Table I. Bulbring and Wajda Anesthesia Test

	Number of squeals out of 5 at time (min)								
	2	5	15	30	45	60	75	90	105
Control (vehicle)	5	5	5	5	5	5	5	5	5
Control (procaine)	0	0	0	2	3	4	5		
P-PEG 400[a]	0	0	0	0	0	2	3	4	5
P-TEG[b]	0	0	0	0	0	0	0	3	4

[a]Procaine attached to polyethylene glycol 400. [b]Procaine attached to tetraethylene glycol.

Table II. Rabbit Eye Corneal Anesthesia

	Grade at min[a]					
	5	10	15	30	45	60
Procaine	0	1	2	2	2	2
Vehicle	2	2	2	2	2	2
P-PEG 400	1	0	0	1	2	2
P-TEG	0	0	0	0	1	2

[a]Gradation of blink response: 0 is no blink, *i.e.*, anesthesia; 1, sluggish response; 2, blink, *i.e.*, no anesthesia.

such as ether, benzene, and chloroform and were insoluble in water.

The procaine derivatives were subjected to two local anesthesia tests. The first one was according to Bulbring and Wadja,[17] in which guinea pigs had their backs shaved and 1% solutions of the substances and a control of vehicle and procaine (as a base) were used. The substances were first dissolved in 1 drop of DMF and made up to volume with sterile distilled water. Intracutaneous injections were made of 0.1-ml volumes to give a weal. Immediately afterward every 5 min a needle was jabbed into the weals five times in succession. The response of a squeal and skin contraction indicate that the animal feels the pain, while absence means local anesthesia. Five areas and three guinea pigs were used per group (Table I).

It can be seen that the effect of procaine lasts in this test between 30 and 45 min, having no effect at 75 min. P-PEG 400 lasts about twice as long and has no activity at 105 min. P-TEG shows even better prolongation of action.

The second test was rabbit eye corneal anesthesia. It determines lipid solubility or penetration effects. In this procaine is weak. Two rabbits were used per group, and 0.5 ml of 1% solutions of substances was instilled into the conjunctional sac of their eyes and held bathing the eye surfaces for 30 sec. Every 5 or 10 min thereafter the eyes were touched with a fine stylus. This normally causes a blink. If there is local anesthesia the eye remains open on being touched with the stylus (Table II).

In this test P-PEG 400 shows a slower onset lasting for 20 min which is twice as good as procaine. P-TEG shows an immediate onset and lasts for 45 min. This result parallels the previous result and shows that P-TEG is a promising prolonged action local anesthetic.

The preliminary pharmacological results indicate that the attachment of procaine to polyethylene glycols has increased the duration of activity of the drug. However, the fact that the shorter polymer showed the longer duration of action may point out that increase of duration of activity is not only a matter of the molecular weight but also of other factors such as different permeabilities through biological membranes, partition coefficients, etc.

Experimental Section

Materials. Tetraethylene glycol and polyethylene glycol 400 (number-average molecular weight = 400), Fluka, were used. Ir spectra were carried out on a Perkin-Elmer 257 instrument and nmr on Varian T-60.

ω,ω'-Dichlorocarbonate of Tetraethylene Glycol. Tetraethylene glycol (19.4 g, 0.1 mol) and a solution (240 ml) of phosgene in toluene (12.4%) was stirred for 48 hr at room temperature. Excess of phosgene and toluene were driven off *in vacuo* at low temperature, leaving the product: 28 g (88%); ir 1778 cm^{-1} (OC(=O)Cl); nmr ($CDCl_3$) δ 3.6 (m, 12, CH_2OCH_2), 4.4 (m, 4, $CH_2OC(=O)Cl$). *Anal.* ($C_{10}H_{16}Cl_2O_7$) C, H, Cl.

The ω,ω'-dichlorocarbonate derivative of polyethylene glycol 400 was similarly prepared in 88% yield. *Anal.* $(C_2H_4O)_{8.7}(C_2Cl_2O_3)$ C, H, Cl.

N,N'-(p-β-Diethylaminocarbethoxyphenyl)-ω,ω'-dicarbamoyltetraethylene Glycol (P-TEG). A solution of procaine hydrochloride (2.73 g, 0.01 mol), triethylamine (6.06 g, 0.06 mol), and chloroform (35 ml) was cooled in an ice bath, and the dichlorocarbonate (1.6 g, 0.005 mol) was added dropwise during 30 min with stirring. The reaction mixture was stirred overnight. Dry ether was added, the precipitated triethylamine hydrochloride was filtered, and the filtrate was evaporated. The oily residue was dissolved in $CHCl_3$ and precipitated by petroleum ether. The procedure was repeated twice. The remaining oil was purified by preparative tlc on silica gel (Merck PF 254) using absolute ethanol as eluent: yield 2.0 g (56%); ir 850 (1,4-C_6H_4-), 1695 (C_6H_5COO-), 1710 (-NHCOO-), 1100 cm^{-1} (-CH_2OCH_2-); nmr ($CDCl_3$) δ 1.0 (t, 12, -CH_3), 2.85 (q, 8, -NCH_2), 6.9 (m, 8, -C_6H_4-), 3.5 (s, 12, -CH_2OCH_2). *Anal.* ($C_{36}H_{54}N_4O_{11}$) C, H, N.

The reaction product between ω,ω'-dichlorocarbonate of polyethylene glycol 400 and procaine hydrochloride was similarly prepared and purified: yield 65%; ir and nmr confirm the structure. *Anal.* $(CH_2CH_2O)_{8.7}(C_{28}H_{38}N_4O_7)$ C, H, N.

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Nitro Analogs of Pyoluteorins

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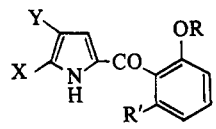
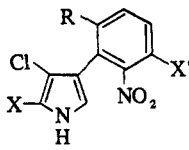
A requirement for antibiotic activity in 2-(2'-hydroxybenzoyl)pyrrole (**1a**) is one or more electron-withdrawing groups X and Y.[1] Compound **1b** is the parent antibiotic pyoluteorin, a metabolite of *Pseudomonas aeruginosa* with antibacterial and antifungal activity.[2] Replacement of the



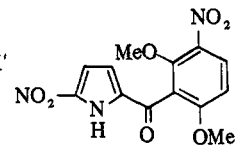
1

a, R = R′ = H
b, X = Y = Cl; R = OH; R = H
c, X = R = H; Y = Cl; R′ = OH
d, X = NO_2; Y = H; R = Me; R′ = MeO
e, X = NO_2; Y = R′ = H; R = Me
f, X = NO_2; R = R = H; R′ = OMe
g, X = NO_2; Y = R = R′ = H
h, X = R = R′ = H; Y = Cl
i, X = R′ = H; Y = Cl; R = Me
j, X = NO_2; Y = Cl; R′ = H; R = Me
k, X = NO_2; Y = Cl; R = R′ = H

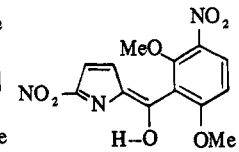
5-chlorine atom by hydrogen gives 5-dechloropyoluteorin (**1c**)[3,4] of much lower activity. Because of the presence of a nitro group in the pyrrolnitrins (**2**),[5-7] which are also metabolites of *Pseudomonas* species, we decided to investigate the effect of a nitro group in place of chlorine on the activity of the pyoluteorins. We therefore nitrated 2-(2′,6′-dimethoxybenzoyl)pyrrole[8] and 2-(2′-methoxybenzoyl)-pyrrole[8] which gave the 5-nitro derivatives **1d** and **1e**, respectively. These were demethylated to 2-(2′-hydroxy-6′-methoxybenzoyl)-5-nitropyrrole (**1f**) and 2-(2′-hydroxybenzoyl)-5-nitropyrrole (**1g**). Preliminary attempts to further demethylate **1f** were unsuccessful but in view of the generally greater activity[1] of monodeoxy compounds (Table I), we decided to concentrate on this series. However, from



2



3a



3b

one preparation of **1d**, an interesting by-product was obtained with mol wt 321 (mass spectrum), indicating the presence of two nitro groups. This was confirmed by the nmr spectrum which showed two AB systems ($J_{3,4}$ = 4.5, $J_{4',5'}$ = 9 Hz). The ir spectrum contained a weak carbonyl band and these facts are consistent with structure **3** in which the enol form **3b** is significant. This is also indicated by the fact that the OH-NH peak is rather broad and the M-OH peak in the mass spectrum at *m/e* 304 is 12% which is higher than usual in the ethers. Also, in the nmr, there was no coupling between H(1) and H(3) or H(4) before or after exchange of H(1) by deuterium, again indicating the predominance of the enol form. We have previously invoked a related enolization as the mechanism in our first synthesis of pyoluteorin (formula **11**, see ref 9).

In our previous work on monodeoxypyoluteorins[8] we prepared 5-dechloromonodeoxypyoluteorin (**1h**) *via* its methyl ether. It had low activity but we were able to utilize the ether **1i** by converting it to its 5-nitro derivative **1j** which was demethylated to the phenol **1k** which had improved activity (Table I).

Discussion

We have previously shown[8] that replacement of the chlorine of monodeoxypyoluteorin by more electropositive halogen, *viz.* bromine and iodine, results in decreasing antibiotic activity. The accompanying lower acidity of the pyrrole NH group was thought to be the reason for this.

A significant increase in the NH acidity, which could cause stronger binding to a receptor site, would be brought about by the substitution of a more strongly electronegative group such as nitro and should therefore be accompanied by improvement of microbiological activity. That this expectation was realized is shown by compound **1g** in which the chlorine atoms of monodeoxypyoluteorin are replaced by a 5-nitro group.

It is further shown (Table I) that when only the 5-chlorine atom is replaced by nitro giving **1k**, the activity is further slightly enhanced, in line with expectation.

Experimental Section

Infrared spectra were run on a Unicam SP 200, nmr spectra [TMS internal standard (τ values quoted)] on a Jeol C60HL machine, and mass spectra on the AEI MS 12 at 70 eV on the probe. Microanalyses are within prescribed limits. Melting points are uncorrected.

2-(2′-Methoxybenzoyl)-5-nitropyrrole (1e). Fuming nitric acid (0.12 ml) was added to 0.4 g of 2-(2-methoxybenzoyl)pyrrole in glacial acetic acid and the mixture was kept at 70–80° for 3 hr. The solvent was evaporated and the residue, in chloroform solution, was washed with sodium bicarbonate solution, dried ($MgSO_4$), and passed down a silica column, eluting with chloroform–petroleum ether (bp 30–60°) (3:2), giving 0.36 g of nitro compound: 75%; long yellow fibers; mp 116.5–117.5° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 276; rel intensity (100, M^+); nmr, see Table II. *Anal.* ($C_{12}H_{10}N_2O_4$) C, H, N.

2-(2′-Hydroxybenzoyl)-5-nitropyrrole (1g). Demethylation of the above methyl ether **1e** with boron trichloride in methylene chloride[8] gave the free nitrophenol (**1g**): mp 146–147° (crystallized from benzene) in 85% yield; mass spectrum *m/e* 232 (70, M^+), 120 (100). *Anal.* ($C_{11}H_8N_2O_4$) C, H, N.

Nitration of 2-(2′,6′-Dimethoxybenzoyl)pyrrole. By the above method, 2-(2′,6′-dimethoxybenzoyl)pyrrole gave the 5-nitro derivative **1d**: yellow cubes; mp 230–234° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 276 (100, M^+); nmr, see Table II. *Anal.* ($C_{13}H_{12}N_2O_5$) C, H, N.

Using acetic anhydride as solvent, a similar nitration, 3 hr at 70–80°, gave in addition to the 5-nitro compound above, a by-product, separable on tlc (silica, ether–petroleum ether, 3:1) as a gum which could not be crystallized. It had only a medium intensity ir band at 1660 cm^{-1} compared with other analogs. The compound was not very stable but on the basis of its spectra it was assigned the structure 2-(2′,6′-dimethoxy-3′-nitrobenzoyl)-5-nitropyrrole (**3**):

Table I. Comparison of Activities[a] of Pyoluteorin Nitro Analogs with Those of Deoxypyoluteorin[8]

Organism	Incubation time	Complete inhibition, μg/ml				Medium
		1g	1k	Deoxypyoluteorin	1f	
Staph. aureus 209-P	18 hr	1.5	0.8	3.1	>100	N. agar
Staph. aureus (resistant)	18 hr	1.5	0.8	3.1	>100	N. agar
Shig. flexneria 2a	18 hr	6.2	3.1	>100	100	N. agar
Shig. flexneri (resistant)	18 hr	6.2	6.2	>100	100	N. agar
Candida albicans	2 days	6.2	25	>100	>100	S. agar
Tricophyton asteroides	7 days	1.5	1.5	6.2	100	S. agar
Tricophyton interdigitale	7 days	1.5	6.2	12.5	100	S. agar
Tricophyton rubrum	7 days	0.8	1.5	6.2	100	S. agar

[a] Plate method, solvent acetone (maximum concentration 10%), nutrient agar medium; culture temperature 37 and 27°, single determinations.

Table II. Nmr Spectra of Methoxy- and Hydroxybenzoylnitropyrroles[a]

Compd	H(3)	H(4)	NH	OH	OMe	Solvent
1e	3.47	3.05	−0.53		6.22	$CDCl_3$
1g	3.35	2.9	Exchanged			DMSO-d_6
1d	3.75	3.08	−3.62		6.38	DMSO-d_6
1f	3.67	3.03	−3.62	0.37	6.38	DMSO-d_6
3	3.6	3.1	−0.5		6.25	$CDCl_3$

[a] In compounds 1, $J_{1,3}$ = 2 Hz; in 3 H(1) is not split.

mass spectrum *m/e* 321 (90, M^+), 179 (100); nmr, see Table II.

5-Nitro-(2′-hydroxy-6′-methoxybenzoyl)pyrrole (1f). Demethylation of the dimethyl ether **1d** by the method above gave 75% of monomethyl ether: mp 139–141° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 262 (65, M^+), 150 (100). *Anal.* ($C_{12}H_{10}N_2O_5$) C, H, N. When boron tribromide was used for demethylation, a minor product was also obtained, shown by mass spectrometry to be a monobromo derivative of **1f** with the bromine in the phenolic ring.

Preparation of 4-Chloro-2-(2′-methoxybenzoyl)-5-nitropyrrole (1j). Nitration of 0.2 g of 4-chloro-2-(2′-methoxybenzoyl)pyrrole in acetic acid as above gave 80 mg of nitro derivative: mp 141–142°; yellow cubes from benzene–petroleum ether; mass spectrum *m/e* 280 (50, M^+), 135 (100). *Anal.* ($C_{12}H_9ClN_2O_4$) C, H, Cl, N.

4-Chloro-2-(2′-hydroxybenzoyl)-5-nitropyrrole (1k). Demethylation of **1j** as above gave the phenol **1k**: 65%; mp 183–185° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 266 (80, M^+), 120 (100). *Anal.* ($C_{11}H_7ClN_2O_4$) C, H, Cl, N.

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Hydroxylamine Derivatives as Potential Inhibitors of Nucleic Acid Synthesis†

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The antineoplastic activity of hydroxyurea **1a** has been the subject of numerous investigations.[1] Currently, its principal clinical utility lies in the treatment of chronic granulocytic leukemia.[2] Its *N*-methyl (**1b**) and *N*-ethyl (**1c**) derivatives have also been studied in considerable detail[3] but neither of these was shown to have a clear-cut chemotherapeutic advantage over the parent compound.

$NH_2CON(R)OH$

1a, R = H
b, R = CH_3
c, R = C_2H_5

The analog of **1a**, hydroxyoxamide (oxamylhydroxamic acid) **2a**, as well as its acetylated derivative acetoxyoxamide **2b**, was shown to be a selective inhibitor of *in vitro* DNA synthesis having a similar level of potency to that of **1a**.[4,5]

$NH_2COCONHOR$

2a, R = H
b, R = $COCH_3$

However, **2a** displays considerably higher toxicity due at least in part to the fact that it readily oxidizes hemoglobin to methemoglobin.[4] Other close structural analogs of **1a** such as 1- and 3-hydroxybiuret (**3a** and **3b**) have also been investigated for antimitotic effects.[6]

$NH_2CON(OH)CONH_2$ **3a** $\qquad$ $NH_2CONHCONHOH$ **3b**

In the present investigation, two modes of modification of **2a** were chosen in an effort to obtain reduced toxicity: (a) insertion of a substituent, preferably a bulky one, on the N′ nitrogen and (b) attaching a group at the hydroxyl which would be less susceptible to hydrolysis than **2b**. Compounds **5–13** (Table I) are representative of the former approach while **14** and **15** are examples of the latter.

Two synthetic routes were evaluated for the preparation of N′-substituted hydroxyoxamides. The more general route involving the reaction of ethyl *N*-hydroxyoxamate (**4**) with the appropriate amine was found to be unsatisfactory since complex mixtures were invariably formed even at low temperature. Therefore, these compounds were prepared from their corresponding esters, the properties of which are summarized in Table II.

Although admittedly equivocal, the structures of compounds **14** and **15** have been assigned the *O*-carbamyl configuration. This is based upon the fact that neither compound gave a positive reaction with aqueous iron(III) chloride. In addition, the infrared spectra of **14** and **15** have a band in common with **2a** and **2b** at 2.94–2.96 μ which is absent in the spectra of **7** and **11**.

Three new pyrimidine-5-carbohydroxamic acids **16–18** were prepared as potential antimetabolites by the reaction of their corresponding esters with hydroxylamine in the presence of excess base. The analogous reaction employing 5-carbethoxycytosine, however, resulted in hydrolysis to the corresponding carboxylic acid.

Each of the compounds presented in Table I was evaluated for inhibition of DNA, RNA, and protein synthesis in Ehrlich ascites tumor cells *in vitro*. Seven compounds showed inhibition of DNA synthesis at concentrations below $10^{-3}M$ and these are presented in Table III together with the corresponding data for **1a** and **2a**. It will be seen that none of the present compounds display the degree of selective inhibition of DNA synthesis which is shown by **1a** and **2a**, although the activity of **14** is considered significant.

With the exception of **8**, none of the derivatives of **2a** (**5–7** and **9–15**) were found to form methemoglobin (*cf.* Experimental Section). However, the structural modifications generally also resulted in loss of selective action. Only compounds **7** and **9** produced "unbalanced growth" of *Escherichia coli* as evidenced by the formation of long, filamentous cell forms which is often associated with selectivity of action against DNA synthesis. It will be noted

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