TABLE I

CONDUCTIVITY OF IRRADIATED LIQUID AMMONIA

Volume NH_3, 8.3 ml.

Exposure, sec.	Dark time, sec.	Target current, μa.	Roentgens[a] × 10^{-7}	Sp. resist × 10^6	r./μa.-sec. × 10^{-6}
Sample 3, temperature −74°; 2 Mev. cathode rays					
0	..	..		1.50	
65	..	3.4	1.8	1.50[b]	0.68
...	30	..	...	1.50	..
...	65	..	...	1.50	..
30	..	10.0	2.4	...	.80
150	..	3.5	4.3	1.45[b]	.82
120	..	3.6	3.5	...	.81

Total exposure time, min.	Current, μa.	Roentgens total	Sp. resist × 10^3
Sample 4, temperature −75° to −72°; 2 Mev. X-rays			
0	...	0	510
2	100	6 × 10^3	490[b]
5	100	1.5 × 10^4	450[b]
Off 5	...	.....	450
6	100	1.8 × 10^4	460[b]
8	100	2.5 × 10^4	...
8'20"	...	.....	460
Off 4	...	.....	460

[a] Dose calculated assuming only one-half ammonia irradiated. [b] Measurement made during irradiation.

tion of about 3 × 10^{-9} mole per liter of alkali metal or free electron in liquid ammonia could have been detected. On the basis of the data, no evidence was obtained for the formation of stabilized free electrons during the irradiation of liquid ammonia under the experimental conditions used.

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CHEMISTRY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, LONG ISLAND, N. Y.

The Effect of Esterification on Anticholinesterases as Determined by Three Different Enzymes

BY HENRY TAUBER AND EDWARD L. PETIT

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The preparation of 50 phosphonic and phosphinic acids has been described recently from our laboratory.[1] These compounds were examined for their anti-plasma cholinesterase activity.[2] Several of the compounds were found to be quite active. A few of the acids were esterified. Most of the esters were much more active against human plasma cholinesterase than the free acids. It is desirable for the development of insecticides to examine the action of anticholinesterases on enzymes of different species. In the present experiments we subjected our most active compounds to a comparative study using three different enzymes, human plasma cholinesterase, pig brain acetylcholinesterase and fly brain acetylcholinesterase. The effect of the esters on the cholinesterase activity of the three different soluble enzyme preparations has also been tested.

Methods and Materials.—The human plasma cholinesterase was the same as in our previous work.[2] The method for the preparation of soluble pig brain acetylcholinesterase has been described recently.[3] A similar procedure was employed for the preparation of acetylcholinesterase from the heads of the house fly (*Musca domestica* L.). An activator buffer–salt solution[3] was used in conjunction with the pig brain and fly brain acetylcholinesterase but not with the human plasma cholinesterase. Details concerning the enzyme inhibitor experiments have been described previously.[2,3] Residual acetylcholine was analyzed by Hestrin's[4] method using Klett–Summerson photoelectric colorimeter.

Inhibition of Three Different Cholinesterases.—It may be seen in Table I that our most active compounds are all

TABLE I

THE EFFECT OF ESTERIFICATION ON ANTICHOLINESTERASES AS MEASURED BY THREE DIFFERENT ENZYMES

Compound	I_{50},[a] moles/l. Plasma ChE	Brain AChE	Fly AChE
(*o*-BrC_6H_4)$C_6H_5PO_2H$	6 × 10^{-6}	7 × 10^{-3}	>5 × 10^{-3}
(*o*-BrC_6H_4)$C_6H_5PO_2CH(CH_3)_2$	1 × 10^{-6}	2.5 × 10^{-4}	2.5 × 10^{-6}
(*o*-BrC_6H_4)$C_6H_5PO_2C_2H_5$	1 × 10^{-5}	2 × 10^{-4}	5 × 10^{-5}
(*o*-BrC_6H_4)$C_6H_5PO_2CH_3$	8 × 10^{-5}	3.1 × 10^{-3}	>1 × 10^{-3}
(*o*-BrC_6H_4)$_2PO_2H$	1 × 10^{-4}	5 × 10^{-3}	>5 × 10^{-3}
(*o*-BrC_6H_4)$_2PO_2C_2H_5$	3 × 10^{-5}	2.5 × 10^{-5}	2 × 10^{-5}
o-$BrC_6H_4PO_3H_2$	4 × 10^{-3}	>5 × 10^{-3}	>5 × 10^{-3}
o-$BrC_6H_4PO(OC_2H_5)_2$	1 × 10^{-5}	1.25 × 10^{-3}	1 × 10^{-3}

[a] The I_{50} values (concentrations required for 50% inhibition) in this table were obtained from graphs in which % inhibition was plotted against the logarithm of the molar concentration of the compounds.

ortho-halogen derivatives. The *m*-halogen derivatives were less active, while the *p*-substituted compounds had no activity. The meta and para compounds are not included in Table I. It may be seen that esterification considerably increased the inhibitory power of the free acids in most instances. The isopropyl ester of (*o*-bromophenyl)-phenylphosphinic acid was more inhibitory than its ethyl ester and methyl ester. Concerning the plasma enzyme the ethyl ester of bis-(*o*-bromophenyl)-phosphinic acid was about 33 times more inhibitory than the free acid and the ethyl ester of *o*-bromobenzenephosphonic acid was 400 times more active than the free acid. When the pig brain enzyme was employed the ethyl ester of bis-(*o*-bromophenyl)-phosphinic acid was 200 times more inhibitory than the free acid and when the fly brain enzyme was tested the ester was at least 250 times more active than the free acid.

Among the 3 enzymes human plasma cholinesterase is much more readily inhibited by all compounds with the exception of ethyl ester of (*o*-bromophenyl)-phenylphosphinic acid, than the pig brain and fly brain acetylcholinesterase. This is not surprising since the plasma cholinesterase and the two brain enzymes belong to 2 different groups of enzymes.

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(1) G. O. Doak and L. D. Freedman, THIS JOURNAL, **73**, 5658 (1951); **74**, 753 (1952); **74**, 2884 (1952); **75**, 683 (1953).

(2) L. D. Freedman, H. Tauber, G. O. Doak and H. J. Magnuson, *ibid.*, in press.

(3) H. Tauber, *ibid.*, **75**, 326 (1953).

(4) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

VENEREAL DISEASE EXPERIMENTAL LABORATORY
U. S. PUBLIC HEALTH SERVICE
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, NORTH CAROLINA

Preparation of a Cyclopentenone by the Stobbe Condensation

BY D. L. TURNER

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The Stobbe condensation with two δ-keto-esters has been shown to give substituted cyclohexen-