv) in dry methanol (35 mL) at room temperature. Methyl iodide (10 mL, ca. 30 equiv) was added to this stirred solution and the sealed reaction vessel was stirred at room temperature for 4 days. The dark purple reaction mixture was concentrated in vacuo and the residue was purified by dry column chromatography (1 × 8 in. silica gel column–ethyl acetate). The mobile band was collected, treated with decolorizing charcoal, and concentrated in vacuo. Two crystallizations from isopropyl ether–petroleum ether gave 0.69 g of 8 (41%) which was identical in all respects with the sample of 8 prepared from **1d**.

Hydrolysis of 1g and 1i in Aqueous Me_2SO. A solution of 0.0025 mol of substrate in 1.0 mL of Me_2SO-d_6 was treated with 50 μL of D_2O at 60 °C in an NMR tube. The solution was maintained at 60 °C and the NMR spectra were recorded at intervals over several days. The reaction was followed by monitoring the disappearance of the acetate methyl signal at δ 1.98 and the appearance of a signal for acetic acid at δ 1.92. Integration of the two signals afforded data on the percent of liberated acetate. Values of $t_{1/2}$ were determined from the slopes of the plotted data; the data (percent decomposition vs. time) were plotted by the least-squares method. The slopes were **1i**, 5.941 (r = 0.9808); **1g**, 2.784 (r = 0.9947); **6g** diacetate, 1.899 (r = 0.9942); **1e**, 1.242 (r = 0.9979); **1c**, 0.3923 (r = 0.9950). A plot of log (slope X/slope H) vs. σ afforded a straight line with slope = −1.493 (r = 0.9960); the values of σ used were −0.27 (X = p-OMe), 0 (X = H), 0.06 (X = p-F), 0.23 (X = p-Cl), and 0.52 (X = 3,4-Cl_2).

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(12) No specific mechanism is implied in this scheme, i.e., S_N1 vs. S_N2, for the reaction with either Y or Z.

Synthesis and Biochemical Evaluation of Nucleosides of Naphthoquinone Heterocycles

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The synthesis, characterization, and biochemical evaluation of 1-β-D-ribofuranosylnaphtho[2,3-*d*]imidazole-4,9-dione (**3**), 2-β-D-ribofuranosylnaphtho[2,3-*d*]pyrazole-4,9-dione (**6**), and 2-β-D-ribofuranosylnaphtho[2,3-*d*]triazole-4,9-dione (**9**) are reported. These quinone nucleosides and the corresponding quinone heterocycles were tested as inhibitors of purine nucleotide biosynthesis in Ehrlich ascites cells. The nucleosides **3** and **9** and naphtho[2,3-*d*]imidazole-4,9-dione were effective inhibitors of hypoxanthine phosphoribosyltransferase.

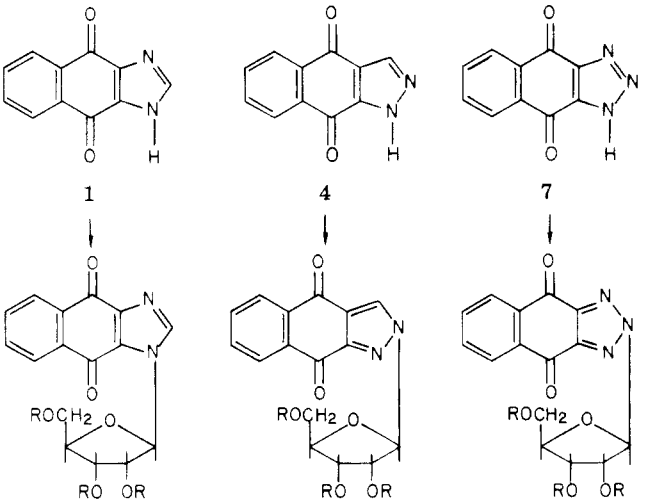
The involvement of quinones in numerous biochemical processes[1] has led to a study of a wide variety of synthetic derivatives. The facile reduction–oxidation of the quinone moiety appears to be the basis for the participation of a number of structurally diverse quinones in electron-transport and oxidative–phosphorylation processes.[1] The biological activity reported for quinones includes enzyme inhibition[2] and activity as antibacterial,[3] antifungal,[4] and anticancer agents.[5,6]

Heterocyclic quinones are one class of such compounds which have been investigated extensively.[7] Since synthetic nucleosides often exhibit enhanced biochemical activity compared to that of the aglycon,[8] it was of interest to investigate the synthesis and properties of nucleosides with the unique features of the quinone moiety.

Recently certain heterocyclic benzoquinone nucleosides were obtained by oxidation of the corresponding benzotriazoles.[9] The synthesis and biochemical activity of some benzoquinone *C*-nucleosides have also been described.[10] In the present work, the synthesis and characterization of ribonucleosides of imidazole, pyrazole, and triazole derivatives of 1,4-naphthoquinones are reported.

Ribosylation of these heterocyclic quinones by the Lewis acid-catalyzed procedure[11] proceeded readily (Scheme I). Thus, treatment of the *N*-trimethylsilyl derivative of naphtho[2,3-*d*]imidazole-4,9-dione[12] (**1**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and stannic chloride afforded the blocked nucleoside **2**. Deacylation of **2** with sodium methoxide gave 1-β-D-ribofuranosylnaphtho[2,3-*d*]-imidazole-4,9-dione (**3**). Similarly, naphtho[2,3-*d*]-pyrazole-4,9-dione[13] (**4**) and naphtho[2,3-*d*]triazole-4,9-dione[14] (**7**) were converted to the corresponding ribonucleosides **6** and **9**, respectively.

Scheme I



The structures of these nucleosides were established on the basis of their carbon-13 and proton NMR data (Table I). Since the imidazole nucleoside **3** is formed from the symmetrical quinone **2** in which both nitrogens are equivalent, ribosylation must give 1-β-D-ribofuranosylnaphtho[2,3-*d*]imidazole-4,9-dione (**3**). The spectral data (Table I) are consistent with this structure. The position of the signal for the anomeric proton of **3** is at lower field

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