THE OCCURRENCE OF METHYLBENZYLAMINE IN THE EXTRACT OF MA HUANG.

BY A. LING CHEN, ELMER H. STUART AND K. K. CHEN.

Since the report of Chen and Schmidt (1) on *l*-ephedrine, a base isolated from the Chinese drug Ma Huang, several other amines closely related to ephedrine have been obtained from the same plant. Thus Chou (2) reported the separation of *d*-pseudoephedrine from Ma Huang, Smith (3, 4) that of nor-*d*-pseudoephedrine and *l*-methylephedrine, Nagai and Kanao (5) that of *d*-methylpseudoephedrine, and Kanao (6) that of *l*-nor-ephedrine. In our examination of the extract of Ma Huang after the removal of *l*-ephedrine, we found a base that boils at a low temperature and forms salts not identical with those of any base of Ma Huang heretofore described. Further study revealed that it is a new subsidiary base and has the formula and properties of methylbenzylamine, $C_6H_5.CH_2.NH(CH_3)$. This report covers our chemical and pharmacological data.

ISOLATION AND IDENTIFICATION OF METHYLBENZYLAMINE.

The extract of Ma Huang, freed as completely as possible from *l*-ephedrine, was made definitely alkaline with potassium hydroxide and vigorously shaken with ether. The residue remaining after the removal of ether from the ethereal layer was distilled under diminished pressure. The portion that distilled over below the boiling point of *d*-pseudoephedrine contained the base in question. The yield was small as compared with that of *l*-ephedrine and *d*-pseudoephedrine, but by working with a large quantity of crude material, a sufficient amount of the base was obtained for this investigation. The base is an oil and forms a hydrochloride with dilute hydrochloric acid in the cold. *Methylbenzylamine hydrochloride*, freed from impurities, crystallizes from an alcohol-ether mixture in fine plates, melting point 175–6° C. (uncorrected), or 180.5° C. (corrected). Emde (7) gave the melting point 174–5° C. It is easily soluble in water, alcohol and chloroform; only slightly soluble in ether, acetone and petroleum ether. Its aqueous solution shows no specific rotation. The hydrochloride of methylbenzylamine yields positive secondary amine reactions, such as the benzene-sulphonyl chloride test and the Simon's test.[1] It is oxidized by an alkaline permanganate solution giving rise to an odor of benzaldehyde. An ether extract of the acidified solution of the oxidized product gives a solid, which when sublimed at 80° C., shows the characteristic crystals of benzoic acid. These crystals melt at 120–121° C. (corrected) and form a flesh-colored precipitate with a solution of ferric chloride.

For analysis, the hydrochloride was dried over H_2SO_4.

4.228 mg. substance: 0.383 cc. N at 33° and 745.4 mm. (micro-Kjeldahl).
3.863 mg. substance: 0.333 cc. N at 33° and 745.4 mm.
3.097 mg. substance: 0.279 cc. N at 33.5° and 744.4 mm.
2.464 mg. substance: 0.217 cc. N at 35° and 744.4 mm.

100.0 mg. substance: 22.31 mg. Cl (Volhard).[2]
100.0 mg. substance: 22.22 mg. Cl.

[1] We are indebted to Dr. Harold W. Coles for his assistance in making the tests.

[2] We are indebted to Mr. Robert M. Lingle for his assistance in making the determinations.

$C_6H_4.CH_2.NH(CH_3).HCl$	Calculated	N 8.88,	Cl 22.50
	Found	N 9.146	
	Found	N 8.665	
	Found	N 9.026	
	Found	N 8.734	
	Found		Cl 22.31
	Found		Cl 22.22

Methylbenzylamine aurichloride can be prepared by adding a solution of auric chloride in dilute hydrochloric acid to an aqueous solution of methylbenzylamine hydrochloride. The golden-yellow precipitate after drying over H_2SO_4 melted at 136–138° C. (uncorrected), or 139–140° C. (corrected). Emde (7) gave the melting point 138° C. *Methylbenzylamine platinichloride*, prepared by the addition of a 10 per cent solution of chloroplatinic acid to an aqueous solution of the base, forms yellow crystals, melting point 192° C. (uncorrected), or 194–195° C. (corrected). Previously reported values for this double salt are 193° C. by Hinsberg (8) and 197° C. by Emde (7).

A portion of the impure base was used for the preparation of several salts by neutralization with their respective acids. With the exception of the sulphate, they appear to be reported for the first time. They gave the following properties:

Methylbenzylamine sulphate crystallized in plates from a mixture of alcohol and acetone. After drying at 105° C., it melted at 144.2° C. (corrected), the melted material becoming a transparent homogeneous mass upon cooling. It is neutral to litmus, hygroscopic, soluble in water and alcohol; only very slightly soluble in ether and acetone.

Methylbenzylamine nitrate crystallized in stout prisms from a mixture of alcohol and ether. After drying at 65° C., it melted at 86.5° C. (corrected). It is soluble in water, alcohol, chloroform and acetone; only very slightly soluble in ether and petroleum ether.

Methylbenzylamine phosphate crystallized in stout prisms from a mixture of alcohol and ether. After drying at 105° C., it melted at 162.5° C. (corrected). This salt contains water of crystallization and it melts at a lower temperature if it is not dehydrated. It is very soluble in water, soluble in alcohol; only slightly soluble in chloroform, acetone, ether and petroleum ether. The aqueous solution is acid to litmus.

Methylbenzylamine oxalate crystallized in angular plates from a mixture of alcohol and ether. After drying at 105° C., it melted at 200.5–201° C. (corrected). It is soluble in water and alcohol; only very slightly soluble in ether, chloroform, acetone and petroleum ether. Its aqueous solution is neutral to litmus.

PHARMACOLOGICAL ACTION OF METHYLBENZYLAMINE AND ITS COMPARISON WITH THAT OF BENZYLAMINE.

Since little information is available concerning the physiological activity of methylbenzylamine, we made a study of its general effects. *A priori*, the compound should possess some sympathomimetic action. The primary amine, benzylamine, was briefly investigated by Barger and Dale (9), and is considered to have a mere trace of sympathomimetic action. We prepared the hydrochloride of benzylamine

(melting point 262.5° C., corrected) from the pure base obtained commercially, and compared its action wherever possible with that of methylbenzylamine.

a. Action on Blood Pressure.—In anesthetized cats, methylbenzylamine as the hydrochloride given intravenously in the dosage of 3 to 3.5 mg. per Kg. raises the arterial blood pressure. Repeated injections do not rapidly diminish the pressor response like ephedrine. Larger doses, say seven or more mg. per Kg. given at once, however, lower the pressure, with complete recovery to the initial level within a short time unless very large doses (35 or more mg. per Kg.) are administered. The blood-pressure raising property of methylbenzylamine is better demonstrated in pithed cats which can tolerate also slightly larger doses (six mg. per Kg.) without showing any depressor action.

The pressor action of methylbenzylamine is much less powerful than that of ephedrine and nor-ephedrine. This is to be expected because the side chain has only one C-atom. When compared equimolecularly with benzylamine, methylbenzylamine was shown to differ only slightly from the primary amine (Fig. 1). In nine observations with six pithed cats the rise of blood pressure was slightly greater after methylbenzylamine than after benzylamine in seven cases and *vice versa* twice. The average difference in the nine determinations with the two compounds was found to be 3.4 mm. Hg in favor of methylbenzylamine, a deviation too small to be considered significant. This is of special interest, for, among the sympathomimetic amines a methylamino-base is usually weaker than the corresponding amino-base, as shown by the work of Barger and Dale (9) and Chen, Wu and Henriksen (10). Only in a few instances is a methylamino-derivative equally active as, or actually stronger than, the amino-base. The following examples may be cited for comparison with regard to their pharmacological action.

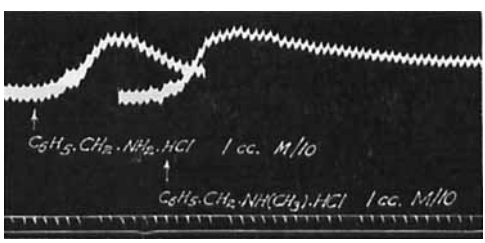


Fig. 1.—Comparison of pressor action between benzylamine and methylbenzylamine. Cat, female, 2.5 Kg., decerebrated and pithed, double vagotomy, artificial respiration, left carotid pressure. The drugs were both injected intravenously. Maximal rise after benzylamine 25 mm. Hg, and that after methylbenzylamine 29 mm. Hg.

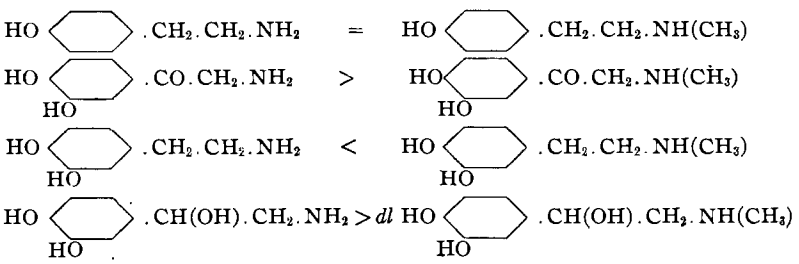
$(CH_3)_2.CH.CH_2.CH_2.NH_2 > (CH_3)_2.CH.CH_2.CH_2.NH(CH_3)$

$C_6H_5.CH_2.CH_2.NH_2 = C_6H_5.CH_2.CH_2.NH(CH_3)$

$C_6H_5.CH(OH).CH_2.NH_2 > C_6H_5.CH(OH).CH_2.NH(CH_3)$

dl $C_6H_5.CH(OH).CH(CH_3).NH_2 >$ *d l* $C_6H_5.CH(OH).CH(CH_3).NH(CH_3)$

dl $C_6H_5.CH(OH).CH(C_2H_5).NH_2 >$ *d l* $C_6H_5.CH(OH).CH(C_2H_5).NH(CH_3)$



b. Action on the Heart.—Methylbenzylamine hydrochloride with different concentrations was perfused through the frog's heart by way of the inferior vena cava according to the method of Howell and Cooke (11) and by means of the Greene cannula (12). Little effect was observed with dilute solutions. A concentration of 1:1,000 produces a transient depression of the rate and amplitude of contraction, which disappears if the drug solution is replaced by the normal Ringer's fluid. Almost identical results were obtained with benzylamine hydrochloride.

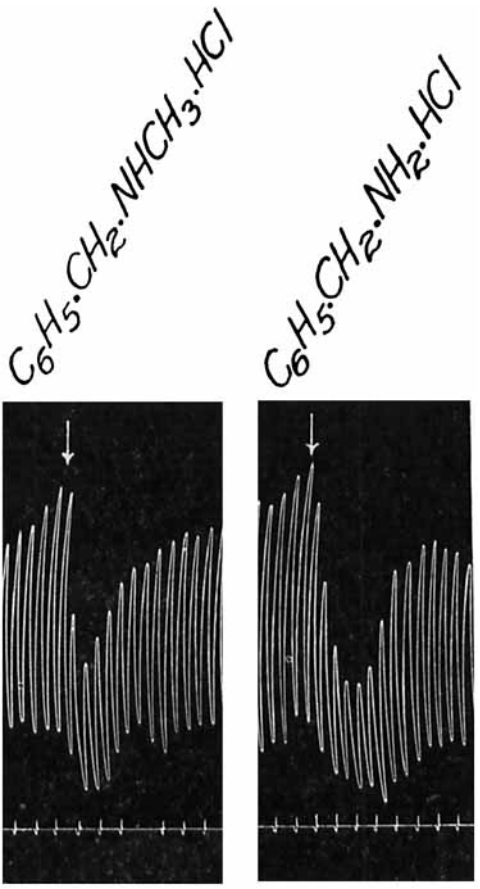


Fig. 2.—Comparison of action of benzylamine and methylbenzylamine on the isolated rabbit's small intestine. A strip was suspended in 50 cc. of Locke's solution maintained at 38° C. At each arrow, 0.5 cc. of a M/10 solution of the drug (as labeled) was added. There was a washing out between the two tests. It should be noted that there was practically no difference in response (inhibition) to benzylamine and methylbenzylamine.

In four cats, myocardiograms were made with intravenous injections of methylbenzylamine hydrochloride. They uniformly showed that pressor doses increased the contractility of the heart muscle, while larger doses which lowered the blood pressure caused a decrease in amplitude of the contraction. Since methylbenzylamine has only a slight action on the blood vessels (see below), the changes in blood pressure appear to be chiefly due to the alteration of the cardiac contraction. Electrocardiograms were obtained on two other cats with methylbenzylamine hydrochloride in large doses (12–23 mg. per Kg.) given intravenously. They illustrated no unusual changes except some exaggeration of the T wave during the fall of blood pressure. The pulse rate was observed in each experiment by either inspection or electrocardiogram. Pressor doses usually caused acceleration, but large doses lowering the blood pressure resulted in temporary slowing of the heart rate.

Benzylamine studied parallelly on the mammalian heart gave closely similar results.

c. Action on Smooth Muscle Organs.—Methylbenzylamine hydrochloride in 1 per cent solution upon instillation in the rabbit's eye, failed to produce any dilatation of the pupil. There is, however, a definite mydriatic action in the same animals after intravenous injections of moderate doses (30 or more mg. per Kg.). The light reflex is not abolished since the pupil promptly constricts upon exposure to strong light. Practically the

same result was obtained with benzylamine. The lack of a hydroxyl group on the α-C-atom from the benzene ring is possibly responsible for the absence of mydriatic action by local application as previously suggested by Chen, Wu and Henriksen (10).

In anesthetized dogs whose kidney volume was recorded, an intravenous injection of methylbenzylamine hydrochloride in the dosage of 4.5 mg. per Kg. resulted in slight constriction of the renal vessels during the rise of blood pressure. The vascoconstricting action of methylbenzylamine is therefore appreciable.

Nineteen strips of the isolated rabbit's small intestine were immersed in Locke's solution maintained at 38° C., and studied by the addition of methylbenzylamine hydrochloride in the concentrations of from 1:10,000 to 1:2,500. In 32 observations, relaxation occurred 15 times, increase in the tonus of contraction 12 times, and increase in the strength of contraction followed by relaxation 5 times. In several experiments, methylbenzylamine was compared with benzylamine in equimolecular concentrations. The response was found to be the same with either drug as shown in Fig. 2.

A similar study was made with four strips of the isolated rabbit's uteri. In nine observations the tonus of the contraction was uniformly increased, although to a small extent. The concentration of methylbenzylamine hydrochloride varied from 1:20,000 to 1:2,500. The action of benzylamine proved to be about the same as that of methylbenzylamine when compared equimolecularly.

We made no attempt to determine precisely the site of action of methylbenzylamine. Since, however, it produces physiological effects similar to, but to a much less degree than, Beta-phenylethylamine and its derivatives, it seems justifiable to call methylbenzylamine a sympathomimetic amine.

d. Toxicity.—In a series of 17 frogs, methylbenzylamine hydrochloride was injected into the anterior lymph sac in a dosage varying from 250 to 1,000 mg. per Kg. The M. L. D. was not precisely determined, but it was found to lie between 500 and 750 mg. per Kg. The chief toxic symptoms are clonic convulsions followed by depression and flaccid paralysis. These convulsions disappear after the medulla, but not after the cerebrum, is destroyed. The point of action is therefore at the upper spinal cord. When the frog has apparently died, autopsy often shows the heart beating very slowly, or that it stops at diastole.

Methylbenzylamine hydrochloride was given intravenously to two rabbits in the dosage of 50 mg. per Kg. Both recovered completely. The symptoms observed were dilatation of the pupils, weakness in the hind legs and fibrillatory movements of the voluntary muscles.

In white rats, benzylamine and methylbenzylamine were parallelly compared by the determination of their M. L. D. as shown in Table I; that for benzylamine hydrochloride being 200, and that for methylbenzylamine hydrochloride 215 mg. per Kg. Methylbenzylamine is slightly less toxic than benzylamine. The toxic symptoms are practically the same with either compound. They consist of dilatation of the pupils, weakness in the legs, clonic convulsions and gasping for air. Death usually occurs within four or five minutes after fatal doses. Recovery from sublethal doses is always complete.

COMMENT.

We have been mindful that methylbenzylamine might be a product of the decomposition of ephedrine during the process of extraction. The formation of methylbenzylamine from ephedrine, however, would involve strenuous oxidation and reduction, and subsequent methylation, according to the usual chemical reactions; and since our method of extraction does not require strong oxidizing or reducing agents, decomposition does not seem probable. Recently, Moore and Moore (13) showed the conversion of ephedrine base (not its salts) to benzal-ephedrine upon exposure to strong sunlight or by the addition of hydrogen peroxide. Our extract was not submitted to similar treatments, and furthermore our compound is not benzal-ephedrine but methylbenzylamine. Unless new evidence arises to prove the contrary, it must be concluded that methylbenzylamine is a normal constituent of Ma Huang.

SUMMARY.

1. Methylbenzylamine has been isolated, and identified as such, from the extract of Ma Huang. Several salts and derivatives of this base have been prepared and described.

2. A pharmacological study of methylbenzylamine has been made.

3. Compared with benzylamine, methylbenzylamine has practically the same physiological activity. The latter is slightly less toxic.

It is our special pleasure to acknowledge our indebtedness to Mr. Horace A. Shonle for his assistance in the virtual identification of our compound.

TABLE I.—TOXICITY OF BENZYLAMINE AND METHYLBENZYLAMINE IN WHITE RATS BY INTRAVENOUS INJECTION.

Hydrochloride of	Dose in mg. per Kg	Number of rats used.	Number died.	Number survived.	M. L. D. in mg. per Kg.
Benzylamine	190	3	0	3	
	195	4	1	3	200
	200	5	3	2	
Methylbenzylamine	205	3	0	3	
	210	5	2	3	215
	215	4	3	1	

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ON THE PHYSICO-CHEMICAL PROPERTIES OF SOLUTIONS OF DIBISMUTHYL MONOSODIUM CITRATE IN ETHYLENEGLYCOL.*

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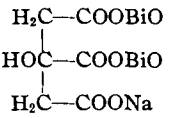
P. Hanzlik recently advocated the use of ethyleneglycol as a solvent and vehicle for bismuth compounds, and for this reason it was attempted to use also as a solvent for dibismuthyl monosodium citrate. This compound is prepared as an anhydride of the formula



which, as such, is insoluble in water and in ethyleneglycol. The hydrate is, however, soluble in both solvents and can be diluted ad libidum. Because it is known that bismuth salts may form compounds with glycerol and different sugars, it appeared possible that a similar compound was also formed with ethyleneglycol.

The freshly precipitated moist hydrate of dibismuthyl monosodium citrate was therefore divided in two portions, one (*a*) was dried as usual, the other (*b*) was dissolved in ethyleneglycol, and the bismuth compound precipitated by means of acetone. After settling (preferably in the icebox), the precipitate was filtered off, washed with acetone, alcohol and ether and dried. The dried material represents a white amorphous powder, freely soluble in water. It does not dissolve in ethyleneglycol directly, but does so freely after hydration. Both samples were analyzed, and showed the same bismuth content:

(*a*) Substance: 0.4763 Bi_2S_3 0.3772 64.3 per cent Bi
(*b*) Substance: 0.4697 Bi_2S_3 0.3711 64.1 per cent Bi

The theoretical bismuth content for dibismuthyl monosodium citrate is 63.0 per cent bismuth, while the hypothetical compound of the type

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