hydrochloride. The mixture was cooled to room temperature; it was then refrigerated until the product precipitated. In most cases a semisolid or oily residue appeared only after the addition of ether. Crystallization was facilitated when the residues were separated and further treated with ether and the sides of the vessel were scratched. The solid thus obtained was removed by filtration and recrystallized to analytical purity from ethanol.

The reactions proceeded with some facility; however, the yields were relatively low for most of the products obtained. Low yields may be attributed to the complexity of the products which arise in the Mannich reaction from the type of by-product formation mentioned earlier. An attempt was made to minimize this by-product formation as described above.

REFERENCES

(1) Mannich, C., and Kresche, W., *Arch. Pharm.*, **250**, 647(1912); through *Chem. Abstr.*, **7**, 2746(1913).
(2) Mannich, C., and Lammering, D., *Ber.*, **55**, 3510 (1922).
(3) Blicke, F. F., and Blake, E. S., *J. Am. Chem. Soc.*, **52**, 235(1930).
(4) Nisbet, H. B., and Gray, C. G., *J. Chem. Soc.*, **1933**, 839.
(5) Levvy, G. A., and Nisbet, H. B., *ibid.*, **1938**, 1053.
(6) Wilson, W., and Kyi, Z. Y., *ibid.*, **1952**, 1321.
(7) Denton, J. J., *et al.*, *J. Am. Chem. Soc.*, **71**, 2048, 2050 2053, 2054(1949); **72**, 3279, 3792(1950).
(8) Weijlard, J., *et al.*, *ibid.*, **78**, 2342(1956).
(9) Mercier, F., Mercier, J., and Sectier, M. R. *J. Physiol. Paris*, **45**, 186(1953); through *Chem. Abstr.*, **47**, 11527(1953).
(10) Issekutz, B., *et al.*, *Acta Physiol. Acad. Sci. Hung.*, **6**, 95(1954); through *Chem. Abstr.*, **49**, 3394(1955).
(11) DaRe, R., *et al.*, *J. Org. Chem.*, **25**, 1097(1960).
(12) Bockstahler, E. R., and Wright, D. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 542(1957).
(13) Florestano, H. J., and Bahler, M. E., *ibid.*, **45**, 320 (1956).
(14) Profit, E., *Chem. Tech. Berlin*, **3**, 210(1951); through *Chem. Abstr.*, **46**, 688(1952).
(15) Profit, E., *Chem. Tech. Berlin*, **4**, 241(1952); through *Chem. Abstr.*, **47**, 10532(1953).
(16) Profit, E., *Chem. Tech. Berlin*, **5**, 13(1953); through *Chem. Abstr.*, **48**, 7608(1954).
(17) Fry, E. M., and Everette, L. M., *J. Org. Chem.*, **24**, 116(1959).
(18) Fuson, R. C., *Chem. Rev.*, **16**, 1(1935).
(19) Burckhalter, J. H., and Johnson, S. H., *J. Am. Chem. Soc.*, **73**, 4835(1951).
(20) Levvy, G. A., and Nisbet, H. B., *J. Pharmacol. Exptl. Therap.*, **65**, 129(1939); through *Chem. Abstr.*, **33**, 2985(1939).
(21) Burckhalter, J. H., *et al.*, *J. Am. Chem. Soc.*, **68**, 1894(1946); **70**, 1363(1948).
(22) Hayes, K., *Chem. Abstr.*, **48**, 12809(1954); U. S. pat. 2,663,710.
(23) Nobles, W. L., Britton, S. B., and Caldwell, H. C., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 641, 644(1954); Nobles, W. L., and Caldwell, H. C., *ibid.*, **44**, 273(1955); Nobles, W. L., and Britton, S. B., *ibid.*, **44**, 717(1955); Nobles, W. L., and Burckhalter, J. H., *ibid.*, **47**, 77(1958).
(24) Nobles, W. L., and Blanton, C. D., *J. Pharm. Sci.*, **51**, 878(1962); Nobles, W. L., and Luts, H. A., *ibid.*, **51**, 273(1962); **54**, 67(1965).
(25) Nobles, W. L., and Blanton, C. D., *ibid.*, **52**, 46(1963); **53**, 521(1964); **53**, 1130(1964); Nobles, W. L., and Thompson, B. B., *ibid.*, **53**, 1554(1964); **54**, 576(1965); **54**, 709(1965).
(26) Blicke, F. F., "Organic Reactions," vol. 1, John Wiley & Sons, New York, N. Y., 1942, p. 303.
(27) Karbe, H., *Arch. Pharm.*, **283**, 48(1950).
(28) Merz, K. W., *Pharmazie*, **11**, 505(1956).
(29) Reichert, B., "Die Mannich-Reaktion," Springer Verlag, Berlin, Germany, 1959.
(30) Hellmann, H., and Opitz, G., "α-Aminoalkylierung," Verlag Chemie, Weinheim, Germany, 1960.
(31) Nobles, W. L., *Pharm. Sci. 4th Ann. Visiting Lecture Ser. Coll. Pharm., Univ. Texas*, Austin, 1961, p. 149; through *Chem. Abstr.*, **58**, 409(1963).
(32) Ogata, Y., and Kawasaki, A., *Yuki Gosei Kagaku Kyakai Shi*, **21**, 79(1963); through *Chem. Abstr.*, **59**, 2625 (1963).
(33) Gilman, H., and Pickens, R. M., *J. Am. Chem. Soc.*, **47**, 245(1925).
(34) Biel, J. H., *ibid.*, **71**, 1306(1949).
(35) LaBarre, J., *et al.*, *Arch. Intern. Pharmacodyn.*, **147**, (No. 3–4) 497(1964); through *Chem. Abstr.*, **60**, 9793(1964).
(36) Clarke, R. L., and Harris, L. S., *J. Med. Pharm. Chem.*, **5**, 77(1962).
(37) *Ibid.*, **5**, 362(1962).
(38) Harris, L. S., Clarke, R. L., and Dembinski, J. R., *Arch. Intern. Pharmacodyn.*, **146**, (No. 3–4) 392(1963); through *Chem. Abstr.*, **60**, 9793(1964).
(39) Mannich, C., and Schütz, M., *Arch. Pharm.*, **265**, 684(1927).
(40) Ruberg, L., and Small, L., *J. Am. Chem. Soc.*, **63**, 736(1941).

Antagonism of Uridine Diphosphate with 3-Methylisoquinoline Compounds

By THOMAS J. HALEY and H. DIX CHRISTENSEN

The ability of four 3-methylisoquinoline compounds to antagonize the spasmogenic effect of uridine diphosphate on goldfish intestine was determined. While there was no significant difference in potency, one compound, 6-ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methyl-isoquinoline, had a lesser effect on acetylcholine contractions and thus may be useful in the differential analysis of tissue extracts containing both uridine diphosphate and acetylcholine.

GADDUM and Szerb (1) showed that the goldfish intestine could be used to estimate the substance P content of tissue extracts. Haley *et al.* (2) adapted the procedure for the determination of acetylcholine. However, both groups observed that tissue extracts contained a substance which stimulated the intestine even in the presence of dichloroisoprenaline, hyoscine, mepyramine, and methysergide. Gaddum and Smith (3) and Gaddum (4) showed that this unknown substance was actually uridine diphosphate. Levy and Michel-Ber (5) pointed out that the spasmogenic effect of uridine triphosphate could be blocked by papaverine. Inasmuch as other naturally occurring substances are blocked by antagonists added to the bathing solution and because such procedures are often better than laborious separations, the authors have studied the antagonism of uridine diphosphate with four synthetic 3-methylisoquinoline derivatives (6).

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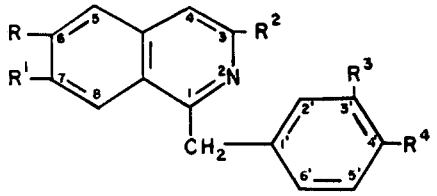
TABLE I.—PAPAVERINE DERIVATIVES EVALUATED



Compd. (Lilly)	R	R′	R^2	R^3	R^4
Dioxyline phosphate	CH_3O	CH_3O	CH_3	CH_3O	C_2H_5O
6,7-Dimethoxy-1-(3′-ethoxy-4′-methoxybenzyl)isoquinoline	CH_3O	CH_3O	H_2	C_2H_5O	CH_3O
6-Ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methylisoquinoline	C_2H_5O	CH_3O	CH_3	C_2H_5O	C_2H_5O
6-Methoxy-7-ethoxy-1-(3′-ethoxy-4′-methoxybenzyl)-3-methylisoquinoline	CH_3O	C_2H_5O	CH_3	C_2H_5O	CH_3O

TABLE II.—ANTISPASMODIC EFFECTS OF 3-METHYLISOQUINOLINE COMPOUNDS

Compd. (Lilly)	ED_{50} and Range, mcg./0.05 ml.[a]	Slope and Range	100% Block mcg./ 0.05 ml.	% Reduction ACh Contraction
Dioxyline phosphate	1.33 (0.89–1.99)	3.42 (1.58–7.42)	4.5	22
6,7-Dimethoxy-1-(3′-ethoxy-4′-methoxybenzyl)isoquinoline	2.10 (1.39–3.16)	1.97 (1.14–3.45)	7.0	36
6-Ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methylisoquinoline	1.15 (0.77–1.69)	3.18 (1.70–5.95)	3.5	16
6-Methoxy-7-ethoxy-1-(3′-ethoxy-4′-methoxybenzyl)-3-methylisoquinoline	1.30 (0.74–2.28)	5.10 (1.02–25.5)	5.0	23

[a] $p = 0.05$.

METHODS

The microbath and goldfish intestinal preparation described by Gaddum and Szerb (1) was used to study the antagonism of uridine diphosphate by the 3-methylisoquinoline derivatives listed in Table I. The intestinal contractions were recorded with a Sanborn linear differential transformer model FTA-1-1 and a Sanborn strain gauge amplifier model 140. The ranges of drug concentrations per 0.05 ml. were: acetylcholine, 4 ng; uridine diphosphate, 250 ng; dioxyline 1 to 20 mcg.; 6-methoxy-7-ethoxy-1-(3′-ethoxy-4′-methoxybenzyl)-3-methylisoquinoline, 4 to 20 mcg.; 6-ethoxy-7-methoxy-1- 3′,4′-diethoxybenzyl)-3-methylisoquinoline, 3 to 10 mcg.; and 6,7-dimethoxy-1-(3′-ethoxy-4′-methoxybenzyl)-isoquinoline, 3 to 20 mcg. All drug additions to the bath were made with a Kensco 50 µl. micropipet. The results obtained were analyzed statistically by the Litchfield-Wilcoxon method (7).

RESULTS AND DISCUSSION

All four of the 3-methylisoquinoline compounds are capable of blocking both uridine diphosphate and acetylcholine but to varying degrees (Table II). Calculation of the potency ratios based upon the ED_{50} values revealed that there was no significant difference in the potency of any of the compounds. However, it takes less of 6-ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methylisoquinoline to produce 100% blockade of uridine diphosphate while affecting the acetylcholine response the least. This latter aspect is of greatest importance because it now becomes possible to utilize 6-ethoxy-7-methoxy-1-(3′,4′-diethoxybenzyl)-3-methylisoquinoline in the bathing solution and thus determine acetylcholine in tissue extracts containing uridine diphosphate. Little can be said concerning structure activity relationships of these compounds because the structural changes are minimal, there were too few compounds evaluated, and the ED_{50} values showed no significant potency differences. One point which must be emphasized, however, is the possible effect of these compounds on other components of tissue extracts. Jaques (8) pointed out that papaverine at a concentration of 1×10^{-6}, could antagonize the spasmogenic effects of substance P on the guinea pig ileum. The authors do not know what our compounds would do under such circumstances, because substance P was not available for testing.

REFERENCES

(1) Gaddum, J. H., and Szerb, J. C., *Brit. J. Pharmacol.*, **17**, 451(1961).
(2) Haley, T. J., Colvin, G., and Efros, M., *J. Pharm. Sci.*, **53**, 1530(1964).
(3) Gaddum, J. H., and Smith, M. W., *Proc. Roy. Soc. London, Ser. B.*, **157**, 492(1963).
(4) Gaddum, J. H., *Brit. J. Pharmacol.*, **23**, 613(1965).
(5) Levy, J., and Michel-Ber, E., *Compt. Rend. Acad. Sci.*, **248**, 2416(1959).
(6) Henderson, F. G., Shipley, R. E., and Chen, K. K., *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 207(1951).
(7) Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharmacol. Expil. Therap.*, **96**, 99(1949).
(8) Jaques, R., *Arch. Expil. Pathol. Pharmacol.*, **245**, 275 (1963).