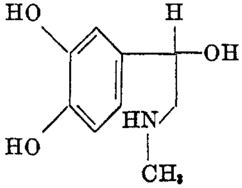
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

THE STRUCTURE OF ADRENOCHROME MONOSEMICARBAZONE

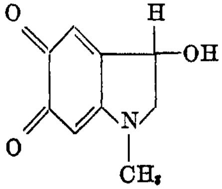
FAUSTO RAMIREZ AND PETER VON OSTWALDEN

Received June 27, 1955

Adrenochrome (1) is a labile oxidation product of adrenalin (A) with hemostatic and other properties; it has been formulated (1) as an *ortho*-quinone derivative of N-methyl-β-hydroxyindoline B.



A



B

Adrenochrome is clinically administered as the stable monosemicarbazone (1g, h) in aqueous solution. This study is concerned with the ultraviolet absorption spectrum of adrenochrome monosemicarbazone in aqueous solutions of different *pH* and with the infrared spectrum of the solid substance. The results of the ultraviolet study are shown in Figure 1 and the pertinent data are recorded in Table I.

For adrenochrome monosemicarbazone, two cross-conjugated resonance interactions can be considered, Ia (*meta*-amino-*ortho*-quinone type) and Ib (*ortho*-quinone semicarbazone type), accounting for the unreactivity of the second carbonyl group (1) and suggesting a weak basicity for the tertiary amino group and the possibility of considerable intramolecular hydrogen bonding ["conjugated chelation" (2a)]. As in the analogous system of 1,2-naphthoquinone phenylhydrazone ⇄ 1-phenylazo-2-naphthol studied by Burawoy (3), the semicarbazone I is capable of tautomerism with an azophenol structure II. For the azophenol structure, two cross-conjugated resonance interactions can be written, IIa and IIb, characteristic of the presence of one electron-attracting group (+E) in *para*- and *ortho*-relationships to two electron-releasing groups (−E).

An ionic equilibrium is possible between the neutral species of adrenochrome monosemicarbazone and the corresponding conjugate acid. The conjugate acid is here written as III, involving the tertiary amino group; this description taken together with that for the neutral azophenol form IIa, implies again a weak basic character for the tertiary amino group. Another ionic equilibrium is possible between the neutral species and the corresponding conjugate base. The conjugate base is written as IV, derivable equally well from either the semicarbazone I or the azophenol II.

The curve at *pH* 1.1 with a maximum at 377 mμ represents essentially the cationic form III. As the *pH* of the solution is increased the maximum decreases

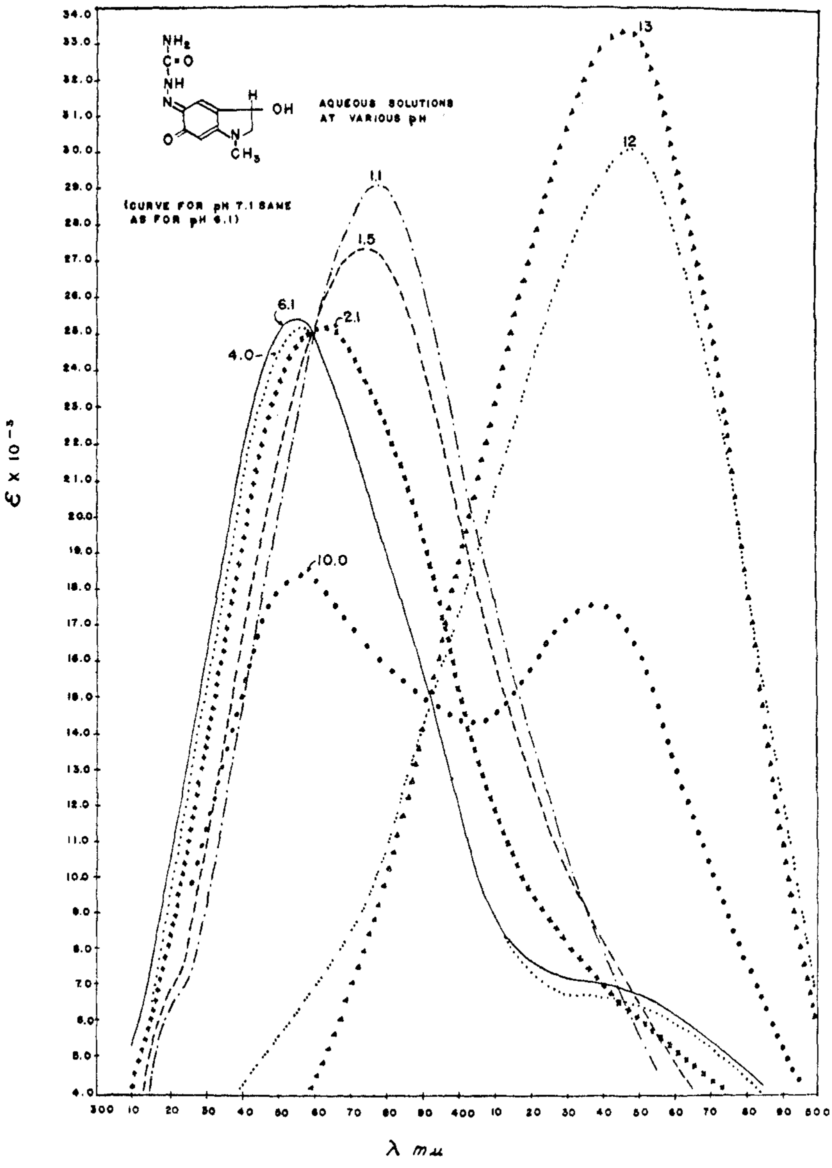


FIG. 1

in intensity and shifts toward shorter wave lengths. At *pH* 4.0, the maximum is found at 357 mμ and remains at this wave length through *pH* 6.1 and 7.1. The curves at *pH* 6.1 and 7.1 are taken to represent essentially the neutral form, presumably the semicarbazone tautomer (3) (*vide infra*). A new maximum at longer wave length is noted with increasing *pH*; this maximum is found at 437 mμ at *pH* 10.0 and shifts toward longer wave length as the *pH* is further increased. The curve at *pH* 13 represents the anionic form IV. Adrenochrome

TABLE I

Ultraviolet Absorption Spectrum of Adrenochrome Monosemicarbazone in Aqueous Solutions at Various pH

pH	λ'_{max}, mμ (ϵ'_{max})	λ''_{max}, mμ (ϵ''_{max})
1.1	377 (29,100)	—
1.5	374 (27,400)	—
2.1	362 (25,200)	—
4.0	356 (25,200)	446 (6,600)
6.1	356 (25,400)	449 (6,800)
7.1	356 (25,800)	445 (7,200)
10.0	358 (18,400)	441 (17,600)
12	—	447 (30,100)
13	—	447 (33,400)

Isosbestic points (wave length at which extinction is the same for the two species in equilibrium) at 360 mμ and 393 mμ.

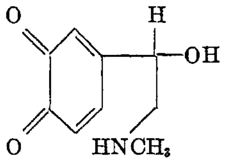
itself (B) is said (1g, h) to exhibit a maximum at 300 mμ;[1] the shift to 357 mμ upon formation of the semicarbazone is reasonable (7).

The isosbestic point (4a, b) at 360 mμ reflects the existence of the equilibrium between cationic and neutral forms (probably III $\rightleftarrows$ II).[2] The isosbestic point at 393 mμ reflects the existence of the equilibrium between neutral and anionic forms (I–II $\rightleftarrows$ IV). From the data, the apparent $p\mathrm{K}_{a1}$ value is 2.0 and the apparent $p\mathrm{K}_{a2}$ value is 10.2 (*cf.* 4a, b; 10).

In the solid state, adrenochrome monosemicarbazone exhibited an infrared spectrum consistent with the semicarbazone formulation I. Bands were present at 3360–3280 cm^{-1} (2.98–3.05 μ; OH), 3120–3040 cm^{-1} (3.21–3.29 μ; bonded NH?), 1690 cm^{-1} (5.92 μ, strongest), 1650 cm^{-1} (6.06 μ, medium), and 1600

---

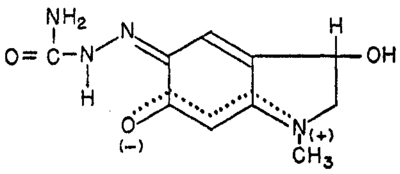
[1] In view of the known behavior of *o*-benzoquinone in aqueous media and of the published ultraviolet absorption data for this quinone (390 mμ, ϵ 1358) and its transformation products (5), the recent report by J. Ruiz-Gijon (6) is surprising. Ruiz-Gijon (6) reported the spontaneous oxidation (9) of adrenaline in slightly alkaline solution to the *o*-benzoquinone derivative adrenoerythrin (C) and to adrenochrome B and gave values for the ultraviolet absorption maxima as follows: adrenoerythrin, 294 and 490 mμ; adrenochrome, 281 mμ. It should be noted that the *oxidation products* of 1,2,4-trihydroxybenzene are known (5) to absorb at 260 and 480–490 mμ.

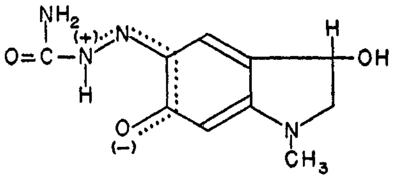


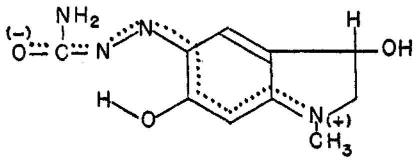
C

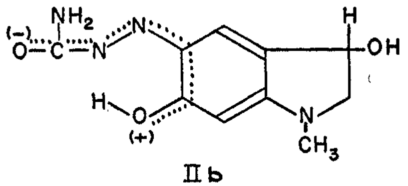
[2] On a purely speculative basis it seems reasonable to consider the 377 mμ maximum as being associated with forms such as III, representing a *hypsochromic* shift of the (unknown) maximum associated with IIa rather than a *bathochromic* shift of the 357 mμ maximum probably associated with Ib. The 447 mμ maximum could be associated with forms such as IV, representing a *bathochromic* shift from Ib (or IIb).

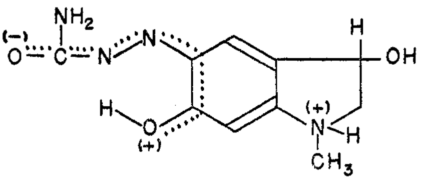
cm^{-1} (6.25 μ, weakest of the C═X). The 6.06 μ band probably corresponds to the urea-type C═O, the weaker 6.25 μ absorption presumably is due to the conjugated C═N. The remaining intense band, on this basis, would appear to be of relatively high frequency for an *o*-quinone derivative (8) (*cf.* *o*-benzoquinone in solution: 1669 cm^{-1}). The inference that the high frequency for this absorption might imply little or no intramolecular hydrogen bonding in I ["conjugated chelation" (2a)] is not warranted in view of the uncertainties (8) associated with the infrared spectrum of amino-*o*-quinones and of the bicyclic nature of the system of I.

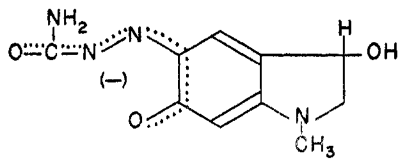

Ia


Ib


IIa


IIb


III


IV

EXPERIMENTAL

Adrenochrome monosemicarbazone. As indicated by the sharpness of the isosbestic points the sample of adrenochrome monosemicarbazone employed was free from ultraviolet-absorbing materials. Clark and Lubs buffer mixtures were used in the region of *p*H 1–10; the *p*H was checked with the glass electrode. The solutions for measurement were prepared by diluting an aliquot volume of a stock solution of the semicarbazone in water with a suitable volume of alkali or buffer solution. The measurements were carried out in a Cary recording spectrophotometer according to well-known procedures (4, 10). The curve in 0.5 *N* hydrochloric acid was not significantly different from that at *p*H 1.1, indicating relatively complete protonation. The curves at *p*H 6.1 and 7.1 were essentially identical and were taken as representing the neutral form.

The apparent pK_{a1} value was calculated from the equation

$$pK_{a1} = pH + \log \frac{\epsilon_{obs} - \epsilon B}{\epsilon BH^{+} - \epsilon_{obs}}$$

where ϵB, ϵBH$^+$ and ϵ_{obs} are the extinction coefficients of the neutral form, the protonated form and mixtures of the two, respectively. The apparent pK_{a2} value was calculated from the equation

$$pK_{a2} = pH + \log \frac{\epsilon A^- - \epsilon_{obs}}{\epsilon_{obs} - \epsilon AH}$$

where ϵAH, ϵA$^-$, and ϵ_{obs} are the extinction coefficients of the neutral form, the anionic form, and mixtures of the two, respectively. The values are probably correct to within 0.2 unit.

SUMMARY

The ultraviolet absorption spectrum of adrenochrome monosemicarbazone in aqueous solutions as a function of *pH*, is discussed. The infrared absorption spectrum of adrenochrome monosemicarbazone in the solid state is given.

NEW YORK 27, N. Y.

REFERENCES

(1) (a) GREEN AND RICHTER, *Biochem. J. (London)*, **31**, 596 (1937); (b) RICHTER AND BLASCHKO, *J. Chem. Soc.*, 601 (1937); (c) MACCARTHY, *Chimie & industrie*, **55**, 435 (1946); (d) BERGEL AND MORRISON, *J. Chem. Soc.*, **48**, (1943); (e) HARLEY-MASON, *J. Chem. Soc.*, 1276 (1950); (f) HARLEY-MASON, *Experientia*, **4**, 307 (1948); (g) BEAUDET, DEBOT, LAMBOT, AND TOUSSAINT, *Experientia*, **7**, 293 (1951); (h) BEAUDET, *Experientia*, **7**, 291 (1951); (i) SCHAYER, *J. Am. Chem. Soc.*, **74**, 2441 (1952).

(2) (a) RASMUSSEN, TUNNICLIFF, AND BRATTAIN, *J. Am. Chem. Soc.*, **71**, 1071 (1949); (b) *Cf.* RAMIREZ AND KIRBY, *J. Am. Chem. Soc.*, **76**, 1037 (1954).

(3) BURAWOY, SALEM, AND THOMPSON, *Chemistry & Industry*, 668 (1952).

(4) (a) CAVALIERI, FOX, STONE, AND CHANG, *J. Am. Chem. Soc.*, **76**, 1119 (1954); (b) SHUGAR AND FOX, *Biochim. Biophys. Acta*, **9**, 199 (1952); (c) MARSHALL AND WALKER, *J. Chem. Soc.*, 1004 (1951).

(5) TARPLEY, Dissertation, Columbia University, New York, 1951.

(6) RUIZ-GIJON, *Farmacognosia* **12**, No. 23, 71 (1952); *Chem. Abstr.*, **47**, 10498 (1953).

(7) GILLAM AND STERN, *Electronic Absorption Spectroscopy*, Edward Arnold Ltd., London, 1954, pp. 52, 100.

(8) JOSIEN, FUSON, LEBAS, AND GREGORY, *J. Chem. Physics*, **21**, 331 (1953).

(9) BEAUDET, *Congr. chim. ind.*, **21**, 439 (1948).

(10) (a) SHUGAR AND FOX, *Bull. soc. chim. Belges* **61**, 44 (1952); (b) SHUGAR AND FOX, *Bull. soc. chim. Belges*, **61**, 293 (1952); (c) ANDON, COX, AND HERINGTON, *Trans. Faraday Soc.*, **50**, 918 (1954).