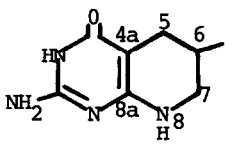
Tetrahedron Letters No. 26, pp 2271 - 2274, 1978. 0040-4039/78/0622-2271$02.00/0

THE MECHANISM OF OXIDATION OF 6-METHYL-5-CARBA-5-DEAZATETRAHYDROPTERIN. EVIDENCE FOR THE INVOLVEMENT OF A 4a-ADDUCT IN THE OXIDATION OF TETRAHYDROPTERINS.

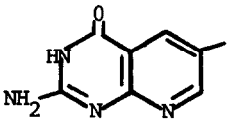
Graeme Moad, Connie L. Luthy and Stephen J. Benkovic*
Department of Chemistry
The Pennsylvania State University
University Park, PA 16802

(Received in USA 28 March 1978; received in UK for publication 2 May 1978)

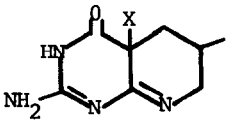
We wish to report the actual isolation and characterization of 4a-adducts derived from 6-methyl-5-carba-5-deazatetrahydropterin (1). The chemical and physical properties of these compounds are directly pertinent to the postulated involvement of analogous species as intermediates in the chemical oxidation of tetrahydropterins and in hydroxylation reactions mediated by enzymes such as phenylalanine and tyrosine hydroxylase.[1-5] Previous attempts[1,5,6] to isolate 4a-(or 8a-) adducts from reactions involving tetrahydropterins have proved fruitless, possibly due to a predisposition of such adducts to undergo elimination and/or rearrangement. The present study was designed to exploit an anticipated resistance to an elimination reaction of adducts formed by electrophilic addition to the 4a-position in the deaza-model (1) relative to the tetrahydropterin system.



1



2



3a X=OH
3b X=Cl
3c X=Br

Compound 1 is readily prepared by catalytic (PtO_2) hydrogenation of the fully oxidized precursor (2)[7] in trifluoroacetic acid. Reaction of 1 with a variety of oxidants, including bromine, N-chloro- and N-bromosuccinimide, and performic acid, involves the initial formation of a 4a-adduct.[8] While these adducts are very labile in aqueous solution at physiological pH, they may be isolated by a suitable choice of reaction conditions.

In a typical experiment 400 mg (2.2 mmol) of 1 in 500 ml of methanol containing 0.5 ml of trifluoroacetic acid was treated with 400 mg (2.2 mmol) of N-bromosuccinimide. After evaporation of the solvent the residue was washed with acetonitrile to afford the analytically

prue bromo-adduct (3c) in quantitative (98%) yield. The synthesis of the chloro-adduct (3b) followed a similar procedure but used N-chlorosuccinimide. The hydroxy adduct (3a) is available from the reaction of 1 with one equivalent of performic acid in formic acid solution. Workup, involving evaporation of the solvent and recrystallization of the residue from methanol-formic acid-ether at room temperature, must be performed rapidly and immediately to minimize decomposition.[9]

The adducts (3a-c) have been characterized by their spectral properties and by analytical data.[10] The ^{13}C NMR spectra are diagnostic for the adduct structure. Particularly notable is the progressive shift of δ_c for the 4a-carbon in the series 3a, 3b, 3c (Figure 1) while δ_c for the remaining carbons of the tetrahydropyridine ring are unchanged. The assignment of the ^{13}C NMR resonances (Figure 1) is based on the published spectra of tetrahydropterin,[11] pyrimidine[12] and piperidine[13] derivatives together with the known substituent parameters[14] for hydroxyl, bromine and chlorine. The resonances due to the 2- and 4-carbons are obscured by the solvent (HCO_2H).[15]

The 100 MHz ^1H NMR spectra of 1 and of the adducts (3a-c) are complex and neither chemical shifts nor coupling constants can be extracted by direct measurement. A sample of 1 labelled with deuterium was prepared by deuteration of 2 in trifluoroacetic acid-d. The mass spectrum shows the product to consist of 15% dideutero-, 60% trideutero-, and 25% tetradeutero-1. A separate experiment showed that 1 slowly incorporated deuterium from solvent on standing in trifluoroacetic acid-d (ca. 5% incorporation after 24 hours) thus accounting for the formation

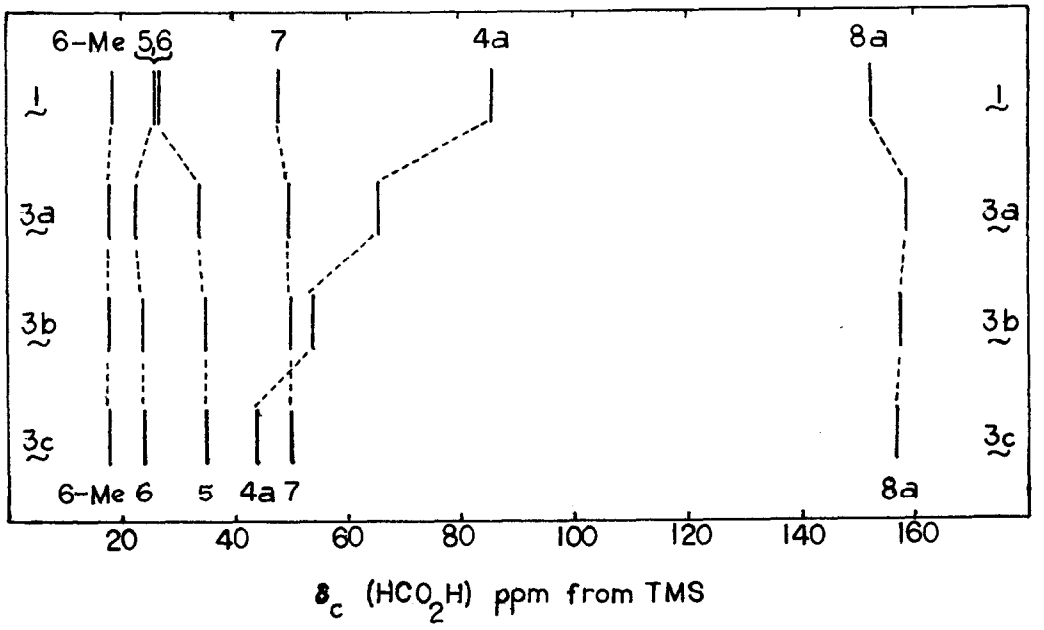


Figure 1. ^{13}C NMR shieldings for the tetrahydropyridine ring carbons of 1 and 3a-c.

of quadrupally labelled 1. The 100 MHz ^{1}H NMR spectrum of the labelled material shows it to be predominantly a single stereoisomer exhibiting three singlets at $\delta(CF_3CO_2H)$ 1.18, 2.13 and 3.14 ppm with a relative intensity of 3.0 : 0.8 : 0.6. The resonances are attributed to the 6-methyl-, 5-, and 7-hydrogens respectively.[16] Samples of the adducts (3a-c) prepared from the deuterated material were each shown by NMR and mass spectroscopy to have identical patterns of labelling; for example, the 100 MHz ^{1}H NMR of the bromo-adduct (3c) spectra shows three singlets at $\delta(CF_3CO_2H)$ 1.31, 2.11 and 3.45 ppm for the 6-methyl, 5-, and 7-hydrogens. Further evidence for the structure of the adducts is provided by the observation that the decomposition of 3c in aqueous solution in the presence of hydride reducing agents ($NaBH_4$, NADH) affords 1.

The ultraviolet spectrum of the hydroxy-adduct (3a) recorded at pH 8.2 (λmax at 245 and 290 nm with $\varepsilon_{245}/\varepsilon_{290}$ = 2.4) bears a striking resemblance to that reported by Kaufman[1] for a tetrahydrobiopterin-derived intermediate formed during the enzyme catalyzed hydroxylation of phenylalanine (λmax at 250 and 295 nm with $\varepsilon_{250}/\varepsilon_{295}$ = 3.1). The deaza-analog (1) has been shown to function as a potent competitive inhibitor against tetrahydrobiopterin for the phenylalanine[17] and tyrosine[18] hydroxylase enzymes. No catalytic activity was detected. The inhibition can be anticipated on the basis of the less favorable oxidation potential of 1. The halfwave potential for 1 has been determined to be ~+0.7V vs. SCE at pH 9 on a glassy carbon electrode relative to ~0.0V for 6,7-dimethyltetrahydropterin under the same conditions.

Further studies are in progress to examine the chemistry and biochemistry of these and other 4a-adducts. It remains to be established whether the adducts (3) will function in or inhibit the enzyme catalyzed reaction.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the National Science Foundation.

REFERENCES

1. S. Kaufman in "The Chemistry and Biology of Pteridines," Proc. 5th Int. Symp., ed. W. Pfleiderer, de Gruyter, Berlin, 1975, p. 291.
2. S. Kaufman and D.B. Fisher in "Molecular Mechanisms of Oxygen Activation," ed. O. Hayaishi, Academic Press, New York, 1975, p. 285.
3. G.I. Dmitrienko, V. Snieckus, and T. Viswanatha, Bioorganic Chem., 6, 421 (1977).
4. G.A. Hamilton in "Molecular Mechanisms of Oxygen Activation," ed. O. Hayaishi, Academic Press, New York, 1975, p. 285.
5. H.I.X. Mager in "The Chemistry and Biology of Pteridines", Proc. 5th Int. Symp., ed. W. Pfleiderer, de Gruyter, Berlin, 1975, p. 753.
6. M. Viscontini and T. Okada, Helv. Chim. Acta, 50, 1492 (1970); G.R. Gapski, S.M. Whitely and F.M. Huennekens, Biochemistry, 10, 2930 (1971); J.A. Blair, A.J. Pearson and A.J. Robb, J.C.S. Perkin II, 18 (1975).

7. E. Stark and E. Breitmaier, Tetrahedron Letters, 29, 2209 (1973).
8. The formation of a 4a-adduct has precedence in the chemistry of 5-alkylbarbiturate and uracil derivatives which react with halogenating agents to afford the respective 5-halo-5-alkyl compounds (cf. D.J. Brown, "The Pyrimidines", Interscience, New York, 1962, p. 213).
9. The hydroxy-adduct is a mixture of diastereomers in the ratio ~2:1. The ^{13}C NMR of the major isomer is shown in Figure 1.
10. Each of the compounds (1 and 3a-c) gave a satisfactory elemental analysis.
11. B. Schircks, J.H. Bieri, and M. Viscontini, Helv. Chim. Acta, 59, 248 (1976); 60, 211 (1977); W. Frick, R. Welser, and M. Viscontini, Helv. Chim. Acta, 57, 2658 (1974).
12. J. Riand, M. Th. Chenon, and N. Lumbroso-Bader, J. Am. Chem. Soc., 99, 6838 (1977).
13. H. Booth and D. Vaughan Griffiths, J.C.S. Perkin II, 842 (1973).
14. G.C. Levy and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Interscience, New York, 1972, p. 47.
15. The low field resonance is assigned to the 8a-carbon on the basis of the fact that this carbon invariably appears at higher field than either of the 2- and 4-carbons in tetrahydropterins and other pterin derivatives.[11]
16. The low field resonance is assigned to the hydrogens α to nitrogen by analogy with the spectra of tetrahydropterins (cf. W.F. Armarego and H. Schou, J.C.S. Perkin II, 2529 (1977).
17. J.E. Ayling, personal communication.
18. S. Kaufman, personal communication.