The Separation of L-Arterenol from Natural U. S. P. Epinephrine

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RECENTLY GOLDENBERG, *et al.* (*3*) obtained evidence by paper chromatography indicating the presence of appreciable quantities of arterenol in crystalline U.S.P. epinephrine derived from adrenal glands. This information, kindly transmitted to us by Dr. Goldenberg in November 1948, suggested the desirability of attempting physical isolation of the arterenol from such samples.

A review of the literature quickly reveals a body of physiological information which can be interpreted to indicate that chromaffin tissue, which constitutes the medulla of the adrenal gland and is the source of natural etinephrine, may elaborate more than one hormone having etinephrine-like properties. In fact, Gaddum and Goodwin (*2*) have confirmed earlier suggestions that the substance released by stimulation of the hepatic nerves of cats may be arterenol. However, direct chemical evidence to support the physiological observations have been lacking, and indeed, only recently have the chemical properties of L-arterenol become known (*4*).

We now wish to report the isolation of pure L-arterenol from several commercial lots of natural epinephrine and also from a sample obtained from the bulk stock of U.S.P. epinephrine reference standard through the kindness of the U.S.P. Board of Trustees.

A 10-g sample of natural U.S.P. epinephrine, containing 17–18 percent arterenol by analysis (*1*) was dissolved by stirring with 8.5 g D-tartaric acid in 75 ml of methanol and 2 ml of water at 25°. When the solution was seeded with L-epinephrine D-bitartrate and let stand overnight a heavy crop of crystals separated. This was collected, washed with a little methanol, and dried *in vacuo,* yielding 15.1 g of L-epinephrine D-bitartrate, mp 147–149°. Analysis of this fraction indicated the presence of about 6 percent arterenol bitartrate.

The methanol liquor and wash were concentrated *in vacuo* at 25–30° and the residue was dissolved in 10 ml water containing 0.2 g $NaHSO_3$. Ammonium hydroxide was added until a faint ammonia odor persisted and the resulting slurry of base was kept at 5° one hour. The precipitated base was collected, washed with a little water, and dried *in vacuo.* There was obtained 1.56 g (mp 190–193° with decomposition), or a total recovery of 98 percent.

This base (1.42 g) was dissolved with 1.25 g L-tartaric acid in 11 ml methanol containing 0.3 ml water. The solution was seeded with L-arterenol L-bitartrate and let stand at 5° overnight. The crystalline precipitate was collected, washed with a little cold methanol, and dried *in vacuo* to give 0.7 g, mp 157–159°. Analysis indicated 95 percent arterenol bitartrate. Recrystallization from 90-percent methanol yielded 0.25 g having mp 161–3°.

Similarly an 80-g sample from the same lot of natural epinephrine gave 7.7 g of once-crystallized L-bitartrate mp 157–159°. When this was dissolved in 7 ml water and 15 ml methanol by warming to 40–50°, and the solution diluted with 80 ml of

TABLE 1

COMPARISON OF NATURAL AND SYNTHETIC L-ARTERENOL*

	Mp °C (corrected)	Mixed mp °C (corrected)	$[\alpha]_D^{25°}$	Analyses		
				% N	% C	% H
L-Arterenol L-bitartrate			Calculated	4.40	45.20	5.33
Natural	163–4	162–3	−40.2	4.37	45.30	5.33
Synthetic	163.5–164.5		−39.6	4.60	45.47	5.58
L-Arterenol D-bitartrate						
Monohydrate			Calculated	4.15	42.73	5.65
Natural	101–102	101–102	−12	3.99	42.86	5.36
Synthetic	102–104		−11		Ref. 2	
L-Arterenol hydrochloride			Calculated	6.80	46.72	5.88
Natural	147.5–148.5	145.6–147	−39.8	7.01	46.83	5.76
Synthetic	146–147		−40		Ref. 2	

* I am indebted to Mr. M. E. Auerbach of these laboratories for the determinations and analyses in this table, as well as for the arterenol assays mentioned in the text. Mr. Auerbach's method for the determination of arterenol in the presence of epinephrine will be the subject of a separate communication.

methanol, crystallization started promptly. After 20 hours at 5° the precipitate was collected, washed with a little cold methanol, and dried *in vacuo,* giving 3.3 g mp 161–3°. After one more crystallization from 30 ml of 90-percent methanol, 2.8 g of pure L-arterenol L-bitartrate, mp 163–4°, was obtained. An additional recrystallization of a portion did not change the melting point. By reworking the liquors, 1.2 g of L-bitartrate, mp 163–4°, was recovered, making a total equivalent to 2.5 percent of the epinephrine sample.

Portions of this pure L-bitartrate were converted to the D-bitartrate monohydrate and to the hydrochloride, as described previously for synthetic L-arterenol (*4*). The identity of these salts with the corresponding synthetic L-arterenol salts is shown in the table.

In summary, natural epinephrine contains appreciable quantities of levo-arterenol, as shown by isolation of the latter compound. The adrenal gland therefore contains arterenol as well as epinephrine. The present study is believed to be the first isolation of levo-arterenol in chemically pure form from biological material and is thus the final step of proof in establishing the hormonal nature of this substance.

References

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The Determination of Arterenol in Epinephrine

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USING A PAPER CHROMATOGRAPHIC TECHNIQUE, Goldenberg, Faber, Alston and Chargaff (*1*) were recently able to demonstrate the presence of arterenol (nor-epinephrine, nor-adrenaline) in supposedly pure crystals (U.S.P. grade) of epinephrine derived from adrenal glands. This most interesting finding prompted investigations which led to the actual isolation of arterenol (*2*) from natural epinephrine, and to the development of a chemical method somewhat more convenient for the assay of arterenol in epinephrine than that offered by paper chromatography. It is the purpose of this note to describe the method, and to report the values found for several lots of natural epinephrine.

Reagents. (1) Buffer, pH 9.6 (Clark and Lubs) 50 cc of a solution which is M/5 in both H_3BO_3 and KCl, plus 36.85 cc M/5 NaOH, diluted to 200 cc. (2) Five-tenths percent sodium β-naphthoquinone-4-sulfonate (Eastman Kodak) in water. This reagent must be used before it is one hour old. (3) One percent aqueous solution of alkyl dimethylbenzylammonium chlorides (Roccal or Zephiran) (benzalkonium chloride). (4) Mixed solvent. Eighty-five volumes C.P. toluene plus 15 volumes redistilled ethylene dichloride. Mix only enough for one day's use. Wash the mixture with a little of the borate buffer, then filter through dry paper to remove droplets of water. (5) Epinephrine control solution. One hundred mg pure synthetic epinephrine base dissolved in 2.5 cc of 5 percent aqueous solution of sodium borate, and diluted to 100 cc with water. (6) Arterenol standard solution. Forty mg of pure arterenol base (or its equivalent of the bitartrate) dissolved in 5 cc of 5 percent aqueous solution of sodium borate, and diluted to exactly 200 cc with water. Store in a cold dark place and discard after 24 hours.

Procedure (setting up the standard curve). To each of five 50-cc glass-stoppered graduated cylinders, add 1 cc of the control epinephrine solution, and to four of these add respectively 0.25, 0.50, 0.75, and 1.00 cc of the standard arterenol solution. To each cylinder add 1 cc. buffer solution, swirl, add 0.5 cc naphthoquinone reagent, swirl; let stand at room temperature for 45 minutes. Add 0.15 cc of the benzalkonium chloride (use of a calibrated dropping bottle is convenient), then add exactly 10 cc of the mixed solvent and shake thoroughly. Let stand for 45 minutes, shaking the mixtures gently at least five times more at regular intervals during this time. After the final shake, the solvent layer (purplish red in the presence of arterenol) should separate clearly. If it is turbid, transfer to a small centrifuge tube, stopper, and centrifuge for a few minutes to clarify. Transfer the extracts to appropriate colorimeter tubes and read the percentage transmittancy in a suitable photo-