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The structure of rubreserine, a decomposition product of physostigmine*

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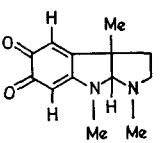
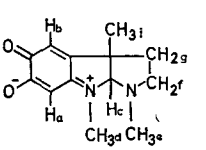
Confirmation of the structure of rubreserine as the resonance hybrid (I)←—›››(II) has been obtained by comparison of its ultraviolet, infrared, and proton magnetic resonance spectra with those of adrenochrome (III)←—›››(IV).

RUBRESERINE, the red oxidation product of physostigmine, has been assigned structure (II) (Coyne & Paterson, 1961). By comparing the ultraviolet, infrared and nuclear magnetic resonance spectra for adrenochrome, known to have the resonance hybrid structure (III) ←—››› (IV) in which the zwitterionic mesomeric structure (IV) makes the major contribution (Harley-Mason, 1948), with the corresponding spectra of rubreserine, support for the resonance hybrid structure (I) ←—››› (II) for rubreserine has now been obtained.

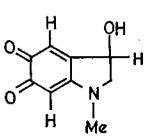
Ultraviolet–visible spectra. A comparison of the spectrum of rubreserine (Coyne & Paterson, 1961) and adrenochrome (Beaudet, 1951; Beaudet, Debot, Lambot & Toussaint, 1951; Marquardt & Carl, 1952; Sabotka & Austin, 1951), both recorded quantitatively, shows that the two spectra are almost superimposable, thus suggesting that the same chromophore is present in both molecules.



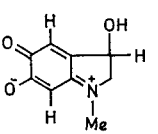
(I)



(II)



(III)



(IV)

Infrared spectra. Both compounds show similar spectra (recorded in Nujol) in the 1700–1550 cm^{-1} region. Rubreserine, ν_{max} 1671 m, 1651 m (shoulder), 1628 m (shoulder), 1614 m (shoulder) and 1600 s cm^{-1}. Adrenochrome, ν_{max} 1671 m, 1661 m, 1635 w, 1616 m and 1590 s cm^{-1} (all frequency measurements are ± 3 cm^{-1}). For adrenochrome, assignments involving C=O, C—O, C=C and C=N stretching have been given to these absorption bands (Heacock & Mahan, 1958). The

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* For the preceding paper in this series see Robinson & Spiteller (1964).

spectrum of adrenochrome had a broad absorption band at 3280 $\pm$ 10 cm^{-1} (m) (O—H stretching) but that of rubreserine, which had been kept *in vacuo* over phosphorus pentoxide for 24 hr before recording the spectrum, showed no absorption in the 4000–3100 cm^{-1} region, contrary to previous observations (Coyne & Paterson, 1961).

Proton magnetic resonance spectra. The spectrum of rubreserine in deuterochloroform had singlets at τ = 8·50, 7·30, 6·90 and 5·64, with intensities 3, 3, 3 and 1 respectively, which are assigned and compared with the corresponding protons in physostigmine (Robinson, 1964) in Table 1. The protons of the two methylene groups in rubreserine form an ABXY system, as do the corresponding protons in physostigmine

TABLE 1. PROTON MAGNETIC RESONANCE DATA

Rubreserine τ	Intensity	Multiplicity	Assignment	Physostigmine τ
3·68 3·52*	1	Singlet	H_a	
4·56 4·64*	1	Singlet	H_b	
5·64	1	Singlet	H_c	5·77
6·90	3	Singlet	H_d	7·00
7·30	3	Singlet	H_e	7·36
6·83–7·24	2	Multiplet half of ABXY system	H_f	7·01–7·30
7·78–8·07	2	Multiplet half of ABXY system	H_g	7·81–8·09
8·50	3	Singlet	H_i	8·53

* Solvent: dimethyl sulphoxide.

(Robinson, 1964), which give rise to two multiplets, each of intensity 2, these are also compared with those for physostigmine and assigned in Table 1. The remaining two protons of rubreserine give rise to two singlets, each of intensity 1, at τ = 4·56 and 3·68. To compare these latter two τ values with those of the corresponding protons in adrenochrome it was necessary to record the spectra in dimethyl sulphoxide, since adrenochrome is only slightly soluble in deuterochloroform. In this solvent the two protons give rise to singlets of equal intensities at τ = 4·64 and 3·52, the corresponding two protons of adrenochrome giving rise to singlets of equal intensities at τ = 4·53 and 3·50.

Experimental

Proton magnetic resonance spectra were recorded on a Varian A.60 spectrometer operating at 60 Mc/sec; tetramethylsilane was used as internal standard and intensities were measured using a planimeter. Infrared spectra were recorded on a Unicam SP.200 spectrophotometer.

Adrenochrome (III)  (IV). This was prepared by oxidation of adrenaline with silver oxide by one of the methods described in the literature (Sabotka & Austin, 1951) [see also (Heacock, Nerenberg & Payza, 1958)].

Rubreserine (I) ←—〉〉〉 (II). Physostigmine (1·00 g) was added to 5% aqueous sodium hydroxide solution (70 ml) in a 250-ml separating funnel. After shaking gently for 10 min some of the physostigmine still remained undissolved and a deep-red colour had developed in the alkaline solution. Chloroform (70 ml) was then added (immediately the remaining solid physostigmine dissolved). After shaking for a further 5 min, the deep-red chloroform layer was separated off; the basic layer was then shaken with further quantities of chloroform (2 × 70 ml) and the combined deep-red chloroform extracts were evaporated under vacuum at room temperature to about 5 ml. Careful addition of light petroleum (b.p. 40–60°) (15–20 ml) dropwise with shaking then effected the crystallisation of rubreserine as bright-red needles (164·7 mg; 12·5%), which after standing over P_2O_5 under vacuum for 24 hr had m.p. 142–145° (reported m.p. 144–145°, Ellis, 1943; 145–146°, Coyne & Paterson, 1961). One recrystallisation from chloroform/light petroleum (40–60°) followed by drying as above, gave bright-red needles, m.p. 145–146°.

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