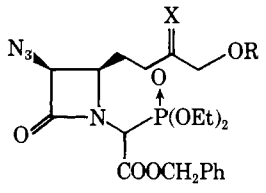
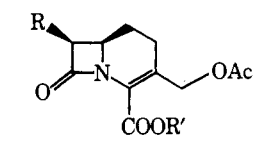
(cis cycloaddition) is totally reversed (to transcycloaddition) if the 4-(3-ethylenedioxy-4-acetoxy)butyl side-chain in **9** is replaced by the 4-SMe group.[1a] Although attempted selective deketalization of the ketal **9** under various conditions failed, treatment with aqueous 10% sulfuric acid in glacial acetic acid (8:1, 2 hr, 50°) resulted in deketalization with concomitant selective ester hydrolysis of the acetate (not the benzyl ester or phosphonate) affording 89% of *cis*-1-(benzyloxycarbonyldiethylphosphono)methyl-3-azido-4-(3-oxo-4-hydroxy)butyl-2-azetidinone (**10**): nmr, ketal, CH_2CH_2, and acetate CH_3 disappeared; ir 2.56 (OH), 4.70 (N_3), 5.62 (β-lactam C=O), 5.72–3.80 (C=O and ester).



9, X =  ; R = Ac

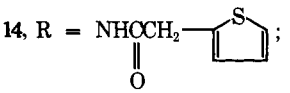
10, X = O; R = H

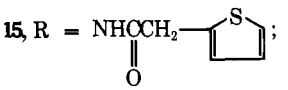
11, X = O; R = Ac



12, R = N_3; R′ = CH_2Ph

13, R = NH_2; R′ = H

14, R =  ; R′ = CH_3

15, R =  ; R′ = Na

Acetylation of the ketol **10** (acetyl chloride, pyridine, methylene chloride, room temperature, overnight) gave the acetoxy ketone **11** in 82.5% yield: nmr (100 MHz) 2.14 (s, CH_3), 1.25 (m; CH_3), 4.13 (m, CH_2), 4.63 (s, CH_2), 4.73 (d, N_3CH, J = 5.5 Hz), 4.99 (d, HCP, J = 24 Hz), 7.32 and 7.34 (Ph); ir 4.70 (N_3), 5.62 (β-lactam C=O), 5.70 (esters). Cyclic olefination of **11** was smoothly effected with sodium hydride in dry glyme (50°, 1.5 hr) to afford on chromatography the bicyclic (±)-benzyl 7β-azido-1-methylenedethiacephalosporanate (**12**) (62%): nmr (100 MHz), 1.98 (s, CH_3), 3.72 (m, CCHN), 4.8 and 5.07 (AB q, CH_2O), 4.85 (d, HCN_3, J = 5.5 Hz), 5.20 (s, CH_2), 7.21 (m, Ph); ir 4.7 (N_3), 5.62 (β-lactam C=O), 5.73 (esters), 6.09 (C=C). Simultaneous hydrogenolysis of the benzyl ester and reduction of azide (10% Pd–C, H_2, aqueous dioxane, 0.5 hr, room temperature, 45 psi) in **12** yielded the zwitterion, (±)-7β-amino-1-methylenedethiacephalosporanic acid (**13**): ir (Nujol), 2.94 (NH_2, OH), 5.57 (β-lactam C=O), 5.74 (acid and ester). Acylation of the amino acid **13** with 2-thienylacetyl chloride and sodium bicarbonate in aqueous acetone at 0° for 1 hr afforded (±)-7β-(2-thienyl)acetamido-1-methylenedethiacephalosporanic acid (**1**), 80% overall yield from the azido benzyl ester **12** (nmr (acetone-d_6) 2.03 (s, CH_3), 3.89 (s, CH_2), 5.50 (dd, NCHC=O, J = 5.5 Hz, J = 9 Hz), 4.8 and 5.07 (AB q, CH_2O), 8.00 (d, NH, J = 9 Hz); ir 5.7 (β-lactam C=O), 5.8 (acid and ester); *m/e* 318 (M^+ − AcOH)), which was further identified as the methyl ester (diazomethane, ethyl acetate, ether) **14** (nmr 2.05 (s, CH_3), 3.8 (s, CH_3 and CH_2), 4.8 and 5.16 (AB q, CH_2), 5.4 (dd, NCHC=O, J = 5.5 Hz, J = 9 Hz), 6.45 (d, NH, J = 9 Hz); *m/e* 392 (M^+)). The sodium salt **15** of the acid **1** was prepared by adding equimolar sodium bicarbonate to the acid **1** in water: nmr (D_2O) 2.07 (s, CH_3), 3.9 (s, CH_2C=O), 5.28 (d, NCHC=O, J = 5 Hz), 4.63 and 4.9 (AB q, CH_2); ir (Nujol) 5.67 (β-lactam C=O), 5.73 (ester), 5.98 (NHC=O), 6.22 (COO^-); uv $\lambda_{max}^{H_2O}$ 238 (ϵ 12,800), 255 (ϵ 10,440).[8] Table I compares the antimicrobial activity of compound **15** with that of 6(*R*),7(*R*)-sodium cephalothin.

Acknowledgment. We are grateful to Dr. R. W. Ratcliffe for stimulating discussion during the course of this work. We also thank Dr. E. H. Thiele for the *in vitro* results reported in this paper.

References and Notes

(1) (a) For Part VII, see L. D. Cama and B. G. Christensen, *J. Amer. Chem. Soc.*, **96**, 7582 (1974). (b) We propose this trivial name for (±)-7β-(2-thienyl)acetamido-1-methylenedethiacephalosporanic acid.
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Decamethoxonium, an Alkylating Analog of Decamethonium

Sir:

Decamethonium (decamethylenebis (trimethylammonium) (I)) binds tightly but reversibly to acetylcholinesterase. In the case of enzyme from the electric eel, the value of the dissociation constant of I is 3×10^{-8} M at low ionic strength.[1] Decamethoxonium (decamethylenebis(dimethyloxonium) (II)) is structurally similar to I, and the trialkyloxonium group is a highly reactive alkylating function.[2] Consequently, we expected that II might be an active-site-directed alkylating agent for acetylcholinesterase. This report describes the synthesis of II and its effect on the esterase, as well as preliminary results with another protein, acetylcholine receptor.

$(CH_3)_3\dot{N}(CH_2)_{10}\dot{N}(CH_3)_3$ I $(CH_3)_2\dot{O}(CH_2)_{10}\dot{O}(CH_3)_2$ II

1,10-Dimethoxydecane was prepared from 1,10-dibromodecane (Aldrich Chemical Co.) by refluxing the dibromo compound with a slight excess of sodium methoxide in methanol for 24 hr. After evaporation of the methanol and addition of water to the residue, the product was extracted into ether and purified by distillation at reduced pressure. II was prepared from 1,10-dimethoxydecane by alkylation with methyl iodide in the presence of silver hexafluorophosphate, after the procedure of Meerwein.[3,4] 1,10-Dimethoxydecane (3.0 ml) and, immediately afterwards, methyl iodide

Table I. The Inactivation of Acetylcholinesterase by Decamethoxonium[a]

Experiment no.	II-$(PF_6)_2$ (μM)	V_i/V_0
1	0.5	<0.05
	0.25	0.05
	0.05	0.37
	0.025	0.46
	0.005	0.69
	0.0025	0.89
2[b]	2.5	<0.05
	2.5	0.50
3[c]	0.50	0.14
	0.50	0.78

[a] A small aliquot of a freshly prepared 20 mM II-$(PF_6)_2$ in dry nitromethane was vigorously mixed with ice-cold water. A minute or less later, a small aliquot of this aqueous solution was added to electric eel acetylcholinesterase (type V, Sigma Chemical Co.) in 1 mM potassium phosphate buffer, pH 7.0, at 2°. After 2 hr or more, an aliquot of the modified enzyme solution was assayed for activity by measuring the rate of hydrolysis of acetylcholine as described in ref 6. The extent of inactivation is expressed as the ratio of the rate with the treated enzyme (V_i) to that with the same amount of untreated enzyme (V_0). The initial concentration of acetylcholinesterase in the modification reaction mixtures, calculated on the basis of the specific activity of the purified enzyme (ref 7), was about 0.0015 μM. When the reagent was allowed to hydrolyze in buffer and then the enzyme was added, no inactivation occurred. [b] The first entry is the result of treatment with II in the presence of 10 mM NaCl, and the second entry is the result of treatment with II in the presence of 10 mM tetramethylammonium chloride. [c] Same as *b*, except 20 mM NaCl and tetramethylammonium chloride.

(3.0 ml) were added to a solution of 7.7 g of dry silver hexafluorophosphate (Cationics, Inc.) in 30 ml of dry 1,2-dichloroethane. The mixture was stirred for 1 hr, and the precipitate (a mixture of silver iodide and II-$(PF_6)_2$) was collected on a sintered-glass funnel. The precipitate was mixed with 10 ml of dry nitromethane. The slurry was filtered through a fine-sintered-glass funnel, and 50 ml of dry 1,2-dichloroethane was added to the filtrate. This mixture was put in a desiccator over P_2O_5, and the desiccator was placed at −20° for several hours. Colorless crystals of II-$(PF_6)_2$ formed. They were collected rapidly by suction filtration and were stored at 2° in a desiccator over P_2O_5. The yield was 3.2 g. The compound was recrystallized from nitromethane-1,2-dichloroethane in the same way. Analysis for C, H, F, and P gave 32.3, 5.7, 43.6, and 11.8%, respectively; the theoretical values are 32.2, 6.1, 43.7, and 11.9%. The pmr spectrum, taken within 15 min after solution in dry deuterated nitromethane, was the following, expressed as δ (apparent multiplicity, relative integrated intensity, assignment to hydrogen atoms in the compound): 0.78 (broad singlet, 12 H, $-[CH_2]_6-$), 1.43 (multiplet, 4 H, OCH_2CH_2), 3.88 (singlet, 12 H, $[CH_3]_2O$), 4.20 (triplet with J = 7 Hz, 4 H, OCH_2). The signals in this spectrum are the ones expected on the basis of the pmr spectrum of the related ion, dimethylethyloxonium ion.[5] II-$(PF_6)_2$ dissolves in water only very slowly. Aqueous solutions were prepared by dissolving it in dry nitromethane, in which it dissolves in a few seconds, and vigorously mixing a small aliquot of the concentrated solution in nitromethane with water. Trialkyloxonium ions react rapidly with water to yield an ether, an alcohol, and hydronium ion.[2] The rate of hydrolysis of 0.15 mM II-$(PF_6)_2$ in 1% nitromethane at pH 7.0 and 5.5° was followed in a pH-stat apparatus. The reaction was first-order with a half-time of 5 min; 2 equiv of base were consumed. We have also synthesized the tetrafluoroborate salt of II by a procedure identical with that for the preparation of the hexafluorophosphate salt, except for the substitution of dry silver tetrafluoroborate (Cationics, Inc.) for silver hexafluorophosphate.[3]

The data in Table I show that II is an extremely potent inactivating agent against eel acetylcholinesterase. It causes about 50% inactivation when present at an initial concentration of 0.025 μM (experiment 1). Tetramethylammonium ion, a competitive inhibitor of the enzyme with a dissociation constant of 1.2×10^{-3} M,[8] blocks the irreversible inactivation by II (experiment 2 and 3). These results suggest that II acts by alkylation of a group at the active site. Other studies[1] have indicated that the high affinity of acetylcholinesterase for decamethonium is due to decamethonium bridging the active site and a peripheral anionic site. Thus, II may also alkylate the peripheral site. We have reported earlier that the monofunctional reagent, trimethyloxonium ion, inactivates the enzyme at concentrations near 1 mM.[6]

Another protein that has a high affinity for decamethonium is acetylcholine receptor. The value of the dissociation constant for I with solubilized receptor from *Electrophorus electricus* is 2×10^{-8} M.[9] The binding of I is competitive with the binding *Naja naja* α-neurotoxin.[10] We have examined the effect of II upon the capacity of purified receptor from *Electrophorus electricus*[10] to bind α-neurotoxin. This activity was measured using radioactively labeled toxin in the antibody precipitation assay that has been reported previously.[11] Treatments of 10^{-8} M receptor at pH 7.4 and 4° with II at initial concentrations of 5.0, 0.50, and 0.05 mM resulted in the loss of 98, 60, and 10% of the toxin-binding activity, respectively. We then determined the ability of decamethonium to protect against inactivation of receptor by II. At a concentration of 5 mM, decamethonium reduced the extent of inactivation produced by 0.5 mM II from 60 to 20%.

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Isolation and Structural Characterization of a μ-Di(η^5:η^1-cyclopentadienyl)dithorium(IV) Complex

Sir:

Considerable interest has recently been focused on those factors which determine the stability of both d and f transition metal to carbon σ bonds.[1-4] Studies of organometallic decomposition mechanisms have principally involved exam-