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Analytical oxidation of reserpine

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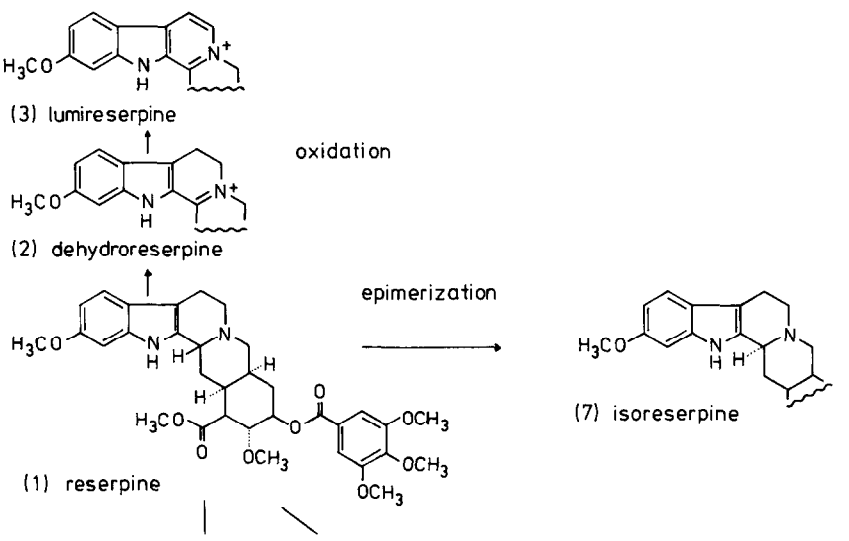
Abstract: Electrochemical (anodic) oxidation of reserpine in an acidic medium results in the formation of 3,4-dehydroreserpine instead of the reported 10-hydroxyreserpine. Electrochemical and chemical oxidation by sodium nitrite lead quantitatively to the same product; few by-products or consecutive products are formed if protected from light. The validity of the conditions of the spectrophotometric assay of the European Pharmacopoeia were confirmed by following the reaction using HPLC with a photodiode-array detector.

Keywords: *Reserpine; electrochemistry; HPLC; photodiode-array detector; 3,4-dehydroreserpine.*

Introduction

Reserpine (**1**) undergoes three different paths of chemical degradation: oxidation to dehydroreserpine (**2**) and subsequently to lumireserpine (**3**); hydrolysis to reserpinic acid (**6**) with intermediates (**4** and **5**); and epimerization in the 3-position to isoreserpine (**7**)



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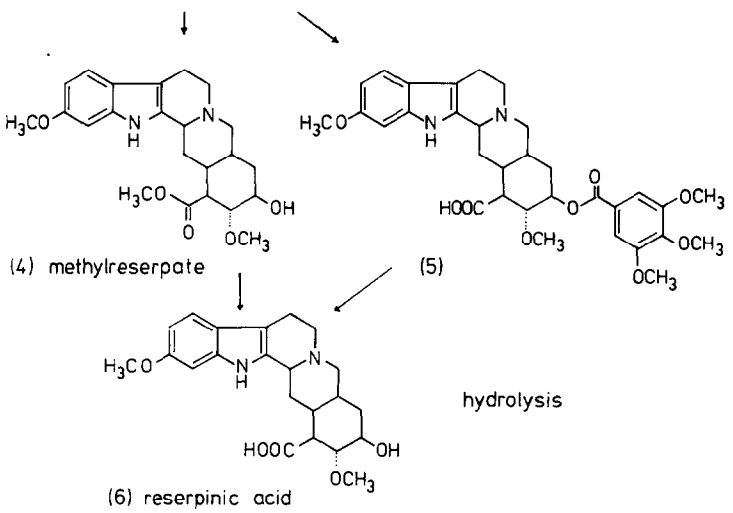
[1, 2]. Furthermore, it is likely that the products of hydrolysis and epimerization will undergo the oxidation process. Therefore, highly selective analytical methods should be used for the assay of reserpine and its pharmaceutical formulations. Using HPLC for the separation with a combination of UV spectrometric and electrochemical detectors, it is possible to distinguish between the different compounds. This paper describes a study of the chemical and electrochemical oxidation of reserpine as a basis for the analysis of reserpine.

Allan and Powell [3] reported the electrochemical oxidation of indolalkaloids at a rotating platinum microelectrode. With methanol–water as the solvent in an acidic medium, only 11-methoxy-substituted indolalkaloids are oxidizable. From these results they postulated a radical mechanism to explain the formation of 10-hydroxy-11-methoxy-indolalkaloids, but no structure elucidation was done.

On the other hand, Krebs and Furtscher [4] reported the formation of 3,4-dehydroreserpine (**2**) by chemical oxidation using hydrogen peroxide [5] or sodium nitrite [6]. The oxidation procedure with $NaNO_2$ is the basis of the spectrophotometric assay of reserpine in the European Pharmacopoeia (Eur. P.).



Experimental

Chemical oxidation

Soldium nitrite (Eur. P.).

Anodic oxidation

Glass microvessel, volume about 180 μl [7]; rotating platinum electrode +1V versus a Ag/AgCl-reference electrode; initial concentration of reserpine, 100 ppm; solvent ethanol–water–5% sulphuric acid (70:25:5, v/v); reaction time, 1 h; yield, 25% in respect to the initial reserpine content; conditions, about 25°C, protected from light.

HPLC

Stationary phase, 5-μm Nucleosil C 18; column, 125 × 4.2 mm, i.d.; mobile phase,

acetonitrile–0.005 M phosphate buffer (pH 2.5) (1:1); flow rate, 1.0 ml min^{-1}; detector, photodiode-array HP 1040 A.

Results and Discussion

Using the oxidation procedure with $NaNO_2$ according to the Eur. P., the oxidation products and the kinetics of this reaction were investigated. Under the chromatographic conditions given below reserpine was eluted at about 4 min. From Fig. 1 it is seen that in the Eur. P. method only one product is formed and that under the specified conditions for time and temperature the method is approximately quantitative.

In a suitable microvessel [7] reserpine was anodically oxidized at an applied potential of +1.0 to +1.1 V versus an Ag/AgCl-reference electrode in ethanol–water–5% sulphuric acid (70:25:5, v/v) as solvent. In the microvessel after 1 h, approximately 25% of the initial reserpine was oxidized. From the chromatogram (Fig. 2) it is evident that there is only one oxidation product eluting at the same retention time as 3,4-dehydroreserpine. Using a photodiode-array detector the chemically and the electrochemically formed oxidation products have identical UV spectra (Fig. 3). Use of other mobile phases led to

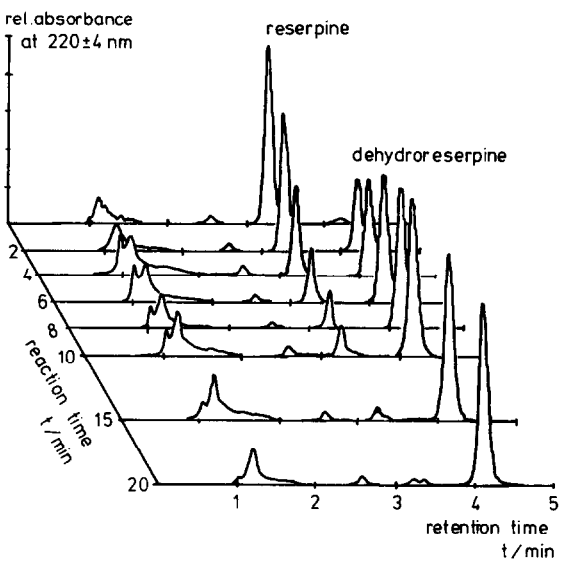


Figure 1
Kinetic experiments of the oxidation of reserpine by $NaNO_2$ according to the Eur. P. by HPLC.

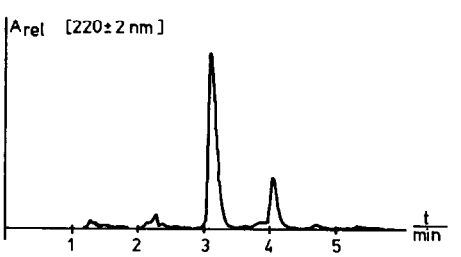


Figure 2
Chromatogram of reserpine and its electrochemical oxidation product.

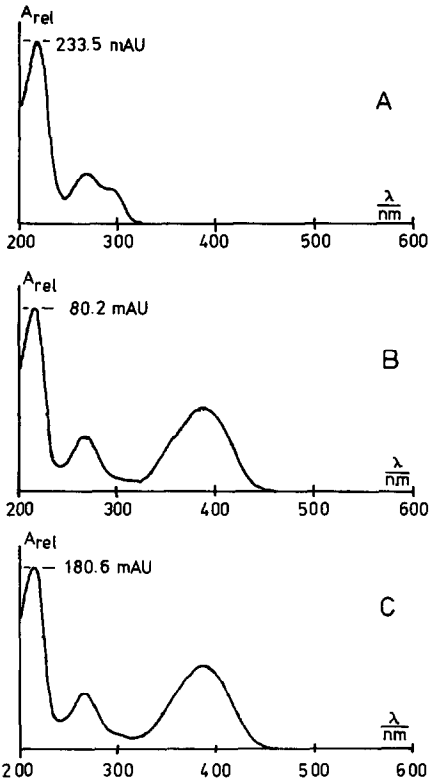
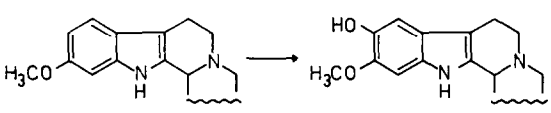


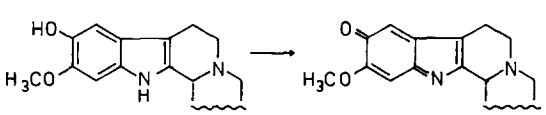
Figure 3
Spectra of reserpine (A) and its oxidation products (B, chemical; C, electrochemical) from the chromatograms by a photodiode-array detector.

no further peaks. Peak purity was confirmed by HPLC using photodiode-array detection. It was deduced that both substances are identical and in both cases 3,4-dehydroreserpine is formed and not the 10-hydroxyreserpine.

Further electrochemical investigations using cyclic voltammetry showed that the resulting oxidation product is electrochemically inactive. If the structure of 10-hydroxy-reserpine (**8**) is correct, further oxidation to the quinoid compound (**9**) would be expected at a lower positive potential, but no peak was observed in voltammetry up to +1200 mV.



(1) (8)



(8) (9)

It was deduced that 3,4-dehydroreserpine was the oxidation product in the chemical as well as in the electrochemical oxidation procedures. This is not very common, for in most cases different reaction products are formed in the two procedures. On the other hand, it is surprising that both oxidation procedures lead approximately quantitatively to only one reaction product when the solutions are protected from light. Furthermore, the results of the kinetics of the chemical oxidation procedure agree with those of Haycock *et al.* [9] and show that the reaction time and temperature in the Eur. P. method are sufficient, and that practically no by-products and consecutive products are formed when different possible oxidation pathways [8] are examined.

References

[1] H. Pötter and R. Voigt, *Pharmazie* **21**, 291–295 (1966).
[2] H. Böhme and K. Hartke (Eds), *Europäisches Arzneibuch Band III Kommentar.* Wissenschaftl. Verlagsgesellsch., Stuttgart (1982).
[3] M. J. Allan and V. J. Powell, *J. Electrochem. Soc.* **105**, 541–544 (1958).
[4] K. G. Krebs and N. Furtscher, *Deutsch. Apoth. Ztg.* **98**, 1341–1344 (1958).
[5] B. Dechene, *J. Am. Pharm. Assoc.* **44**, 657–660 (1955).
[6] C. R. Szalkowsky and W. J. Mader, *J. Am. Pharm. Assoc.* **45**, 613–617 (1956).
[7] S. Ebel, F.-J. Placke and P. Surmann, *Fresenius Z. Anal. Chem.* **321**, 660–665 (1985).
[8] F. L. Weisenborn and H. E. Applegate, *J. Am. Chem. Soc.* **78**, 2021–2022 (1956).
[9] R. P. Haycock, P. B. Sheth, T. Higuchi, W. J. Mader and G. J. Papariello, *J. Pharm. Sci.* **55**, 826–828 (1966).

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