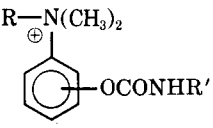
Synthesis of Some Dialkyl Aminoalcohols and Some of Their Disubstituted Carbamates as Potential Anticholinesterases

By VADLAMANI K. PRASAD and OLE GISVOLD

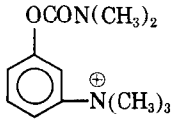
1,3- and 1,4-Dialkylaminocyclohexanols were made by the reduction of the corresponding phenols. The quaternary salts of the dialkylcarbamates of the above alcohols were prepared for pharmacological screening as anticholinesterases. Dialkylcarbamates of 2-pyridylcarbinol have been synthesized and subsequently quaternized. Also the synthesis of the methiodide of 3-dimethylamino-1-acetoxycyclohexene-1 has been described. These saturated analogs of some of the anticholinesterases were prepared to further delineate structure-activity relationships in this field.

THE EARLIEST reversible inhibitor of acetylcholinesterase, physostigmine or eserine, was isolated in an amorphous form by Jobst and Hesse (1). It was Engel-Hart *et al.* (2) who demonstrated that acetylcholine in tissues was inactivated by acetylcholinesterase and that physostigmine was highly specific in inhibiting acetylcoholinesterase. The rapid progress in the development of synthetic anticholinesterases followed the structural elucidation of physostigmine (3). Stedman *et al.* (3) reasoned that the pyrrolidine rings in physostigmine may not be essential for activity. They assumed that methylcarbamates of simple phenols might yield active compounds, and they subsequently demonstrated that type formula (I) produced active compounds.



I

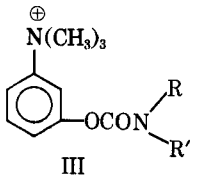
Stedman *et al.* (3) noted that the *meta* substituted quaternary compounds were more active than the corresponding tertiary compounds, whereas quaternization of the *ortho* and *para* analogs led to inactive congeners. Stedman and Stedman (4, 5) showed that the monosubstituted phenyl carbamates were unstable in aqueous solution. The disubstituted phenyl carbamates are quite stable and Aeschlimann and Reinert (6) prepared neostigmine (II) which was found to be a clinically useful competitive cholinesterase inhibitor.



II

Blaschko *et al.* (7) studied the influence of the structural variation of the quaternary head as well as various other modifications of neostigmine. Stepwise replacement of the methyl groups of neostigmine by ethyl groups led first to an increase in anticholinesterase activity, reaching a maximum until the diethyl-methyl derivative, and then decreased sharply in activity with the triethyl derivative.

Aeschlimann and Stempel (8) reported on the activities of a comprehensive series of disubstituted carbamate analogs (III) of neostigmine, where they modified the carbamate group and found that substitution of both the carbamate methyl groups by larger aliphatic or aromatic groups does not appear to be favorable for activity. Also they came to the conclusion that there may be a position of additional attachment of the described compounds on the receptor surface.

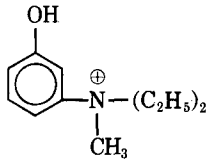


III

Bloch (9) showed that some activity was present when the carbamate group in II was replaced by an ester group, and he suggested that the carbamate grouping is not essential for activity. Subsequently, it was shown that in some cases the carbamate ester group is not necessary for anticholinesterase activity. Thus, McFarlane (10), Cohen and Unna (11), and other groups of workers found IV to be the most active compound as a cholinesterase inhibitor which is approximately

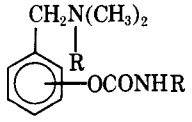
Received February 1, 1965, from the College of Pharmacy, University of Minnesota, Minneapolis.
Accepted for publication May 26, 1965.
Abstracted from a thesis submitted by Vadlamani K. Prasad to the Graduate School, University of Minnesota, Minneapolis, in partial fulfillment of Doctor of Philosophy degree requirements.

equal to neostigmine in activity. Increasing or decreasing the size of the quaternary head resulted in a decrease in anticholinesterase activity.



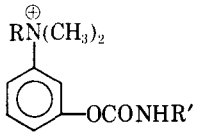
IV

Even though the above substituted aminophenols produced active compounds, Stedman demonstrated that the cationic head need not be directly connected to the aromatic ring. Thus Stedman made and tested (12) benzyl-substituted compounds (V).

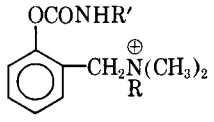


V

This series showed that methyl urethanes of the general structure V had the following order of activity: *ortho* > *para* > *meta*. The observation that the quaternary derivatives of the general structures VI and VII are the more active compounds in the two different series indicates that the increase in activity may be related to a formal charge and not to inductive effects of the quaternary nitrogen atom. Also the interatomic distances between the quaternary nitrogen and the carbonyl group in the derivatives of *meta* phenols are approximately the same as those found in VII.



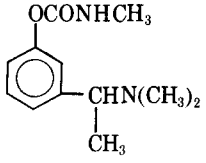
VI



VII

R = H or alkyl
R′ = alkyl

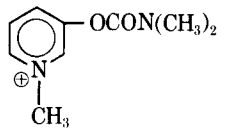
The next advance came with the synthesis (13, 14) of further benzyl-substituted compounds. This series led to the development of the first useful synthetic miotic agent, miotine (VIII).



VIII

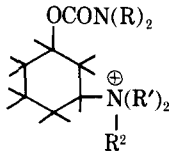
Wilson (15) showed that choline, as an inhibitor, is no more active than trimethylpropylammonium ion and therefore the hydroxyl group of choline does not bind to acetylcholinesterase. But Baldridge *et al.* (16) and Friess and Baldridge (17), comparing the dissociation constants of acetylcholinesterase complexes with choline, *cis*- and *trans* - 2 - (hydroxycyclohexyl) trimethylammonium and 2-(hydroxycyclopentyl) trimethylammonium, concluded that a hydroxyl function can contribute to binding on the enzyme surface. The latter studies are not comparable to those of Wilson and therefore furnish no basis for comparisons of activities.

Wuest and Sakal (18) reported on a large number of quaternized carbamic esters of 3-pyridol (1X) which exhibited anticholinesterase and parasympathomimetic activity. In the present study some

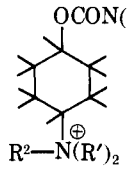


IX

of the saturated analogs (X) of neostigmine and the corresponding 1,4 isomers (XI) were synthesized, in which the groups R, R′, and R² were varied. Although neostigmine is used clinically, it has not been shown whether the aromatic ring system in the neostigmine type of compounds is essential for maximum anticholinesterase activity. As was mentioned earlier, Friess and his group (17) found that very powerful cholinergic compounds and also extremely active



X



XI

acetylcholinesterase inhibitors can result from nonaromatic systems. In both cases the *cis* isomers were more active than the *trans* isomers.

Substituted six-membered saturated ring systems usually exist in the most stable conformation *in vitro*. However, *in vivo*, this conformation may vary in order to be more complimentary to the receptor site to exert a biological effect. Thus, the derivatives of X and XI may be expected to exhibit anticholinesterase activity because each of these compounds can be converted to a more favorable conformation to give a better fit at the enzyme surface. Also, these compounds possess a cationic head which would be attracted to the anionic site of the enzyme by coulombic forces and the carbonyl carbon would be electrostatically attracted to the esteratic site of the cholinesterase enzyme surface. Thus, these compounds would comply with the dual requirement for maximum complimentariness, as was proposed by Wilson and Nachmansohn (19) at the enzyme surface to compete with the natural substrate acetylcholine.

DISCUSSION

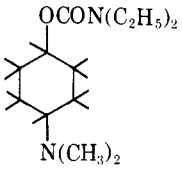
Compounds with the general structures X and XI were synthesized from the corresponding dialkylaminocyclohexanols. These alcohols were obtained by the reduction of *m*- and *p*-dialkylaminophenols. *m*-Diethylaminophenol[1] and *m*-dimethylaminophenol[2] were commercially available. *p*-Dimethyl-

[1] Aldrich Chemical Co.
[2] Eastman Organic Chemicals.

aminophenol was made by the methylation of the corresponding aminophenol with dimethyl sulfate using the procedure of Crossley (20).

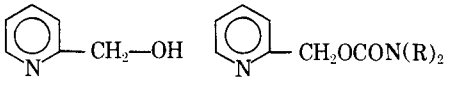
The *cis* isomer of 3-dimethylaminocyclohexanol was obtained by the reduction of *m*-dimethylaminophenol. To confirm that the catalytic hydrogenation of *m*-dimethylaminophenol gave the *cis*-3-dimethylaminocyclohexanol, the latter was stereoselectively synthesized by employing the procedure of Traynelis and Dadura (21). The *cis*-3-dimethylaminocyclohexanol obtained by either route was esterified with dimethylcarbamoyl chloride and subsequently quaternized with methyl iodide. The methiodides thus obtained had the same melting point and mixed melting point.

The hydrogenation of *p*-dimethylaminophenol gave a product that apparently was a mixture of the *cis*- and *trans*-4-dimethylaminocyclohexanol. However, two diethylcarbamates were obtained (XII); one was isolated as a liquid and the other as a semisolid (isomers A and B). These two gave different methobromides when reacted with methyl bromide. The stereochemistry of these was not determined.



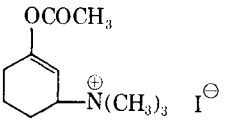
XII

Because pyridostigmine (VII) is a very active compound, the quaternary salts of the dialkylcarbamates (XIV) of 2-pyridylcarbinol (XIII) were prepared for biological screening. Compound XIII was commercially available. The dialkylcarbamates of the so far described dialkylaminoalcohols were prepared by a slight modification of Aeschlimann's procedure (22).



XIII XIV

Compound XV also was synthesized with the hope that this would exhibit parasympathomimetic activity. The attempted synthesis of its tertiary base has been reported (23). Compound XV was prepared from cyclohexanone *via* its enol acetate.[3] Allylic bromination, followed by displacement with iodine, gave the iodide which readily reacted with dimethylamine to give the expected tertiary amine. The latter was readily quaternized with methyl iodide.



XV

The quaternary derivatives of 3-dialkylaminocyclohexanols were prepared to test for anticurare activity since the corresponding aromatic derivatives were found to be extremely active. The pharmacological testing of the compounds synthesized may provide some evidence as to the necessity of the aromatic ring system in some of the most active reversible cholinesterase inhibitors.

EXPERIMENTAL

All melting points were taken with a Thomas-Hoover Unimelt apparatus. The melting points and the boiling points are uncorrected. The microanalysis was performed by the Micro-Analytical Laboratory, School of Chemistry, University of Minnesota, Minneapolis.

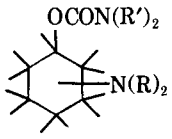
Dialkylaminocyclohexanols.—Commercially available dialkylaminophenols were first purified by standard techniques. The hydrogenation procedure is a slight modification of that of Billman and Buehler (24). The following procedure is representative of the modification. *m*-Dimethylaminophenol (80 Gm., 0.58 mole) was dissolved in 200 ml. of absolute ethanol. Raney nickel (25) (10 Gm.) was added, and the product was reduced with hydrogen at 1400 p.s.i. and bomb temperatures of 100–110°. The hydrogenation was continued until no more absorption of hydrogen occurred. The catalyst was filtered off and the ethanol removed in vacuum. The brown liquid was dissolved in ether and then washed with two 5-ml. portions of 2% sodium hydroxide and finally with 5 ml. of ice cold water. The ether extract was dried with Drierite, the solvent removed, and the residue then distilled under vacuum. The product boiling at 74° was collected. The above procedure was employed to prepare 1,3-diethylaminocyclohexanol and 1,4-dimethylaminocyclohexanol. The physical constants of these have been reported (26).

Dialkylcarbamates of Dialkylaminocyclohexanols.—In a typical operation, a mixture of 1.0 mole of the dialkylaminocyclohexanol and 1.5 moles of the dialkylcarbamoyl chloride[4] was refluxed in an oil bath at 110–120°. After 4 hr. of refluxing, the contents of the flask were cooled and the yellow oil washed several times with anhydrous ether or ethyl acetate. A portion of the hydrochloride of the ester was recrystallized from a mixture of ethanol and ethyl acetate to obtain an analytical sample (Table I). The other portion of the crude hydrochloride was dissolved in ice cold water and basified with a saturated solution of potassium carbonate. The separated oil was extracted with ether; the ether extract was washed once with 5

[3] In their investigations on the free radical bromination of enol acetates employing *N*-bromosuccinimide, Mousseron and Jacquier [*Bull. Soc. Chim.*, **1951**, 106] have reported on the preparation of 3-acetoxycyclohex-2-enyl bromide from cyclohex-1-enyl acetate. It is evident from their findings that the allylic position attacked by the bromine radical is the third allylic position and not the first allylic position of cyclohex-1-enyl acetate. The structural evidence for 3-acetoxycyclohex-2-enyl bromide was given by reacting the bromo compound with the sodium salt of diethyl malonate. The resulting cyclohexanone-3-malonic acid was not isolated in a pure form but was decarboxylated directly to cyclohexanone-3-acetic acid. The experimental details of the proof of structure are given in the above reference. The authors have employed the same experimental conditions as those of Mousseron and Jacquier in the preparation of the desired 3-acetoxycyclohex-2-enyl bromide, b.p. 56°/0.8 mm. Mousseron and Jacquier reported the b.p. 115–118°/18 mm. The experimental conditions employed by Machinskaya and Barkash [*Zhur. Obschei Khim.*, **26**, 848(1956); and *Chem. Abstr.*, **50**, 14588 (1956)] could possibly have led to the formation of 3-bromo-2-acetoxycyclohexene, wherein the bromine radical might have attacked the first allylic position and not the third allylic position of cyclohex-1-enyl acetate.

[4] K and K Chemical Co.

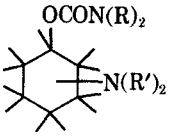
TABLE I.—HYDROCHLORIDES OF 3- AND 4-DIALKYLCARBAMYLOXY-*N,N*-DIALKYLCYCLOHEXYLAMINE



Substituent on Position	R	R'	M.p., ° C.	Formula	Anal., % Calcd.	Found
3[a]	$-CH_3$	$-C_2H_5$	136–150	$C_{13}H_{27}ClN_2O_2$	C, 56.04 H, 9.71	C, 56.32 H, 9.79
3[a]	$-C_2H_5$	$-C_2H_5$	138–139	$C_{15}H_{31}ClN_2O_2$	C, 58.75 H, 10.12	C, 58.99 H, 10.12
3[a]	$-C_2H_5$	$-CH_3$	186–187	$C_{13}H_{27}ClN_2O_2$	C, 56.04 H, 9.70	C, 56.07 H, 9.74
4	$-CH_3$	$-CH_3$	194–197	$C_{11}H_{23}ClN_2O_2$	C, 52.80 H, 9.18	C, 52.58 H, 9.02
4	$-CH_3$	$-C_2H_5$	228–229	$C_{13}H_{27}ClN_2O_2$	C, 56.02 H, 9.68	C, 55.37 H, 9.67

[a] Hygroscopic crystals. Crystallized from a mixture of ethanol and ether.

TABLE II.—DIALKYLCARBAMATES OF DIALKYLAMINOCYCLOHEXANOLS



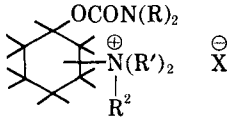
Substituent on Position	R	R'	B. p.
3	$-C_2H_5$	$-C_2H_5$	116° (0.4 mm.)
3	$-CH_3$	$-CH_3$	98° (0.5 mm.)
3	$-CH_3$	$-C_2H_5$	105–106° (0.4 mm.)
4	$-CH_3$	$-CH_3$	108–109° (0.9 mm.)
4	$-CH_3$	$-C_2H_5$	132–133° (2.0 mm.)

ml. of a saturated solution of sodium sulfate. The ether extract then was dried with anhydrous sodium sulfate, the solvent removed, and the liquid distilled in vacuum (Table II).

Quaternary Salts.—The quaternary salts of the dialkylaminocyclohexanols and their dialkylcarbamates in general were prepared by adding a solution of the ester in anhydrous ether to an excess of the appropriate alkyl halide in dry ether. In some cases the quaternary salts precipitated out within a few hours, but in certain instances the salt was obtained only after refluxing the mixture for 2 to 4 hr. (Tables III and VI).

Dialkylcarbamates of 2-Pyridylcarbinol.—These were made essentially by the same procedure as described above, except that the mixture of the alcohol and dialkylcarbamoylchloride had to be refluxed on a water bath for 2 hr. The dialkylcarbamate hydrochlorides were extremely hygroscopic and could not be isolated. The isolation of the basic ester is as described earlier, but the solvent used in the extraction procedure was a mixture of ether and chloroform (1:2) (Table IV). The quaternary salts in this series were made as described earlier (Table V).

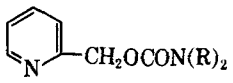
TABLE III.—QUATERNARY DERIVATIVES OF 3- AND 4-(DIALKYLCARBAMYLOXY)-*N,N*-DIALKYLCYCLOHEXYLAMINE[a]



Substituent on Position	R	R'	R^2	X	M.p., °C.	Formula	Anal., % Calcd.	Found
3	$-CH_3$	$-CH_3$	$-CH_3$	I	168	$C_{12}H_{25}IN_2O_2$	C, 40.44 H, 7.02	C, 40.30 H, 7.21
3	$-C_2H_5$	$-CH_3$	$-CH_3$	Br	183–186	$C_{14}H_{29}BrO_2N_2$	C, 49.85 H, 8.61	C, 49,80 H, 8.70
3	$-CH_3$	$-CH_3$	$-C_2H_5$	I	178–179	$C_{13}H_{27}IN_2O_2$	C, 42.16 H, 7.30	C, 41.99 H, 7.68
3	$-C_2H_5$	$-CH_3$	$-CH_3$	I	113–115	$C_{16}H_{33}IN_2O_2$	C, 46.60 H, 8.00	C, 46.52 H, 8.13
4	$-CH_3$	$-CH_3$	$-CH_3$	Br	231–232	$C_{12}H_{25}BrN_2O_2$	C, 46.60 H, 8.07	C, 46.26 H, 8.07
4 (Isomer A)	$-C_2H_5$	$-CH_3$	$-CH_3$	Br	243–244	$C_{14}H_{29}BrN_2O_2$	C, 49.85 H, 8.60	C, 49.81 H, 8.50
(Isomer B)	$-C_2H_5$	$-CH_3$	$-CH_3$	Br	159–161	$C_{14}H_{29}BrN_2O_2$	C, 49.85 H, 8.60	C, 49.59 H, 8.35

[a] Solvent used for crystallizations, a mixture of ethanol and ethyl acetate. The 1,3-disubstituted quaternary derivatives in which (*a*) R,R', and R^2 is ethyl, (*b*) R is methyl and R', R^2 is ethyl, and (*c*) R and R^2 is methyl and R' is ethyl could not be obtained in crystalline form.

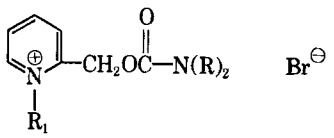
TABLE IV.—2-PYRIDYLMETHYL CARBAMATES



R	B.p.
$-CH_3$	105° (0.8 mm.)
$-C_2H_5$	117–118° (1.2 mm.)

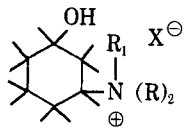
Methiodide of 3-Dimethylamino-1-acetoxycyclohexene-1.—1-Acetoxy-1-cyclohexene was prepared according to Bedoukian (27). This was brominated with *N*-bromosuccinimide using the procedure of Mousseron and Jacquier (23). The bromo compound (6.0 Gm., 0.028 mole) was refluxed on a water bath with a solution of 4.2 Gm. (0.028 mole) of sodium iodide in 50 ml. of dry acetone. After refluxing for 2 hr., the reaction mixture was cooled in an ice bath and the precipitated sodium bromide filtered, and the acetone removed under vacuum. Without further purification, the iodo compound was taken up in anhydrous ether and to this was added an excess of dimethylamine in anhydrous ether. The precipitate obtained was filtered off, and the ether from the filtrate was removed under vacuum, and the remaining brown liquid was taken up in ice cold water. This was basified with a solution of potassium carbonate, and the separated oil was extracted with ether. The ether extract was first dried with anhydrous sodium sulfate and then with calcium hydride. To this ether solution was added a cold ether solution of methyl iodide. The methiodide that formed was recrystallized

TABLE V.—QUATERNARY HALIDES OF 2-PYRIDYLMETHYL(*N,N*-DIALKYL)CARBAMATES



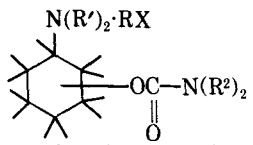
R	R_1	M.p., ° C.	Formula	Anal., % Calcd.	Found
$-CH_3$	$-CH_3$	177 dec.	$C_{10}H_{15}BrN_2O_2$	C, 43.64 H, 5.46	C, 43.76 H, 5.33
$-CH_3$	$-CH_2C_6H_5$	168–170	$C_{16}H_{19}BrN_2O_2$	C, 54.70 H, 5.42	C, 54.98 H, 5.70
$-C_2H_5$	$-CH_2C_6H_5$	131–133	$C_{18}H_{23}BrN_2O_2$	C, 57.01 H, 6.07	C, 57.35 H, 6.29
$-C_2H_5$	$-CH_3$	127°	$C_{12}H_{19}BrN_2O_2$	C, 47.53 H, 6.27	C, 47.16 H, 6.21

TABLE VI.—3-HYDROXYCYCLOHEXYLTRIALKYLAMMONIUM HALIDES



R	R_1	X	M.p., ° C.	Formula	Anal., % Calcd.	Found
$-CH_3$	$-CH_3$	I	229–232	$C_9H_{20}ONI$	C, 37.89 H, 7.01	C, 38.02 H, 7.00
$-C_2H_5$	$-CH_3$	I	149–151	$C_{11}H_{24}ONI$	C, 42.18 H, 7.65	C, 42.21 H, 7.55
$-CH_3$	$-C_2H_5$	Br	193–195	$C_{10}H_{22}ONBr$	C, 47.62 H, 8.73	C, 47.34 H, 8.69

TABLE VII.—PHARMACOLOGICAL TESTING



Compd.	Substituted on Position	RX	R′	R^2	Concn., *M*	% Inhibition
1	3	CH_3Br	$-CH_3$	$-C_2H_5$	1.4×10^{-3}	37
2	3	CH_3I	$-C_2H_5$	$-C_2H_5$	9×10^{-4}	38
3	3	C_2H_5I	$-CH_3$	$-CH_3$	9×10^{-4}	14
4	3	CH_3I	$-CH_3$	$-CH_3$	9×10^{-4}	24
5[a]	4	CH_3Br	$-CH_3$	$-C_2H_5$	9×10^{-4}	25
6[a]	4	CH_3Br	$-CH_3$	$-C_2H_5$	9×10^{-4}	55
7	4	CH_3Br	$-CH_3$	$-CH_3$	9×10^{-4}	22

[a] Compounds *5* and *6* are isomers A and B previously described.

from a mixture of ethanol and ether. The final product was obtained as a monohydrate, m.p. 158–160°.

Anal.—Calcd. for $C_{11}H_{22}NIO_3$: C, 38.5; H, 6.41. Found: C, 38.26; H, 6.40.

Pharmacological Testing[5]

Some of the carbamates were screened as follows for cholinesterase inhibitory activity. An 0.1-ml. quantity of inhibitor solution was added to 1.0 ml. of 0.003 *M* acetylcholine bromide in 0.1 *M* NaCl, 0.02 *M* $MgCl_2$, 0.02 *M* phosphate buffer pH 7.0 at 25°. The reaction was started by adding 10 μl. of enzyme solution (electric eel acetylcholinesterase) and stopped 2 min. later with alkaline hydroxylamine. The remaining ester was estimated by the hydroxamic acid formed. The results are as shown in Table VII. Compound *6* exhibited the maximum inhibitory activity.

To see whether carbamylation occurs, 0.1 ml. of 6×10^{-4} *M* inhibitor *6* and 10 μl. of enzyme solution were incubated for 2, 4, and 14 min. before enzyme assay with 1 ml. of solution containing acetylcholine. The inhibition was about 7% in all cases. This value is consistent with reversible inhibition. If it is assumed that the diethyl carbamyl enzyme does not hydrolyze within a few minutes, the above results indicate that little or no carbamylation occurred within 15 min.

[5] The authors are grateful to Professor I. B. Wilson, College of Physicians and Surgeons, Columbia University, New York, N. Y., for the pharmacological screening of some of the compounds.

REFERENCES

(1) Jobst, J., and Hesse, O., *Ann.*, **129**, 115(1869).
(2) Engel-Hart, E., and Loewi, U. O., *Arch. Exptl. Pathol. Pharmacol.*, **150**, 1(1930).
(3) Stedman, E., and Barger, J., *J. Chem. Soc.*, **127**, 247(1925).
(4) Stedman, E., and Stedman, E., *Biochem. J.*, **25**, 1147(1931).
(5) *Ibid.*, **26**, 1214(1932).
(6) Aeschlimann, J. A., and Reinert, M., *J. Pharmacol. Exptl. Therap.*, **43**, 413(1931).
(7) Blaschko, H. E., *et al.*, *ibid.*, **4**, 29(1949).
(8) Aeschlimann, J. A., and Stempel, A., *Fetschr. Emil Barell*, **1946**, 306.
(9) Bloch, H., *Arch. Exptl. Pathol. Pharmacol.*, **193**, 292(1939).
(10) McFarlane, D. W., *et al.*, *J. Pharmacol. Exptl. Therap.*, **100**, 382(1950).
(11) Cohen, H. L., and Unna, K. R., *ibid.*, **103**, 340(1951).
(12) Stedman, E., *Biochem. J.*, **23**, 17(1929).
(13) White, A. C., and Stedman, E., *J. Pharmacol. Exptl. Therap.*, **41**, 259(1931).
(14) Easson, L. H., and Stedman, E., *Biochem. J.*, **27**, 1257(1933).
(15) Wilson, I. B., *J. Biol. Chem.*, **197**, 215(1952).
(16) Baldridge, H. D., Jr., *et al.*, *J. Am. Chem. Soc.*, **77**, 739(1955).
(17) Friess, S. L., and Baldridge, H. D., *ibid.*, **78**, 2482 (1956).
(18) Wuest, H. M., and Sakal, E. H., *ibid.*, **73**, 1210(1951).
(19) Wilson, I. B., and Nachmansohn, D., *Advan. Enzymol.*, **12**, 259(1951).
(20) Crossley, M. L., *J. Am. Chem. Soc.*, **74**, 573(1952).
(21) Traynelis, V. J., and Dadura, J. G., *J. Org. Chem.*, **26**, 1813(1961).
(22) Aeschlimann, J., U. S. pat. 2,449,440 (1948); through *Chem. Abstr.*, **43**, 1439(1949).
(23) Mousseron, M., *et al.*, *Bull. Soc. Chim. France*, **1954**, 1246; *ibid.*, **1951**, 106.
(24) Billman, J. H., and Buehler, J. A., *J. Am. Chem. Soc.*, **75**, 1345(1953).
(25) Mozingo, R., "Organic Synthesis" coll. vol. 3, John Wiley & Sons, Inc., New York, N. Y., 1955, p. 181.
(26) Heckel, H., and Adams, R., *J. Am. Chem. Soc.*, **47**, 1713(1925).
(27) Bedoukian, P. Z., *ibid.*, **67**, 1430(1945).

Photobinding and Photoreactivity of Riboflavin in the Presence of Macromolecules

By H. B. KOSTENBAUDER, P. P. DELUCA*, and C. R. KOWARSKI

The rate of aerobic photobleaching of riboflavin by visible light is considerably enhanced by the presence of macromolecules such as PVP, polysorbate 80, and sodium decyl sulfate. The catalysis by the macromolecules is attributed in part to a reversible binding of excited riboflavin molecules to macromolecules to produce longer-lived excited species. Methods are described which permit direct determination of enhanced binding of riboflavin to macromolecules during irradiation. Evidence is presented to indicate involvement of a triplet state in both the photobinding and the photodecomposition.

IN THE course of an investigation of binding of dyes to macromolecules in aqueous solution, the macromolecules were observed to have considerable influence on the light stability of the dyes. Although Oster and co-workers (1–6) had previously reported an enhanced rate of photofading for certain dyes in the presence of polymers such as polyacrylic acid, polymethacrylic acid and polyvinylpyrrolidone, and Scott *et al.* (7) reported that the color loss of some FDA certified dyes in aqueous solution at elevated temperatures was accelerated in the presence of nonionic surfactants, the nature of the observed catalysis was not obvious.

The enhanced photosensitivity of dyes in the presence of macromolecules is of considerable

Received April 7, 1965, from the School of Pharmacy, Temple University, Philadelphia, Pa.
Accepted for publication June 28, 1965.
Presented in part to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.
This investigation was supported by research grant RG-9957 from the Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
Preliminary report: *Nature*, **199**, 999(1963).
* Walter G. Karr Fellow, Smith Kline & French Laboratories, Philadelphia, Pa., 1959–1962. Present address: Ciba Pharmaceutical Products, Inc., Summit, N. J.