was removed from the org layer under reduced pressure, and the residue was recrystd from *i*-PrOH to give **43**.

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Quaternary Pilocarpine Derivatives Acting as Acetylcholine Antagonists

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Several quaternary *d*-pilocarpine derivatives have been prepared in order to investigate the influence of structural changes on the biological activity of this alkaloid. The effect of the substituents in the reagent, as well as of the temp and the solvent (its dielectric constant), on the rate of the quaternization has been studied, and the products have been analyzed by various spectroscopic means. The anticholinergic activities of the compounds are reported, and a relation has been sought in connection with the structural changes.

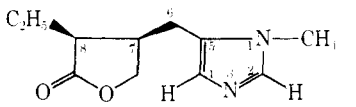
Pilocarpine (I) is the main alkaloid obtained from the leaves of the South American shrubs *Pilocarpus jaborandi* and *Pilocarpus microphyllus* Stapf. The structures of pilocarpine and its isomer, isopilocarpine, were determined by Jowett[1] and both were synthesized by several routes.[2] The absolute configuration of pilocarpine has been established as being 7*R*,8*S*.[3] *d*-Pilocarpine, one of the oldest parasympathomimetic drugs,[4] may act as an anticholinergic in certain systems.[5]

The purpose of this study was: (a) to develop methods for the addition of various groups to the alkaloid by quaternization at N-3 and determine the various conditions influencing the reaction and the stability of the products; (b) study some aspects of the relative reactivity of the alkaloid with various halo organic reagents; (c) test the pharmacological activity of the new compds as a function of structural change. It has been reported that quaternization of atropine and scopolamine with different substituted phenacyl bromides induces changes in their pharmacological activities.[6]

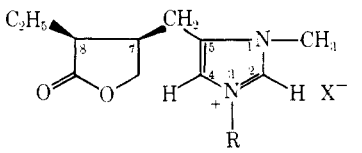
Results and Discussion

The free base of *d*-pilocarpine (I) was treated with different halo organic compds producing a series of quaternary deriv with the general structure II (Table I).

The effect of the substituents in the halo organic reagents, the temp, and the solvent influence the optimal time of the reaction. The data collected in Table I show a marked decrease in the rate of quaternization in Me_2CO medium passing from Et to *n*-Pr (**1-3**), but in contrast to previous observations,[7,8] with *n*-BuBr practically no reaction took place. In a solvent with higher polarity (2-methoxyethanol) only 6 days were required for completion of the reaction. It was observed that *n*-PrI was about twice as reactive as the bromide, whereas with *i*-PrBr no quaternization would take place. It is therefore difficult to distinguish between electronic and steric effects in these reactions.



I



II

In the case of benzyl halides the reactivity is relatively greater, and is influenced by the character and the position of the substituent. Electron-releasing groups in the para position (**7**, **10**, **13**) enhance the displacement of the halogen, the reaction becoming more sluggish with a Me group. With ortho substituents of the same character (**9**, **11**) steric hindrance makes the reaction slower by far. An electron-attracting group, such as NO_2, at the para position induces a decrease of the rate of the reaction, bromide **15** being more reactive than chloride **16**. In contrast, when NO_2 is at the meta position (**14**) the reaction is faster. When Ph is further away from the side-chain halogen atom (**5**), no conjugation between the ring and the side-chain halogen is possible, and the reaction becomes sluggish; it could be accelerated, however, by using a solvent with high dielectric constant (ε) such as 2-methoxyethanol ($\varepsilon \sim 40$). The comparatively high reactivity with the phenacyl bromides (**19-23**) may be explained by the activating effect of the carbonyl group.[9]

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TABLE I

QUATERNARY *d*-PILOCARPINE DERIVATIVES. PREPARATION METHODS, PHYSICAL AND ANALYTICAL DATA

No.	R	X	Method[a]	Reaction time	Yield, %	Mp, °C	$[\alpha]^{22}D$, deg[b]	Formula[c]
1	C_2H_5	Br	A[d]	5 hr[e]	88	Hygr[f]	+80	$C_{13}H_{21}BrN_2O_2$
2	*n*-C_3H_7	Br	B[g]	7 days	87	Hygr[f]	+77	$C_{14}H_{23}BrN_2O_2$
3	*n*-C_3H_7	J	A[g]	5 days	85	Hygr[f]	+61	$C_{14}H_{23}IN_2O_2$
4	*n*-C_4H_9	Br	D[h]	6 days[i]	90	110–111	+62	$C_{15}H_{25}BrN_2O_2$
5	$C_6H_5CH_2CH_2$	Br	D	36 hr	84	172–173 dec	+53.5	$C_{19}H_{25}BrN_2O_2$
			C[j]	2 days	73			
6	$C_6H_5CH_2$	Br	B[k]	14 hr	94	126	+59.5	$C_{18}H_{23}BrN_2O_2$
7	*p*-$BrC_6H_4CH_2$	Br	C[h]	6 hr	87	159	+52	$C_{18}H_{22}Br_2N_2O_2$
8	*m*-$ClC_6H_4CH_2$	Br	B[h]	36 hr	75	155	+53.5	$C_{18}H_{22}BrClN_2O_2$
9	2,6-$Cl_2C_6H_3CH_2$	Cl	B[h]	4 days	82	146	+52.5	$C_{18}H_{21}Cl_3N_2O_2$
10	3,4-$Cl_2C_6H_3CH_2$	Cl	B[h]	24 hr	88	183–185 dec	+52	$C_{18}H_{21}Cl_3N_2O_2$
11	*o*-$CH_3C_6H_4CH_2$	Br	B[l]	4 days	88	115	+54	$C_{19}H_{25}BrN_2O_2$
12	*m*-$CH_3C_6H_4CH_2$	Br	B[l]	2 days	79	115	+53.5	$C_{19}H_{25}BrN_2O_2$
13	*p*-$CH_3C_6H_4CH_2$	Br	B[l]	36 hr	94	187–188 dec	+53.5	$C_{19}H_{25}BrN_2O_2$
14	*m*-$NO_2C_6H_4CH_2$	Br	A[k]	36 hr	80	180–181 dec	+53	$C_{18}H_{22}BrN_3O_4$
15	*p*-$NO_2C_6H_4CH_2$	Br	A[g]	7 days	84	157	+54.5	$C_{18}H_{22}BrN_3O_4$
16	*p*-$NO_2C_6H_4CH_2$	Cl	C	8 days	96	45–47	+60	$C_{18}H_{22}ClN_3O_4$
			A[g]	9 days	75			
17	$C_6H_5OCH_2CH_2$	Br	D[k]	5 days	85	82	+52.5	$C_{19}H_{25}BrN_2O_3$
18	$C_6H_5OCH_2CH_2CH_2$	Br	D	7 days	86	51	+50	$C_{20}H_{27}BrN_2O_3$
			C[k]	9 days	73			
19	$C_6H_5COCH_2$	Br	A[m]	36 hr	90	48–50 110 dec	+58	$C_{19}H_{23}BrN_2O_3$
20	*p*-$BrC_6H_4COCH_2$	Br	A	10 hr	74	~220 dec	+54	$C_{19}H_{22}Br_2N_2O_3$
			C[h]	6 hr	86			
21	*p*-$FC_6H_4COCH_2$	Br	C[l]	3 days	84	90–91	+57	$C_{19}H_{22}BrFN_2O_3$
22	*p*-$NO_2C_6H_4COCH_2$	Br	C[l]	6 days	86	139–141 dec	+55.5	$C_{19}H_{22}BrN_3O_5$
23	*p*-$C_6H_5C_6H_4COCH_2$	Br	D	5 days	89	178–179 dec	+55	$C_{25}H_{27}BrN_2O_3$
			C[n]	6 days	69			
24	Pilocarpine-$(CH_2)_4$-pilocarpine[o]	2Br	C[h]	3 days	88	75–77	+68	$C_{26}H_{40}Br_2N_4O_4$

[a] The solvents used for trituration, washing, or crystn were: $CHCl_3$, Et_2O, Me_2CO, and C_6H_6. [b] All measurements were made in EtOH for 0.5% concn, only for 1 and **17** the concn was 1%; the observed optical rotation of *d*-pilocarpine is +108.5° (*c* 1.05); mol rotations were within a small range, 210–252. [c] All compds were analyzed for C, H, N, and the results were within ±0.4% of the theoretical value. Only for 11, **12**, **19**, and **21** the results were within ±0.6%; the value of the anionic part was detd by potentiometric titrn and was within ±0.5%. [d] Trituration and washing with Et_2O (several times). [e] Complete crystn, 24 hr. [f] Highly hygroscopic, detn of mp impossible. [g] The crude product was extracted with $CHCl_3$. The oil fraction undissolved in $CHCl_3$ was triturated with dry Et_2O. [h] Several times trituration with Me_2CO–dry Et_2O (2:5), washing with Et_2O. [i] In Me_2CO medium there was no reaction after 9 days. [j] Trituration with Et_2O–C_6H_6 (1:1), crystn by dissoln in min vol of gently warmed EtOH, cooling, and pptn with Et_2O. [k] Trituration with $CHCl_3$–Et_2O (2:5), crystn by dissoln in min vol of $CHCl_3$ and pptn by addition of Et_2O to turbidity, cooling, and trituration, washing with Et_2O. [l] Trituration with $CHCl_3$–Et_2O (1:5), crystn by dissoln in abs EtOH and pptn by addn of Et_2O. [m] Trituration in the following sequence, C_6H_6, $CHCl_3$–Et_2O (2:5), and finally Et_2O. [n] Trituration with Me_2CO, crystn by dissoln in min vol of abs MeOH distn and addn of Et_2O with trituration of the residue. [o] Two moles of *d*-pilocarpine bonded through a $(CH_2)_4$ chain.

The introduction of a substituent at the para position having a mesomeric positive effect, such as a Br atom (**20**), increases the rate of the reaction, as has been observed with similar reactions using pyridine.[10] In the case of an F atom (**21**) the effect is much weaker. The introduction of the electron-attracting *p*-NO_2 group (**22**) causes an opposite effect, thereby decreasing the rate of the quaternization. A Ph group at the same position (**23**) also slows down the reaction.

The influence of the solvent on the reaction rate is well described,[11–13] in our case solvents were carefully selected in order to prevent side reactions with the reagents; dry Me_2CO ($\varepsilon \sim 21.4$ at 20°) proved to be satisfactory, and 2-methoxyethanol ($\varepsilon \sim 40$) was used in order to study its effect on the rate of quaternization and to accelerate the reaction whenever needed (**4**, **5**, **18**, **23**).

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The structures of the quaternary products were studied in detail by nmr and mass spectrometry, and an account of the analysis of the spectra and a description of the characteristic fragmentation pattern will be given separately.

Pharmacology.—Pilocarpine (0.6 μg) caused contraction of guinea pig isolated ileum, and the height of responses was approximately equiv to that elicited by 20 ng of ACh. The antagonistic activity of the compds toward ACh-induced spasm is shown in Table II. Compds **7**, **10**, **20**, and **23** were most potent, whereas **24** was devoid of activity. Compds **19** and **26** (2 μg) and **21** and **22** (1 μg) did not antagonize ACh, and at higher concns contracted the gut by themselves.

Table II also shows the materials tested against organophosphate intoxication. None of them conferred protection. The animals treated with the test compds, as well as untreated animals, died within 9 min after poisoning. In contrast, 5 out of 6 mice of a group injected with 25 mg/kg of atropine and pralidoxime methanesulfonate prior to TEPP survived.

d-Pilocarpine is known to act as a cholinomimetic

TABLE II
ANTICHOLINERGIC ACTIVITY OF QUATERNARY DERIVATIVES OF *d*-PILOCARPINE

Compd	R	X⁻	Antagonism[a] to acetylcholine guinea pig ileum	Dose,[b] mg/kg, administered before TEPP poisoning in mice
1	C_2H_5	Br	20	100
2	*n*-C_3H_7	Br	35	20
3	*n*-C_3H_7	J	15	20
4	*n*-C_4H_9	Br	15	100
5	$C_6H_5CH_2CH_2$	Br	15	100
6	$C_6H_5CH_2$	Br	30	50
7	*p*-$BrC_6H_4CH_2$	Br	85	50
8	*m*-$ClC_6H_4CH_2$	Br	27	
9	2,6-$Cl_2C_6H_3CH_2$	Cl	31	
10	3,4-$Cl_2C_6H_3CH_2$	Cl	90	50
11	*o*-$CH_3C_6H_4CH_2$	Br	39	
12	*m*-$CH_3C_6H_4CH_2$	Br	30	
13	*p*-$CH_3C_6H_4CH_2$	Br	78	30
14	*m*-$NO_2C_6H_4CH_2$	Br	60	50
15	*p*-$NO_2C_6H_4CH_2$	Br	25	50
16	*p*-$NO_2C_6H_4CH_2$	Cl	50	50
17	$C_6H_5OCH_2CH_2$	Br	35	100
18	$C_6H_5OCH_2CH_2CH_2$	Br	16	
19	$C_6H_5COCH_2$	Br	0	100
20	*p*-$BrC_6H_4COCH_2$	Br	90	1
21	*p*-$FC_6H_4COCH_2$	Br	0	
22	*p*-$NO_2C_6H_4COCH_2$	Br	0	
23	*p*-$C_6H_5C_6H_4COCH_2$	Br	100	50
24	Pilocarpine-$(CH_2)_4$-pilocarpine[c]	2Br	0	100
25	*p*-$BrC_6H_4CH(OH)CH_3$[d]	Br	35	
26	*p*-$C_6H_5C_6H_4CH(OH)CH_3$[d]	Br	0	

[a] Antagonism is expressed as per cent redn of the control response to acetylcholine. [b] Dose which did not cause observable symptoms when injected together with pralidoxime methanesulfonate to control mice. [c] Two moles of pilocarpine bonded through a $(CH_2)_4$ chain. [d] Reduced products of the compds **20** and **23**, resp.

and anticholinergic.[5] In the guinea pig isolated ileum prepn used in the present work, *d*-pilocarpine was found to act as a cholinomimetic; whereas when changes were made in the imidazole moiety, compds were obtained possessing anticholinergic activity. This activity was weak for compds with aliphatic groups attached to the imidazole ring (**1-4**) or with a Ph group at the end of an ethylene chain (**5**). For a benzyl group, however, the activity increased (**6**), and introducing a Br atom at the para position of the Ph ring (**7**) increased the anti-ACh action even more. Furthermore, it seems that besides Br other electron donors such as Cl and CH_3, at the para position (**10**, **13**), augmented the activity. On the other hand, substitution at the ortho position (**9**, **11**) did not change the antagonistic effects when compared with the unsubstituted benzyl group.

When the group was phenacyl alone (**19**) or substituted at the para position of the benzene ring with electron-attracting groups (**22**), the compds remained pilocarpine-like. However, with electron-donor substituents at the para position, such as **20** and **23**, the activity was reversed, becoming strongly anticholinergic.

It seemed that quaternization alone does not confer anticholinergic activity, since **24** containing 2 pilocarpine moieties bonded by a $(CH_2)_4$ bridge, was inactive. The influence of the group attached to a quaternary N has been described earlier.[14]

The fact that none of the compds tested, even those strongly antagonizing ACh *in vitro*, replaced atropine in organophosphate poisoning could be explained by the difficulty that the quaternized drugs have in crossing the blood–brain barrier. Thus, in contrast to atropine, they could hardly reach cholinergic receptors in the CNS.

Experimental Section

Mps were taken on a Fisher-Johns apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Infracord, Model 137 spectrometer equipped with a NaCl prism in KBr pellets; whenever the compds were hygroscopic the film method was used. Uv spectra were determined with a Cary Model 15 spectrophotometer in aq solns. Nmr spectra were recorded on a Varian A-60 for approximately 15% soln in D_2O or $(CD_3)_2SO$ (TMS). The mass spectra were taken on an Atlas CH 4 mass spectrometer by the direct inlet method. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter using a Na light source; and the values refer to EtOH solns.

For tlc, chromatoplates coated with cellulose (Riedel De Haën, 0.25 mm) were used with a solvent mixt of *n*-BuOH–AcOH–H_2O (4:0.5:5). The plates were developed with an iodoplateate soln. In certain cases (redn products **25**, **26**), silica gel chromatoplates were used with $CHCl_3$–MeOH (4:1) and developed by spraying with 2% AcOH followed by iodoplateate. The equiv wts of the products were detd by potentiometric titrn. The elemental anal. were performed by the microanalytical laboratory of our Institute under the direction of Mr. R. Heller.

Chemical Methods.—*d*-Pilocarpine free base was prepd from its HCl salt (Plantex Ltd., Nathanya, Israel) by adding NH_4OH (sp gr 0.91) to the aq soln up to pH 7.7, thereby avoiding opening of the lactone ring. The soln was then extd several times with $CHCl_3$, and the org layer was washed with distd H_2O and dried (Na_2SO_4). After filtration, the solvent was thoroughly removed affording the pure base as a heavy oil. For all expts it was of the utmost importance to carefully dry the alkaloid in a desiccator over P_2O_5 for 48 hr. The halo organic reagents were of high purity and, if necessary, specially purified. Dry Me_2CO and 2-methoxyethanol were used as solvents for the reaction and during purification or crystn of the products. The expts were carried out in carefully dried, air-tight vessels avoiding moisture. The course of the reaction was followed by nmr spectroscopy and by titrimetric detn of the anionic part.

The quaternary derivs of *d*-pilocarpine were synthesized using the following methods according to the thermal stability and the soly of the halo organic reagents; high temps were avoided since isomerization of the alkaloid may then take place.

In addition to being generally sol in H_2O, the quaternary derivs dissolve in alcohols, in propylene glycol, and sometimes in Me_2CO; they are, however, rather insol in Et_2O, $CHCl_3$, C_6H_6, and *n*-C_6H_{14}. The compds with substd phenacyl groups (**20–23**) are sparingly sol in H_2O.

Method A.—*d*-Pilocarpine (0.02 mole) was mixed with 0.025 mole of the halo organic reagent, and the mixt was heated in an oil bath until a homogeneous soln was formed. The temps required were 60° for **14** and **19**, 90° for **16**, and 115° for **15** and **20**. After cooling the melt, 30 ml of dry Me_2CO was added, and the mixt was heated to reflux until complete soln was obtained. Following the required reaction period, Me_2CO was removed under reduced pressure, and the product was purified and eventually crystd as described in Table I. In the case of **1** and **3** a spontaneous exothermic reaction developed upon mixing the reagents, and the products were obtd directly from the mixt (transparent crystals for **1**, oil for **2**).

Method B.—*d*-Pilocarpine (0.02 mole) was dissolved in Me_2CO (10 ml) with stirring under anhyd condns; then a soln of 0.025 mole of the halo organic compd in 10 ml of Me_2CO was added dropwise (in the case of **2**, 0.03 mole of the halo organic reagent was used). After 10 min an addnl quantity of Me_2CO (10 ml)

(14) J. M. van Rossum and E. J. Ariëns, *Experientia*, **13**, 161 (1957).

was added, and the flask was tightly closed and left at room temp. At the end of the required reaction time the solvent was removed under reduced pressure, and the product was purified as described in Table I.

Method C.—In a flask as above and using a condenser, *d*-pilocarpine (0.02 mole) was dissolved in Me_2CO (10 ml) with constant stirring while heating to about 50°. A freshly prepd soln of 0.025 mole of the halo organic reagent in 10 ml of Me_2CO was then slowly added and the mixt was heated to reflux for 30 min (reflux was not necessary for **9**). After cooling, the soln was transferred to a dry flask with 20 ml of dry Me_2CO and kept tightly closed at room temp for the required period. The Me_2CO was then removed under reduced pressure above a H_2O bath, and the residue was dried. For purification of the products see Table I. In the case of **23**, *d*-pilocarpine was added to a suspension of the halo org reagent in dry Me_2CO at reflux temp, and the product was collected by filtration.

Method D.—In a flask equipped as above, a soln of halo org reagent (0.02 mole) in 2-methoxyethanol (20 ml) was heated to 50° with stirring; then a soln of *d*-pilocarpine (0.02 mole in 25 ml of the same solvent) was added dropwise. The mixt was heated with stirring to 80° for 30 min and left tightly closed at room temp for the required period. The solvent was removed at 80° under reduced pressure, and the residue was dried over P_2O_5. For **4** no heating was required and the molar ratio was 1.5:1.

Whenever min amounts of the HBr of the pilocarpine were obtd as a side product, the sepn from the quaternary compds was accomplished from an aq soln at pH 7.5. The free pilocarpine was extd with $CHCl_3$ leaving the quaternary compd in the aq layer which was then lyophilized.

Compd 25 (**R** = *p*-$BrC_6H_5C_6HOHCH_2$).—3-(*N*-*p*-Bromophenacyl)-*d*-pilocarpinium bromide (**20**) (0.24 g, 5×10^{-4} mole) was dissolved in MeOH (30 ml), and $NaBH_4$ (0.12 g, 3×10^{-3} mole) was added and stirred for 1 hr. The soln was then filtered and adjusted to pH ~ 7 (with HCl 1:4). The solvent was removed under reduced pressure, and the residue was dried overnight in a desiccator over P_2O_5. This residue was then dissolved in a min quantity of abs EtOH and filtered. After evapn of the solvent, the yellowish product (0.29 g, 93%) was redissolved in abs EtOH, decolorized with activated C (Darco G 60; 15 min at 30°), and filtered, and the solvent was removed under reduced pressure above a water bath. The white product was stored over P_2O_5; ν_{max}^{film} 3450 (broad) and 1095 cm^{-1} (OH); $[\alpha]^{22}D$ + 21° (*c* 0.25).

Compd 26 (**R** = *p*-$C_6H_5C_6H_4CHOHCH_2$).—3-(*N*-*p*-Phenylphenacyl)-*d*-pilocarpinium bromide (**23**) (0.24 g, 5×10^{-4} mole) was dissolved in MeOH (20 ml) with stirring, and $NaBH_4$ (0.12 g, 3×10^{-3} mole) was added. The mixt was kept for 90 min at 30°, work-up as above. For the sepn and purifn, dry $CHCl_3$ was used producing a yellowish hygroscopic solid (0.22 g, 90%) which was stored in a desiccator over P_2O_5: ν_{max}^{film} 3450 (broad), and 1095 cm^{-1} (OH); $[\alpha]^{22}D$ + 21.6° (*c* 0.25).

Pharmacological Methods.—Anticholinergic activity was detd on a piece of guinea pig terminal ileum suspended in a 5-ml organ bath filled with Tyrode soln at 35°. Contractions were induced by 20 ng of ACh at intervals of 2 min. The height of the contraction was estimated before and during the presence of the test compd. In the case of **21** and **22** the concn was 1 μg, for **19** and **26** it was 2 μg, and for all other compds, 10 μg. Amounts refer to final concn in ml of bathing fluid. Antagonism was expressed as % redn of the control response to ACh, and represents the average of 2 assays in 2 separate prepns.

A number of compds were examined as substitutes to atropine in exptl organophosphate poisoning. Groups of 6 male mice of 20-g body weight were used. The test compds were injected ip at a preselected dose which did not cause observable abnormalities. This was immediately followed by adminstration of 40 mg/kg of pralidoxime methanesulfonate. Five min later, animals were injected sc with 3 × LD_{50} of tetraethyl pyrophosphate (TEPP). Solns of the materials were made up in saline immediately before use and injected at a max vol of 0.2 ml. Compds 20 and 23 were dissolved in propylene glycol and injected in a vol of 0.05 ml.

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Benzimidazo[2,1-*b*]quinazolin-12-ones. A New Class of Potent Immunosuppressive Compounds

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The benzimidazo[2,1-*b*]quinazolin-12-ones constitute a novel group of compounds, only the parent unsubstituted tetracyclic compound being known previously. They were prepared as part of a search for new immunosuppressive agents and have proven markedly active in the sheep erythrocyte antibody in mice (SEAM) assay. They are much more active than azathioprine in this assay.

Apart from establishing some of the general structural requirements for maximal immunosuppressive activity in benzimidazo[2,1-*b*]quinazolin-12-ones, we attempted to modify the *in vivo* transport of these compounds by making carrier derivatives. These derivatives were designed with a view to their undergoing cleavage *in vivo* to an active immunosuppressive component and an inactive carrier portion which served only to alter the *in vivo* distribution of the active component.

It is clear that the activity of a presumably reversible compound cannot be ascribed, with any certainty, to the fact that the expected cleavage did indeed precede the demonstrated activity. Immunosuppression may well have arisen from the intact molecule. Studies with labeled compounds would be required to distinguish between these two modes of action. As will be seen, there are results which are consistent with the idea that cleavage of the reversible carrier compounds is, at least in some instances, a prerequisite to immunosuppression.

Details of the sheep erythrocyte antibody in mice (SEAM) assay used in these laboratories have been described elsewhere,[1] except to add that the dose levels used constituted the geometric series 50, 25, 12.5, 6.2, 3.1, 1.6. . . mg/kg. For better readability, the activities of the compounds are discussed throughout the paper rather than in the form of a single table. The number in parentheses next to a molecular diagram in the minimum dose, in mg/kg, which, when administered intraperitoneally at three optimal times (72, 48, and 24 hr

(1) C. J. Paget, K. Kisner, R. L. Stone, and D. C. DeLong, *J. Med. Chem.*, **12**, 1010 (1969).