Mass Spectral Analysis of Substituted Pteridines and Their Reduced Analogs

V. P. Williams and J. E. Ayling

Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California 90024

Received May 14, 1973

The reduced forms of substituted pteridines exhibit cofactor and inhibitory properties with the enzyme, phenylalanine hydroxylase (Ayling, *et al.*, *Biochemistry*, 12, 2045 (1973)). Mass spectrometry has been used as a method for structure confirmation and to determine the state of purity and the extent of reduction. The structural analysis was facilitated by the use of several homologous classes of compounds. Reduced pteridines have not previously been analyzed by mass spectrometry and only a limited amount of data, much of which was from trimethylsilyl or acetyl derivatives, has been published on the mass spectral analysis of the non-reduced compounds. Detailed mass spectral fragmentation modes are presented for the reduced and non-reduced forms of the following compounds: 4-aminopteridine, 4(3*H*)pteridinone, 6,7-dimethyl-4(3*H*)pteridinone, 2,4-diamino-6,7-dimethylpteridine, 6,7-dimethyl-2,4-(1*H*,3*H*)pteridinedione, 2-amino-4(3*H*)pteridinone, 2-amino-6,7-dimethyl-4(3*H*)pteridinone, 2-amino-6-(L-erythro-1,2-dihydroxypropyl)-4(3*H*)pteridinone, 2-amino-4,6(3*H*,5*H*)pteridinedione, 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione, 2-amino-4,7(3*H*,8*H*)pteridinedione, 2-amino-6-methyl-4,7(3*H*,8*H*)pteridinedione.

Introduction.

2-Amino-5,6,7,8-tetrahydro-6-(L-erythro-1,2-dihydroxypropyl)-4(3*H*)pteridinone (tetrahydrobiopterin) (Fig. 3e) is the natural cofactor of enzymatic aromatic amino acid hydroxylations. In order to clarify the mechanism of participation of the cofactor in hydroxylation reactions, an examination was made of the structure-action relationship of a number of substituted pteridines in the reaction with phenylalanine hydroxylase (1). The structure and state of reduction of each of the compounds used in the enzymatic study were verified by mass spectrometry.

Mass spectral analysis of the pteridines and their corresponding reduced analogues are presented in this report. Oxidized and reduced pteridines exhibited different mass spectral fragmentation patterns, and together they yield considerable structural information. Many of the reduced pteridines are very rapidly re-oxidized upon exposure to air. Therefore, in order to avoid re-oxidation, it was essential to analyze the labile reduced compounds in their native form. In addition, the structural information that can be obtained from the native compounds is greater than that from the trimethylsilyl or acetylated derivatives (2-6).

For convenience the spectra are classified into four groups: (1) pteridines substituted at the 4-position: 4-aminopteridine, 4(3*H*)pteridinone, 6,7-dimethyl-4(3*H*)-pteridinone; (2) pteridines with the same substituent at the 2- and 4-positions: 2,4-diamino-6,7-dimethylpteridine, 6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione; (3) 2-amino-4-(3*H*)pteridinones (pterins) with either hydrogen or an alkyl substituent at positions 6 and 7: 2-amino-4(3*H*)-pteridinone (pterin), 2-amino-6,7-dimethyl-4(3*H*)pteridinone, 2-amino-6-(L-erythro-1,2-dihydroxypropyl)-4(3*H*)-pteridinone (biopterin); (4) pterins with a keto substituent in the pyrazine ring: 2-amino-4,6(3*H*,5*H*)pteridinedione, 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione, 2-amino-4,7(3*H*,8*H*)pteridinedione, 2-amino-6-methyl-4,7-(3*H*,8*H*)pteridinedione.

Results.

1. Pteridines Substituted in the 4-Position.

The mass spectrum of 4-aminopteridine (Fig. 1a) is characterized by the successive loss of three molecules of HCN to give the fragment ions at m/e 120, 93, and 66 (metastable transitions (m*) 98.0, 72.0 and 46.8, respectively).

An alternative fragmentation pathway is observed (Scheme I) due to the initial loss of 42 mass units [$-NH_2CN$] from the molecular ion to give m/e 105 which in turn loses a molecule of HCN to yield the ion at m/e 78 (m*57.9). Although no metastable was observed for the transition m/e 147 → m/e 105, a similar sequence has been reported for 4-methylpteridine which decomposes by the consecutive loss of CH_3CN and HCN (7).

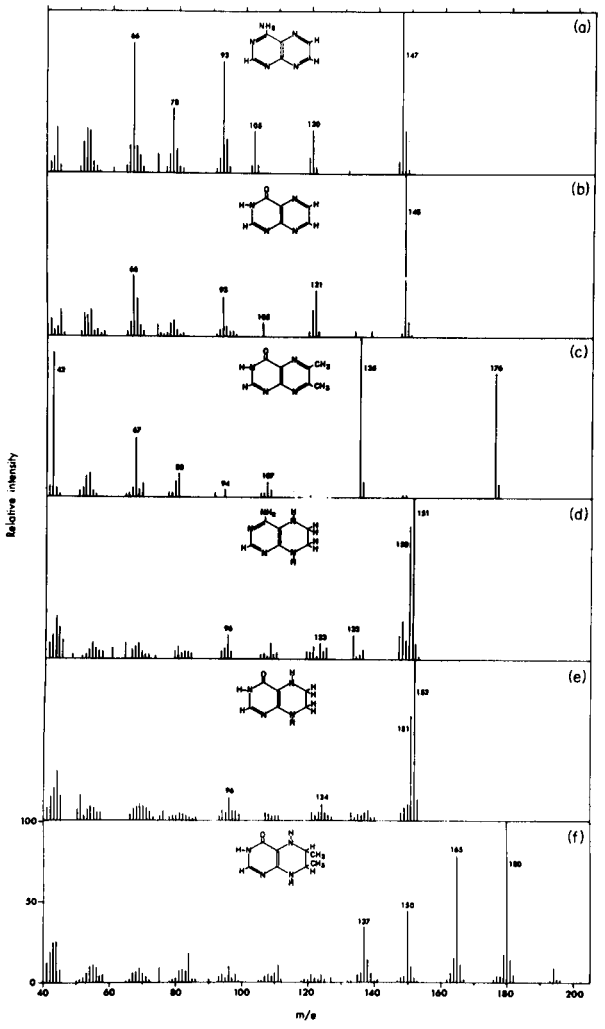
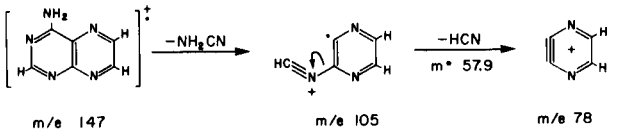


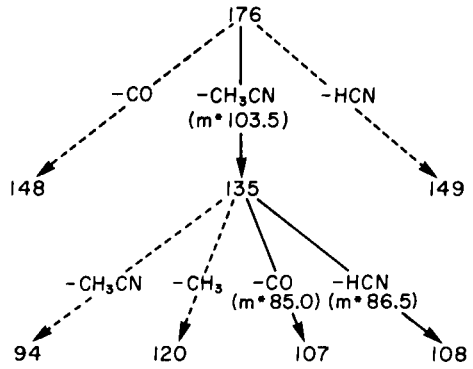
Figure 1. Mass spectra of 4-substituted pteridines: (a) 4-aminopteridine; (b) 4(3*H*)pteridinone; (c) 6,7-dimethyl-4(3*H*)pteridinone; (d) 4-amino-5,6,7,8-tetrahydropteridine; (e) 5,6,7,8-tetrahydro-4(3*H*)pteridinone; (f) 5,6,7,8-tetrahydro-6,7-dimethyl-4(3*H*)pteridinone.



SCHEME I

4(3*H*)Pteridinone (Fig. 1b) behaves in a similar fashion to 4-aminopteridine in that fragment ions at m/e 121 and 105 correspond to the initial loss of HCN and NHCO from the molecular ion at m/e 148. However, the cleavage of the N-3, C-4 unit from the molecular ion is not as prominent as that for 4-aminopteridine. This difference is probably the result of a competition between the loss of the N-3, C-4 unit and the ejection of CO (m/e 120). After the loss of CO the spectrum shows the elimination of two molecules of HCN (m/e 93 and 66), similar to 4-aminopteridine. Goto, *et al.* (7) have also reported a mass spectrum for 4(3*H*)pteridinone, which differs from that shown in Fig. 1b by the presence of an intense ion at m/e 44 attributed by them to the rearranged product $[NH_2CO]^+$. The spectrum reported here is identical to that of Clark, *et al.* (8) who suggested that m/e 44 may originate from an impurity. However, it is also likely that this ion arose from sample decomposition in the heated glass inlet system used by Goto, *et al.* (7).

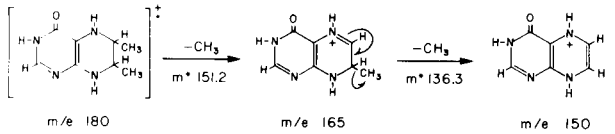
The mass spectral fragmentation of 6,7-dimethyl-4(3*H*)-pteridinone (Fig. 1c) is markedly different from that of 4(3*H*)pteridinone. The characteristic feature in this spectrum, which has also been reported by Clark, *et al.* (8) is the loss of CH_3CN from the pyrazine ring to give the base peak at m/e 135 (m*103.7). Other fragment ions of low intensity are present, which correspond to eliminations of HCN, CO, CH_3CN and CH_3 from both the molecular ion and the base peak. These transitions are outlined in Scheme II. The intense ion at m/e 42 is probably due to $CH_3C \equiv \overset{+}{N}H$, but no accurate mass measurement was performed to confirm this structure.



SCHEME II

4-Amino-5,6,7,8-tetrahydropteridine and 5,6,7,8-tetrahydro-4(3*H*)pteridinone give simple mass spectra characterized by an intense $[M-H]^+$ ion (Figs. 1d and 1e). Fragmentation ions due to the losses of HCN are not as prominent as in the oxidized compounds. However, weak ions are observed at m/e 123 (m*100.9) and 124, (Fig. 1d and 1e), respectively, which are attributable to

the loss of HCN from the $[M-H]^+$ ions. One additional feature in the spectrum of 4-amino-5,6,7,8-tetrahydropteridine is the loss of 17 mass units (m*117.9) from m/e 150, indicating the loss of NH_3. The parallel loss of H_2O from 5,6,7,8-tetrahydro-4(3*H*)pteridinone is not observed. Figure 1f illustrates the effect of methyl substitution in the 6- and 7-positions upon the mode of fragmentation. Both methyl substituents are eliminated in a two-step process as detailed in Scheme III. CO and HCN are eliminated from m/e 165 to yield the ions m/e 137 (m*113.7) and 138 respectively.



SCHEME III

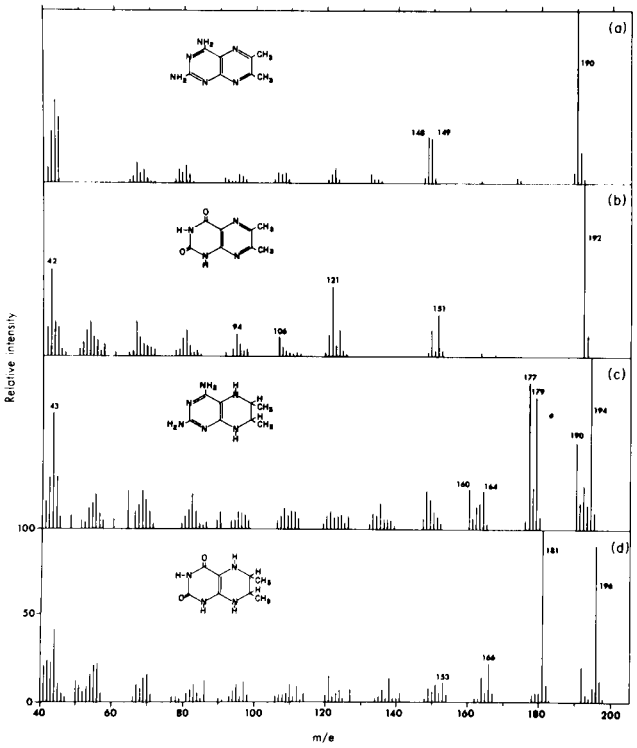
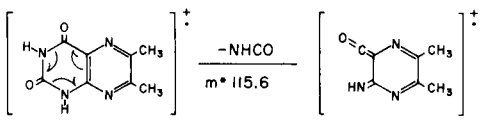


Figure 2. Mass spectra of pteridines with the same substituents at the 2- and 4-positions: (a) 2,4-diamino-6,7-dimethylpteridine; (b) 6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione; (c) 2,4-diamino-5,6,7,8-tetrahydro-6,7-dimethylpteridine; (d) 5,6,7,8-tetrahydro-6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione.

2. Pteridines with the Same Substituents at the 2- and 4-Positions.

In contrast to the mass spectrum of 6,7-dimethyl-4(3*H*)-pteridinone (Fig. 1c), compounds substituted also in the 2-position (Fig. 2a,b) give mass spectra where both the pyrazine and pyrimidine rings fragment to an equal extent. Both 2,4-diamino-6,7-dimethylpteridine (Fig. 2a) and 6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione (Fig. 2b) lose CH_3CN. The resulting fragment ions at m/e 149 (m*116.9) and 151 (m*118.9) respectively, are only about 25% of the intensity of the corresponding molecular ions which are the base peaks in each spectrum. Fragmentation of the pyrimidine ring occurs in a similar fashion to that observed for 4-substituted pteridines, *i.e.* the N-3,C-4 unit cleaves. This is evident for 2,4-diamino-6,7-dimethylpteridine from the transition m/e 190 → m/e 148 (m*115.1) (Fig. 2a). The parallel loss of 43 mass units [-NHCO], in 6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione can arise in at least two ways. For example, in addition to the loss of the N-3, C-4 unit, a second mode of cleavage may proceed *via* a retro Diels-Alder decomposition (9), (Scheme IV).



SCHEME IV

The overall fragmentation of 6,7-dimethyl-2,4(1*H*,3*H*)-pteridinedione is more complex than that for 2,4-diamino-6,7-dimethylpteridine, but the majority of the fragmentation processes can be supported by metastable transitions. There is a prominent loss of CO from the two fragment ions at m/e 151 and 149 to give m/e 123 and 121 (m*100.3 and 98.4 respectively). Subsequently, m/e 121 loses 27 mass units to give m/e 94 (m*73.0); this ion must arise by a complex rearrangement and loss of HCN. One unusual fragmentation, as evidenced from the metastable at m/e 92.9, is the loss of 15 mass units [$-CH_3$] from m/e 121.

The mass spectra of the corresponding 5,6,7,8-tetra-reduced analogues are shown in Figs. 2c and 2d. Both spectra indicate the presence of parent compound resulting from air oxidation during the transfer of the sample to the direct insertion probe. The spectra of both 2,4-diamino-5,6,7,8-tetrahydro-6,7-dimethylpteridine and 5,6,7,8-tetrahydro-6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione show two consecutive losses of methyl groups to give the pairs of ions: m/e 179, 164, and 181 (m*167.1) and 166 (m*152.1) respectively. The spectrum of 2,4-diamino-5,6,7,8-tetrahydro-6,7-dimethylpteridine shows an intense

ion at m/e 177, attributable to the direct loss of NH_3 from the molecular ion. The loss of 17 mass units from m/e 177 to give the ion at m/e 160 (m*144.6), indicates the ejection of a second molecule of NH_3. 5,6,7,8-Tetrahydro-6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione loses OH (m*148.6) and CO (m*129.1) from the $[M\text{-}CH_3]^+$ ion in the transitions, m/e 181 → 164 and 181 → 153, respectively.

3. 2-Amino-4(3*H*)pteridinones (Pterins) with Either Hydrogen or an Alkyl Substituent at Positions 6 and 7.

2-Amino-4(3*H*)pteridinone (pterin) gives a mass spectrum, in which the molecular ion (m/e 163) is the base peak (Fig. 3a). Although the fragment peaks are of low intensity, the large number of metastable transitions allow an unequivocal fragmentation pattern to be established. This is illustrated in Scheme V. The molecular ion (m/e 163) decomposes by the loss of CO, HCN and NHCN.

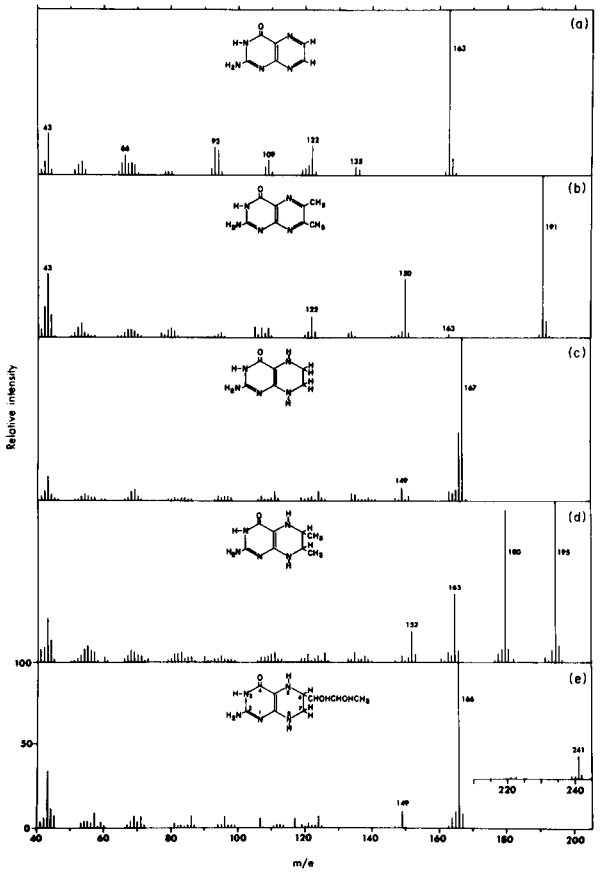
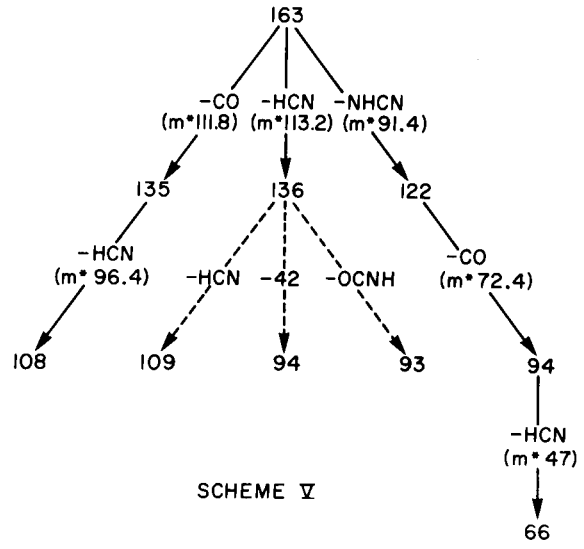
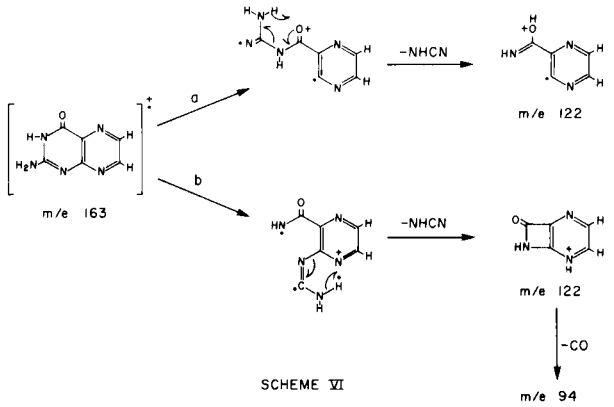


Figure 3. Mass spectra of 2-amino-4(3*H*)pteridinones (pterins) with either hydrogen or an alkyl substituent at positions 6 and 7: (a) 2-amino-4(3*H*)pteridinone (pterin); (b) 2-amino-6,7-dimethyl-4(3*H*)pteridinone; (c) 2-amino-5,6,7,8-tetrahydro-4(3*H*)pteridinone; (d) 2-amino-5,6,7,8-tetrahydro-6,7-dimethyl-4(3*H*)pteridinone; (e) 2-amino-5,6,7,8-tetrahydro-6-(L-erythro-1,2-dihydroxypropyl)-4-(3*H*)pteridinone (tetrahydrobiopterin).



SCHEME V

Clearly, CO arises from C-4, and HCN from N-5, C-6 or C-7, N-8. However, the loss of NHCN must be due to the scission of the N-1, C-2 unit, together with concommitant hydrogen transfer. There would be two likely receptor sites for the transferred hydrogen atom, (a) the 4-keto group and (b) the N-8 position. Both (a) and (b) could proceed *via* a six-membered transition state, (Scheme VI).



SCHEME VI

Of the two alternatives shown in Scheme VI, pathway (b) is more likely, since m/e 122 further decomposes by the loss of CO. The $[M\text{-}HCN]^+$ ion at m/e 136 appears to lose a second molecule of HCN and the N-3, C-4 unit [-NHCO], but neither transition is supported by metastable peaks. The remainder of the spectrum is due to the further losses of CO and HCN (see Scheme V).

The spectrum of 2-amino-6,7-dimethyl-4(3*H*)pteridinone (Fig. 3b) is dominated by the loss of CH_3CN to

give the ion at m/e 150 (m*107.8) showing once again the pronounced effect of substitution in the pyrazine ring upon the fragmentation. The only other fragment ion of any significance is that due to the loss of CO in the transition 150 → 122 (m*99.0).

The mass spectra of the corresponding 5,6,7,8-tetrahydro derivatives (Figs. 3c and d) show fragmentation peaks due to cleavage of the substituents in the reduced pyrazine ring. 2-Amino-5,6,7,8-tetrahydro-4(3*H*)pteridinone (Fig. 3c) gives an intense $[M-H]^+$ ion (m*165.0) whereas 2-amino-5,6,7,8-tetrahydro-6,7-dimethyl-4(3*H*)-pteridinone (Fig. 3d) loses both methyl groups in the sequence m/e 195 → m/e 180 → m/e 165 (m*166.6 and 151.1 respectively). The ion at m/e 180 probably loses CO to give m/e 152. Both of these tetrahydropteridines, and also tetrahydrobiopterin (see below), show small fragment ions in the spectra due to loss of NH_3 (166 → 149, Fig. 3c; 180 → 163, Fig. 3d; 166 → 149, Fig. 3e).

The spectrum of the synthetic natural cofactor, tetrahydrobiopterin (2-amino-5,6,7,8-tetrahydro-6-(L-erythro-1,2-dihydroxypropyl)-4(3*H*)pteridinone) (Fig. 3e) is characterized by an analogous loss of the dihydroxypropyl side chain to give the base peak m/e 166 (no metastable

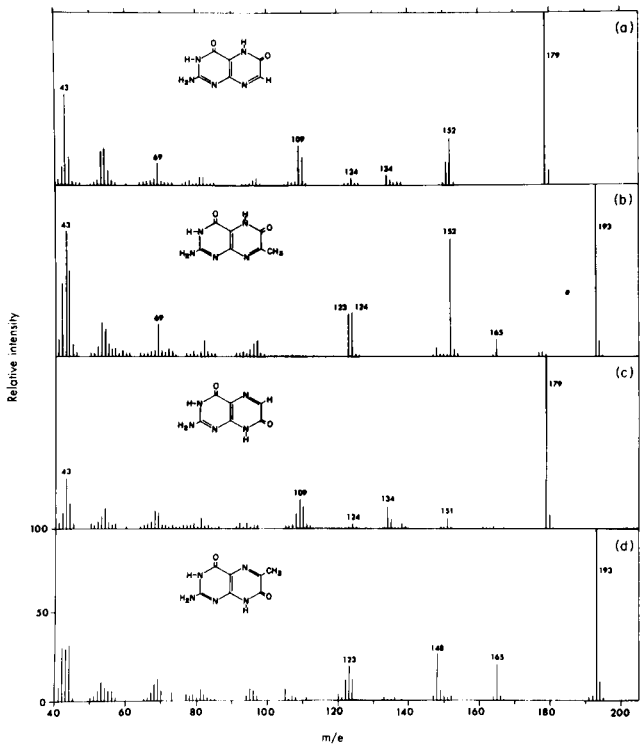


Figure 4. Mass spectra of pterins with a keto substituent in the pyrazine ring (pteridinediones): (a) 2-amino-4,6-(3*H*,5*H*)pteridinedione; (b) 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione; (c) 2-amino-4,7(3*H*,8*H*)pteridinedione; (d) 2-amino-6-methyl-4,7(3*H*,8*H*)pteridinedione.

detected), thus proving the presence of this substituent in the pyrazine ring. In contrast, the published spectrum for unreduced biopterin, which lacks a molecular ion and gives only the $[M-H]^+$ peak, is so complex that precise information on the pyrazine ring substituents is not easily obtained (10).

4. Pterins with a Keto Substituent in the Pyrazine Ring (Pteridinediones).

Each of the non-reduced compounds in this class gives a molecular ion which is the base peak. The fragmentation mode is clearly dependent upon the position of the keto substituent in the pyrazine ring. Both 2-amino-4,6(3*H*,5*H*)pteridinedione and 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione are characterized by the prominent cleavage of the C-7, N-8 moiety, *i.e.* m/e 179 → 152 (-HCN, m*129.1), and m/e 193 → 152 ($-CH_3CN$,m*119.9) (Figs. 4a,b). This fragmentation is insignificant in 2-amino-4,7(3*H*,8*H*)pteridinedione and 2-amino-6-methyl-4,7(3*H*,8*H*)pteridinedione (Figs. 4c,d). Another feature which distinguishes the 4,6(3*H*,5*H*)pteridinediones from the 4,7(3*H*,8*H*)pteridinediones is the homologous ions at m/e 122 and 108 in the spectra of the 6-methyl and non-methyl substituted 4,7(3*H*,8*H*)pteridinediones. The probable origin of these two ions is the loss of [-NHCO] from the $[M-CO]^+$ ion (see below). The corresponding fragment ions are not observed in the spectra of the two 4,6(3*H*,5*H*)pteridinediones.

All four spectra show fragment ions that arise by the loss of CO from the molecular ions to give m/e 165 for the methyl substituted 2-aminopteridinediones and m/e 151 (m*127.4) for the non-methylated 2-aminopteridinediones. The $[M-CO]^+$ ions in turn lose 17 mass units (NH_3) to give m/e 148 and m/e 134. A detailed interpretation of these spectra is hindered by the lack of detectable metastable transitions which in part is due to the low volatility of the compounds. However, some insight into the subsequent fragmentation can be gained from a comparison of the spectra for the methyl substituted 2-aminopteridinediones with those for the unsubstituted 2-aminopteridinediones. In the former, there are pairs of ions at m/e 123 and 124 (Figs. 4b,d) whereas the latter have pairs of ions at m/e 109 and 110 (Figs. 4a,c). Since these two pairs differ from each other by one methylene group and are common to all spectra, it seems very likely that they are derived from the pyrimidine ring by the loss of 41 and 42 mass units from the $[M-CO]^+$ ion. At least, with the loss of 41 mass units, a rearrangement process involving hydrogen transfer in the pyrimidine ring must occur (*e.g.* Scheme VI), since the expected fragmentation of this ring ($-NH_2CN$, from the N-1, C-2 moiety and -NHCO from the N-3, C-4 moiety) would result in the loss of 42 and 43 mass units. The loss of

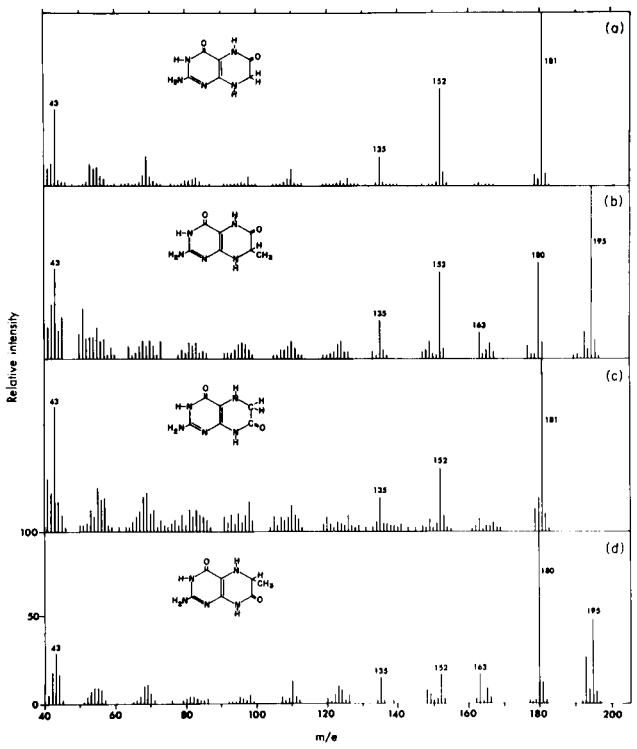


Figure 5. Mass spectra of dihydropterins with a keto substituent in the pyrazine ring (dihydropteridinediones): (a) 2-amino-7,8-dihydro-4,6(3*H*,5*H*)pteridinedione; (b) 2-amino-7,8-dihydro-7-methyl-4,6(3*H*,5*H*)pteridinedione; (c) 2-amino-5,6-dihydro-4,7(3*H*,8*H*)pteridinedione; (d) 2-amino-5,6-dihydro-6-methyl-4,7(3*H*,8*H*)pteridinedione.

42 mass units could also arise from a similar hydrogen rearrangement, involving the N-3, C-4 unit, or, alternatively, could be derived from a direct loss of the N-1, C-2 unit.

The non-methyl substituted 2-aminopteridinediones (Figs. 4a,c) both give small peaks at m/e 124 which must arise by the loss of CO and HCN from the molecular ion (m/e 179). It seems more likely that CO is eliminated before HCN since the ratio of m/e 124:151 in both spectra is similar, whereas the ratios of m/e 124:152 are very different (Figs. 4a,c).

2-Amino-7,8-dihydro-7-methyl-4,6(3*H*,5*H*)pteridinedione and 2-amino-5,6-dihydro-6-methyl-4,7(3*H*,8*H*)pteridinedione (Figs. 5b,d) lose the pyrazine methyl group, but the non-methyl substituted analogues do not show as significant $[M-H]^+$ ions as do reduced compounds of the other groups (Fig. 5a,c). The $[M-CH_3]^+$ ion forms the base peak in the spectrum of the 6-methyl compound (Fig. 5d), whereas with the 7-methyl isomer the base peak is the molecular ion (Fig. 5b). The pyrazine ring of these reduced compounds decomposes by the loss of CH_2NH (m/e 181 → 152) (Figs. 5a,c) or CH_3CHNH (m/e 195 → 152) (Figs. 5b,d); these transitions are more prominent with the 6-keto isomers. The product ions, at m/e 152, in each instance lose 17 mass units (NH_3). In contrast to the nonreduced compounds, CO is not eliminated from the pyrazine ring.

Common Fragmentations.

Unreduced Pteridines.

All of the non-reduced pteridines studied here having double methyl substitution in the pyrazine ring, eliminate CH_3CN from the molecular ion. This is in agreement with the original observation made with 6,7-dimethylpteridine (7), that substitution in the pyrazine ring directs the principal fragmentation to that part of the molecule. However, although 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione follows this general behavior, the spectrum of 2-amino-6-methyl-4,7(3*H*,8*H*)pteridinedione shows no loss of CH_3CN. This suggests that the labile CH_3CN arises from the C-7, N-8 moiety.

The spectra of ketopteridines contain fragment ions due to the loss of CO. Even if the pyrazine ring is alkyl substituted CO is lost from the pyrimidine ring. However, when the pyrazine ring also carries a keto group, CO is eliminated from that ring, only. An analogous fragmentation has been reported for 6-pteridinone and 7-pteridinone, which lose CO to give purine (7).

Reduced Pteridines.

Although the structural information contained in the spectra of the reduced pteridines is less than for the non-reduced compounds, exact information concerning the nature of the pyrazine ring substituents is more readily obtained. Characteristically, the reduced compounds eliminate the pyrazine ring substituents.

All the reduced pteridines examined, which have amino substituents in the pyrimidine ring, show fragment ions which originate by the loss of NH_3. The analogous loss of OH and H_2O from the corresponding ketopteridines is not observed. In these compounds the loss of CO is favored. This difference is probably due to the strong tendency of a hydroxyl substituent to be in the keto form (11,12).

EXPERIMENTAL

Pteridines were obtained from the following sources: 2-amino-5,6,7,8-tetrahydro-6,7-dimethyl-4(3*H*)pteridinone from Calbiochem; 6,7-dimethyl-4(3*H*)pteridinone, 6,7-dimethyl-2,4(1*H*,3*H*)pteridinedione, 2-amino-6-methyl-4,7(3*H*,8*H*)pteridinedione, 2-amino-4,6(3*H*,5*H*)pteridinedione, 2-amino-4,7(3*H*,8*H*)pteridinedione, 2-amino-6,7-dimethyl-4(3*H*)pteridinone and 4(3*H*)pteridinone from Aldrich; 2,4-diamino-6,7-dimethylpteridine, 2-amino-7-methyl-4,6(3*H*,5*H*)pteridinedione from Alfred Bader Co., 2-amino-4(3*H*)pteridinone from Sigma Chemical Co. 4-Aminopteridine,

biopterin and 5,6,7,8-tetrahydrobiopterin were generously donated respectively by Dr. C. Bayley, Cyclo Chemical Corp., Los Angeles, Dr. A. R. Maas, Smith, Kline, and French Laboratories, Philadelphia, and Dr. K. J. M. Andrews, Roche Products Ltd., Hertfordshire, England. Where necessary, pteridines were purified by thin-layer chromatography and/or on silica columns using one of the solvent systems: butanol/saturated aqueous phenol, 5:1; butanol/5*N* acetic acid, 2:1; methanol/water/concentrated ammonia, 100:9:1; 10% aqueous ammonia. The tetrahydropteridines were produced by catalytic hydrogenation in trifluoroacetic acid (13) as described elsewhere (1).

Mass spectra were determined by using an A. E. I. MS 902 mass spectrometer. The ionizing energy was 70 eV and ionizing current was 500μA. Samples were introduced to the spectrometer on a direct insertion probe. All pteridines were volatilized at an ion source temperature between 180° and 250°.

Acknowledgments.

Supported by USPHS Grants HL-12745, HD-05061, HD-04612, MCH-927 and FR 5354, the California State Department of Mental Hygiene and the Mental Retardation Program, NPI, UCLA.

We wish to acknowledge the technical assistance of Mr. Clifford Fried in running some of the mass spectra.

REFERENCES

(1) J. E. Ayling, G. R. Boehm, S. C. Textor and R. A. Pirson, *Biochemistry*, 12, 2045 (1973).

(2) T. Lloyd, S. Markey and N. Weiner, *Anal. Biochem.*, **42**, 108 (1971).

(3) W. J. A. Vanden Heuvel, J. L. Smith, P. Haug and J. L. Beck, *J. Heterocyclic Chem.*, 9, 451 (1972).

(4) K. Kobayashi and M. Goto in "Chemistry and Biology of Pteridines," K. Iwai, M. Goto, M. Akino and Y. Iwanami, Eds., p. 57, Int. Acad. Printing Co. Ltd., Tokyo, 1970.

(5) J. W. Serum, P. Haug, T. Urushiba and H. S. Forrest, *Z. Anal. Chem.*, **262**, 110 (1972).

(6) K. Kobayashi, T. Kinoshita and M. Goto, *Nippon Kagaku Zasshi*, 91, 1096 (1970).

(7) T. Goto, A. Tatematsu and S. Matsuura, *J. Org. Chem.*, 30, 1844 (1965).

(8) J. Clark, R. Maynard and C. Smith, *Org. Mass Spectrom.*, 5, 993 (1971).

(9) H. Budzikiewicz, C. Djerassi and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco (1967), p. 591-592.

(10) Y. Iwanami and M. Akino, *Tetrahedron Letters*, 31, 3219 (1972).

(11) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 3443 (1956).

(12) A. R. Katritzky, *Advan. Heterocyclic Chem.*, 1, 341 (1963).

(13) A. Bobst and A. Viscontini, *Helv. Chim. Acta*, **49**, 875 (1966).