A PROCEDURE FOR ESTIMATING THE RACEMISATION OF ADRENALINE OR NORADRENALINE IN DILUTE SOLUTION BY MEANS OF AN ION EXCHANGER

By Hans Hellberg

From the Chemical Department, the State Pharmaceutical Laboratory, Stockholm

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It is well known that the lævorotatory form of adrenaline is physiologically much more active than the dextrorotatory. Current textbooks of pharmacology state the quotient between the activities to be from 12 to 20. Therefore the pharmacopœias have definite requirements as to the rotatory power of (−)-adrenaline. In addition to the base certain pharmacopœias describe the (−)-adrenaline acid tartrate and usually require a definite optical rotation of the base isolated from the salt. On the other hand tests for rotatory power are never seen in the monographs of common dilute adrenaline solutions (1 :1000). This certainly depends on the angle of rotation of such a dilute adrenaline solution being too small (0·05° per dm.) to be usable as a test and the isolation or concentration of the base being difficult.

As early as 1933 Haddock[1] showed that acidic adrenaline solutions were easily racemised. In 1944 Berry and West[2] published an investigation of the stability of adrenaline solutions but without clearly separating the problem of racemisation from the problem of oxidation. They demonstrated that the adrenaline solution had maximum stability at *p*H 4 when kept in hermetically closed containers but found that solutions exposed to the air must have a lower *p*H in order not to become gradually oxidised. To-day *p*H values down to 2 seem to be usual for adrenaline solutions. However, here the risk of racemisation increases and a number of investigations have appeared in order to elucidate this question. Hald[3] worked with spray solutions 1 : 10 and Rosenblum, Goldman and Feldman[4] with more dilute solutions. Kisbye and Schou[5] have examined the question thoroughly and calculated the velocity constants for the racemisation at different *p*H values and temperatures. None of the investigations states clearly the stability of an adrenaline solution as to racemisation at room temperature but from the figures given by Kisbye and Schou it is possible to estimate approximately that a noticeable racemisation at least takes place in an adrenaline solution of *p*H 2 during storage for years. Certain pharmacopœias have considered this risk of racemisation. Thus the Danish pharmacopœia does not allow adrenaline solutions 1 : 1000 to have a *p*H below 2·2 and to be stored for more than a year. The U.S.P. XIV requires a biological assay of adrenaline solutions. In the Swedish state control of pharmaceutical specialities we have hitherto been of the opinion that a thorough examination of dilute adrenaline solutions with regard to the risk of racemisation ought to include a biological assay.

As to noradrenaline the problems are principally the same, but the risk of racemisation is not necessarily so great, because a solution of noradrenaline is stable to oxidation at higher *p*H values than adrenaline.

The need of a physical or physico-chemical test for racemisation in dilute adrenaline solutions has of course been felt for a long time. Rosenblum, Goldman and Feldman[4] suggested a concentration of the solution by vacuum evaporation. According to a statement made by Miller at a lecture[6] in Stockholm in 1953 the use of the melting point of the stable triacetyl derivative as a criterion of racemisation is contemplated in the U.S.A. Directions for the synthesis of this derivative have been given by Welsh.[7]

Some time ago there arose again in this laboratory the need for testing adrenaline solutions 1 : 1000 in respect to racemisation. The method described below was then worked out. The principal features are that the adrenaline is adsorbed on a suitable ion exchanger and subsequently eluted in a considerably concentrated form. The optical rotation of the solution obtained is determined in the usual way and the adrenaline content by a suitable photometric method.

As to the general theory of ion exchangers reference is made to Samuelson's monograph[8]. From papers by Winters and Kunin[9] and by Büchi and Furrer[10] it is evident that Amberlite IRC-50 (a cation-exchange resin with carboxyl groups as active centra) in its neutralised, ammonium-saturated form, has a certain exchange capacity towards alkaloidal cations and that a comparatively small quantity of hydrochloric acid is needed for the elution of the alkaloid.

Basic Experiments

The ion exchanger, Amberlite IRC-50, 44 mesh, used in this investigation, was transformed from its proton-form to its ammonium-form by treatment with an excess of 2M ammonia. The product was washed and air-dried. 2·5 g. of the material formed a column 9 × 130 mm. in a chromatographic tube. In order to minimise the "dead" volume below the column the outlet was a capillary provided with a stopcock.

The adsorption step. From 100 ml. of an 1 : 1000 adrenaline solution containing only 0·6 ml. of M hydrochloric acid all the adrenaline was retained by the resin and could not be washed out with water. However, if 0·75 per cent. of sodium chloride was incorporated with the solution about 10 per cent. of the adrenaline leaked through the column continuously. The further addition of 0·05 per cent. of sodium metabisulphite and 0·5 per cent. of chlorbutol to the solution did not alter the adsorption of adrenaline considerably. The quantity retained from 1 : 1000 solutions of current compositions thus seemed to be sufficient for the purpose intended.

The elution step. M hydrochloric acid was not strong enough to elute the adrenaline into a sufficiently small volume of liquid and 5M acid caused a noticeable racemisation in the eluate during the experiment. 2M hydrochloric acid, however, was quite satisfactory. Using this acid as elutriant the break-through curves for the hydronium ions and the chloride ions and the elution curve for 100 mg. of adrenaline were determined on an exchanger column of the size described. The break-through curves were obtained in experiments without adrenaline. The eluting liquid was

allowed to drip off with a velocity of 1 ml./minute and was collected in portions of 2 ml., free acid and chloride being determined in each by common titrimetric methods and the adrenaline by a photometric procedure. The curves obtained are represented in Figure 1. From this diagram it is possible to estimate the quantities of the different constituents to be found in the eluate at alternative ways of dividing it. By choosing the fraction 10 to 14 ml. it would be possible to obtain a concentration factor of up to 14 in respect of adrenaline. In our standard method, described below, the fraction 7 to 16 ml. (= 10·0 ml.) is taken out, which means a concentration factor of 8 or 9. The contents of electrolytes, as read from Figure 1, will be 0·2 8 M in respect of hydrochloric acid and 1·3 M in respect of ammonium chloride.

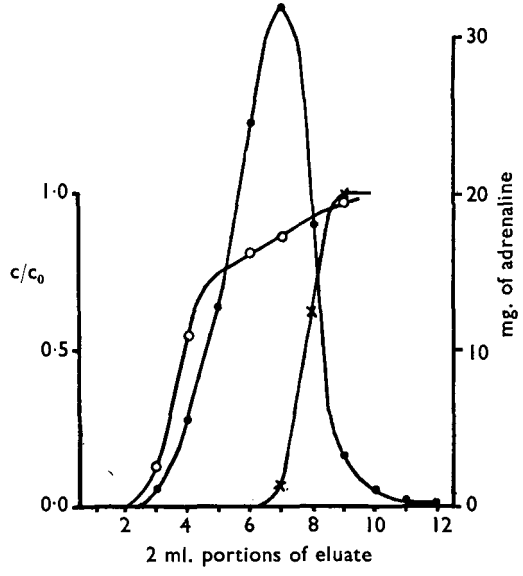

FIG. 1. Elution of ammonium-saturated Amberlite IRC-50, 44 mesh, with hydrochloric acid, 2M. Column 130 × 9 mm. Break-through curves for Cl^- —○— and H^+ —x—. Elution curve for 100 mg. of adrenaline —●—.

c = concentration of effluent.
c_0 = concentration of inffluent.

Parenthetically it may be mentioned that the sum of the adrenaline contents of the eluate portions pointed to a complete recovery of the adrenaline adsorbed on the column within a moderate quantity of eluate. Experiments to prove this observation were not made, because it was of minor importance for this work.

The risk of racemisation during the test. In the elution step the adrenaline is brought into contact with such strong hydrochloric acid that a noticeable racemisation is theoretically possible. In practice, however, the danger is not so great, because the first portion of hydrochloric acid (about 12 ml. in the standard experiment) is consumed at the exchange of ammonium ions in the column, but depending on the fraction of eluate chosen the concentration of hydrochloric acid may approach 2M. To get an idea of the risks some model experiments were made with adrenaline dissolved in different mixtures of 2M hydrochloric acid and 2M ammonium chloride. The results are shown in Figure 2, from which it is clear that the racemisation does not play any appreciable role even at such a

high concentration of hydrochloric acid as 1·5 M, because the determination of the optical rotation is accomplished within half an hour, counting from the beginning of the elution. As mentioned above the concentration of hydrochloric acid in the eluate will not be higher than 0·28 M in the standard method formulated below.

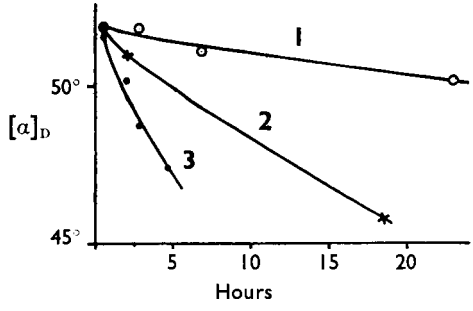

FIG. 2. Racemisation of adrenaline in mixtures of hydrochloric acid 2M and ammonium chloride 2M. Room temperature:

1. 1 vol. of 2M hydrochloric acid + 19 vol. 2M ammonium chloride.
2. 1 vol. of 2M hydrochloric acid + 3 vol. 2M ammonium chloride.
3. 3 vol. of 2M hydrochloric acid + 1 vol. 2M ammonium chloride.

Standard value for the specific rotation of (−)-adrenaline. Most pharmacopœias agree that the specific rotation of (−)-adrenaline shall be from −50° to −53° in 0·3 to 1 M hydrochloric acid. The products accepted by Swedish pharmacies during the last 6 years have shown an average specific rotation of −50·9° (−48·8° to −52·5°)[11]. As a standard value of the specific rotation of (−)-adrenaline −52° may serve, but when the real optical rotation of the raw material is known, it may sometimes be better to use this value in judging the solution.

A comparison between the specific angles of rotation for adrenaline in 0·5 M hydrochloric acid and in the actual solution of hydrochloric acid

TABLE I

COMPARISON OF THE SPECIFIC ROTATION OF ADRENALINE IN 0·5 M HYDROCHLORIC ACID AND IN SOLUTIONS CONTAINING HYDROCHLORIC ACID AND AMMONIUM CHLORIDE

Sample	2 per cent. of adrenaline in 0·5M hydrochloric acid; 2 dm. tube			1 per cent. of adrenaline in a solution 0·28 M as regards hydrochloric acid and 1·3 M as regards ammonium chloride; 2 dm. tube		
1	−48·3° −49·1°	−48·8°	Av. = −48·7°	−47·8°	−49·1°	Av. = −48·4°
2	−50·3° −50·7°	−50·7° −50·7°	Av. = −50·6°	−50·4° −49·3° −50·4°	−49·7° −50·1°	Av. = −50·0°

and ammonium chloride (0·28 M HCl and 1·3 M NH_4Cl) showed that for practical purposes it is possible to calculate with the same specific rotation (Table 1).

The possibility of removing interfering anions as sulphite or tartrate. In the exchange operation this was investigated separately. Both sulphite and tartrate are common ingredients in adrenaline solutions. Noticeable amounts of sulphite disturb certain methods of assay of adrenaline or

noradrenaline, and tartrate, if present, has to be eliminated because it has an optical rotation of its own.

Solutions, containing 100 mg. of sodium metabisulphite, 0·8 ml. of M hydrochloric acid and 0·75 g. of sodium chloride in 100 ml. were treated according to the standard method. In the hydrochloric acid eluates sulphite was determined iodometrically. In the 10 ml. portion of the eluate, which is used for the determination of the rotation, less than 0·1 mg. of the sulphite was found. Analogous experiments in which 100 mg. of ammonium acid tartrate was substituted for the metabisulphite were also made. Tartaric acid in the eluate was determined photometrically with dinitrophenylhydrazine after transformation of the tartaric acid to glyoxylic acid by periodate according to Adelberg.[12] In the actual 10 ml. portion of eluate less than 0·2 mg. of the tartrate was found. This means that the interfering anions sulphite and tartrate are practically removed from the adrenaline during the exchange process.

Final Methods

The basic experiments reported above lead to the following procedure, in this paper designated the standard method:—

Material. Amberlite IRC–50, 44-mesh, in ammonium-saturated form. 2 M hydrochloric acid.

Procedure. 2·5 g. of the exchange resin is poured into a chromatographic tube making a column of 9 × 130 mm. Adrenaline solution corresponding to 100 mg. of adrenaline is allowed to flow through the column with a velocity of 2 ml./minute and then the exchanger is washed with 100 ml. of water. The column is eluted by 2M hydrochloric acid, which is allowed to pass at a velocity of 1 ml./minute. The first 6 ml. is discarded and the following 10 is collected in a volumetric flask. The angle of rotation of this solution is determined immediately in a tube of the greatest possible length and afterwards the adrenaline content is assayed by a suitable method. The specific optical rotation is calculated.

One disadvantage of this method is that it requires a comparatively large amount of test solution. However, it is possible to diminish the scale. To-day we apply the method on a 3:10 scale, observing that the length of the column is kept at about 130 mm. by choosing a tube only about 5 mm. wide. For the determinations 3 ml. of the eluate (3rd to 5th ml.) are taken out. By the application of micro technique to the determination of optical rotation it may be possible to reduce the scale further.

For noradrenaline it is also possible to use the method in its standard form as well as in its diminished form.

Results. If the specific optical rotation of the adrenaline in a solution is too low by a certain percentage the decrease in the content of the lævorotatory form is only one half that percentage. Therefore it is not a great drawback that the results of the proposed methods are subject to an error of 3 to 5 per cent., arising partly from the determination of optical rotation, partly from the photometric assay of adrenaline.

In Tables II and III are collected the results obtained by the proposed methods on pure substances, on mixtures of (−)-adrenaline and (±)-adrenaline and on actual preparations taken from the Swedish market.

TABLE II

APPLICATION OF THE PROPOSED METHODS TO ADRENALINE, ADRENALINE ACID TARTRATE AND NORADRENALINE ACID TARTRATE

	Specific rotation of the base determined according to the proposed methods				
	Standard method			Diminished scale	
Adrenaline sample 1	−47·4°	−48·7°	−47·0°†		
$[\alpha]_D^{20°}$ = −48·7°, c = 2; 0·5 M HCl ..	−48·1°†	−46·4°†	−48·6°*		
Adrenaline sample 2	−52·5°*	−51·9°*		−50·6°†	
$[\alpha]_D^{20°}$ = −50·6; c = 2; 0·5 M HCl ..					
Adrenaline acid tartrate	−52·0°	−50·2°	−50·0°	−51·7°	−51·7°
Base, isolated according to B.P. 1953, had $[\alpha]_D^{20°}$ = −52·4°; c = 2; 0·5 M HCl ..					
Mixtures of (−)-adrenaline and (±)-adrenaline					
Calculated specific rotation: −25·1° ..				−25·1°	
−34·3° ..				−34·0°	
Noradrenaline acid tartrate					
Base, isolated as from the corresponding adrenaline salt, had $[\alpha]_D^{20°}$ = −45·7°; c = 2; 0·5 M HCl ..	−44·0°			−44·9°	

* Figure obtained from a solution containing 0·8 per cent. of M hydrochloric acid and 0·75 per cent. of sodium chloride.

† Figure obtained from a solution containing 0·8 per cent. of M hydrochloric acid, 0·75 per cent. of sodium chloride, 0·05 per cent. of sodium metabisulphite and 0·5 per cent. of chlorbutol.

Otherwise the figures are obtained from solutions in pure water; in the case of adrenaline base, containing the minimum quantity of hydrochloric acid.

TABLE III

APPLICATION OF THE PROPOSED METHODS TO ACTUAL PREPARATIONS ON THE SWEDISH MARKET

Preparations	Specific rotation of the base	*p*H	Age
Adrenaline solution (1:1000)—			
Factory No. 1	−45·4°	2·7	Less than 1 year
Factory No. 2	−42·6°	2·0	8 months
	−29·7°	2·1	3 years
Factory No. 3	−48·2°	2·7	Not known
Noradrenaline solution—			
1:500	−41·7°	3·2	Not known

SUMMARY

1. A procedure is described for the determination of the degree of racemisation of adrenaline and noradrenaline in dilute solutions.

2. The principal features are that the base is adsorbed on an ammonium-saturated cation exchange resin of carboxyl type (Amberlite IRC–50) and subsequently eluted by 2 M hydrochloric acid in a considerably concentrated form. The optical rotation and the adrenaline content of the concentrated solution are then determined by usual methods.

3. The interfering anions sulphite and tartrate are practically removed from the adrenaline during the exchange process.

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