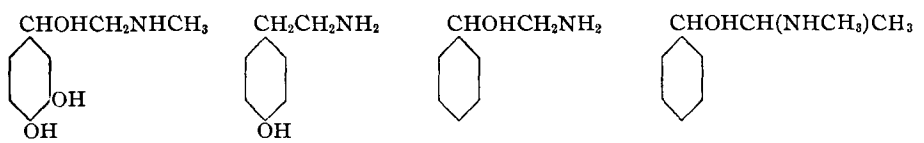
	I.	II.		I.	II.
Mg	0.046 p. c.	0.042 p. c.	CO_3	2.01 p. c.	2.03 p. c.
Ca	0.24 " "	0.24 " "	SO_4	0.27 " "	0.31 " "
Fe	0.073 " "	0.067 " "	SiO_3	1.42 " "	1.37 " "
Al	0.242 " "	0.240 " "	Undetermined	5.71 " "	5.73 " "
Cl	0.24 " "	0.22 " "			

A CHEMICAL EXAMINATION OF PARA-HYDROXYPHENYL METHYLAMINO ETHANOL HYDROCHLORIDE.*,†

BY SAMUEL M. GORDON.

The practitioner of medicine now has available as vasoconstrictor agents a number of substances. The best known of these is epinephrine, first isolated by Abel in 1897; then later by Takamine and others in pure form. The relative ease with which epinephrine undergoes oxidation and the relatively pronounced therapeutic action of very small doses of epinephrine have stimulated interest in the search for compounds having similar pharmacologic properties. The search in part has been for compounds with less pronounced pharmacologic action and greater resistance to the oxidizing influences of the air. Such work has led to the preparation of many interesting compounds. But it is worth noting that of the many compounds prepared under this stimulus, epinephrine still remains the only official drug of this group. The outstanding workers in this field have been the Edinburgh investigators, Barger and Dale. It was these workers who in a large measure clearly elucidated the relation between chemical constitution and pharmacologic properties in the group of compounds related to β-phenylethylamine. As a result of such investigations there has been made available to the clinical worker tyramine (originally isolated from ergot, but now prepared synthetically), ephedrine from the Chinese plant Ma-Huang,[1] and a more recent introduction, phenylamino ethanol, a synthetic product, for which standards have already appeared,[2] and others which are of more or less academic interest at present.

A comparison of the structural relations of these four compounds may serve to bring out the chemical similarities and conversely the differences, due to molecular structure.[3,4]



Epinephrine Tyramine Phenylamino Ethanol Ephedrine

* Contribution from the American Dental Association, Bureau of Chemistry and the A. M. A. Chemical Laboratory. † The work reported herein was completed in June 1929.

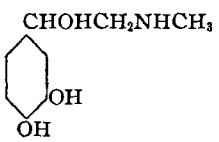
[1] The work of Chen and others has revived an interest in this long forgotten Chinese drug. Synthetic forms are now on the market, but up to now only the laevorotatory compound from the plant has been acceptable for inclusion in New and Nonofficial Remedies. Standards have been developed to rule out as far as possible all isomers except laevo ephedrine.

[2] Samuel M. Gordon, JOUR. A. PH. A., 17 (1928), 1195; also Miller and Piness, *J. Am. Med. Assocn.*, 91 (1928), 1033; and "Report of Council on Pharmacy and Chemistry," *Ibid.*, 91 (1928), 1037.

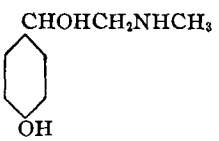
[3] Samuel M. Gordon, *loc. cit.* [4] J. B. Peterson, *Ind. Eng. Chem.*, 20 (1928), 388.

An examination of these formulas will readily reveal that the similarities in structure, while identical in the main characters, are not enough graduated to reach strictly comparable results; and indeed may serve to indicate the need for even a more systematic study of this group of compounds, before the whole story is told. The student with a leaning toward synthetic organic chemistry and pharmacology will still find here a fruitful harvest for his endeavors.[1] Newer introductions will, as is to be expected, introduce complications in the elaboration of standards of identity and purity. Axiomatically it may be stated that the closer one compound approaches another in chemical constitution the distinction will often be found quantitative rather than qualitative. This is well illustrated in the standards described at the end of this paper for the drug which is the subject of this report.

Within the past few years, there has been introduced into materia medica a substance more closely related to epinephrine than the substances previously mentioned. This compound is 4-oxyphenyl methylamino-ethanol hydrochloride. Structurally it resembles 3,4-dioxy-phenyl-methyl-amino-ethanol (Epinephrine) in all respects, but that it lacks a hydroxyl group in position 3 of the benzene nucleus. The former may be considered as a derivative of phenol; the latter of catechol. These formulas placed side by side will bring out the close similarity of the two compounds.



Epinephrine



"Desoxy Epinephrine"

It has been claimed that "desoxy epinephrine"[2] is less prone to oxidation than is epinephrine, and that pharmacologically it reacts in the same manner as epinephrine but in a lesser degree; that is, a larger dose (50–100 times) of the new compound is required to bring about an equal response.[3,4,5,6,7]

The pharmacological experiments reported by Ehrismann were made with a specimen of "desoxy epinephrine" supplied by the reputed discoverer of the compound, H. Legerlotz; those carried out by F. Lasch were made with a sample supplied by "der Wiener Chemischen Fabrik Syngala." With the exceptions noted, an examination of the literature failed to reveal any mention of a compound with

---

[1] Compare K. K. Chen and Carl F. Schmidt, "Ephedrine and Related Substances," Baltimore, 1930. This monograph appeared after the experimental work recorded herein and the report was finished.

[2] The name "desoxy epinephrine" is proposed because of its relation to epinephrine.

[3] O. Ehrismann, *Deut. med. Wochschr.*, 53 (1927), 1263; through *Chem. Abs.*, 21 (1927), 342.

[4] F. Lasch, *Arch. exptl. Path. Pharmakol.*, 124 (1927), 231.

[5] U. Von Euler and G. L. Liljestrand, *Skand. Arch. Physiol.*, 55 (1929), 1; through *Chem. Abs.*, 23 (1929), 2213.

[6] K. K. Chen and Carl F. Schmidt, *Medicine Monographs*, 17 (1930), 91.

[7] Compare, *e. g.*, "The Comparative Actions of *d*- and *l*-Epinephrine."

the formula assigned to para-hydroxyphenylmethylamino ethanol[1] and details concerning its preparation and properties are meagre.

More recently specimens of salts of para hydroxyphenylmethylamino ethanol and various dosage forms containing it were submitted to the Council on Pharmacy and Chemistry of the American Medical Association for consideration for inclusion in "New and Nonofficial Remedies." As is customary, the aid of the A. M. A. Chemical Laboratory was invoked to examine the product and to elaborate standards of purity and identity. Details concerning the preparation of the compound were not submitted, but it is probable from a consideration of U. S. Patent No. 1,680,055 issued to H. Legerlotz that "desoxy epinephrine" is prepared by reduction of the corresponding ketone.

The product available for examination was furnished by Frederick Stearns and Company, Detroit, Michigan, through the Council on Pharmacy and Chemistry. The sample of "desoxy epinephrine" was a fine, white, odorless, crystalline powder, possessing a bitter taste. It was readily soluble in cold water and alcohol. It melted at 149.5–151° C., corrected (U. S. P. X Method). It was optically inactive. Dr. A. J. Walcott, professor of Mineralogy and Crystallography at Northwestern University, reports that two different crystalline forms are present in unequal proportions.[2]

Chemically, para hydroxyphenylmethylamino ethanol is of interest because of its close relation to epinephrine. As already pointed out the two compounds are

[1] A. Kaufmann (*Ber. d. Chem. Ges.*, 46 (1913), 1826) gives the name homorenon to the same formula as is assigned to paraoxyphenylmethylamino ethanol, but no details concerning it are found in this article. A search through the chemical literature previous to 1913 did not throw light on the subject. Fränkel ("Arzneimittel Synthese," Berlin, 1927, 6th Edition, page 268) carried this formula and name into his textbook. But in another place (*loc. cit.*, page 468) he states: "Homorenon wird Äthylamino-3-4-desoxyacetophenon genannt." Gehes Codex (IV Auflage, 1926, page 429), gives the same formula to Homorenon. Hager's "Handbuch der Pharmazeutischen Praxis," Berlin 1929, page 826, states homorenon "war das Hydrochloride des dioxyphenyl äthylamino, $C_6H_3(OH)_2CH(OH)CH_2NHC_2H_5HCl$)."

[2] "A microscopic examination of oxyphenylmethylaminoethanol reveals two distinct crystalline substances. For convenience these will be referred to as (*a*) and (*b*). There is a much larger per cent of (*a*) than of (*b*). The optical properties of each were found to be as follows:

INDICES OF REFRACTION FOR SODIUM LIGHT.

$\beta = 1.610 \pm 0.002$	$\alpha = 1.500 \pm 0.001$
$\gamma = 1.664 \pm 0.002$	$\beta = 1.554 \pm 0.002$
α not determined	γ not determined
(*a*)	(*b*)
Biaxial	Biaxial
Optically negative	*Optically positive*
Optic angle large	Optic angle small
Birefringence strong	Birefringence strong
Inclined extinction	Parallel extinction

Fig. 1. Fig. 2.

Fig. 3.

"Figures 1 and 2 represent the crystal habits occurring most commonly for (*a*). Figure 3 represents the crystal habit for (*b*) somewhat idealized."

identical in all respects but that the new compound has one hydroxyl less in the nucleus. This would make it less prone to destruction by oxidation. Such appears to be the case at ordinary temperature. However, when the hydrochloride in solid form is exposed to higher temperature but far below the melting point of the hydrochloride, an almost complete decomposition ensues. From drying experiments performed at 100° C., it would appear that the compound is stable until a "nucleus" for decomposition forms, after which decomposition with rapid loss of weight sets in. The following table is illustrative of these remarks.

TABLE I.—LOSS ON HEATING.

		Weight of specimen, Gm.	Time in hours. 6.	12.	24.	48.	72.	96.
Drying at 100° C.	Loss in weight	0.3716	0.0004	0.0004	0.0007*	0.0039	0.0245**	0.0391†
	Loss in per cent		0.10	0.10	0.18	1.04	6.59	10.52
Drying at 120° C.	Loss in weight	0.3662	0.0346††	0.0355	0.0358	0.0362	0.0362	.....
	Loss in per cent		9.44	9.69	9.77	9.88	9.88	.....

* Decomposition begins—specimen turns buff colored.

** Marked decomposition.

† Decomposed completely.

†† The specimen had markedly decomposed.

Loss over phosphorous pentoxide, potassium hydroxide, sulphuric acid and calcium chloride was negligible.

This behavior is somewhat analogous to epinephrine but it is markedly different from phenylamino ethanol sulphate which was found to be stable even after 120 hours at 120° C.[1]

The substance gives typical phenolic reactions with ferric chloride and Millon's reagent. The latter fact may be adduced as evidence of the para position of the hydroxyl group in the benzene nucleus. The color obtained with ferric chloride is different enough to be used for differentiation of para hydroxyphenylmethylamino ethanol and epinephrine. The new compound gives an amethyst-violet color while epinephrine, as is well known, yields a green color.

The qualitative similarity of the new compound is brought out by the application of certain well-known color tests for epinephrine.

It is well known that epinephrine brings about marked color changes when treated with certain agents. A commonly applied color test is that with Folin's phosphotungstic acid reagent.[2] Under proper conditions it is claimed that one part of epinephrine in 3,000,000 of water can be detected by the blue color formed. The reaction with "desoxy epinephrine" under similar conditions is far less sensitive. The intensity of color formation of the two compounds is large enough to serve as a means of qualitative differentiation. Under comparable conditions one-tenth mg. of epinephrine hydrochloride yielded a decided blue color while five mg. of desoxy epinephrine yielded only a faint suggestion of blue.

A dilute aqueous solution (1 per cent) of the hydrochloride does not yield precipitates with the ordinary alkaloidal reagents, tannic acid excepted, which yields a light brown precipitate. In moderately concentrated solutions it yields a yellow

[1] Samuel M. Gordon, *loc. cit.*

[2] O. Folin, W. B. Cannon and W. Denis, *J. Biol. Chem.*, 13 (1912), 480.

crystalline precipitate with platinic chloride (chloroplatinic acid solution). The platinic chloride melts at different temperatures from 123–140° depending on the rate of heating.

Similar to epinephrine but different from ephedrine or phenylamino ethanol salts, the free base is precipitated by ammonia water. Whereas ephedrine can be quantitatively shaken out with ether,[1] and phenylamino ethanol is decomposed in dilute alkali solutions, para-hydroxy-phenyl methylamino ethanol is too insoluble in ordinary organic solvents, such as ether or chloroform, to permit it to be quantitatively shaken out. The free base is apparently slightly soluble in water at room temperature, which vitiates against basing an assay method on this procedure. Several experiments indicated a recovery of about 64 per cent. Theory requires 82.09 per cent. This point was not investigated further, but it may be of some significance in the light of the crystallographic examination already mentioned.

TABLE II.—RECOVERY OF THE FREE BASE.*

Gm. hydrochloride taken	0.3641	0.3663
Gm. base recovered	0.1844	0.1956
Gm. base required by theory	0.2989	0.3008
Per cent of theory recovered	63.0	65.0

* The hydrochloride was transferred to a small beaker and dissolved in 2 cc. of water, 5 cc. of ammonia water were added and the liquid stirred until crystals separated. The beaker and contents were set aside for one hour; the residue filtered through a tared porous crucible and washed with 25 cc. of water, previously saturated with the free base. The residue was dried at 100° C. to constant weight.

The free base melts with decomposition at 184–185° C. The rate of heating is important. If the temperature of the bath is slowly raised, according to the directions in U. S. P. X, the compound begins to decompose at almost 160° and completely decomposes between 168° and 176° C. When the bath is heated at the rate of 5–6° per minute much of the preliminary decomposition is avoided and the compound melts at the higher temperature. The free base isolated assayed for nitrogen by the Kjeldahl method yields the theoretical per cent of nitrogen. From the melting point determinations carried out it would appear more proper to speak of the final decomposition point rather than the melting point.

The hydrochloride is apparently stable in the presence of alkali. When boiled with 10 per cent sodium hydroxide in an apparatus arranged for the recovery of volatile bases, almost all of the standard acid used was recovered on titration.

TABLE III.—RESULTS OF THE QUANTITATIVE DETERMINATIONS.

	Found.	Theory calculated for $HO_6H_4CHOHCH_2NHCH_3.HCl$.
Loss by drying at 100° C. (6 hours)	0.10*	...
Nitrogen (N)	6.89	6.86
Nitrogen in free base	8.32	8.38
Chlorine (Cl^-)	17.57	17.42
Free base	64.0	82.09
Ash	0.09	...

* Figures are reported in terms of percentage.
See Table II.

[1] J. B. Peterson, *Ind. Eng. Chem., loc. cit.;* also New and Nonofficial Remedies, 1930, page 170.

When treated with benzoyl chloride and sodium hydroxide a dibenzoyl compound melting at 176° C. is obtained.

Parahydroxyphenylmethylaminoethanol hydrochloride can be distinguished from the corresponding ketone with Legal's reagent—the ketone yields a green color to the final solution, while desoxy epinephrine remains colorless.

Quantitative determinations showed the hydrochloride to be of a good grade of purity. The results of the quantitative determinations are shown in Table III.

Based in part on the information supplied by the manufacturer and in part on the examination of the specimen of parahydroxy phenyl methylamino ethanol hydrochloride described above, the following standards have been suggested:

Hydroxyphenylmethylaminoethanol Hydrochloride.—Parahydroxyphenylmethylaminoethanol Hydrochloride.—Racemic-α-4-hydroxyphenyl-β-methylamino ethanol hydrochloride.—Racemic-α-hydroxy-β-methylamino-4-hydroxy ethyl benzene hydrochloride.—$HOC_6H_4CHOHCH_2NHCH_3.HCl$.—The hydrochloride of an alkaloid obtained synthetically.

Hydroxyphenylmethylaminoethanol Hydrochloride occurs as fine, odorless, white crystals. It is readily soluble in water and cold alcohol. The aqueous solution is neutral to litmus. It is optically inactive. It melts between 149° and 151° C. with decomposition. (The rate of heating must be strictly according to the method of U. S. P. X.)

Dissolve 0.005 Gm. of hydroxyphenylmethylaminoethanol hydrochloride in 1 cc. of water and add 0.1 cc. of copper sulphate solution followed by 1 cc. of sodium hydroxide solution (20 per cent): a blue-violet color forms at once; the color is not extracted by ether. To 1 cc. of an aqueous solution containing 0.005 Gm. of hydroxyphenylmethylaminoethanol hydrochloride add one drop of mercurous nitrate solution and boil: a blood-red color develops (*distinction from ephedrine and phenylamino ethanol*). To 1 cc. of an aqueous solution containing 0.005 Gm. of hydroxyphenylmethylaminoethanol hydrochloride add one drop of ferric chloride solution (10 per cent): an amethyst-purple color develops (*distinction from phenylaminoethanol, ephedrine and epinephrine*). To 1 cc. of an aqueous solution containing 0.005 Gm. of synephrin, contained in a Nessler tube, add 2 cc. of phosphotungstic acid reagent (*J. Biol. Chem.*, 13 (1912) 477), followed by 10 cc. of sodium carbonate solution (saturated) and dilute to the 50-cc. mark: a faint suggestion of blue color is apparent when viewed longitudinally. At the same time and in the same manner treat 0.1 cc. of 1:1000 (0.0001 Gm.) epinephrine hydrochloride: a decided blue color forms (*distinction from epinephrine*). To 3 cc. of an aqueous solution containing 0.015 Gm. of hydroxyphenylmethylaminoethanol hydroehloride add 1 cc. of alcoholic potassium hydroxide solution followed by 3 drops of chloroform and boil: no odor of carbylamine is observed (*absence of primary amines*). To 0.10 Gm. of hydroxyphenylmethylaminoethanol hydrochloride dissolved in 1.0 cc. of water, add 3 to 5 drops of ammonia water: on rubbing the walls of the container with a glass rod, the free base separates. Filter by suction, wash well with water and dry on a porous plate: the dried base melts with decomposition at 184–185° C. The temperature of the bath must be raised at the rate of from 5 to 6 degrees per minute. To 1 cc. of a solution containing 0.005 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, add 0.1 cc. of diluted nitric acid followed by 0.1 cc. of silver nitrate solution: a white precipitate appears; on boiling, an orange color appears in the liquid.

Dissolve 0.05 Gm. of hydroxyphenylmethylaminoethanol hydrochloride in from 30 to 40 cc. of distilled water, add 1 cc. of diluted hydrochloric acid and 1 cc. of barium chloride solution: no turbidity should result (*absence of sulphate*). Dissolve 0.2 Gm. of hydroxyphenylmethylaminoethanol hydrochloride in 10 cc. of distilled water: the solution yields a negative test for heavy metals when tested according to the U. S. P. X method (see U. S. P. X, page 439). To 1 cc. of a solution containing 0.02 Gm. of hydroxyphenylmethylaminoethanol hydrochloride add 5 drops of a freshly prepared solution of sodium nitroprusside (1 per cent), then 1 cc. of sodium hydroxide solution followed by 10 drops of glacial acetic acid: the final solution is colorless (*absence of corresponding ketone*).

Dissolve about 0.3 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed, in 200 cc. of water; heat to boiling and add 10 cc. of diluted nitric acid followed by sufficient silver nitrate to precipitate all the chloride; allow to stand for six hours; transfer to a tared Gooch crucible; wash well with hot water; then with cold water and dry at 120° C., cool in a desiccator and weigh: the chloride (Cl^-) calculated from the silver chloride weighed is not less than 17.30 nor more than 17.60 per cent. Transfer about 0.35 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed, to a 500-cc. Kjeldahl flask and determine the nitrogen content according to the method described in Medical War Manual No. 6, Laboratory Methods of the U. S. Army, page 221: the percentage of nitrogen corresponds to not less than 6.7 per cent nor more than 7.0 per cent. Incinerate 0.1 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed: the ash is not more than 0.0001 Gm.

A COMPARISON OF THE CHEMICAL AND BIOLOGICAL ASSAYS OF OLEUM CHENOPODII.*

BY WILLIAM F. REINDOLLAR AND JAMES C. MUNCH.

In the lower portion of Carroll County, Maryland, is a narrow strip of land four miles wide and fifteen miles long which is known as the "oil belt" and which produces the greater part of the Oleum Chenopodii, or the American wormseed oil of commerce. Here the plants are grown, harvested and steam-distilled to obtain the volatile oil which occurs mainly in the seed, of which it constitutes three to three and a half per cent. Distillation is carried out during a period ranging from two to six weeks, in October and November depending upon the size of the crop, weather conditions, etc. The oil is distilled with live steam under pressure, allowed to separate from the condensed water, and is drawn off as "normal" oil of wormseed. The product is somewhat soluble in water and this, together with the fact that a portion remains mechanically suspended in the lower liquid, has led, in recent years, to the practice of collecting and redistilling the water. While this second distillation yields a much smaller quantity of oil, this redistilled product has been found to be much richer in ascaridol and of higher specific gravity than the normal oil. It is termed by the growers "high-test" oil of wormseed.

During the autumn of 1929 authentic samples of normal and high-test oil were collected at the stills. These were examined to determine if any relationship exists between the physical constants and/or ascaridol content, and certain pharmacological reactions. The following U. S. P. physical constants were determined:

(*a*) Specific gravity at $\frac{25°}{25°}$ C.
(*b*) Specific rotation.
(*c*) Refractive index at 20° C.
(*d*) Solubility of one volume of oil in 70% alcohol.

Although certain inaccuracies have been shown by Paget (1) to exist in the U. S. P. assay method for ascaridol, and these findings have been confirmed by Nelson (2) and Broughton (3), yet in view of its official character, and present status as an arbiter of quality, it was thought best to adhere to the Pharmacopœial

---

* Scientific Section, A. PH. A., Baltimore meeting, 1930.