SCIENTIFIC SECTION

THE PHARMACOLOGICAL ACTION OF TEN AMINES RELATED TO EPHEDRINE AND TRYPTAMINE.*

BY K. K. CHEN AND A. LING CHEN.

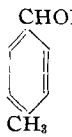
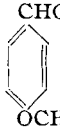
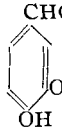
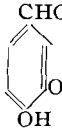
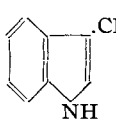
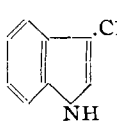
In 1929 one of us (K. K. C.) in association with Wu and Henriksen (1) reported results on a series of 27 amines related to ephedrine and drew certain conclusions regarding the relationship between pharmacological action and chemical constitution. Since then 10 additional amines have been made available to us, and a similar investigation has been carried out with them on animals. As shown in Table I, the first 4 substances are ephedrine derivatives and the next 5 contain an indole ring. The last member, cino-bufotenine, is a constituent of the Chinese toad poison *Ch'an Su*, raises the arterial blood pressure and has been shown by Jensen and Chen (2) to be an amine derived from an indole. The chief object of this paper is to furnish (*a*) a comparison of the physiological activity of the different compounds with particular reference to epinephrine and ephedrine, and (*b*) a further elucidation of the relationship between chemical structure and pharmacological effects.

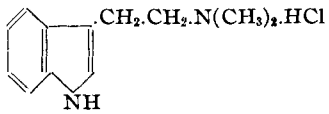
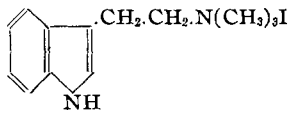
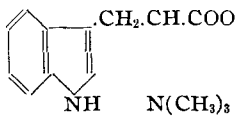
Of the 10 compounds listed in Table I, we are indebted to Dr. R. H. F. Manske, National Research Council, Ottawa, Canada, for Nos. 6, 7, 8 and 9; to Dr. O. Schaumann, I. G. Farbenindustrie A. G., Frankfurt a. Main-Hoechst, Germany, for Nos. 3 and 4; to Dr. R. W. Jackson, Yale University, New Haven, for No. 9; and to Dr. W. H. Hartung, Sharp and Dohme, Baltimore, for Nos. 1 and 2. We also wish to acknowledge our gratitude to Dr. H. Jensen, Johns Hopkins Medical School, for suggestions of study and for his assistance in securing hypaphorine and the four tryptamines from the proper authorities. Cino-bufotenine was prepared by ourselves as a flavianate.

Before the presentation of the data, a brief résumé of the status of these compounds may be desirable. Both *p*-methyl- and *p*-methoxy-*nor*-ephedrines have been shown by Hartung and his associates (3) (4) (5) to have a weaker pressor action than phenylpropanolamine. Compound No. 3, *3,4*-dihydroxy-*nor*-ephedrine, was suggested for further study by Chen, Wu and Henriksen (1), and reports have since been made by Schaumann (6), Raymond-Hamet (7), Hartung and his associates (5) and Tainter (8). Compound No. 4, *3,4*-dihydroxy-ephedrine, is relatively new, and its physiological effects have been studied by Schaumann (6) and Raymond-Hamet (7). Tryptamine, or indole-ethylamine, has been subjected to animal experimentation by Ewins and Laidlaw (9), Guggenheim and Löffler (10), and Guggenheim (11). The methyl derivatives of tryptamine are the results of Manske's recent work (12). Hypaphorine occurs in the seeds of *Erythrina hypaphorus* (13) or *E. variegata var. orientalis* (14), and its synthesis has been perfected by Romburgh and Barger (15). A metabolic investigation with this product has been made by Jackson (16). Hypaphorine is of special interest in the present series because Wieland, Hesse and Mittasch (17) believe that it is closely

* From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

TABLE I.—PHARMACOLOGICAL ACTIVITY OF COMPOUNDS RELATED TO EPHEDRINE AND TRYPTAMINE.

Compound No.	Name.	Formula.	M. P. ° C. Corrected.	Pressor Action in Pithed Cats.	Rabbit's Pupil.	Isolated Rabbit's Intestine.	Isolated Guinea Pig's Uterus.
1	*p*-Methyl-*nor*-ephedrine HCl		205–206	+	Dilated	Slightly stimulated	Stimulated
2	*p*-Methoxy-*nor*-ephedrine HCl		224.5	+	No response (4% solution)	Slightly stimulated	Stimulated
3	*3,4*-Dihydroxy-*nor*-ephedrine HCl		179–180	1/4 of epinephrine	Definitely dilated	Inhibited	Stimulated
4	*3,4*-Dihydroxy-ephedrine HCl		189	1/40 of epinephrine	Definitely dilated	Inhibited	Stimulated
5	Tryptamine HCl		252–253	+++	No response	Slightly stimulated	Stimulated
6	Methyl-tryptamine HCl		180	++	No response	Slightly stimulated	Stimulated

7	Dimethyl-tryptamine HCl		Hygroscopic	+	No response	Slightly stimulated	Stimulated
8	Trimethyl-tryptamine Ammonium Iodide		197	$^1/_{21}$ of epinephrine	No response	Definitely stimulated	Stimulated
9	Hypaphorine		238	No response	No response	No response	No response
10	Cino-bufotenine Flavianate	$C_{13}H_{23}O_2N_2.C_{10}H_6O_8N_2S$	200.5	$^1/_{10}$ of epinephrine	Slightly constricted	Definitely stimulated	Stimulated

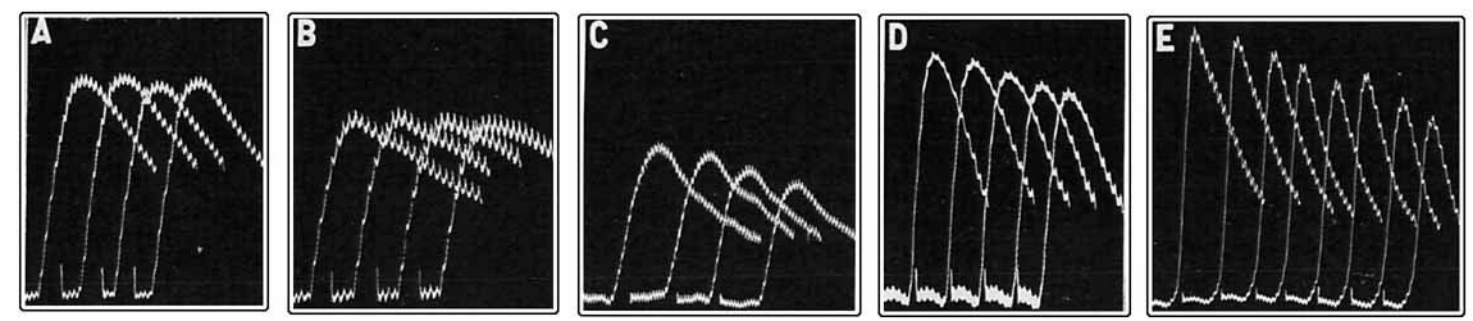


Fig. 1.—Action on blood pressure by repeated injections with five amines.

A. *3,4*-Dihydroxy-*nor*-ephedrine HCl, 1:5000, 0.4 cc. for each injection in a pithed and vagotomized cat, female, weighing 1.78 Kg.
B. *3,4*-Dihydroxy-ephedrine HCl, 1:500, 0.4 cc. for each injection in a pithed and vagotomized cat, female, weighing 2.088 Kg.
C. Tryptamine HCl, 1:500, 0.4 cc. for each injection in a pithed and vagotomized cat, female, weighing 2.552 Kg.
D. Trimethyl-tryptamine ammonium iodide, *M*/320 (ca. 1:1000), 0.4 cc. for each injection in a pithed and vagotomized cat, female, weighing 1.746 Kg.
E. Cino-bufotenine flavianate, 1:4000, 0.8 cc. for each injection in a pithed and vagotomized cat, male, weighing 3.3 Kg.

related to cino-bufotenine ("bufotenidine"). If that were the case, it might also be expected to have a pressor action like cino-bufotenine (18).

The present investigation comprises studies with the above products on the blood pressure in pithed cats, and on smooth muscle organs—rabbits' pupils, isolated guinea pigs' uteri and rabbits' intestines. On several occasions, perfusion experiments were made in frogs to determine the cardiac or vascular changes. The results are summarized in Table I.

A. PRESSOR ACTION.

The rise of blood pressure as observed in pithed cats is probably the best indicator of the inherent property of amines of this group. In two experiments, it was found that both *p*-methyl- and *p*-methoxy-*nor*-ephedrines had only a trace of pressor action. When compared equimolecularly with *nor*-ephedrine and *p*-hydroxy-*nor*-ephedrine, they were shown to be much less active. It is thus clear that the replacement of H on the benzene ring at the *p*-position, or on the OH group at the *p*-position, by a simple alkyl group such as a methyl radical is unfavorable to the pressor action.

3,4-Dihydroxy-*nor*-ephedrine (racemic) is the most powerful of the whole list. In 10 pithed cats, the average pressor action was determined to be $^1/_4$ that of epinephrine. A 1:5000 solution of *3,4*-dihydroxy-*nor*-ephedrine matched very closely with one of 1:20,000 of epinephrine in the majority of animals. Unlike ephedrine, with this synthetic product there is no prolongation of action and no loss of effectiveness on repeated intravenous injections (see Fig. 1).

The same ratio of activity between *3,4*-dihydroxy-*nor*-ephedrine and epinephrine, that is, 1:4, was obtained by perfusion experiments in frogs for cardiac stimulation or vaso-constriction. The minimal concentration of *3,4*-dihydroxy-*nor*-ephedrine that increased both the rate and the amplitude was 1:2,500,000, while that of epinephrine which produced the same effect was 1:10,000,000. Similarly, the minimal effective concentration of the former for vaso-constriction was 1:2,000,000 and that of the latter 1:8,000,000. It has been our impression that the vaso-constricting action in frogs of *3,4*-dihydroxy-*nor*-ephedrine is relatively less prompt than that of epinephrine.

Tainter (8) and Hartung and co-workers (5) working with Hartung's product concluded that *3,4*-dihydroxy-*nor*-ephedrine has $^1/_{12}$ the activity of epinephrine, or indirectly, it is $^1/_3$ as active as the German preparation which we studied. It is possible that Hartung's compound had not attained its highest purity since he stated that he had failed to find a suitable solvent for recrystallization (5).

Compound No. 4, *3,4*-dihydroxy-ephedrine (racemic), is $^1/_{10}$ as active as *3,4*-dihydroxy-*nor*-ephedrine, or $^1/_{40}$ as active as epinephrine, but is decisively more powerful than ephedrine in animals by intravenous injections. In 10 pithed cats, a small volume (say 0.4 cc.) of a 1:500 solution of *3,4*-dihydroxy-ephedrine caused practically the same rise of blood pressure as that produced by an equal volume of a 1:5000 solution of *3,4*-dihydroxy-*nor*-ephedrine, or a 1:20,000 solution of epinephrine. Like the latter, *3,4*-dihydroxy-ephedrine has a brief action, and repeated administration does not diminish the sensitivity of animals to it, as shown in Fig. 1.

Of the four tryptamines, the order of activity on the blood pressure was found to be as follows: dimethyl-tryptamine < methyl-tryptamine < tryptamine < tri-

methyl-tryptamine ammonium iodide. Four cats were used in the determination. The fact that tryptamine is stronger than methyl- and dimethyl-tryptamines, and *3,4*-dihydroxy-*nor*-ephedrine stronger than *3,4*-dihydroxy-ephedrine, is in keeping with our previous observation (1) that primary amines are more powerful than secondary or tertiary amines. The ammonium iodide of trimethyl-tryptamine has a marked pressor action. An *M*/320 solution matched very closely in two pithed cats a 1:20,000 solution of epinephrine—making the ratio of activity approximately 1:21. This increase of potency is to be expected for Barger and Dale (19) discovered some time ago that the quaternary ammonium salts are much more powerful than the corresponding less highly methylated amines. They further observed that these substances have a nicotine-like effect. The quantity of the present compound available was not sufficient to permit a thorough study, but *a priori* one would be tempted to assume that it would have the same type of action, that

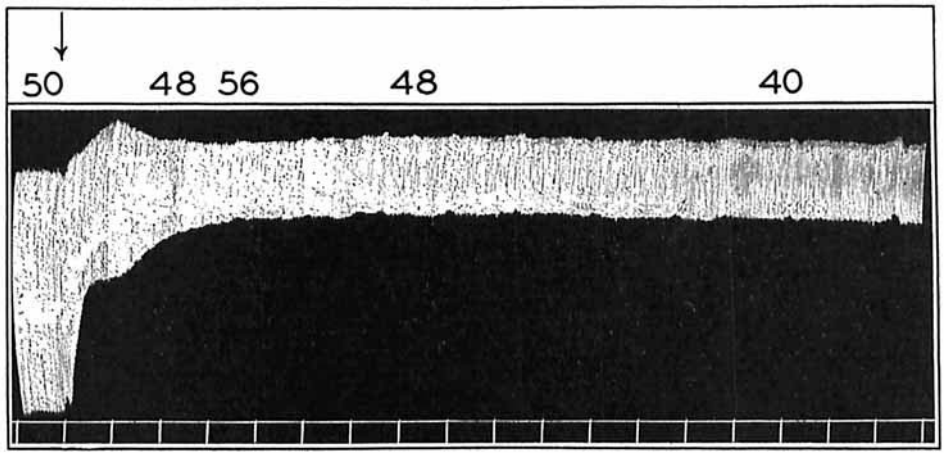


Fig. 2.—Action of trimethyl-tryptamine ammonium iodide on frog's heart.

Frog, male, weighing 69 Gm., and pithed, was perfused (at arrow) through the inferior vena cava with a 1:5000 solution of trimethyl-tryptamine ammonium iodide. The figures refer to ventricular rates per minute.

is, a nicotine-like effect. Both tryptamine and trimethyl-tryptamine ammonium iodide gradually lose a small fraction of their blood pressure raising property upon repeated intravenous injections (Fig. 1).

Hypaphorine in the dosage of 5 mg. did not raise the blood pressure, while cino-bufotenine in the form of a flavianate has $^1/_{10}$ the pressor action of epinephrine (18). This appears to indicate that cino-bufotenine is more likely a derivative of tryptamine than of hypaphorine. Furthermore, trimethyl-tryptamine ammonium iodide, like cino-bufotenine, has a tendency to increase the tone of the frog's heart when perfused through the inferior *vena cava*, as shown in Fig. 2. There is also a gradual diminution of the action on the blood pressure with cino-bufotenine (see Fig. 1).

B. ACTION ON SMOOTH MUSCLE ORGANS.

From Table I, it may be noted that, with the exception of *p*-methoxy-*nor*-ephedrine, other ephedrine derivatives all have a mydriatic action. The results in

general agree with our former observation that an OH group on the C-atom next to the benzene ring is essential for the dilatation of the pupil. The tryptamines and hypaphorine have practically no action on the pupil. Cino-bufotenine, however, constricts it (18).

On the isolated rabbit's intestine the majority of substances under investigation exert a stimulating action, most marked with trimethyl-tryptamine ammonium iodide and cino-bufotenine flavianate (Table I). Like epinephrine, both *3,4*-dihydroxy- and *3,4*-dihydroxy-*nor*-ephedrines inhibit intestinal movements. Hypaphorine is the only one of these amines that is devoid of any action.

All the compounds, except hypaphorine, contract the isolated virgin guinea pig's uterus. Epinephrine was tested on those strips of uteri with which *3,4*-dihydroxy- and *3,4*-dihydroxy-*nor*-ephedrines were studied, and was found to be oxytocic also. In other words, the similarity between the two dihydroxy-ephedrines and epinephrine is qualitatively very great.

SUMMARY.

A series of 10 amines, 4 of which are ephedrine derivatives and the remaining 6 contain an indole ring, have been studied pharmacologically.

If the blood pressure is taken as the criterion, an introduction of a methyl or methoxy radical at the *p*-position in the *nor*-ephedrine molecule results in a reduction of activity. The replacement of two OH groups for H at the *3,4*-positions on the benzene ring greatly increases the intensity but abolishes the prolongation of the action. *3,4*-Dihydroxy-*nor*-ephedrine has $^1/_4$ and *3,4*-dihydroxy-ephedrine $^1/_{40}$ the activity of epinephrine (natural). Repeated intravenous injections of both compounds elicit the same responses as those produced by the first injection. They dilate the pupil and inhibit intestinal movements.

Tryptamine is more powerful than methyl- or dimethyl-tryptamines, but decisively less active than trimethyl-tryptamine ammonium iodide. The last one has $^1/_{21}$ the activity of epinephrine, as far as the blood pressure is concerned.

p-Methyl- and *p*-methoxy-*nor*-ephedrines and all the tryptamines investigated stimulate isolated rabbits' intestines and guinea pigs' uteri. They have practically no action on rabbits' pupils, except *p*-methyl-*nor*-ephedrine which dilates them slightly.

Hypaphorine does not produce effects similar to those of the tryptamines.

Physiologically, cino-bufotenine, which has $^1/_{10}$ the pressor action of epinephrine, is more like a derivative of tryptamine than of hypaphorine.

BIBLIOGRAPHY.

(1) K. K. Chen, C. K. Wu and E. Henriksen, *J. Pharmacol. & Exper. Therap.*, 36 (1929), 363.
(2) H. Jensen and K. K. Chen, *Ber. deut. chem. Gesellsch.*, 65 (1932), 1310.
(3) W. H. Hartung and J. C. Munch, *J. Am. Chem. Soc.*, 51 (1929), 2262.
(4) W. H. Hartung, J. C. Munch, W. A. Deckert and F. Crossley, *Ibid.*, 52 (1930), 3317.
(5) W. H. Hartung, J. C. Munch, E. Miller and F. Crossley, *Ibid.*, 53 (1931), 4149.
(6) O. Schaumann, *Arch. exptl. Path. Pharmakol.*, 160 (1931), 127.
(7) Raymond-Hamet, *Compt. rend. acad. sci.*, 192 (1931), 300.
(8) M. L. Tainter, *Arch. intern. pharmacodyn. et thér.*, 41 (1931), 365.
(9) A. J. Ewins and P. P. Laidlaw, *Biochem. J.*, 7 (1912), 18.

(10) M. Guggenheim and W. Löffler, *Biochem. Z.*, 72 (1916), 325.
(11) M. Guggenheim, *Die biogenin Amine*, Julius Springer, Berlin, 2nd Edition, 1924, 335.
(12) R. H. F. Manske, *Can. J. Research*, 5 (1931), 592.
(13) M. Greshoff, *Mededeeliigen uit Lands Plantantuin*, 7 (1890), 29 (cited by Romburgh and Barger).
(14) J. Marañon and J. K. Santos, *Philippine J. Sci.*, 48 (1932), 563.
(15) P. v. Romburgh and G. Barger, *J. Chem. Soc.*, 99 (1911), 2068.
(16) R. W. Jackson, *J. Biol. Chem.*, 84 (1929), 1.
(17) H. Wieland, G. Hesse and H. Mittasch, *Ber. deut. chem. Gesellsch.*, 64 (1931), 2099.
(18) K. K. Chen, H. Jensen and A. L. Chen, *J. Pharmacol. & Exper. Therap.*, 43 (1931), 13.
(19) G. Barger and H. H. Dale, *J. Physiol.*, 41 (1910-1911), 19.

PHYTOCHEMICAL NOTES.*[1]

No. 109. On the Non-Preëxistence of Azulene in Milfoil.

BY KATHERINE GRAHAM.[2]

The preëxistence of azulene in plants was first questioned by Tschirch and Hohenadel in 1895 (1), when they observed that sagapen yielded a yellow oil upon extraction with petroleum ether and that this oil became blue during fractionation. Not knowing whether the formation of the blue substance was due to the exposure of the volatile oil extracted with petroleum ether or to resin extracted at the same time, they prepared a resin-free volatile oil by steam distillation. This also was faintly yellow and only upon fractional distillation involving higher temperatures, *viz.*, abt. 200°, did they obtain a blue fraction. They, therefore, arrive at the conclusion that "without doubt, the blue oil is a pyrogenic decomposition product" (2). In 1917, however, Tschirch expresses himself as still in doubt as to whether the azulene is formed during the process of distillation (3).

Herzenberg and Ruhemann made a similar investigation in 1927 (4). They found that chamomile yielded only a small amount of a yellow oil to petroleum ether but that the extracted plant yielded a blue oil upon steam distillation. From this they concluded that azulene did not preëxist in the plant and that its formation was from sesquiterpenes by fermentative action especially since they had isolated a sesquiterpene which yielded a blue color by dehydrogenation. However, the experiment performed does not support this conclusion. If the azulene were formed from sesquiterpenes, it would not then be obtained from the extracted marc, from which the sesquiterpenes had been removed. Furthermore, fermentative action would not be expected during steam distillation, where the temperature is much above the thermal death point of enzymes. Nor is dehydrogenation likely to take place during steam distillation.

The existence of azulene in the plant may more logically be explained by the assumption of an acid addition product since it is known that azulene readily forms such a product which is not soluble in petroleum ether. The union of azulene with either phosphoric acid or an acid phosphate would presumably give

* From the laboratory of Edward Kremers.
[1] Scientific Section, A. PH. A., Miami meeting, 1931.
[2] Fritzsche Bros. Fellow.