Chemistry of the Aminochromes. Part XIV. Noradrenochrome[1,2]

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The preparation, in pure crystalline form, of noradrenochrome and its monosemicarbazone, together with some of their more important properties is described.

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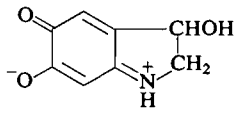
Despite the fact that adrenochrome, the important red quinonoid oxidation product of adrenaline, has been known in crystalline form since 1937 (1), noradrenochrome (**1**), the corresponding aminochrome (*cf.* refs. 2, 3) obtained from noradrenaline (**2**), has not been fully characterized in the solid state. The properties of solutions containing variable amounts of **1** prepared by the oxidation of solutions of **2** have been described fairly extensively (*cf.* refs. 2, 3). These solutions may also have contained other substances, such as unchanged **2** and its initial, uncyclized oxidation product, noradrenaline quinone (4, 5). Noradrenochrome (**1**) has been obtained as "red rings on the side of the flask" after solutions containing **1**, prepared by the oxidation of **2** with silver oxide in acetonitrile (6), were concentrated to dryness *in vacuo*. However, the preparation of crystalline **1** has not yet been reported. This note describes the preparation, characterization, and properties of this interesting oxidation product of noradrenaline (**2**).

Noradrenochrome (**1**) can be obtained as a deep reddish-violet crystalline solid on careful concentration, at low temperatures, of its solutions in methanol, prepared by oxidation of suspensions of **2** (free base) by silver oxide. Extreme care must be taken to remove all traces of silver from the solutions containing **1** prior to their concentration. This has been achieved by filtration of the solution, firstly through a bed of anhydrous sodium sulfate (*cf.* 7) and



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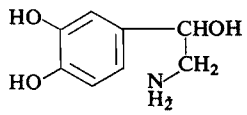

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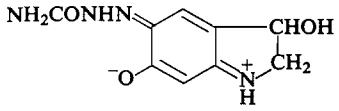

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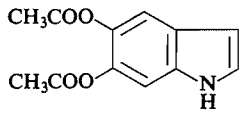

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secondly through a Dowex-1 (Cl^-) resin bed (*cf.* 8). There appeared to be no advantage in centrifuging the solution (*cf.* 9) prior to crystallization of the aminochrome.

A crystalline derivative of **1**, the monosemicarbazone **3**, has been prepared directly from the aminochrome. This compound has not previously been described in the literature. The aminochrome monosemicarbazones have considerable importance in medicine as haemostatic agents (*cf.* refs. 3, 10).

Noradrenochrome (**2**) can be easily reduced in aqueous solution by ascorbic acid (*cf.* refs. 2, 3) to 5,6-dihydroxyindole, which can readily be isolated as its diacetyl derivative (*i.e.* **4**).

Experimental

The melting points were determined on either a Leitz Hot-Stage instrument or a Fisher–Johns apparatus and are uncorrected. The i.r., u.v., n.m.r., and mass spectra were determined on Perkin–Elmer model 237, Bausch and Lomb 505, Varian A-60-A, and Dupont/C.E.C. 21-110B instruments respectively.

Noradrenochrome (1)

Freshly prepared silver oxide (22.5 g) was added to a suspension of L-noradrenaline base (5 g, Schuchardt) in

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dry methanol (500 ml) at 32°. The reaction mixture was vigorously shaken for 6 min at this temperature (it was possible to maintain the temperature at 32° without external heating due to the mildly exothermic nature of the reaction), after which time it was filtered firstly through an anhydrous sodium sulfate bed (height = 2 cm; diameter = 11 cm) and secondly through a Dowex-1 × 8 (Cl^-) resin[7] bed (height = 3 cm; diameter = 10 cm). The filtrate was concentrated below 20° *in vacuo* to small bulk (*ca.* 30 ml), until crystals began to form in the solution. Noradrenochrome was obtained in dark reddish-violet needles [150 mg (3%); totally decomposed without melting by 105°] on filtration of the solution; $\lambda_{max}(H_2O)$ nm(ε): 217(22 350), 293(8580), 488(3190); ν_{max}(KBr): 3320 (sh), 3120, 1676, 1663, 1622, 1580, 1530 cm^{-1}.

Anal. Calcd. for $C_8H_7NO_3$: C, 58.18; H, 4.24; N, 8.48. Found: C, 58.39; H, 4.08; N, 8.56.

Noradrenochrome Monosemicarbazone (3)

Semicarbazide hydrochloride (27 mg) in acetate buffer (1.5 ml) at p*H* 5 was added to a stirred solution of noradrenochrome (20 mg) in water (1.5 ml). The crude monosemicarbazone (15 mg) was obtained in brownish-orange needles after the solution had been allowed to stand at room temperature for 1.5 h. Pure noradrenochrome monosemicarbazone [9 mg (34%); totally decomposed without melting by 221°] was obtained in fine orange needles after recrystallization of the crude product from water; λ_{max}(0.1 *N* HCl): nm(ε): 373(29 000); $\lambda_{max}(H_2O)$ nm(ε): 440(sh), 351(27 000); λ_{max}(0.1 *N* NaOH) nm(ε): 441 mμ (27 700).

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.65; H, 4.54; N, 25.22. Found: C, 48.84; H, 4.46; N, 25.03.

Reduction of Noradrenochrome (1) by Ascorbic Acid (cf. 11); Preparation of 5,6-Diacetoxyindole (4)

Ascorbic acid (400 mg) was added to a mixture of noradrenochrome (75 mg) in water (5 ml) and peroxide-free ether (15 ml). The system was vigorously shaken until the red color was totally discharged (about 1 min). The ether phase was removed and dried (Na_2SO_4) and a mixture of acetic anhydride (2 ml) and dry pyridine (2 ml) added. After removal of the ether *in vacuo* the reaction mixture was allowed to stand at room temperature for 2 h, and was then evaporated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 (15 ml) and washed in turn with the following reagents: 0.1 *N* HCl (15 ml), saturated aqueous sodium bicarbonate (3 × 15 ml), and water (2 × 15 ml). The CH_2Cl_2 solution was dried (Na_2SO_4) and on evaporation to dryness gave a white crystalline solid (47 mg), which gave 5,6-diacetoxyindole [38 mg (36%), m.p. 139–142°] on recrystallization from heptane containing a small quantity of benzene, lit. m.p. 139–140° (12), 130–133° (13). The n.m.r., τ($CDCl_3$): 1.6 (broad s, *N*—H), 2.74 (s, H_4), 2.96 (s, H_7), 3.08 (t, H_2), 3.70 (t, H_3), 7.73 (s, CH_3). After shaking with D_2O the peak at τ 1.6 disappeared and the triplets at τ 3.08 and 3.70 collapsed to doublets ($J_{2,3}$ = 2.7 Hz).[8] Mass spectrum: $M^{+\cdot}$ 233.0684 ± .0007; Calcd. for $C_{12}H_{11}NO_4$ 233.06881.

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1. D. E. Green and D. Richter. Biochem. J. **31**, 599 (1937).
2. R. A. Heacock. Chem. Rev. **59**, 181 (1959).
3. R. A. Heacock. Advan. Heterocycl. Chem. **5**, 205 (1965).
4. A. Kodja and S. Bouchilloux. C. R. Soc. Biol. **153**, 1407 (1959).
5. A. Kodja and S. Bouchilloux. Biochim. Biophys. Acta, **41**, 345 (1960).
6. P. Marquardt and E. Carl. Naturwiss. **39**, 210 (1952).
7. H. Sobotka and J. Austin. J. Amer. Chem. Soc. **73**, 3077 (1951).
8. R. A. Heacock, C. Nerenberg, and A. N. Payza. Can. J. Chem. **36**, 853 (1958).
9. A. Feldstein. Sci. **128**, 28 (1958).
10. H. Sobotka, N. Barsel, and J. D. Chanley. Fortschr. Chem. Org. Naturst. **14**, 217 (1957).
11. G. L. Mattok and R. A. Heacock. Can. J. Chem. **42**, 484 (1964).
12. R. A. Heacock, M. E. Mahon, and B. D. Scott. Can. J. Chem. **39**, 231 (1961).
13. R. J. S. Beer, K. Clarke, H. G. Khorana, and A. Robertson. J. Chem. Soc. 2223 (1948).
14. W. Powell and R. A. Heacock. Can. J. Chem. **47**, 2102 (1969).

[7]The Dowex resin was pretreated by the method described in the literature (8).

[8]Long range coupling between H_3 and H_7 was also apparent, although the coupling constant ($J_{3,7} \simeq 0.7$ Hz) was difficult to measure accurately.