Pterins.† Part 2.[1] Stereochemistry of Catalytic Reduction of 6-Methyl- and 6,7-Dimethyl-pterin and of 2,4-Diamino-6-methylpteridine

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Catalytic addition of two molecules of hydrogen to 7-deuterio-6-trideuteriomethylpterin yields a 0.8 : 1 mixture of *cis*- and *trans*-7-deuterio-6-trideuteriomethyl-5,6,7,8-tetrahydropterin. Similar reduction of 2,4-diamino-7-deuterio-6-(partial)trideuteriomethylpteridine gives a 1 : 1 mixture of *cis*- and *trans*-2,4-diamino-7-deuterio-6-(partial)trideuteriomethyl-5,6,7,8-tetrahydropteridine. Catalytic reduction of 6,7-dimethyl-and 6,7-bis(trideuteriomethyl)pterin, on the other hand, is stereospecific and forms only the *cis*-5,6,7,8-tetrahydro-derivatives. Reduction of 6,7-dimethylpterin with sodium in ethanol provides a 1 : 1 mixture of *cis*- and *trans*-6,7-dimethyl-5,6,7,8-tetrahydropterin. The stereochemistry of these products was deduced from ^{1}H n.m.r. spectroscopy.

THE 5,6,7,8-tetrahydro-derivatives of 6-methyl- (1) and 6,7-dimethyl-pterin (2) are substrates for the mono-oxygenase enzyme systems which hydroxylate (*S*)-phenylalanine to (*S*)-tyrosine,[2,3] (*S*)-tyrosine to (*S*)-3,4-dihydroxyphenylalanine (dopa),[2,4] and (*S*)-tryptophan to (*S*)-5-hydroxytryptophan.[5] The first-mentioned system, which has been studied in great detail,[2,3] consists of at least two separate enzymes: a hydroxylase which oxidises phenylalanine, and an NADPH-requiring dihydropterin reductase which reduces the dihydropterin formed back to its tetrahydro-derivative; and the cycle is then repeated. The tetrahydro-derivatives of the substrates (1) and (2) can replace the natural coenzyme 5,6,7,8-tetrahydrobiopterin (3) very effectively.[2,3] Much evidence has been presented in support of the intermediate ' quinonoid ' 6,7-dihydropterin (4), which could not be isolated because it rearranges rapidly in the absence of enzymes to the isomeric biologically inactive 7,8-dihydropterin.[2,3,5,6] The available data indicate that the chiral centre at C-6 and the chiral or prochiral centre at C-7 are unaffected in the enzymic cycle. We require, however, more direct evidence regarding the relative configuration of the two centres during the cycle. We also want to know the relative and absolute stereospecificities at these two centres when the substrate (1) is reduced by folate reductases.

In order to solve these problems we needed a method to identify the stereochemistry of addition of hydrogen to C-6 and -7 in 6-methyl- and 6,7-dimethyl-pterin. The ^{1}H n.m.r. approach chosen was based on the knowledge that the vicinal coupling constants for the *cis*-2- and 3-protons (*ca.* 2.7 Hz) in substituted 1,2,3,4-tetrahydroquinoxalines were consistently smaller than those for the corresponding protons (*ca.* 8 Hz) in the *trans*-isomers.[7] We describe here the preparation and spectra of 6,7-dimethyl- (5), 6,7-bis(trideuteriomethyl)- (8), 6-methyl- (6), 7-deuterio-6-methyl- (7), 6-trideuteriomethyl- (9), and 7-deuterio-6-trideuteriomethyl- (10) 5,6,7,8-tetrahydropterins, and of 2,4-diamino-7-deuterio-6-trideuteriomethyl-5,6,7,8-tetrahydropteridine. The relative stereochemistry of hydrogen addition to C-6 and

† Pterin is 2-aminopteridin-4(3*H*)-one. For a discussion of the term see ref. 2, p. 2.

[1] Part 1, W. L. F. Armarego and B. A. Milloy, *Austral. J. Chem.*, 1977, in the press.

[2] R. L. Blakley, ' The Biochemistry of Folic Acid and Related Pteridines,' North-Holland, Amsterdam, 1969, p. 293.

[3] S. Kaufman, *Adv. Enzymol.*, 1971, **35**, 245.

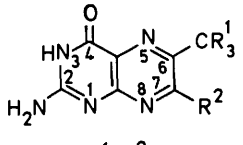
[4] H. Ozawa and K. Suzuki, *J. Pharm. Soc. Japan*, 1971, **91**, 1250.

[5] V. Massey and P. Hemmerich, ' The Enzymes,' vol. XII, ed. P. D. Boyer, Academic Press, New York, 1975, p. 240.

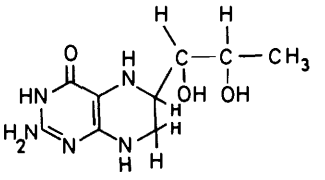
[6] K. G. Scrimgeour, ' Chemistry and Biology of Pteridines,' ed. W. Pfleiderer, de Gruyter, Berlin, 1975, p. 731.

[7] R. Aguilera, J-C. Duplan, and C. Nofre, *Bull. Soc. chim. France*, 1968, 4491.

-7 to form these tetrahydro-derivatives can now be deduced from ^{1}H n.m.r. data.



(1) $R^1=R^2=H$
(2) $R^1=H$, $R^2=Me$



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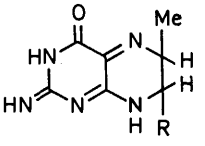

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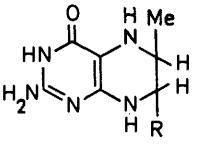
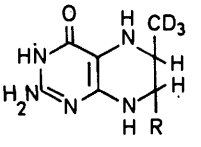


(5) R=Me
(6) R=H
(7) R=D



(8) $R = CD_3$
(9) R = H
(10) R = D

The methyl protons of 6,7-dimethylpterin, which is readily prepared from 2,4,5-triamino-6-hydroxypyrimidinium sulphate and biacetyl,[8] are barely exchanged by deuterium in deuteriated acid. In deuteriated aqueous base, on the other hand, the exchange is much faster and 6,7-bis(trideuteriomethyl)pterin with >98% isotopic purity is obtained. Catalytic reduction of this product in 3N-hydrochloric acid gave 6,7-bis(trideuteriomethyl)-5,6,7,8-tetrahydropterin hydrochloride (8) in high yield without observable loss of deuterium. The ^{1}H n.m.r. spectrum of this compound [see below and Figure 1(c)] shows that the configuration is entirely *cis*. While this work was in progress Weber and Viscontini [9] demonstrated by a different method that the catalytic reduction of 6,7-dimethylpterin in trifluoroacetic acid gave exclusively the *cis*-tetrahydro-derivative (5) (see below).

The catalytic reduction of 6-methylpterin to 6-methyl-5,6,7,8-tetrahydropterin provides only one product, although the relative stereochemistry of the hydrogen atoms that add to C-6 and C-7 can be *cis* or *trans*. The stereochemistry of the addition can, however, be determined by the reduction of 7-deuterio-6-methylpterin. We have analysed the ^{1}H n.m.r. spectrum of 6-methyl-5,6,7,8-tetrahydropterin hydrochloride in D_2O. The axial and equatorial C-7 proton signals are clearly separated and their coupling constants with H-6 are observable [see Figure 2(a) and below]. The spectrum of the tetrahydro-derivative of 7-deuterio-6-trideuteriomethylpterin should therefore readily show the steric relation of the protons that have added to C-6 and -7.

Our first approach to the synthesis of 7-deuterio-6-methylpterin was by direct deuteriation of 6-methylpterin. Here, as in 6,7-dimethylpterin, we found that deuteriated acid caused very little exchange, other than of the labile hydrogen atoms on the nitrogen atoms. Deuteriated alkali exchanged all the protons on the 6-methyl group, but there was negligible exchange of H-7 under the variety of conditions tried. We then turned to Taylor's unambiguous pteridine synthesis; [10,11] *viz.* (11; $R^1 = R^2 = H$) ⟶ (12; $R^1 = R^2 = H$, $R = CO_2Et$) ⟶ (13; $R^1 = R^2 = H$) ⟶ (1). Perdeuteriopyruvaldehyde oxime (deuteriated oximinoacetone) (11; $R^1 = R^2 = D$) of high deuterium content was prepared as described [12] from ethyl aceto-acetate but using

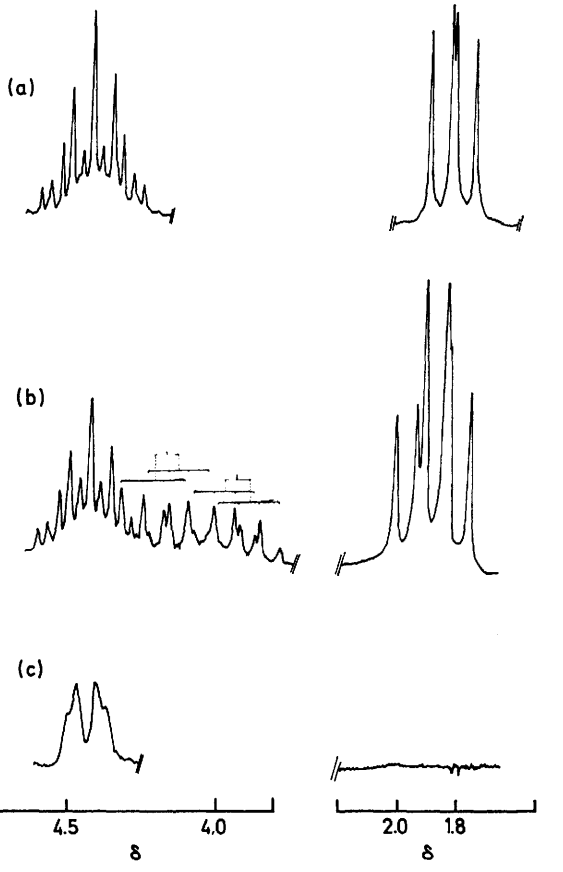


FIGURE 1 ^{1}H N.m.r. spectra of (a) *cis*-6,7-dimethyl-5,6,7,8-tetrahydropterin hydrochloride in D_2O at 100 MHz; (b) *cis*- and *trans*-6,7-dimethyl-5,6,7,8-tetrahydropterin hydrochloride in 0.5N-DCl at 100 MHz; (c) *cis*-6,7-bis(trideuteriomethyl)-5,6,7,8-tetrahydropterin hydrochloride in D_2O at 100 MHz

[8] H. I. X. Mager, R. Addink, and W. Berends, *Rec. Trav. chim.*, 1967, **86**, 833; C. K. Cain, M. F. Mallette, and E. C. Taylor, *J. Amer. Chem. Soc.*, 1946, **68**, 1996.
[9] R. Weber and M. Viscontini, *Helv. Chim. Acta*, 1976, **59**, 2379.
[10] E. C. Taylor, K. L. Perlman, I. P. Sword, M. Séquin-Frey, and P. A. Jacobi, *J. Amer. Chem. Soc.*, 1973, **95**, 6407.
[11] E. C. Taylor, K. L. Perlman, Y-H. Kim, I. P. Sword, and P. A. Jacobi, *J. Amer. Chem. Soc.*, 1973, **95**, 6413.
[12] L. Vanino, 'Handbuch der Präparative Chemie,' Ferdinand Enke Verlag, Stuttgart, 1936, vol. II, p. 834.

completely deuteriated reagents in deuterium oxide. The direct deuterium exchange reactions

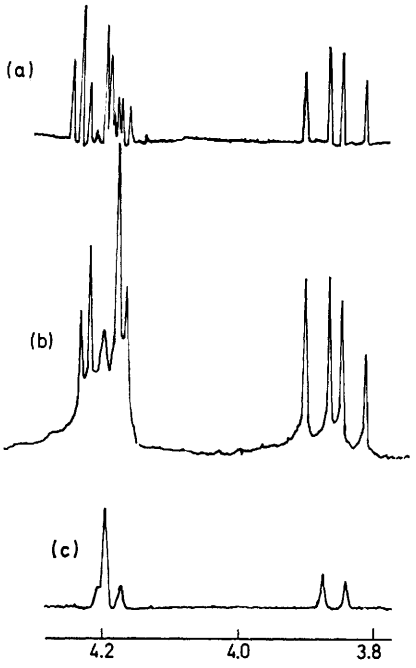
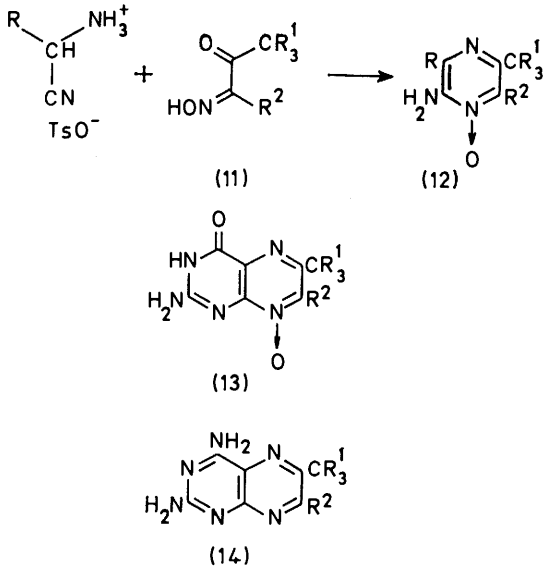


FIGURE 2 ^{1}H N.m.r. spectra of (a) 6-methyl-5,6,7,8-tetrahydropterin hydrochloride in D_2O at 270 MHz; (b) 6-trideuteriomethyl-5,6,7,8-tetrahydropterin hydrochloride in D_2O at 270 MHz; (c) *cis*- and *trans*-7-deuterio-6-trideuteriomethyl-5,6,7,8-tetrahydropterin hydrochloride in D_2O at 270 MHz

of the oxime were less satisfactory, and in deuterium oxide containing sodium deuterioxide, the protons of the methyl group of the oxime were exchanged more



rapidly than the aldehydic proton. Although almost complete exchange of the aldehydic proton was possible, it was found that, during exchange of the CD_3 group to give back a CH_3 group with aqueous alkali, much self-condensation of the oxime had taken place. This was revealed by an increase in the number of *C*-methyl signals in the ^{1}H n.m.r. spectrum. The reaction of ethyl α-aminocyanoacetate toluene-*p*-sulphonate salt with the deuteriated oxime proceeded smoothly and gave 2-amino-6-deuterio-3-ethoxycarbonyl-5-trideuteriomethylpyrazine 1-oxide (12; R = CO_2Et, $R^1 = R^2 = D$) in which only a small percentage of the deuterium on the methyl group and none of the 6-D had been exchanged. The deuteriated ester reacted with guanidine in the presence of sodium methoxide but provided 6-methylpterin 8-oxide (13; $R^1 = R^2 = H$) in which almost complete exchange of deuterium at C-7 and in the methyl group had occurred. A repetition of this reaction with deuteriated reagents and deuteriated solvents would be prohibitive in cost. Alternative syntheses were therefore sought. Attempts to prepare the pterin 8-oxide (13; $R^1 = R^2 = H$) from 2-amino-3-ethoxycarbonyl-5-methylpyrazine 1-oxide (12; R = CO_2Et, $R^1 = R^2 = H$) by condensation with cyanamide or methyl isothiouronium sulphate, or from 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide with cyanamide or cyanogen bromide, or from 3-carbamoyl- or 3-ethoxycarbonyl-2-amino-5-methyl-pyrazine with guanidine and sodium methoxide were uniformly unsuccessful. These data imply that in the formation of the pteridine 8-oxide, guanidine reacts first with the nitrile or ester function in (12; R = CN or CO_2Et), and intramolecular cyclization then takes place onto the 2-amino-group. If this is not the case then the pyrazine 1-oxide (12; R = CO_2Et, $R^1 = R^2 = H$) should be less reactive than 2-amino-3-ethoxycarbonyl-5-methylpyrazine because of the stronger basicity of the amino-group in the latter. Predicting that the nitrile function would be more reactive than the ester function in this system, we condensed 2-amino-3-cyano-5-methylpyrazine with guanidine as above and obtained 2,4-diamino-6-methylpteridine (14; $R^1 = R^2 = H$) in good yields (*cf.* ref. 11). When the reaction was repeated with 2-amino-3-cyano-6-deuterio-5-methylpyrazine [obtained from the oxime (11; $R^1 = R^2 = D$) by deoxygenation of the derived 1-oxide (12; R = CN, $R^1 = R^2 = D$)], it provided 2,4-diamino-6-methylpteridine (14) in which C-7 was completely deuteriated and the 6-methyl group was *ca.* 65% deuteriated. This result is not surprising because we know that the hydrogen atoms on the methyl group in 6-methylpterin are exchanged in the presence of sodium deuterioxide. Catalytic reduction of the diaminopteridine gave 2,4-diamino-7-deuterio-6-(partial)-trideuteriomethyl-5,6,7,8-tetrahydropteridine hydrochloride which had a ^{1}H n.m.r. spectrum consistent with a product from a mixture of *cis*- and *trans*-addition of hydrogen at C-6 and -7 (see below). The integrals indicated that the 6-methyl group was 65% deuteriated.

2,4-Diamino-6-methylpteridine was relatively stable in warm hydrochloric acid but, as in the hydrolysis of 2,4-diamino-6-bromomethylpteridine hydrobromide to 6-bromomethylpterin hydrobromide,[13] it was converted

[13] J. A. Montgomery, J. D. Rose, C. Temple, jun., and J. R. Piper, 'Chemistry and Biology of Pteridines,' ed. W. Pfleiderer, de Gruyter, Berlin, 1975, p. 485.

into 6-methylpterin hydrobromide on heating with aqueous 48% hydrobromic acid. A much more satisfactory preparation of 6-methylpterin involved heating and recrystallising the 2,4-diamino-derivative from 2N-sodium hydroxide, which yielded the sodium salt of 6-methylpterin from which the free base can be isolated in a pure state by acidification. A similar recrystallisation of 2,4-diamino-7-deuterio-6-(partial)trideuteriomethylpteridine from aqueous 2N-sodium hydroxide gave 7-deuterio-6-methylpterin, whereas heating and recrystallising from 2N-sodium deuterioxide gave the deuterio-compound (1; $R^1 = R^2 = D$) in excellent yields. Catalytic reduction of these compounds furnished the tetrahydro-derivatives (7) and (10), respectively, without loss of deuterium at C-7.

^1H N.m.r. data of 5,6,7,8-tetrahydropteridines at 100 MHz [a]

5,6,7,8-Tetrahydropterin hydrochloride	H-6	H-7	CH_3	Solvent
cis-6,7-Dimethyl	4.34 [b] (J 3.1, 6.7)	4.43 [b] (J 3.1, 6.7)	6-Me [b] 1.79 (J 6.7) 7-Me [b] 1.87 (J 6.7)	D_2O
cis-6,7-Bis(trideuteriomethyl)	4.34 [b] (J 3.1)	4.43 [b] (J 3.1)		0.5N-DCl
trans-6,7-Dimethyl	3.94 [c] (J 8.3, 6.8)	4.16 [d] (J 8.3, 6.8)	6-Me [c] 1.87 (J 6.8) 7-Me [d] 1.95 (J 6.8)	0.5N-DCl
6-Trideuteriomethyl [e,f] (15; $R^1 = CD_3$, $R^2 = H_C$)	H_A 4.19$_5$ (J_{AB} 3.6, J_{AC} 9.0)	H_B 4.19$_8$ (J_{AB} 3.6, J_{BC} −14.2); H_C 3.85$_6$ (J_{AC} 9.0, J_{BC} −14.2)		D_2O
6-Methyl	(as above and J 6.4)	(as above and J 6.4)	6-Me 1.98 (J 6.4)	D_2O
6-Methyl [g]	H_A (J_{AB} 3, J_{AC} 10.0)	H_B 4.43 (J_{AB} 3, J_{BC} 13); H_C 4.05 (J_{AC} 10.0, J_{BC} 13)		0.5N-DCl
trans-7-Deuterio-6-trideuteriomethyl [f] (16)	H_A 4.19 (J 9.0)	H_B 3.86 (J 9.0)		D_2O
cis-7-Deuterio-6-trideuteriomethyl [f] (17)	H_A 4.20	H_B 4.20		D_2O
2,4-Diamino-5,6,7,8-tetrahydropteridine hydrochloride				
6-Methyl	4.19 [h]	7-*ax* 3.84 (J_{vic} 10.2, J_{gem} −14.5) 7-*eq* 4.20 (J_{vic} 3.1, J_{gem} −14.5)	6-Me 2.03 (J 6.3)	D_2O
trans-7-Deuterio-6-(partial)trideuteriomethyl	4.19 (J 10.2)	3.84 (J 10.2)	6-CHD_2 2.03br (d)	D_2O
cis-7-Deuterio-6-(partial)trideuteriomethyl	4.20	4.20	6-CHD_2 2.03br (d)	D_2O

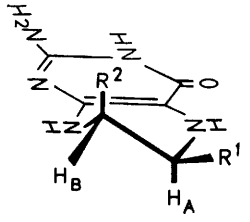
[a] Concentration 20 mg in 0.5 ml; tetramethylsilane as external standard. [b] Assignments taken from ref. 9. [c,d] Tentative, assignments may be reversed. [e] Computer-simulated spectrum from the experimental signal positions (by M. J. Whittaker), error ±0.1 Hz. [f] Data at 270 MHz are almost identical with these although the spectrum appears simpler (see Figure 2). [g] From ref. 14. [h] Complex multiplet.

^1H N.m.r. Spectra and Stereochemistry.—The spectrum of 6,7-dimethyl-5,6,7,8-tetrahydropterin hydrochloride in deuterium oxide [Figure 1(a) and Table] has eleven lines (theory: fourteen lines) for H-6 and -7, and two doublets for Me-6 and -7. The coupling constants for the methyl doublets (6.7 Hz) are almost identical, making the vicinal coupling constant for H-6 and -7 (3.1 Hz) readily observable. The small coupling constant suggests a *cis*-stereochemistry consistent with a half-chair conformation (15; $R^1 = R^2 = Me$) or equilibrating half-chair conformations (15) ⇌ (15a), as has previously been postulated for such systems.[7,9,14] The spread of the signals for *cis* H-6 and -7 (*ca.* 0.5 p.p.m.) could conceivably be masking signals from the *trans*-isomer which may be a contaminant. However, the absence of the latter isomer in the crude and purified samples was confirmed by the spectrum of 6,7-bis(trideuteriomethyl)-5,6,7,8-tetrahydropterin hydrochloride [Figure 1(c)] which was prepared under identical conditions. The J value and chemical shifts of the two doublets are consistent with the values derived from the multiplet of the non-deuteriated isomer. Our deductions and those reported [9] were made only from the knowledge that the coupling constant observed between H-6 and -7 was small. We confirmed this beyond doubt by preparing a *ca.* 1 : 1 mixture of *cis*- and *trans*-6,7-dimethyl-5,6,7,8-tetrahydropterin hydrochloride by reduction of 6,7-dimethylpterin with a large excess of sodium in ethanol. The ^1H n.m.r. spectra of the isomers were clearly separated [see Figure 1(b)] and the coupling constant between H-6 and -7 in the *trans*-isomer was significantly larger than that in the *cis*-isomer (see Table).

The spectrum of 6-methyl-5,6,7,8-tetrahydropterin hydrochloride and its 6-deuterio-derivative in 0.5N-deuterium chloride at 100 MHz had been reported previously [15] and the J values were deduced by computation. The quartet for H-7 was assigned from the spectrum of the 6-deuteriated derivative. We attempted unsuccessfully to convert the ABC pattern of signals from H-6, H_{ax}-7 and H_{eq}-7 into a first-order spectrum by varying the solvents [*e.g.* $CF_3{\cdot}CO_2H$, D_2SO_4, $(CD_3)_2SO$, $(CD_3)_2N{\cdot}CDO$, and DCl]; however our spectrum of the hydrochloride in deuterium oxide was similar to the one in 0.5N-deuterium chloride reported. The complicating factor in this spectrum is the further coupling of H-6 with the 6-methyl group which makes the theoretical spectrum of H-6 consist of sixteen lines. In a further attempt to obtain a first-order spectrum we measured this compound

[14] R. A. Archer and H. S. Mosher, *J. Org. Chem.*, 1967, **32**, 1378.
[15] R. Weber and M. Viscontini, *Helv. Chim. Acta*, 1975, **58**, 1772.

at 270 MHz [Figure 2(a)]. The signal pattern for H-6 and -7 [*i.e.* in (15; R^1 = Me, R^2 = H)] at this field was slightly different from the one at 100 MHz, but there appeared to be very little dispersion of H-6 and H_{eq}-7. The pattern was, however, simplified in the spectrum of



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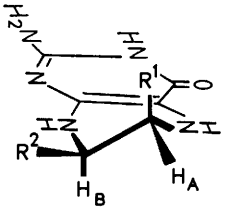

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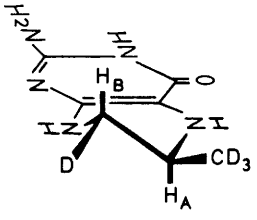

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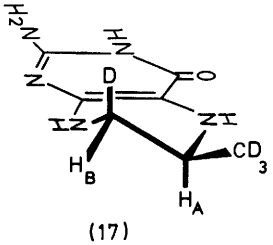

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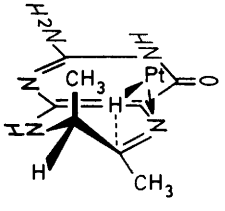

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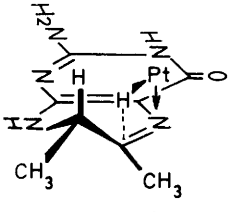

(19)

6-trideuteriomethyl-5,6,7,8-tetrahydropterin hydrochloride (9) [Figure 2(b)]; the quartet from H_C in (15; R^1 = CD_3, R^2 = H_C) is clearly visible and the downfield signals contain the quartet from H_B (see simulated spectrum in Table) confirming that the equilibrium (15; R^1 = CD_3, R^2 = H_C) ⇌ (15a; R^1 = CD_3, R^2 = H_C) is largely in favour of the conformer with the 6-methyl group pseudoequatorial, 6-H_A pseudoaxial, 7-H_B pseudoequatorial, and 7-H_C pseudoaxial (see ref. 15). In this example also the spectra in deuterium oxide at 100 and at 270 MHz were similar, with a very small separation in chemical shifts between H_A and H_B [in formula (15; R^1 = CD_3, R^2 = H_C)]. The computer-simulated spectra (kindly computed by M. J. Whittaker) together with those reported for 6-methyl-5,6,7,8-tetrahydropterin hydrochloride in 0.5N-deuterium chloride are in the Table. The data indicate that these spin systems are more like an AA'B than an ABC system, and the computed J_{AB}, J_{AC}, and J_{BC} values are very close (within ±0.1 Hz) to the values measured directly from the spectrum at 270 but not at 100 MHz. The former spectrum is closer to a first-order spectrum with regard to H_{ax}-7 and H_{eq}-7, but the signals from H-6 are still too broad to be assigned by inspection. The differences in J values between this work and that reported [15] may be partly due to the effect of solvent on the equilibrium (15) ⇌ (15a).

It is now possible, with the knowledge of the above assignments, to determine the stereochemistry of addition of hydrogen to C-6 and -7 in 7-deuterio-6-trideuteriomethylpterin. The spectrum of the tetrahydro-derivative obtained from catalytic reduction [Figure 2(c)] consists of two doublets (*trans* J 9.0 Hz) and a singlet inside the downfield doublet. The chemical shifts of these signals, when compared with those of 6-trideuteriomethyl-5,6,7,8-tetrahydropterin hydrochloride, confirm that the compound is a mixture consisting of the *trans*-isomer (a quartet) in the predominant conformation (16) and the *cis*-isomer (a singlet) in the predominant conformation (17), with almost similar chemical shifts for H_A and H_B in the *cis*-isomer, although the *trans*- (16) and *cis*- (17) conformers predominate. The spectra give time-averaged lines for each isomer due to the conformers in equilibrium. The slightly broadened singlet from the *cis*-isomer in the spectrum [Figure 2(c)] is not centred between the doublet from the *trans*-isomer and can be explained if the position of equilibrium of the conformers is slightly different in the two isomers. This difference can be caused by the effect of the deuterium atom on the conformational equilibria, or a deuterium isotope effect on the chemical shift of the geminal proton, and is probably not of steric origin. The *cis* : *trans* ratio calculated from the integrals is 0.8 : 1.

The spectrum of 2,4-diamino-6-methyl-5,6,7,8-tetrahydropteridine hydrochloride is quite similar to that of the pterin (6), *i.e.* an AA'B pattern, and the chemical shift of the C-7 protons can be assigned by inspection from the above knowledge. 2,4-Diamino-7-deuterio-6-(partial)-trideuteriomethylpteridine gave on catalytic reduction in 3N-hydrochloric acid the corresponding tetrahydro-derivative, which had a spectrum similar to that of the pterin (10). The *cis* : *trans* ratio in this case was *ca* 1 : 1.

The difference in the stereospecificity between the catalytic additions of hydrogen to 6,7-dimethylpterin and to 6-methylpterin deserves some comment. Undoubtedly two reduction steps are involved: addition of one molecule of hydrogen across the 7,8-double bond followed by addition across the 5,6-double bond. We have checked this point with 6-methylpterin by measuring the ^{1}H n.m.r. and u.v. spectra of samples withdrawn after the absorption of 0.4, 0.9, 1.5, and 2.0 mol. equiv. of hydrogen. The spectra showed that the ratios of 6-methylpterin to 6-methyl-7,8-dihydropterin to 6-methyl-5,6,7,8-tetrahydropterin were 0.7 : 1.0 : 0.0; 0.1 : 1.0 : 0.0; 0.0 : 1.0 : 0.8; and 0.0 : 0.0 : 1.0, respectively. A sample withdrawn after absorption of 1 mol. equiv. of hydrogen was evaporated and the product converted into the dithionite salt. This proved identical with authentic 6-methyl-7,8-dihydropterin dithionite. In 6,7-dimethylpterin addition of hydrogen across the 7,8-double bond is slower than in the above and gave ratios of pterin to dihydropterin to tetrahydropterin of 0.4 : 1.0 : 0.4; 0.1 : 0.8 : 1.0; and 0.0 : 0.0 : 1.0 after absorption of 1.0, 1.5, and 2.0 mol. equiv. of hydrogen, respectively. The intermediate was shown to be 6,7-dimethyl-7,8-dihydropterin

by aeration of a sample at the end of the reduction, giving a u.v. spectrum similar to the one obtained from the sample withdrawn after absorption of 1.5 mol. equiv. of hydrogen. The ^{1}H n.m.r. spectra are also consistent with these findings. Aeration of 6,7-dimethyl-5,6,7,8-tetrahydropterin is known to furnish the 7,8-dihydro-derivative.[16] The stereospecificity of *cis*-addition in 6,7-dimethylpterin can be explained by adsorption of the molecule on the reduced catalyst and the addition of one molecule of hydrogen across the 7,8-double bond. Then either the dihydro-substrate is held on the catalyst and a second molecule of hydrogen is added stereospecifically, or more likely it is desorbed, and readsorbed stereospecifically because of the encumbrance of the 7-methyl group [*i.e.* structure (19) would be more favoured in the transition state than structure (18)]. In the case of 6-methylpterin, after the 7,8-dihydro-compound is formed it must be released into the solution and readsorbed on the catalyst, almost randomly because of lack of steric hindrance near C-7, and reduced further across N(5)–C(6).

EXPERIMENTAL

Elemental analyses were determined by the Australian National University Analytical Service Unit; values for H + D were calculated as before.[17] I.r. spectra of solids (KBr) were measured with a Unicam SP 1000 spectrometer, and u.v. spectra with a Unicam SP 1800. ^{1}H N.m.r. spectra were obtained with Varian T60A and HA100 spectrometers (tetramethylsilane as internal or external lock). 270 MHz Spectra were measured with a Brucker HFX-270 spectrometer by the National NMR Centre (Dr. A. J. Jones). *J* Values are in Hz. Mass spectra (by Dr. J. K. MacLeod and staff) were measured with an A.E.I. MS9 instrument. Deuterium oxide (>99.9%) was purchased from the Australian Atomic Energy Commission. All evaporations were carried out at <30 °C and 18 mmHg.

6,7-Dimethylpterin (7 g) was converted into the *sodium salt* (82%) by recrystallisation from 2N-sodium hydroxide (150 ml). The salt was washed with a little cold water then ethanol and dried at 100 °C, and had m.p. >360 °C (decomp.) (Found: C, 45.3; H, 3.9; N, 32.6; Na, 11.1. $C_8H_8N_5ONa$ requires C, 45.1; H, 3.8; N, 32.85; Na, 10.8%). The sodium salt of 6,7-bis(trideuteriomethyl)pterin was prepared in 70% yield by heating 6,7-dimethylpterin (250 mg) at 100 °C in 2N-sodium deuteroxide in deuterium oxide (25 ml) for 24 h in a sealed tube and isolated as above. It had no proton n.m.r. signals in 2N-sodium deuterioxide or 2N-deuterium chloride.

6-Methylpterin Sodium Salt.—Crude 6-methylpterin, prepared from 2,5,6-triamino-4-hydroxypyrimidinium sulphate as before [18] but on a 130 g scale, was shown by ^{1}H n.m.r. spectroscopy in 2N-DCl–D_2O to contain 36% of 7-methylpterin [δ 2.77 (7-Me), 2.80 (6-Me), 8.81 (6-H), and 8.93 (7-H)]. The pure sodium salt of 6-methylpterin was obtained by recrystallisation of the mixture from 10 parts of 2N-sodium hydroxide (50% recovery) and had m.p. >360 °C (decomp.) [Found (after drying at 150 °C for 12 h): C, 41.4; H, 3.3; N, 34.4; Na, 11.3. $C_7H_6N_5NaO,0.25H_2O$ requires C, 41.3; H, 3.2; N, 34.4; Na, 11.3%]. The free base was prepared by acidifying an aqueous solution of the salt, and collecting and washing (H_2O and EtOH) by centrifugation because conventional filtration was exceedingly slow. This compound and its tetrahydro-derivative were identical with samples from an unequivocal synthesis.[10]

6-Trideuteriomethylpterin Sodium Salt.—6-Methylpterin (1 g) in 2N-sodium deuterioxide (70 ml) was heated in a bomb at 100 °C for 24 h. The *sodium salt* (600 mg) that crystallised on cooling was collected, washed with a little water and ethanol, and dried. It had m.p. >360 °C (decomp.) (Found: C, 39.7; H + D, 4.7; N, 32.9; Na, 10.7. $C_7H_3D_3N_5NaO,0.5H_2O$ requires C, 39.8; H + D, 4.7; N, 33.2; Na, 10.9%). Prolonged heating of the sodium deuterioxide solution at 120 °C did not cause H-7 to be displaced by deuterium.

7-Deuterio-6-methylpterin Sodium Salt.—2,4-Diamino-7-deuterio-6-(partial)trideuteriomethylpteridine (200 mg; see below) in 2N-sodium hydroxide (75 ml) was stirred at 100 °C for 9 h. The solution was concentrated until a solid crystallised, and was cooled. The yellow *sodium salt* (120 mg) was collected as above. A further 60 mg of salt was obtained from the mother liquors. The u.v. spectra and t.l.c. properties were identical with those of authentic non-deuteriated 6-methylpterin, and only a sharp 6-methyl signal was present in the ^{1}H n.m.r. spectrum (Found: Na, 11.3. $C_7H_5DN_5NaO$ requires Na, 11.5%).

7-Deuterio-6-trideuteriomethylpterin Sodium Salt.—This was prepared as above from 2,4-diamino-7-deuterio-6-(partial)trideuteriomethylpteridine in 2N-sodium deuterioxide (18 h at 100 °C) and had m.p. >360 °C (decomp.) (Found: C, 40.8; H + D, 5.2; N, 34.4. $C_7H_{1.5}D_{4.5}N_5NaO,0.1H_2O$ requires C, 40.9; H + D, 5.2; N, 34.1%). The u.v. spectra and t.l.c. behaviour were identical with the above, but the product had no ^{1}H n.m.r. signals in 2N-deuterium chloride.

Deuteriated Hydroxyiminoacetone.—Ethyl acetoacetate (23.8 g) was added to a solution of sodium (4.6 g, 1.1 mol. equiv.) in deuterium oxide (300 ml) followed by sodium nitrite (13.2 g). The mixture was set aside for 24 h, acidified to pH 1 with concentrated hydrochloric acid, and extracted thoroughly with ether and dried (Na_2SO_4). Evaporation gave the *deuteriated oxime* (14.5 g, 87%), which was sublimed at 50 °C and 0.5 mmHg, and had m.p. 65—66 °C (lit.,[19] 65 °C for non-deuteriated compound prepared by nitrosation of acetone); $\nu_{max.}$ 1 660 (CO), 1 445, and 980 cm^{-1} (Found: C, 39.7; H + D, 10.0; N, 15.2. $C_3HD_4NO_2$ requires C, 39.55; H + D, 9.95; N, 15.4%); *m/e* 92 (7%, $C_3D_5NO_2^{\cdot+}$), 91 (100, $C_3HD_4NO_2^{\cdot+}$), 90 (24, $C_3H_2D_3NO_2^{\cdot+}$), 89 (7, $C_3H_3D_2NO_2^{\cdot+}$), and 87 (0); the *CH*:NOH signal was absent in the ^{1}H n.m.r. spectrum.

2-Amino-6-deuterio-3-ethoxycarbonyl-5-(partial)trideuteriomethylpyrazine 1-Oxide.—The preceding deuteriated oxime (2.2 g) and ethyl α-aminocyanoacetate toluene-*p*-sulphonate salt [20] (7.6 g) in methanol (10 ml) were stirred at 35 °C for 24 h. The solution was evaporated and diluted with water (40 ml); the pH was adjusted to 9 and the mixture extracted with chloroform. Evaporation of the extract gave the *deuteriated pyrazine oxide* (3.3 g), m.p. 132—133.5 °C after sublimation at 140 °C and 0.5 mmHg (lit.,[9] m.p. 132.5—133.5 °C for non-deuteriated pyrazine oxide); *m/e* 185 (28%, $C_8H_7D_4$-

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$N_3O_2^{\cdot+}$), 184 (71, $C_8H_8D_3N_3O_2^{\cdot+}$), 183 (100, $C_8H_9D_2N_3O_2^{\cdot+}$), 182 (85, $C_8H_{10}DN_3O_2^{\cdot+}$), and 181 (0); $\nu_{max.}$ 3 470, 3 335 (NH), 1 690 (CO), and 1 610 cm^{-1}; u.v. data identical with lit. values [10] for non-deuteriated oxide; δ (60 MHz; $CDCl_3$) 1.45 (3 H, t, *J* 7, Me), 2.47 (2 H, s, 5-Me) and 4.51 (2 H, q, *J* 7, CH_2), and 7.3br (2 H, s, NH_2), with no signal for H-6 (Found: C, 48.3; H + D, 6.7; N, 21.1. $C_8H_9D_2N_3O_3$ requires C, 48.2; H + D, 6.6; N, 21.1%).

2-*Amino*-3-*cyano*-6-*deuterio*-5-*trideuteriomethylpyrazine* 1-*Oxide*.—The preceding deuteriated hydroxyiminoacetone (5.8 g) and α-aminomalononitrile toluene-*p*-sulphonate salt [21] (16.9 g) in propan-2-ol (100 ml) were stirred at 25 °C for 4 h. The pyrazine oxide that separated contained some toluene *p*-sulphonic acid which was removed by dissolving the mixture in water, adjusting the pH to 9, and extracting with chloroform. Evaporation gave the *deuteriated pyrazine oxide* (74%), m.p. 188.5—189 °C, after sublimation at 125 °C and 0.1 mmHg (lit.,[11] m.p. 187—188 °C for non-deuteriated oxide); u.v. data identical with those of non-deuteriated oxide; $\nu_{max.}$ 3 405, 3 310 (NH), 2 150 (CN), 1 645, and 1 625 cm^{-1}; *m/e* 156 (2%), 155 (13), 154 (100, $C_2H_2D_4N_4O^{\cdot+}$), 153 (39, $C_6H_3D_3N_4O^{\cdot+}$), 152 (5, $C_6H_4D_2N_4O^{\cdot+}$), 151 (1), and 150 (0) (Found: C, 46.7; H + D, 6.3; N, 36.2. $C_6H_2D_4N_4O$ requires C, 46.75; H + D, 6.5; N, 36.3%).

2-*Amino*-3-*carbamoyl*-5-*methylpyrazine* 1-*Oxide*.—2-Amino-3-cyano-5-methylpyrazine 1-oxide [11] (5 g) in sulphuric acid (*d* 1.8; 25 ml) was heated at 100 °C for 15 min; the product was poured into water and neutralised with aqueous ammonia, and the yellow solid was collected and sublimed at 180—190 °C and 0.1 mmHg to give the *amide* (84%), m.p. 230—230.5 °C (lit.[10] 218—219 °C; lit.,[22] 235—236 °C), as prepared from α-aminocyanoacetamide and hydroxyiminoacetone; $\nu_{max.}$ 3 470, 3 440, and 3 400 (NH), 1 695, 1 670 (amide), 1 620 (C=N), and 1 170 cm^{-1} (N–O) (Found: C, 43.1; H, 4.8; N, 33.5. Calc. for $C_6H_8N_4O_2$: C, 42.8; H, 4.8; N, 33.3%). The amide could not be hydrolysed further by prolonged heating in sulphuric acid.

2-*Amino*-3-*cyano*-6-*deuterio*-5-(*partial*)*trideuteriomethylpyrazine*.—This was prepared (46% yield) by deoxygenation of the 1-oxide with phosphorus trichloride (with one quarter of the proportion used in the reported procedure [11]), and after addition of water it was necessary to concentrate the reaction mixture until turbid, and cool. The pyrazine had m.p. 174.5—175.5 °C after sublimation (lit.,[10] 172—173 °C for non-deuteriated pyrazine); u.v. spectrum identical with that of non-deuteriated pyrazine; $\nu_{max.}$ 2 140 (CN) and 1 215s cm^{-1} (Found: C, 53.1; H + D, 5.6; N, 41.3. $C_6H_{4.5}D_{1.5}N$ requires C, 53.1; H + D, 5.6; N, 41.3%), H-6 signal absent in the ^1H n.m.r. spectrum.

2,4-*Diamino*-7-*deuterio*-6-(*partial*)*trideuteriomethylpteridine*.—The preceding deuteriated pyrazine (5 g) was added to a methanolic solution of guanidine [from guanidine hydrochloride (4.1 g) dissolved in methanol (350 ml) containing sodium (2.6 g)], and the mixture was stirred at room temperature. No solid had separated after 16 h, and the solution was boiled under reflux for 18 h. The yellow solid that separated on cooling (4.7 g, 73%) was collected, washed with water, and dried. The *pteridine* had m.p. >360 °C (decomp.), and its t.l.c. properties and u.v. spectra were identical with those of authentic non-deuteriated diaminopteridine. The ^1H n.m.r. spectrum (solvent 2N-deuterium chloride) showed no peak for H-7, and the 6-methyl signal was weak and broad because of geminal H–D coupling (Found: C, 46.7; H + D, 5.6; N, 46.4. $C_7H_6D_2N_6$,0.15H_2O requires C, 46.5; H + D, 5.7; N, 46.5%).

2,4-*Diamino*-6-*methyl*-5,6,7,8-*tetrahydropteridine Hydrochloride*.—2,4-Diamino-6-methylpteridine [11] (2.5 g) was added to a pre-reduced suspension of platinum oxide (250 mg) in 3N-hydrochloric acid (250 ml) and shaken with hydrogen at 20 °C and 720 mmHg. After absorption of the theoretical amount of hydrogen (3 h), the catalyst was filtered off, and the filtrate evaporated. The residue was recrystallised from ethanol containing a little ethanolic hydrogen chloride and gave (quantitative) the *tetrahydropteridine hydrochloride*, m.p. >230 °C (decomp.), $\lambda_{max.}$ (pH 2) 219 (log ε 4.21) and 274 nm (4.15) [Found (after drying at 100 °C for 6 h): C, 21.3; H, 5.0; Cl, 45.2. $C_7H_{12}N_6$,5HCl,1.75H_2O requires C, 21.3; H, 5.2; Cl, 45.0%]. The HCl content decreased on further heating but the salt darkened in colour. The ^1H n.m.r. data are in the Table.

cis- *and* trans-2,4-*Diamino*-7-*deuterio*-6-(*partial*)*trideuteriomethyl*-5,6,7,8-*tetrahydropteridine Hydrochloride*.—This mixture was prepared as above from the preceding deuteriated diaminopteridine and had m.p. 238—240 °C (decomp.); its u.v. spectrum was identical with that of the non-deuteriated pteridine salt (Found: C, 27.7; H + D, 6.8; Cl, 27.1. Calc. for $C_7H_{10}D_2N_6$,2.3HCl,2H_2O: C, 27.8; H + D, 6.8; Cl, 27.0%).

6-*Trideuteriomethyl*-5,6,7,8-*tetrahydropterin Hydrochloride*.—This derivative, m.p. >260 °C (decomp.) (Found: C, 31.2; H + D, 7.0; Cl, 24.8. $C_7H_8D_3N_5O$,1.9HCl,H_2O requires C, 31.0; H + D, 6.65; Cl, 24.7%) was prepared by catalytic reduction of 6-trideuteriomethylpterin as above; the ^1H n.m.r. spectrum is in Figure 2(b) and in the Table. Similarly *cis*- and *trans*-7-deuterio-6-methyl-5,6,7,8-tetrahydropterin hydrochloride, m.p. >260 °C (decomp.) (Found: C, 30.5; H + D, 6.1; Cl, 22.9. Calc. for $C_7H_{10}DN_5O$,1.8HCl,1.5H_2O: C, 30.7; H + D, 6.2; Cl, 23.0%) and *cis*- and *trans*-7-deuterio-6-trideuteriomethyl-5,6,7,8-tetrahydropterin hydrochloride, m.p. >260 °C (decomp.) (Found: C, 34.1; H + D, 6.6; Cl, 23.6. Calc. for $C_7H_7D_4N_5O$,1.6HCl: C, 34.4; H + D, 6.8; Cl, 23.7%) [^1H n.m.r. spectrum in Figure 2(c) and the Table] were prepared by catalytic reduction as above. The u.v. spectra and t.l.c. behaviour of these salts were identical with those of authentic 6-methyl-5,6,7,8-tetrahydropterin hydrochloride. The HCl and H_2O contents of the crystals varied with the drying conditions and the nitrogen figures were all consistently *ca.* 1% too low.

cis-6,7-*Bis*(*trideuteriomethyl*)-5,6,7,8-*tetrahydropterin Hydrochloride*.—This derivative, m.p. >300 °C (decomp.) (Found: C, 35.0; H + D, 7.8; Cl, 24.6; N, 25.1. $C_8H_7D_6N_5O$,1.9HCl,0.25H_2O requires C, 34.9; H + D, 7.3; Cl, 24.6; N, 25.4%), was prepared by catalytic reduction of 6,7-bis(trideuteriomethyl)pterin sodium salt as above and had the same u.v. and t.l.c. properties as the authentic non-deuteriated salt. The ^1H n.m.r. spectrum is in Figure 1(c) and the Table.

cis- *and* trans-6,7-*Dimethyl*-5,6,7,8-*tetrahydropterin Hydrochloride*.—To the sodium salt of 6,7-dimethylpterin (426 mg) under dry nitrogen in boiling ethanol was added sodium until the u.v. spectrum of a sample at pH 2 indicated that reduction was complete. A total of 17 g of sodium was added during a reflux period of 48 h. The solution was cooled in an ice-bath and acidified with ethanolic hydrogen chloride

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(120 ml) under nitrogen. Sodium chloride was removed by repeated concentration and filtration. The *ca.* 1 : 1 mixture of *cis*- and *trans*-6,7-dimethyltetrahydropterin hydrochloride (282 mg) obtained from the mother liquors was crystallised from ethanol containing a little ethanolic hydrogen chloride under nitrogen. The u.v. data of the mixture, m.p. >250 °C (decomp.), were identical with those of the *cis*-isomer; the ^{1}H n.m.r. spectrum is in Figure 1(b) and the Table (Found: C, 36.2; H, 5.8; Cl, 24.8. Calc. for $C_8H_{13}N_5O,1.85HCl,0.1H_2O$: C, 36.3; H, 5.7; Cl, 24.8%). The *trans*-isomer was clearly less stable to aerial oxidation than the *cis*-isomer, with a $t_{\frac{1}{2}}$ value at 20 °C and pH 2 ($\lambda_{analyt.}$ 219 nm) of 40 min, to be compared with 5.2 h for the pure *cis*-isomer. Chromatographic separation of the mixture has not yet been achieved.

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