

RESEARCH PAPERS

SPECTROPHOTOMETRIC STUDIES OF THE IODATE AND PERSULPHATE OXIDATIONS OF ADRENALINE AND NORADRENALINE

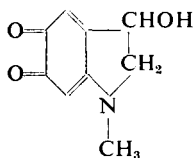
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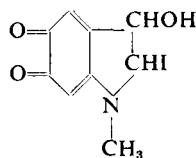
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INTRODUCTION

THE colorimetric determination of adrenaline has been the subject of many papers, a useful review of which has been made by Jackerott¹. Most of the suggested methods depend on the oxidation of adrenaline to a coloured substance, but one of the major difficulties in the use of the oxidation process is the necessity for the careful control and standardisation of the working conditions. It was therefore decided to subject the method to a spectrophotometric study, with a view to following the course of the reaction under different series of conditions. For this purpose the two oxidants chosen were potassium persulphate and potassium iodate which are probably the most popular. If potassium persulphate is used, the red primary oxidation product formed is adrenochrome, while in the case of oxidation by potassium iodate an iodine-substituted adrenochrome is obtained; this latter compound has been identified by Richter and Blaschko², as 2-iodo-3-hydroxy-1-methyl-2:3-dihydroindole-5:6-quinone.



Adrenochrome



Iodo-adrenochrome

Adrenochrome and its iodo-derivative have very similar, but not identical, absorption spectra, the difference being easily detectable by visual inspection of their solutions. The possibility of the occurrence of noradrenaline in a sample of pharmaceutical adrenaline made it necessary to study the behaviour on oxidation of this substance also. It was found that the oxidation of noradrenaline proceeds at a much slower rate than that of adrenaline, and this fact made it possible to obtain an approximation of the percentages of the two substances present when in admixture.

Von Euler and Hamburg³, using iodine as oxidant (which produces adrenochrome and not iodo-adrenochrome) found that at *pH* 4.0 adrenaline is quantitatively converted to adrenochrome in $1\frac{1}{2}$ minutes, but that only 10 per cent. of noradrenaline is so converted. They also

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found that 3 minutes treatment with iodine at pH 6.0 resulted in maximal formation of adrenochrome and noradrenochrome. These facts were used by them to estimate noradrenaline in the presence of adrenaline. For the accurate determination of noradrenaline in adrenaline the method of Auerbach and Angell⁴ is probably the best available at the moment, but it is a somewhat lengthy process, and the short simple method outlined in this paper, while making no pretence of superseding that of the two authors mentioned does, it is claimed, give an estimate of the amounts of noradrenaline and adrenaline in a mixture sufficiently accurate for any practical purpose.

METHODS OF OXIDATION

The method of persulphate oxidation employed was the accepted procedure originally proposed by Ewins⁵ and later modified by Barker, Eastland and Evers⁶, in which a buffer solution of pH 5.5 is used, containing 0.239 per cent. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.937 per cent. $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.0 per cent. sodium chloride and 0.2 per cent. potassium persulphate; the oxidation is effected by mixing equal volumes of adrenaline solution and buffer at 22° C. and allowing to stand for 30 minutes. For the iodate oxidation a modification of the procedure described by Jackerott was used; 5 ml. of a dilute adrenaline solution are mixed with 1 ml. of 5 per cent. phosphoric acid and 4 ml. of 0.0125 molar potassium iodate solution, the mixture is heated in a boiling water bath for 60 seconds and cooled immediately: it is extremely important that the water in the bath should actually be boiling. The standard adrenaline solution was prepared either from adrenaline bitartrate or from adrenaline base using the calculated quantity of 0.1 N sulphuric acid required to effect solution. A solution containing 10 mg. per cent. was found to be convenient because when diluted for oxidation with an equal volume of reagent, the final concentration of 5 mg. per cent. is suitable for use in the spectrophotometer; the expression $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ proved to be very useful for the comparison of spectrophotometric data.

SPECTROSCOPIC PROPERTIES OF ADRENOCHROME AND IODO-ADRENOCHROME

It was decided to prepare both adrenochrome and its iodine-substituted derivative and determine their spectroscopic characteristics. Adrenochrome was prepared by a method described by Harley-Mason⁷, and the iodo-compound by one published by Richter and Blaschko²; repeated preparation of these compounds by the methods quoted, gave the same products in so far as they quantitatively exhibited the same absorption spectra. Analyses of the compounds obtained by Harley-Mason⁷, and Richter and Blaschko², are given by these authors in the original papers, and in both, agree quite well with theoretical considerations.

Adrenochrome gave maxima at 220, 302 and 485 $\mu\mu$, the ratios of the absorption values at these peaks being 5.53:2.45:1.00; the iodo-compound gave maxima at 233, 305 and 520 $\mu\mu$, with ratios of 5.83:2.94:1.00. The $E_{1\text{ cm.}}^{1\text{ per cent.}}$ values obtained for the visible peaks were

231 for adrenochrome and 111 for its iodine derivative. The $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ λ_{max} values obtained in the visible region when adrenaline was subjected to persulphate and iodate oxidations respectively were approximately 0.930 and 0.810; as 1 g. of adrenaline produces on oxidation 0.978 g. of adrenochrome or 1.666 g. of iodo-adrenochrome, it therefore follows that the average conversions obtained at the times of measurement for the persulphate and iodate oxidations, under the conditions described

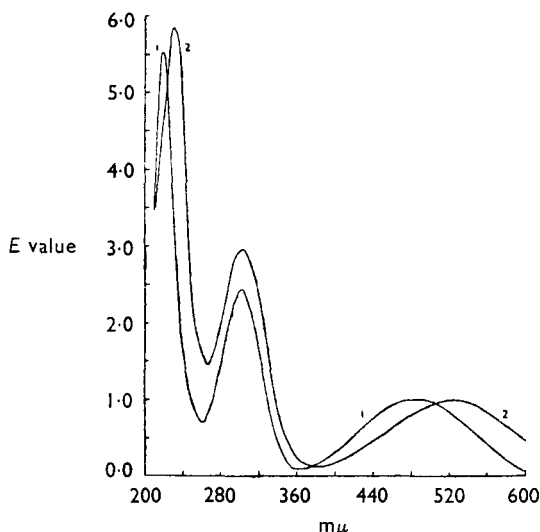


FIG. 1. Absorption spectra of adrenochrome (1) and its iodine-substituted derivate (2).

in these methods, represented approximately 82 and 88 per cent. of the theoretical total conversions respectively. The absorption curves of adrenochrome and its iodo-derivative are illustrated in Figure 1, and are identical in characteristics with those obtained in the persulphate and iodate assays under the conditions at which they are carried out. The colorimetric assay is obviously based upon the absorption band in the visible spectrum, but there is no reason why the other two peaks in the ultra-violet region should not be used for a spectrophotometric assay. The peak in the far ultra-violet region, however, seems to be unsuitable because of the irrelevant absorption frequently encountered at these wavelengths; the peak in the 300 $m\mu$ region could be used with the advantage of a threefold increase in sensitivity over the peak in the visible region.

PECULIARITIES OF IODATE AND PERSULPHATE OXIDATIONS

It will be convenient at this stage to consider in detail some aspects that are peculiar to either the persulphate or iodate oxidations.

(a) Potassium Persulphate Oxidation

Using the standard method of potassium persulphate oxidation at pH 5.5, the peak in the visible spectrum for adrenochrome was slightly displaced from 485 to 490 $m\mu$, consequently all measurements of $E\lambda_{\text{max}}$ were taken at this wavelength. It has long been known that the oxidation process is greatly affected by variation of temperature. At 22° C. the

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oxidation reached its maximum after about 20 minutes and then gradually the indicated adrenochrome concentration began to fall, so that the reading taken at 30 minutes was slightly less than the maximum value obtained; but for obvious reasons it represents a point of greater stability for measurement in a comparative assay. The results obtained for the

TABLE I
POTASSIUM PERSULPHATE OXIDATION AT 22° C. FOR DIFFERENT SAMPLES
OF ADRENALINE AND NORADRENALINE

Sample	$E_{1\text{ cm.}}^{0.5\text{ mg. per cent. } 490\text{ m}\mu\text{ value}}$			
	Time of oxidation in minutes			
	20	25	30	35
(-)-Adrenaline (synthetic)	0.940	0.937	0.925	0.915
	0.940	0.930	0.912	0.902
(-)-Adrenaline (A)	0.940	0.935	0.920	0.912
	0.935	0.935	0.920	0.910
	0.930	0.947	0.935	0.920
	0.915	0.937	0.947	0.925
.. (B)	0.922	0.910	0.890	0.880
	0.915	0.920	0.912	0.900
.. (C)	0.912	0.900	0.885	0.872
	0.910	0.920	0.910	0.887
Adrenaline B.P. (suspected to contain noradrenaline)	0.763	0.817	0.852	0.870
	0.820	0.855	0.870	0.880
(-)-Noradrenaline	0.137	0.153	0.175	0.200
	0.133	0.159	0.191	0.220
	0.130	0.157	0.186	0.219

$E_{1\text{ cm.}}^{0.5\text{ mg. per cent. } 490\text{ m}\mu\text{ values}}$ of various samples of adrenaline and noradrenaline are given in Table I. The oxidation of noradrenaline proceeded at a much slower rate than that of adrenaline, and this fact is illustrated by Figure 2. In the same diagram the effect of the presence of metals upon the oxidation is shown; copper accelerated and iron retarded the oxidation of both adrenaline and noradrenaline. For mixtures of adrenaline and noradrenaline the $E_{1\text{ cm.}}^{0.5\text{ mg. per cent. } 490\text{ m}\mu\text{ value}}$ fell as the proportion of noradrenaline in the mixture increased and some results obtained for such mixtures are given in Table II.

TABLE II
POTASSIUM PERSULPHATE OXIDATION AT 22° C. SHOWING $E_{1\text{ cm.}}^{0.5\text{ mg. per cent. } 490\text{ m}\mu\text{ VALUES}}$
FOR MIXTURES OF ADRENALINE AND NORADRENALINE

Mixture		$E_{1\text{ cm.}}^{0.5\text{ mg. per cent. } 490\text{ m}\mu\text{ value}}$			
		Time of oxidation in minutes			
		20	25	30	35
Adrenaline	Noradrenaline				
95	5	0.922	0.922	0.920	0.912
90	10	0.898	0.902	0.898	0.895
85	15	0.825	0.853	0.864	0.871
80	20	0.768	0.806	0.826	0.838

(b) Potassium Iodate Oxidation

With iodate oxidation the experimental conditions are not so critical, for we found that the concentrations of potassium iodate and phosphoric acid could be varied within wide limits and reproducibility still be achieved.

Furthermore, unlike the persulphate oxidation the effects of copper and iron were found to be negligible. Luhr and Rutschel⁸ compared the results of the iodate method with biological examinations and found that

there was good agreement between them. The time of immersion in the water bath and the necessity for maintaining a good "rolling boil" were really the only critical factors in the oxidation. Under the conditions of iodate oxidation and the measurement of the absorption in phosphoric acid solution the absorption maximum in the visible spectrum was displaced slightly from 520-5 to 530 $m\mu$, and accordingly $E\lambda_{max}$ values were measured at this wavelength.

The relationship between the $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ 530 $m\mu$ value and the time of oxidation is illustrated by Figure 3; in the case of adrenaline the absorption increased rapidly to reach its maximum at about 60 seconds and there was little significant difference between the values obtained over the range 60 to 80 seconds.

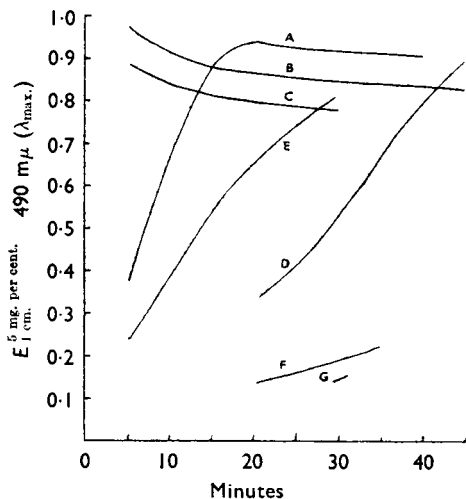


FIG. 2. Potassium persulphate oxidation at 22° C. showing relationship of the absorption at 490 $m\mu$ ($\lambda_{max.}$) with time for adrenaline and noradrenaline and the effect of copper and iron.

- A. Adrenaline, normal curve.
- B. " 10 p.p.m. copper.
- C. " 1 " " "
- D. " 10 " " "
- E. Noradrenaline, 10 p.p.m. copper.
- F. " normal curve.
- G. " 10 p.p.m. iron.

Even at 120 seconds the value was only 10 per cent. below its peak value, on the other hand, the oxidation of noradrenaline proceeded at a much slower rate. The results obtained with mixtures of these two substances are set out in Table III; a mixture of 20 per cent. noradrenaline and 80 per cent. adrenaline had a higher absorption value at 120 seconds than at 60 seconds; for a 15:85 mixture the absorption was much the same at 60 and 120 seconds, but its maximum value was reached at 100 to 110 seconds; a mixture containing 10 per cent.

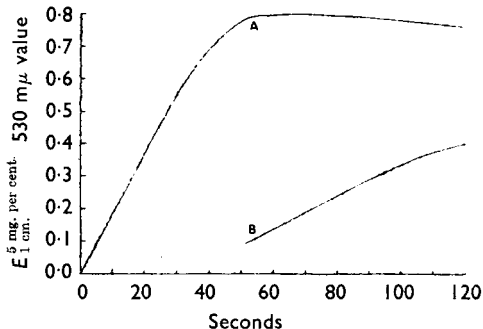


FIG. 3. Potassium iodate oxidation showing relationship between $E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ 530 $m\mu$ and time of oxidation for A, adrenaline and B, noradrenaline.

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of noradrenaline had its period of constant absorption prolonged over the range 60 to 100 seconds. Much useful information on a particular sample of adrenaline B.P. (which may contain noradrenaline) can be obtained from a study of the effects of different periods of oxidation upon both the magnitude of $E\lambda_{\max}$ and the shape of the absorption curve itself. It should be possible from an examination of data of this type to detect the presence of 10 per cent. of noradrenaline in a sample of adrenaline

TABLE III
POTASSIUM IODATE OXIDATION OF SYNTHETIC (—)-ADRENALINE, (—)-NORADRENALINE AND MIXTURES OF THE TWO

Substance		$E_{1\text{ cm.}}^{5\text{ mg. per cent. } 530\text{ m}\mu}$ value								
		Time of oxidation in seconds								
		50	60	70	80	90	100	110	120	180
(—)-Adrenaline (synthetic)		0.786	0.800	0.797	0.796	0.790	0.778	0.772	0.766	0.710
(—)-Noradrenaline		0.087	0.137	0.200	0.241	0.275	0.326	0.376	0.398	0.464
Mixtures of the above										
Adrenaline	Noradrenaline									
80	20	0.658	0.668	0.678	0.677	0.678	0.680	0.680	0.683	0.658
85	15	0.660	0.696	0.696	0.700	0.702	0.705	0.706	0.698	0.666
90	10	0.691	0.738	0.734	0.732	0.732	0.730	0.720	0.720	0.703
95	5	0.728	0.766	0.766	0.768	0.763	0.761	0.755	0.750	0.702

B.P. If, however, one starts with a solid which is known to be a mixture of adrenaline and noradrenaline and nothing else, then it is possible from a determination of the E value at λ_{\max} alone to assess the proportions of the two substances in the mixture. The complete absorption curves obtained for varying times of oxidation from 1 to 40 minutes for both adrenaline and noradrenaline are given in Figures 4 and 5. In the case of adrenaline there was a rapid rise to a maximum at about 1 minute after which the peaks fell slowly at first, accompanied by a corresponding rise in the absorption at λ_{\min} around $390\text{ m}\mu$; the absorption in this region rose rapidly as the peaks in the visible and ultra-violet regions fell. A very good internal check of the stage of oxidation reached may be obtained by examining the relationship between the absorption at λ_{\max} and λ_{\min} . This also illustrates the fact that under controlled conditions one obtains the typical red coloured primary oxidation product; the occurrence of secondary oxidation is shown by the more yellowish solutions obtained when this stage is reached. With noradrenaline the maximum absorption was reached in about 3 minutes, but its magnitude was still only just over 60 per cent. of that reached by adrenaline at its maximum; as time increased there was a general rise in the absorption on the short wave side of the peak as that of the peak itself fell, in much the same manner as for adrenaline.

In Table IV we record data for the $E_{1\text{ cm.}}^{5\text{ mg. per cent. } 530\text{ m}\mu}$ values obtained by 1 minute iodate oxidation on various samples of adrenaline supplied as laboratory reagents, including a sample of synthetic (—)-adrenaline and also one of (+)-adrenaline together with samples of both (\pm)- and

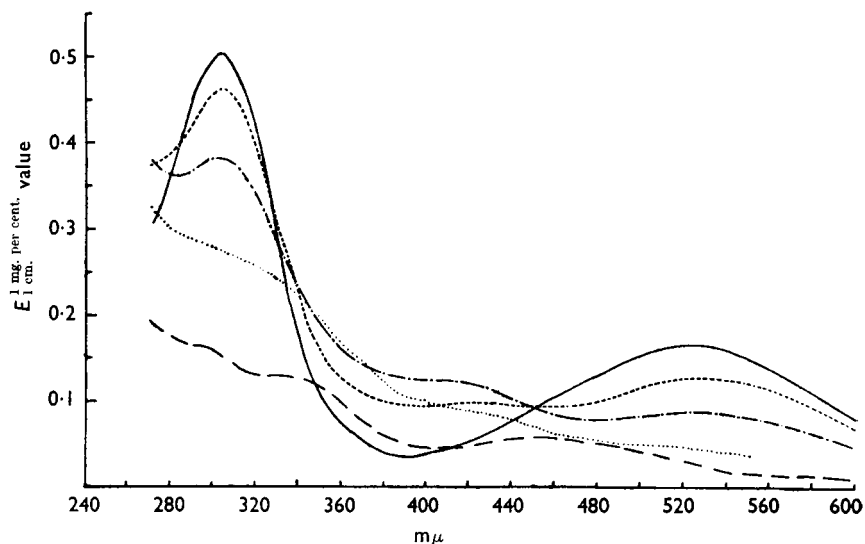


FIG. 4. Potassium iodate oxidation of adrenaline showing the effect of the time of oxidation on the $E_{1 \text{ cm.}}^{1 \text{ mg. per cent.}}$ value.

————— 1 minute. 20 minutes.
 - - - - - 5 minutes. — — — 40 " "
 - 10 " "

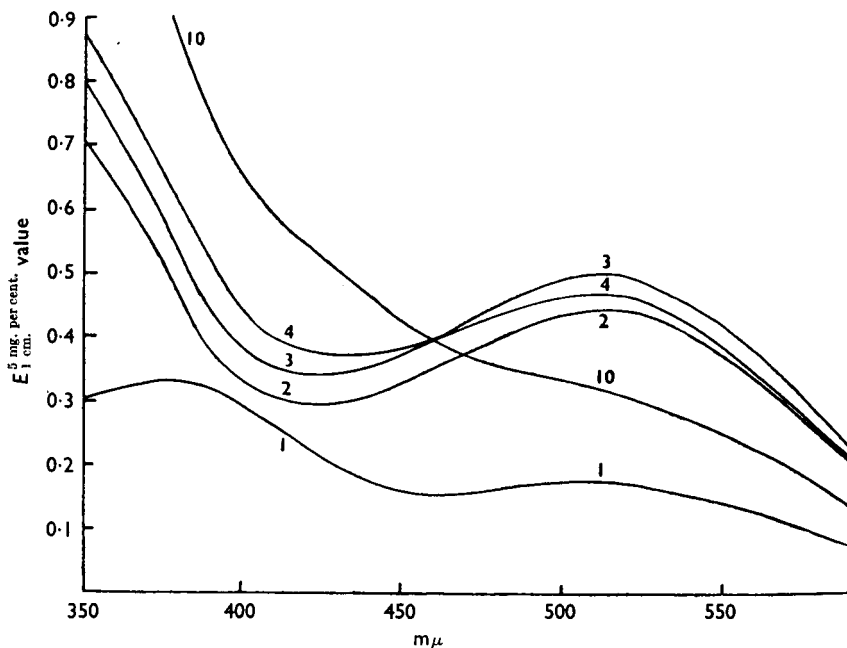


FIG. 5. Potassium iodate oxidation of noradrenaline showing the relationship between absorption and time of oxidation (given in minutes by the figures on the curves).

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TABLE IV

 POTASSIUM IODATE OXIDATION OF VARIOUS SAMPLES OF ADRENALINE AND
 NORADRENALINE; $E_{1\text{cm.}}^{1\text{mg. per cent.}}$ 530 $m\mu$ VALUES AT 1 MINUTE

Sample							
(-)-Adrenaline (synthetic)	0-810	0-812	0-815	0-805	0-812		
(-)-Adrenaline	0-812	0-815	0-800	0-800			
(-)-Adrenaline (A)	0-820	0-835	0-830	0-812	0-805	0-805	
.. (B)	0-798	0-792	0-812	0-805			
.. (C) (i)	0-800	0-803	0-822	0-813	0-820		
.. (ii)	0-800	0-805					
.. (D)	0-805	0-810					
(-)-Adrenaline bitartrate	0-812	0-812	0-815	0-815			
Adrenaline B.P. (suspected to contain noradrenaline)	0-736	0-730	0-730	0-738	0-735	0-750	0-750
(-)-Noradrenaline bitartrate	0-135	0-138					
(±)-Noradrenaline bitartrate	0-137	0-132	0-150	0-148			

(-)-noradrenaline. The agreement although not exact is extremely good, and most of the samples of adrenaline appear to be free from noradrenaline except one supplied as of B.P. quality. This gave a significantly lower E value, which on detailed examination proved to be due to the presence of noradrenaline, estimated very approximately at somewhere in the 10 to 15 per cent. region.

 THE APPROXIMATE ASSESSMENT OF ADRENALINE AND
 NORADRENALINE IN MIXTURES

Since the rates of oxidation of adrenaline and noradrenaline by potassium iodate are significantly different and the changes in the absorptions of the pure substances with time are known, it is possible to calculate the proportions present in a mixture by comparing the changes in its absorption at a suitable wavelength between two times t_1 and t_2 .

If an exact determination of the noradrenaline and adrenaline content of a mixture is required the method of Auerbach and Angell already referred to should be used; there are, however, times when an approximation is all that is required, in such cases the following method is suitable because the necessary information can be obtained in minutes rather than in hours required for the application of the former method.

Assume that the following data are available.

Adrenaline: ratio of absorption at t_2 to absorption at t_1 is R_A

Noradrenaline: ratio of absorption at t_2 to absorption at t_1 is R_N

Mixture: ratio of absorption at t_2 to absorption at t_1 is R_s .

Let x be the absorption due to adrenaline at t_1 ,

then, xR_A is the absorption due to adrenaline at t_2 ,

and, if y is the absorption due to noradrenaline at t_1 ,

then, yR_N is the absorption due to noradrenaline at t_2 .

If, b is the absorption of the mixture at t_1 , then $b = x + y$,

and, if a is the absorption of the mixture at t_2 , then $a = xR_A + yR_N$.

Now $R_s = a/b = (xR_A + yR_N)/(x + y)$.

$$\text{Hence } x = \frac{R_s - R_N}{R_A - R_N} \cdot b = \frac{R_s - R_N}{R_A - R_N} \cdot (b - x).$$

Thus
$$x = \frac{(R_N b - a)}{(R_N - R_A)}$$

The $E_{1 \text{ cm.}}^{5 \text{ mg. per cent.}}$ 530 $m\mu$ values obtained for the potassium iodate oxidations of adrenaline and noradrenaline at different values of t from 1 to 7 minutes are given in Table V; each value represents the mean of 24 determinations. If the data are examined in order to choose

TABLE V

$E_{1 \text{ cm.}}^{5 \text{ mg. per cent.}}$ 530 $m\mu$ VALUES FOR THE POTASSIUM IODATE OXIDATION OF ADRENALINE AND NORADRENALINE AT DIFFERENT VALUES OF t

t in minutes	1	2	3	4	5	6	7
(-)-Noradrenaline	0.141	0.384	0.458	0.450	0.414	0.379	0.351
(-)-Adrenaline	0.811	0.767	0.719	0.676	0.619	0.580	0.529

TABLE VI

APPLICATION OF THE METHOD TO MIXTURES OF ADRENALINE AND NORADRENALINE OF KNOWN COMPOSITION

Composition of mixture, per cent.		$E_{1 \text{ cm.}}^{5 \text{ mg. per cent.}}$ 530 $m\mu$ value		Determined composition, per cent.	
Adrenaline	Noradrenaline	1 minute	5 minutes	Adrenaline	Noradrenaline
90	10	0.728	0.562	89	6
		0.732	0.575		
		0.730	0.577		
		0.730	0.586		
		0.732	0.572		
		0.730	0.570		
	Mean 0.730	0.574			
75	25	0.637	0.562	75	23
		0.645	0.565		
		0.640	0.557		
		0.642	0.548		
		0.644	0.555		
		0.636	0.568		
	Mean 0.641	0.559			
50	50	0.460	0.497	49	46
		0.460	0.490		
		0.462	0.493		
		0.458	0.488		
		0.460	0.485		
		0.453	0.490		
	Mean 0.459	0.491			
25	75	0.312	0.468	25	77
		0.300	0.468		
		0.308	0.473		
		0.308	0.473		
		0.316	0.472		
		0.302	0.473		
	Mean 0.308	0.471			
10	90	0.203	0.442	11	91
		0.203	0.442		
		0.215	0.442		
		0.215	0.431		
		0.230	0.440		
		0.230	0.442		
	Mean 0.216	0.440			
Unknown sample labelled B.P.		0.725	0.602	87	14
		0.727	0.606		
		0.727	0.592		
		0.728	0.590		
		0.720	0.594		
		0.728	0.590		
		0.728	0.590		
			Mean 0.726		

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suitable values for t_1 and t_2 we find that the ratios of the absorptions at 7 minutes to those at 4 minutes are the same for the two substances, hence t_1 must be less than 4 minutes; the times 1 and 5 minutes seem to be as good as any and these were the values for t_1 and t_2 selected. Using the standard data,

$E_{1\text{ cm.}}^{5\text{ mg. per cent.}}$ 530 $m\mu$; adrenaline 1 minute 0.811, 5 minutes 0.619;
noradrenaline 1 minute 0.141, 5 minutes 0.414:

so that, R_A is 0.763 and R_N is 2.936, the general equation becomes:

$$x = (2.936b - a)/2.173.$$

The above method was then applied to mixtures of adrenaline and noradrenaline and the results obtained are shown in Table VI, and the agreements with theory were good. It must be stressed, however, that the solutions used were of the pure substances and consequently deductions made by application of the method to impure extracts would not of necessity be valid.

SUMMARY

1. A spectrophotometric study of the persulphate and iodate oxidations of adrenaline and noradrenaline has been made; adrenochrome and its iodine-substituted derivative have been prepared and studied.

2. The spectrophotometric examination of the coloured solutions given by adrenaline on oxidation seems in certain cases to offer a more logical approach to the use of this reaction for the assay of the hormone since the shape of the absorption curve gives an internal check on whether or not the primary oxidation stage has been passed and secondary oxidation commenced.

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