Ethyl 3β-Acetoxy-20-aza-24-norchol-5-en-23-oate (3a). Formalin (0.6 ml) was added to a solution of **2a** (0.5 g) in MeOH (10 ml) and the mixture stirred for 12 hr. $NaBH_4$ (200 mg) was added to the externally cooled reaction mixture and the contents were allowed to stir 10–15 min before being poured into H_2O. The resulting precipitate was filtered and air-dried. Tlc examination of this material revealed that starting material was still present along with other minor products and the mixture was chromatographed on alumina (Woelm neutral, activity II). Elution with $CHCl_3$–hexane (3:7) afforded the desired compound **3a** (300 mg): mp 79–80°; [α]D −69° *Anal.* ($C_{26}H_{41}NO_4$) C, H.

Ethyl 20-Aza-3β-hydroxy-21-norchol-5-en-24-oate (2b). Dehydroepiandrosterone acetate (**1**, 25 g) was treated with ethyl β-aminopropionate (22.5 g) as in the procedure for **2a**. Reduction with $NaBH_4$ (2.7 g) gave a semisolid which resisted crystallization from common solvents and was hydrolyzed directly to the acid with 10% ethanolic Na_2CO_3. Resterification of a portion of the crude acid (5 g) in 1% ethanolic HCl gave **2b** (3 g): mp 75.5° (Et_2O–*n*-hexane); [α]D −24°. *Anal.* ($C_{24}H_{39}NO_3$) C, H.

20-Aza-24-norchol-5-en-23-oic Acid (3b). A. Hydrolysis of **3a** (250 mg) with 5% ethanolic KOH and recrystallization from EtOH gave **3b** (200 mg): mp 278–280°; *m/e* 361. *Anal.* ($C_{22}H_{35}NO_3 \cdot 0.5H_2O$) C, H.

B. To a solution of *N*-methyl-17β-aminoandrost-5-en-3β-ol[9] (**4**, 1 g) in Me_3CN–C_6H_6 (5:1, 50 ml) were added ethyl chloroacetate (0.3 ml), NaI (0.2 g), and Et_3N (0.3 ml), and the mixture was refluxed for 1 hr. The reaction mixture was allowed to cool, poured into H_2O, and extracted with $CHCl_3$. The residue obtained after removal of the solvents *in vacuo* was hydrolyzed with 5% methanolic KOH overnight and made acidic (pH 1), and the HCl salt of **3b** was filtered. Distilled H_2O was then added to the HCl salt, the solution was made basic with NH_4OH, boiled until neutral, and cooled, and the precipitate was filtered. Recrystallization from EtOH gave **3b**, mp 276–278°, whose ir spectrum was identical with that obtained in method A above.

Ethyl 20-Aza-3β-hydroxychol-5-en-24-oate (3c). To a solution of *N*-methyl-17β-aminoandrost-5-en-3β-ol[9] (**4**, 7 g) in absolute EtOH (300 ml) was added ethyl acrylate (2.32 g) and the mixture was allowed to stand in the dark for 7 days. Removal of the solvent *in vacuo* gave an amorphous solid. A portion (2 g) was chromatographed on silica gel in order to remove a small amount of starting material. Elution with EtOAc–C_6H_6 (1:9) gave the desired material **3c** (1 g): mp 91–92°; [α]D −35°. *Anal.* ($C_{25}H_{41}NO_3$) C, H.

20-Aza-3β-hydroxychol-5-en-24-oic Acid (3d). Hydrolysis of **3c** (4 g) with 10% Na_2CO_3 and work-up as indicated for **3b** (method B) gave **3d** (1.2 g): mp 208–210° (H_2O–MeOH); [α]D −44° (MeOH). *Anal.* ($C_{23}H_{37}NO_3$) C, H.

Ethyl (20*S*)-3β-Hydroxy-22-azachol-5-en-24-oate (9a). To a solution of the 20β-amine[10] **7** (2 g) in MeCN (75 ml) were added NaI (800 mg), ethyl chloroacetate (0.4 ml), and Et_3N (0.4 ml). After the mixture had refluxed for 1 hr, it was poured into dilute HCl (10%, 200 ml) in order to hydrolyze the THP ether. The mixture was then extracted with $CHCl_3$, dried (Na_2SO_4), evaporated, and chromatographed on alumina (Woelm neutral, activity II). Elution with hexane–$CHCl_3$ (8:2) (300 ml) gave the desired compound **9a** (1 g): mp 110–112° (hexane); [α]D −59°. *Anal.* ($C_{25}H_{41}NO_3$) C, H.

(20*S*)-3β-Hydroxy-22-azachol-5-en-24-oic Acid (9b). Hydrolysis of **10a** with methanolic KOH and the usual work-up gave the 20β-amino acid **9b**, mp 284–286°. *Anal.* ($C_{23}H_{37}NO_3$) C, H.

***N,N*-Bis(carboethoxymethyl)-20β-aminopregn-5-en-3β-ol (10).** Treatment of the 20β-amine **7** (250 mg) with excess ethyl chloroacetate (1 ml), NaI (200 mg), and Et_3N (0.5 ml) in a MeCN solution (20 ml) and use of a work-up similar to **9a** afforded the dialkylated product **10** (150 mg): mp 158–160° (MeOH); [α]D −52°. *Anal.* ($C_{29}H_{47}NO_5$) C, H.

Ethyl (20*R*)-3β-Hydroxy-22-azachol-5-en-24-oate (11a). To a solution of the 20α-amine **8** (1 g) in MeCN–C_6H_6 (1:1, 50 ml) were added ethyl chloroacetate (0.4 ml), NaI (0.3 g), and Et_3N (0.3 ml), and the mixture was refluxed for 30 min, cooled, and poured into H_2O. The oily suspension was extracted into $CHCl_3$ and washed with H_2O. The organic layer was evaporated and the residue redissolved in ethanolic HCl (3%, 10 ml) and left at room temperature overnight. The mixture was then neutralized with dilute NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and the residue was chromatographed on alumina (Woelm neutral, activity II). Elution with petroleum ether (bp 30–60°)–$CHCl_3$ (1:1, 200 ml) gave the desired compound **11a** (150 mg): mp 62–64° (MeOH); [α]D −17°; *m/e* 403. *Anal.* ($C_{25}H_{41}NO_3 \cdot 0.5H_2O$) C, H. Further elution with $CHCl_3$ gave yellow oils which were discarded due to the complexity of the tlc examination.

(20*R*)-3β-Hydroxy-22-azachol-5-en-24-oic Acid (11b). Hydrolysis of **11a** with methanolic KOH and the usual work-up gave the 20α-amino acid **11b**, mp 260–262° (EtOH). *Anal.* ($C_{23}H_{37}NO_3$) C, H.

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Polyethylene Glycol Derivatives of Procaine

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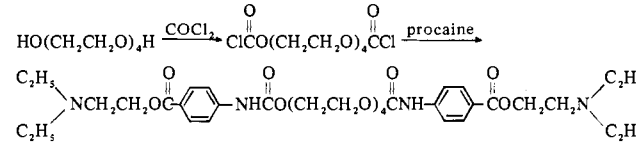
Recently much interest was manifested in synthetic polymers having biological activity as a means of increasing the duration of activity of drugs.[1-3] Synthetic polymers were used not only as carriers for the drugs but also for their biological activity as a whole.[4-6]

In the present work we studied the attachment of procaine hydrochloride, which is a well-known local anesthetic, to polyethylene glycols as a possible means of prolonging its activity. Previous approaches toward changing the duration of anesthesia of procaine-like compounds were based on carrying out changes in the molecular skeleton, such as increase of the aminoalkyl group, or the intermediate alkylene chain[7-9] and the introduction of alkyl groups into the 4-amino group, which provided prolonged duration of local anesthesia.[10-12]

We chose polyethylene glycols as the carrier polymers because they are known to be nontoxic,[13-16] are soluble in both water and organic solvents, and are available in various well-defined molecular weights.

Use was made of the two terminal hydroxyl groups for attaching the procaine to the polymer. These groups were converted to the corresponding chlorocarbonates, by reaction with phosgene in toluene, which on reaction with procaine gave polyethylene glycols having the procaine attached by carbamate linkages as follows.



The chlorocarbonates were freshly prepared before every reaction, due to slow decomposition. The polyethylene glycol derivatives of procaine were oils which dissolved in solvents

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