

**439.** *Complexes formed by Adrenaline and Related Compounds with Transition-metal Ions. Part I. Acid Dissociation Constants of the Ligands*

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The first and second dissociation constants of adrenaline, noradrenaline, 2-amino-1-phenylethanol, and catechol have been determined in aqueous solution at 25° by means of a glass electrode. The further ionisation of adrenaline and of noradrenaline in aqueous solution is considered, and the interference that arises in quantitative work from the oxidation of these compounds to adrenochrome and melanin-like compounds is discussed.

ALTHOUGH the chemical and pharmacological properties of adrenaline and noradrenaline have been extensively studied (see, *e.g.*, ref. 1, and references therein), their properties as metal-complexing agents, although mentioned,<sup>2,3</sup> do not appear to have been studied in detail. This is in part due to the readily oxidisable nature of adrenaline,<sup>4</sup> and, in fact, it

<sup>1</sup> West, *J. Pharm. Pharmacol.*, 1955, **7**, 81.

<sup>2</sup> Green, Mazur, and Shorr, *J. Biol. Chem.*, 1956, **220**, 237.

<sup>3</sup> Vogeler, *Arch. exp. Path. Pharmacol.*, 1940, **194**, 281.

<sup>4</sup> Bu'Lock and Harley-Mason, *J.*, 1951, 712.

has been suggested that the oxidation of adrenaline by copper(II) ions proceeds *via* a copper-adrenaline complex.<sup>5</sup> Other reported reactions of adrenaline include those with uranyl ions,<sup>6</sup> selenium dioxide,<sup>7</sup> and tetrathiocyanatomercurate(II).<sup>8</sup>

In order to facilitate the study of the relatively complicated adrenaline and noradrenaline, the present work was extended to cover some related, simpler ligands, and these are listed, together with convenient abbreviations, in Table 1. The abbreviations take into

TABLE 1  
Adrenaline and related ligands

Ligand	Abbreviation	Atoms available for co-ordination
Adrenalinium ion .....	adH <sub>4</sub> <sup>+</sup>	One N, three O
Noradrenalinium ion .....	nadH <sub>4</sub> <sup>+</sup>	One N, three O
2-Amino-1-phenylethanol cation .....	apeH <sub>2</sub> <sup>+</sup>	One N, one O
Catechol .....	catH <sub>2</sub>	Two O

account the theoretical maximum number of ionisable protons in aqueous solution, disregarding any practical evidence, since in this way any subsequent ionisation that may be dependent on complex-formation is allowed for.

Unfortunately, the series of ligands studied is incomplete, as we were unable to synthesise 2-methylamino-1-phenylethanol in sufficient yield or purity for accurate measurements to be made with it. Of the simpler ligands chosen, only catechol has been studied previously.<sup>9</sup>

Adrenaline and noradrenaline are comparatively stable in acid solution, but decompose slowly in solutions of high pH, as is indicated by the development of a brown colour. However, the extent of this decomposition was very small over the time taken for a titration, so that corrections of the type used in the study of the  $\alpha$ -hydroxyamidinium ions<sup>10</sup> were unnecessary.

## RESULTS

It was not possible to titrate solutions more than about  $10^{-2}$ M in ligand, because of precipitation; in the case of adrenaline, there was always precipitation in the pH range 9–10, but this became obvious as the titration proceeded. The resulting gap in the pH data could, however, be minimised by adding excess of sodium hydroxide just before the precipitation point, and back-titrating with 0.1M-hydrochloric acid.

The average number of protons abstracted from each ligand,  $g$ , was then calculated, and the titration curves were plotted, as shown in Figure 1. In the case of adH<sub>4</sub><sup>+</sup> and of nadH<sub>4</sub><sup>+</sup>, the curves are not symmetrical about  $g = 0.5$ , and are neither asymptotic to  $g = 1.0$  nor to  $g = 2.0$ , but are merely inflected at  $g = 2.0$ . This indicates two dissociation constants, fairly close together,<sup>10</sup> with further ionisation at high pH. The values of these constants were calculated by means of a curve-fitting method,<sup>11</sup> a "strip" technique<sup>12</sup> being employed when two  $pK$  values differed by less than 2.5; the relevant  $pK$  values are given in Table 2, and the corresponding theoretical curves shown in Figure 1. It was not possible to make more than an estimate of  $pK_3$  and  $pK_4$ .

There is clear evidence from Figure 1 that, in the case of apeH<sub>2</sub><sup>+</sup> and of catH<sub>2</sub>, both protons are ionised, and that the constants are fairly widely separated from each other (Table 2). The accuracy of the fit of the theoretical curves to the experimental results for these dibasic acids, at pH values of 11 and above, adds weight to the conclusion that more than two protons are removed from adH<sub>4</sub><sup>+</sup> and from nadH<sub>4</sub><sup>+</sup> (Figure 1).

<sup>5</sup> Bouvet, *Bull. Soc. Chim. biol.*, 1949, **31**, 1301.

<sup>6</sup> Cohen, *Compt. rend.*, 1946, **222**, 890.

<sup>7</sup> Giral, *Ciencia*, 1948, **9**, 127.

<sup>8</sup> Morvay, Császár, and Almasy, *Z. analyt. Chem.*, 1958, **162**, 187.

<sup>9</sup> Timberlake, *J.*, 1957, 4987.

<sup>10</sup> Gould and Jameson, *J.*, 1962, 296.

<sup>11</sup> Rossotti and Rossotti, *Acta Chem. Scand.*, 1955, **9**, 1166.

<sup>12</sup> Rossotti, Rossotti, and Sillén, *Acta Chem. Scand.*, 1956, **10**, 203.

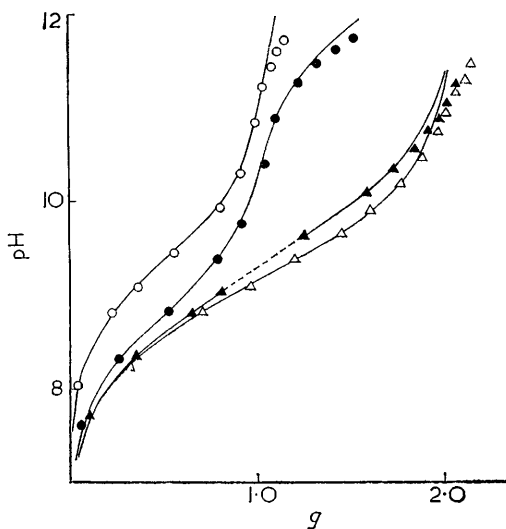


FIGURE 1. pH Titrations of the ligands. Theoretical curves calculated for  $pK_1$  and  $pK_2$  in Table 2, and some experimental points

○ =  $\text{catH}_2$ . ● =  $\text{apeH}_2^+$   
 △ =  $\text{nadH}_4^+$ . ▲ =  $\text{adH}_4^+$

TABLE 2

Acid dissociation constants for the ligands, measured at an ionic strength of 0.1 at 25°

Ligand	$pK_1$	$pK_2$	$pK_3$	$pK_4$
$\text{adH}_4^+$	$8.66 \pm 0.01$	$9.95 \pm 0.05$	ca. 13	ca. 13
$\text{nadH}_4^+$	$8.64 \pm 0.01$	$9.70 \pm 0.05$	ca. 13	ca. 13
$\text{apeH}_2^+$	$8.79 \pm 0.01$	$11.90 \pm 0.05$	—	—
$\text{catH}_2$	$9.37 \pm 0.01$	$12.8 \pm 0.2$	—	—

#### DISCUSSION

*Dissociation of the Acids.*—The values of  $pK_1$  and  $pK_2$  for  $\text{adH}_4^+$  and for  $\text{nadH}_4^+$  (Table 2) are in good agreement with those reported by Lewis<sup>13</sup> (for  $\text{adH}_4^+$   $pK_1 = 8.71$ ,  $pK_2 = 9.90$ ; for  $\text{nadH}_4^+$   $pK_1 = 8.73$ ,  $pK_2 = 9.78$ ), although he used a less rigorous method of computation; the results for  $\text{catH}_2$  confirm those of Timberlake<sup>9</sup> ( $pK_1 = 9.45$ ,  $pK_2 = 12.8$ ). A comparison of  $pK_1$  for  $\text{catH}_2$  with  $pK_2$  for  $\text{adH}_4^+$  and for  $\text{nadH}_4^+$  (Table 2) strongly suggests that the last two values refer to the ionisation of one of the phenolic groups in each case. Similarly,  $pK_1$  for  $\text{adH}_4^+$  and for  $\text{nadH}_4^+$  agrees well with  $pK_1$  for  $\text{apeH}_2^+$  and with the values for the ionisation of the  $-\text{NH}_3^+$  proton in related substances such as ethanolamine.<sup>14</sup> What, on the other hand, is perhaps a little surprising is the close similarity between the ionisation of the primary amine group of  $\text{nadH}_4^+$  and that of the secondary amine group of  $\text{adH}_4^+$ , although this is in accord with the findings of Lewis.<sup>13</sup>

pH Values above 11 are of considerably decreasing accuracy, and in the method of computation employed, the hydroxyl-ion concentration becomes a significant term in the calculation of  $g$ . Nevertheless, there is little doubt that further ionisation of  $\text{adH}_4^+$  and of  $\text{nadH}_4^+$  takes place in aqueous solution (Figure 1). Unfortunately, the values of  $pK_2$  for  $\text{catH}_2$  and for  $\text{apeH}_2^+$  (Table 2) suggest that  $pK_3$  and  $pK_4$  for  $\text{adH}_4^+$  and for  $\text{nadH}_4^+$  would be not only close together, but rather high for accurate evaluation. The titration curves indicate that, if this reasoning is valid,  $pK_3 \approx pK_4 \approx 13$  (Figure 1; Table 2).

*Oxidation of Adrenaline and Noradrenaline.*—The oxidation of adrenaline and noradrenaline to adrenochrome and a mixture of melanin-like compounds, even in a stream of nitrogen, decreased the accuracy of the present work, although the effects could be minimised by carrying out the titrations in as short a time as possible, especially as high pH

<sup>13</sup> Lewis, *Brit. J. Pharmacol.*, 1954, **9**, 488.

<sup>14</sup> Bjerrum, Schwarzenbach, and Sillén, "Stability Constants," Part I, *Chem. Soc. Special Publ.*, No. 6, 1957, p. 9.

values were encountered towards the end of a titration. Even in the case of catechol, there was a suspicion of autoxidation, although only at high pH, and a similar spectrum to that of a catechol polymer reported by Hathway and Seakins,<sup>15</sup> in the autoxidation of catechol, was obtained when the pH was much above 10. This is similar to the spectra of melanin obtained from adrenaline and noradrenaline, and indeed both polymerisations proceed *via* the quinone.<sup>4,15</sup>

It was found that the oxidation products existed in even freshly prepared solutions, this being shown by the characteristic spectra (Figure 2a). Solutions less than 0.002M in

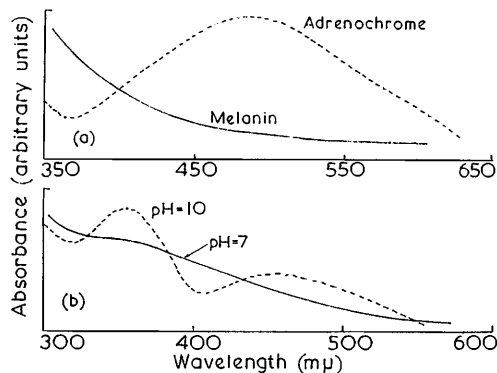
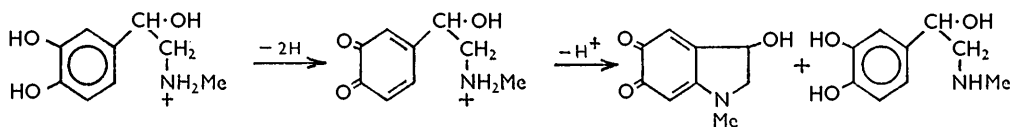
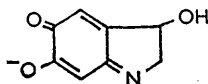


FIGURE 2. (a) Spectra of adrenochrome and melanin. (b) Effect of pH on the spectrum of noradrenaline

adrenaline were visibly pink at high pH, and gave the characteristic spectrum of adrenochrome, an effect not noted in the case of noradrenaline. At a pH of about 7, there was no indication of adrenochrome being formed, whereas, at about pH 10, its spectrum (Figure 2a) was clearly seen. Bu'Lock and Harley-Mason<sup>4</sup> have suggested that, in alkaline solution, adrenaline and noradrenaline can exist in an indole form; the  $\text{adH}_4^+$  is oxidised to give a quinone, which cyclises to give an indole or goes to adrenochrome:



In the case of noradrenaline, at pH 7, a spectrum similar to the melanin spectrum obtained from adrenaline (Figure 2a) was found (Figure 2b), whereas, at pH 10, a completely different spectrum was obtained (Figure 2b), probably owing to the auto-oxidation of noradrenaline occurring in a different manner from that of adrenaline. This bears out Bu'Lock and Harley-Mason's work,<sup>4</sup> which suggests that addition of alkali to  $\text{nadH}_4^+$  gives, *via* the corresponding oxidised quinone, the anion



The ultraviolet absorption spectra obtained for the unoxidised materials were in agreement with those reported by Buzard and Nytch<sup>16</sup> for noradrenaline and by Morvay *et al.*<sup>8</sup> for adrenaline.

The other ligands studied were much more stable towards oxidation, and normal precautions to exclude oxygen sufficed to eliminate any oxidation during a titration.

<sup>15</sup> Hathway and Seakins, *J.*, 1957, 1562.

<sup>16</sup> Buzard and Nytch, *J. Biol. Chem.*, 1959, **234**, 884.

## EXPERIMENTAL

*Apparatus.*—pH Measurements were made by using a "Doran" precision pH-meter reading to 0.001 pH unit and reproducible to 0.003 unit. An "Alkacid" glass electrode was used in conjunction with a saturated calomel electrode having a ground-glass junction. All leads were shielded, and an oil-filled thermostat bath kept the titration cells at  $25.0^\circ \pm 0.1^\circ$ . Solutions were made up in either water (in the case of catechol) or in dilute hydrochloric acid (in the case of the other ligands), in order to start the titrations with the fully protonated species, and the overall ionic strength was adjusted to 0.1 with potassium chloride. The solutions were stirred by means of a stream of oxygen-free nitrogen, which also minimised the extent of oxidation. 0.05M-Potassium hydrogen phthalate was used as a primary standard (pH = 4.005 at 25°), with 0.01M-borax as a secondary standard for the higher pH range<sup>17</sup> (pH = 9.174, measured against 0.05M-potassium hydrogen phthalate).

The spectrophotometer used was an "Optica" CF4DR.

*Preparation of Materials.*—2-Amino-1-phenylethanol was recrystallised from cyclohexane containing a little benzene, and had m. p. 52–54°. Adrenaline was recrystallised from a mixture of benzene and dimethylformamide, and had m. p. 212° (decomp.). Noradrenaline was recrystallised from a similar solvent, and had m. p. 191° (decomp.). Catechol was sublimed twice at 40 mm. to give a white product, m. p. 104°.

Carbonate-free sodium hydroxide was prepared by adding sufficient barium chloride to give complete precipitation of barium carbonate,<sup>18</sup> and was standardised against AnalaR potassium hydrogen phthalate.

All solutions were made up with freshly boiled deionised water that had been allowed to cool under a stream of nitrogen, and were protected by means of soda-lime guard tubes.

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<sup>17</sup> Bates, "Electromeric pH Determinations," Wiley, New York, 1954.

<sup>18</sup> Vogel, "A Textbook of Quantitative Inorganic Analysis," 3rd edn., Longmans, 1961, p. 242.