*Journal of Chromatography*, 278 (1983) 245—254
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1877

# GAS CHROMATOGRAPHY—MASS SPECTROMETRY OF TRIMETHYLSILYL PTERIDINES*

THOMAS KUSTER and ALOIS NIEDERWIESER*

*Department of Pediatrics, University of Zürich, Steinwiesstrasse 75, CH-8032 Zürich (Switzerland)*

(Received July 29th, 1983)

## SUMMARY

The trimethylsilyl derivatives of about 70 naturally occurring as well as synthetic pteridines have been investigated by glass capillary gas chromatography—mass spectrometry. On the apolar SE-52 phase used, the retention time of the compounds, tabulated in methylene units, was influenced more by the polarity than by the molecular weight. The electron-impact mass spectra of most compounds showed intense $M^{+\cdot}$ and $M^{+}$—15 ions and characteristic fragmentation patterns of 5,6,7,8-tetrahydropteridines, 7,8-dihydropteridines, sepia analogues and fully oxidized pteridines. The methylene units, together with the five most intense fragments tabulated, provide a good basis for the identification of these compounds in biological samples and for structure elucidation of unknown pteridines.

## INTRODUCTION

Investigation of the butterfly wing pigments, at the beginning of this century, led to the identification of several compounds with the same heterocyclic ring structure, which is called pteridine (Fig. 1, A). Although the term pterin was used earlier as a collective name for the butterfly wing pigment, nowadays, according to IUPAC [1], it is used exclusively for the 2-amino-4-hydroxypteridine residue (Fig. 1, B). In the last few years, due to their important role as cofactors in various metabolic pathways, pterins have gained increasing interest in biochemistry and medicine [2—4] and therefore different methods of isolating and separating these compounds have been developed. Very promising quantitative results were reported especially with high-performance liquid chromatography [5, 6]. The inherent poor selectivity of

*Dedicated to Prof. H.-Ch. Curtius on the occasion of his 60th birthday.

0378-4347/83/$03.00 

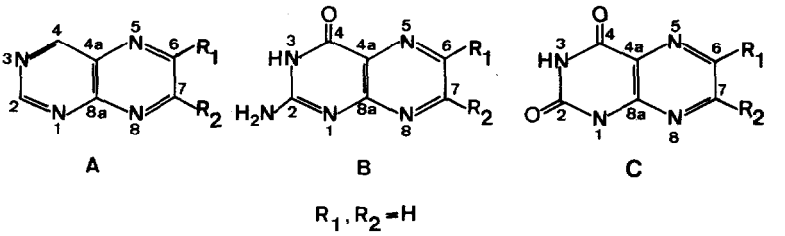


Fig. 1. Structural formulae of the investigated compounds: A = pteridine, B = 2-amino-4-hydroxypteridine (pterin), C = 2,4-dihydroxypteridine (lumazine).

chromatographic methods, however, prevents identification of unknown compounds in many cases. Mass spectrometry (MS) in combination with gas chromatography (GC) is well suited to solve such problems.

The first report on an MS analysis of acetylated and trimethylsilylated pteridines was by Kobayashi and Goto in 1970 [7], and Lloyd et al. [8] investigated the GC—MS behaviour of some pterins in 1971. Röthler and Karobath [9] presented in 1976 a mass-fragmentographic assay for biopterin and neopterin in human urine.

In the course of our work with pterins, we are often confronted with compounds which have not been known to occur in humans. In such cases, a library of reference compounds would be very helpful in structure elucidation and thus we present here the GC and MS data for 67 trimethylsilyl (TMS) derivatives. This data base has been used for pteridine analysis in various biological materials; an example can be found in ref. 10.

## EXPERIMENTAL

The gas chromatograph used was a Fractovap 2900 (Carlo Erba, Milan, Italy) with a Grob-type split—splitless injector and a 20 m × 0.3 mm SE-52 glass capillary column (H. Jaeggi, Trogen, Switzerland). The injector temperature was 275°C; the carrier gas was helium at 1.2 bars. The temperature program was 3 min at 180°C to 270°C at a rate of 4°C per min. The GC—MS interface was an open split and direct coupling device with a fused-silica transfer line [11, 12]. The mass spectrometer was a VG-16F single-focusing magnetic field instrument. Electron-impact ionization with 30 eV at an ion source temperature of 200°C. Accelerating voltage was 4 kV and the scan range *m/z* 100—750 with a cycle time of 2 sec (scan time 1.4 sec). A Finnigan Incos 2000 data system was used.

Most reference compounds were generous gifts from Prof. W. Pfleiderer, University of Constance (F.R.G.), Prof. M. Viscontini, University of Zürich (Switzerland) and Dr. B. Schircks, Wettswil (Switzerland). Some 7,8-dihydropteridines were prepared by reduction of the fully oxidized compounds with dithionite and some 5,6,7,8-tetrahydropteridines by catalytic reduction [13].

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was from Regis Chemical Co., acetonitrile from Fluka, Buchs, Switzerland.

For derivatization, a mixture, 200 μl, of acetonitrile—BSTFA (1:1, v/v) was added to 20—50 μg of the dry sample, sonicated for 30 sec and heated for 1 h at 100°C. For GC—MS, 1 μl was injected at an inlet split ratio of 1:10.

## RESULTS AND DISCUSSION

Table I lists the investigated compounds which are either pteridine (A), pterin (B) or lumazine (C) derivatives by increasing methylene units together with the number of TMS groups, the molecular weights and the five most prominent ions in the mass spectra. Fig. 2 shows the total ion current chromatogram of a set of reference compounds together with the even-numbered straight chain alkanes ($C_{18}$—$C_{28}$) used for the determination of the methylene units.

*Gas chromatography*

From the results in Table I, the following general rules concerning the GC behaviour of TMS pteridines on SE-52 could be deduced:

(1) Lumazines have shorter retention times than pterins (Table I, Nos. 1/2, 3/15, 4/22, 14/25, 30/44, 48/60).

(2) 7-Substituted isomers elute faster than the corresponding 6-substituted isomers (see hydroxylumazines No. 3/4, xanthopterins No. 15/22 and pterin carboxylic acids No. 39/43), confirming the observation reported for 6- and 7-biopterins [9].

(3) The retention times are influenced more by polarity than by the molecular weight (MW) of the compound. Thus, leukopterin-$(TMS)_4$, MW 483 (No. 25) elutes faster than 2′-deoxysepiapterin-$(TMS)_2$, MW 365 (No. 27), since silylation of the two oxo groups in positions 6 and 7 via enolization lowers the polarity of the compound compared with No. 27 bearing a free polar imino group in position 8.

(4) Derivatives with an additional TMS group (e.g. on the exocyclic 2-amino group) elute slower than the parent compound. Here, the molecular-weight criterion gains in importance (see e.g. Nos. 2/11, 22/29 or 45/53 and 55). The increments for one TMS group, however, are not constant. Thus, in tetrahydro-6,7-dimethylpterin (Nos. 8 and 9), it is only 0.11 methylene unit whereas in xanthopterin (Nos. 22 and 29), it is found to be 1.17, reflecting the superimposed influence of the polarity upon the retention time.

(5) *erythro*-Biopterin elutes faster than *threo*-biopterin (No. 44/47), whereas opposite behaviour is found in neopterin (No. 56/60) probably due to reversed polarities.

(6) In the series of biopterin (Nos. 44, 37, 53) and neopterin (Nos. 60, 52, 62 and 56, 65), the 7,8-dihydro compounds have shorter retention times than the aromatic and the 5,6,7,8-tetrahydro species, which is another indication that the chromatographic behaviour results from a mixture of polarity and molecular-weight effects.

The described analyses have been performed over a period of about six months. Due to the ageing of the column, there can be some variation in the determination of the methylene units in that the retention times tend to become lower with time. An example of this is Fig. 2: the column has been in use for two years and the methylene units are about 0.3 lower than those in Table I.

TABLE I

METHYLENE UNITS (MU) AND ELECTRON-IMPACT (30 eV) MASS SPECTRAL DATA OF PTERIDINE TRIMETHYLSILYLETHER (TMS) DERIVATIVES

| Ref. No. | Type* | $R_1$ | $R_2$ | Compound name | No. of TMS | MU | MW | Base peak | Mass spectral data: *m/z* (relative intensity) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C | —H | —H | Lumazine | 2 | 18.64 | 308 | 308 | 293(47),147(46),100(26),309(25) |
| 2 | B | —H | —H | Pterin | 2 | 20.17 | 307 | 292 | 307(87),293(32),308(26),147(17) |
| 3 | C | —H | —OTMS | 7-Hydroxylumazine | 3 | 20.40 | 396 | 396 | 381(54),397(36),382(18),398(15) |
| 4 | C | —OTMS | —H | 6-Hydroxylumazine | 3 | 20.60 | 396 | 396 | 381(50),397(36),395(23),100(20) |
| 5 | A | —H | —H | Pteridine | 2 | 20.77 | 306 | 291 | 306(78),219(31),234(22),292(14) |
| 6 | B | —$CH_3$ | —H | 6-Methylpterin | 2 | 20.84 | 321 | 306 | 321(82),307(36),322(25),308(14) |
| 7 | C | —$CH_3$ | —OTMS | 6-Methyl-7-hydroxylumazine | 3 | 20.86 | 410 | 410 | 395(98),411(34),396(33),397(17) |
| 8 | B | —$CH_3$ | —$CH_3$ | 5,6,7,8-Tetrahydro-6,7-dimethylpterin | 2 | 20.94 | 339 | 339 | 324(62),340(26),325(17),309(13) |
| 9 | B | —$CH_3$ | —$CH_3$ | 5,6,7,8-Tetrahydro-6,7-dimethylpterin | 3 | 21.05 | 411 | 411 | 396(90),412(36),397(32),413(16) |
| 10 | B | —$CH_3$ | —H | 5,6,7,8-Tetrahydro-6-methylpterin | 2 | 21.06 | 325 | 325 | 310(50),326(27),327(10),294(9) |
| 11 | B | —H | —H | Pterin | 3 | 21.28 | 379 | 364 | 379(54),365(31),147(22),292(19) |
| 12 | B | —$CH_3$ | —H | 5,6,7,8-Tetrahydro-6-methylpterin | 3 | 21.29 | 397 | 325 | 397(77),382(67),310(62),326(30) |
| 13 | B | —$CH_3$ | —$CH_3$ | 6,7-Dimethylpterin | 2 | 21.60 | 335 | 320 | 335(81),321(31),336(23),322(10) |
| 14 | C | —OTMS | —OTMS | Leukolumazine | 4 | 21.64 | 484 | 469 | 484(95),470(44),395(36),485(33) |
| 15 | B | —H | —OTMS | Isoxanthopterin | 3 | 21.69 | 395 | 380 | 395(67),381(49),396(43),382(32) |
| 16 | B | —H | —H | 5,6,7,8-Tetrahydropterin | 4 | 21.71 | 455 | 455 | 456(45),292(25),440(24),307(19) |
| 17 | B | —$CH_3$ | —OTMS | 6-Methylisoxanthopterin | 3 | 21.71 | 409 | 394 | 409(35),395(34),405(14),393(13) |
| 18 | C | —OTMS | —H | 7,8-Dihydro-6-hydroxylumazine | 4 | 21.77 | 470 | 470 | 471(58),397(37),469(35),455(32) |
| 19 | B | —$CH_3$,—$CH_3$ | —H | 5,6,7,8-Tetrahydro-6,6-dimethylpterin | 3 | 21.78 | 411 | 411 | 396(64),412(36),397(21),381(13) |
| 20 | B | —CHO | —H | 6-Formylpterin | 2 | 21.89 | 335 | 335 | 320(87),336(35),321(19),100(18) |
| 21 | B | —$CH_3$ | —H | 6-Methylpterin | 3 | 21.93 | 393 | 378 | 393(75),379(47),394(30),380(20) |
| 22 | B | —OTMS | —H | Xanthopterin | 3 | 22.15 | 395 | 380 | 935(83),381(61),396(42),382(30) |
| 23 | B | —H | —OTMS | Isoxanthopterin | 4 | 22.52 | 467 | 452 | 380(52),467(45),453(36),395(28) |
| 24 | B | —$CH_3$ | —OTMS | 6-Methylisoxanthopterin | 4 | 22.57 | 481 | 466 | 465(75),467(31),394(28),481(15) |
| 25 | B | —OTMS | —OTMS | Leukopterin | 4 | 22.68 | 483 | 100 | 147(76),468(72),469(43),483(26) |
| 26 | B | —OTMS | —H | 7,8-Dihydroxanthopterin | 4 | 22.80 | 469 | 469 | 470(60),454(37),471(29),468(26) |
| 27 | B | —CH(OTMS)$C_2H_5$ | —H | 2′-Deoxysepiapterin | 2 | 22.81 | 365 | 365 | 366(36),350(20),308(14),293(10) |
| 28 | C | —COOTMS | —H | Lumazine-6-carboxylic acid | 3 | 23.00 | 424 | 424 | 409(53),425(38),410(21),426(12) |
| 29 | B | —OTMS | —H | Xanthopterin | 4 | 23.32 | 467 | 452 | 467(86),453(45),468(32),454(22) |
| 30 | C | —CH(OTMS)CH(OTMS)$CH_3$ | —H | *erythro*-Biolumazine | 4 | 23.48 | 526 | 410 | 411(39),338(27),147(26),117(21) |
| 31 | B | —CH(OTMS)$C_2H_5$ | —H | 2′-Deoxybiopterin | 3 | 23.56 | 437 | 408 | 437(63),409(46),422(43),438(20) |
| 32 | C | —COOTMS | —OTMS | 7-Hydroxylumazine-6-carboxylic acid | 4 | 23.69 | 512 | 512 | 497(47),513(32),498(31),395(20) |
| 33 | B | —$CH_2$OTMS | —H | 6-Hydroxymethylpterin | 3 | 23.70 | 409 | 394 | 409(96),395(44),410(44),396(22) |
| 34 | B | —OTMS | —$NH_2$ | 7-Aminoxanthopterin | 4 | 23.79 | 482 | 467 | 482(56),100(55),466(48),468(44) |
| 35 | C | —CH(OTMS)$C_2H_5$ | —H | 7,8-Dehydro-2′-deoxy-sepialumazine | 3 | 23.89 | 436 | 436 | 421(35),435(33),437(32),347(18) |
| 36 | B | —CH(OTMS)$C_2H_5$ | —H | 2′-Deoxysepiapterin | 3 | 23.98 | 437 | 437 | 438(46),380(15),365(13),422(12) |
| 37 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | 7,8-Dihydro-*erythro*-biopterin | 4 | 24.09 | 527 | 410 | 527(49),411(30),437(29),528(23) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 38 | B | —C(OTMS)C(OTMS)$CH_3$ | —H | Sepiapterin | 3 | 24.48 | 453 | 453 | 336(58),452(57),309(24),438(21) |
| 39 | B | —H | —COOTMS | Pterin-7-carboxylic acid | 3 | 24.52 | 423 | 408 | 423(98),409(41),424(40),147(11) |
| 40 | A | —H | —CH(OTMS)$C_2H_5$ | 2,4-Diamino-7-(1′-hydroxypropyl)pteridine | 4 | 24.56 | 510 | 379 | 380(30),291(19),306(11),495(5) |
| 41 | B | —H | —CH(OTMS)$C_2H_5$ | 5,6,7,8-Tetrahydro-7-(1′-hydroxypropyl)pterin | 4 | 24.62 | 513 | 513 | 512(72),382(56),514(48),498(25) |
| 42 | A | —H | —CH(NHTMS)$C_2H_5$ | 5,6,7,8-Tetrahydro-2,4-diamino-7-(1′-aminopropyl)pteridine | 4 | 24.76 | 509 | 380 | 381(32),291(31),306(30),307(15) |
| 43 | B | —COOTMS | —$CH_3$ | Pterin-6-carboxylic acid | 3 | 24.88 | 423 | 423 | 408(99),409(55),424(54),425(30) |
| 44 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | L-*erythro*-Biopterin | 4 | 24.88 | 525 | 409 | 410(46),101(35),408(34),117(25) |
| 45 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | (6*R*)-5,6,7,8-Tetrahydro-L-*erythro*-biopterin | 3 | 24.90 | 457 | 238 | 457(27),237(10),239(7),442(6) |
| 46 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | 7,8-Dihydro-L-*erythro*-biopterin | 5 | 24.96 | 599 | 599 | 482(85),600(47),483(42),509(37) |
| 47 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | D-*threo*-Biopterin | 4 | 24.98 | 525 | 409 | 410(37),408(25),337(21),117(18) |
| 48 | C | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | D-*erythro*-Neolumazine | 5 | 25.09 | 614 | 410 | 409(99),411(37),412(22),599(15) |
| 49 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | 2-N,N-Dimethyl-L-erythro-biopterin | 3 | 25.41 | 481 | 365 | 364(51),101(30),147(26),366(24) |
| 50 | B | —C(OTMS)C(OTMS)$CH_3$ | —H | Sepiapterin | 4 | 25.48 | 525 | 525 | 526(56),408(28),435(26),510(25) |
| 51 | B | —OTMS | —COOTMS | Xanthopterin-7-carboxylic acid | 4 | 25.66 | 511 | 496 | 100(75),511(44),510(43),147(42) |
| 52 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | 7,8-Dihydro-D-*erythro*-neopterin | 5 | 26.72 | 615 | 410 | 337(69),409(61),615(52),411(44) |
| 53 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | (6*R*)-5,6,7,8-Tetrahydro-L-*erythro*-biopterin | 4 | 25.76 | 529 | 310 | 529(55),117(33),530(32),311(29) |
| 54 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | (6*S*)-5,6,7,8-Tetrahydro-L-*erythro*-biopterin | 3 | 25.83 | 457 | 238 | 457(31),222(16),239(16),117(15) |
| 55 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | (6*R*)-5,6,7,8-Tetrahydro-L-*erythro*-biopterin | 5 | 26.04 | 601 | 382 | 601(59),600(49),409(32),602(31) |
| 56 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | L-*threo*-Neopterin(monapterin) | 5 | 26.37 | 613 | 613 | 598(81),409(68),614(60),599(52) |
| 57 | B | —CH(OAc)CH(OAc)$CH_3$ | —H | 1′,2′-Diacetyl-L-*erythro*-biopterin | 2 | 26.43 | 465 | 465 | 336(86),363(85),450(60),405(54) |
| 58 | B | —C(OTMS)$CHCH_2$OTMS | —H | 2′-Deoxy-3′-hydroxysepiapterin | 4 | 26.52 | 525 | 525 | 435(40),526(34),527(21),510(16) |
| 59 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | 7,8-Dihydro-*erythro*-neopterin | 6 | 26.53 | 687 | 687 | 688(56),494(50),689(36),482(27) |
| 60 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | D-*erythro*-Neopterin | 5 | 26.58 | 613 | 409 | 408(50),410(37),613(17),598(13) |
| 61 | B | —CH(OTMS)CH(OTMS)$CH_3$ | —H | (6*S*)-5,6,7,8-Tetrahydro-L-*erythro*-biopterin | 4 | 26.77 | 529 | 310 | 529(59),311(27),530(25),238(20) |
| 62 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | (6*R*)-5,6,7,8-Tetrahydro-D-*erythro*-neopterin | 5 | 26.87 | 617 | 310 | 617(93),618(37),619(28),311(26) |
| 63 | B | —C(OTMS)C(OTMS)$CH_2$OTMS | —H | 3′-Hydroxysepiapterin | 5 | 27.06 | 613 | 613 | 614(78),615(47),598(22),616(20) |
| 64 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | (6*R*)-5,6,7,8-Tetrahydro-D-*erythro*-neopterin | 6 | 27.28 | 689 | 689 | 382(87),690(80),688(74),691(41) |
| 65 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | (6*R*)-5,6,7,8-Tetrahydro-L-*threo*-neopterin | 5 | 27.54 | 617 | 310 | 617(69),618(33),619(25),311(22) |
| 66 | B | —CH(OTMS)CH(OTMS)$CH_2$OTMS | —H | (6*S*)-5,6,7,8-Tetrahydro-D-*erythro*-neopterin | 5 | 27.91 | 617 | 310 | 617(71),618(37),311(25),238(16) |
| 67 | B | —C(OTMS)$CHCH_2$OTMS | —H | 2′-Deoxy-3′-hydroxysepiapterin | 5 | 28.16 | 597 | 507 | 597(81),435(72),508(47),436(41) |

*A = pteridine derivative, B = pterin derivative, and C = lumazine derivative (see Fig. 1 for formulae).

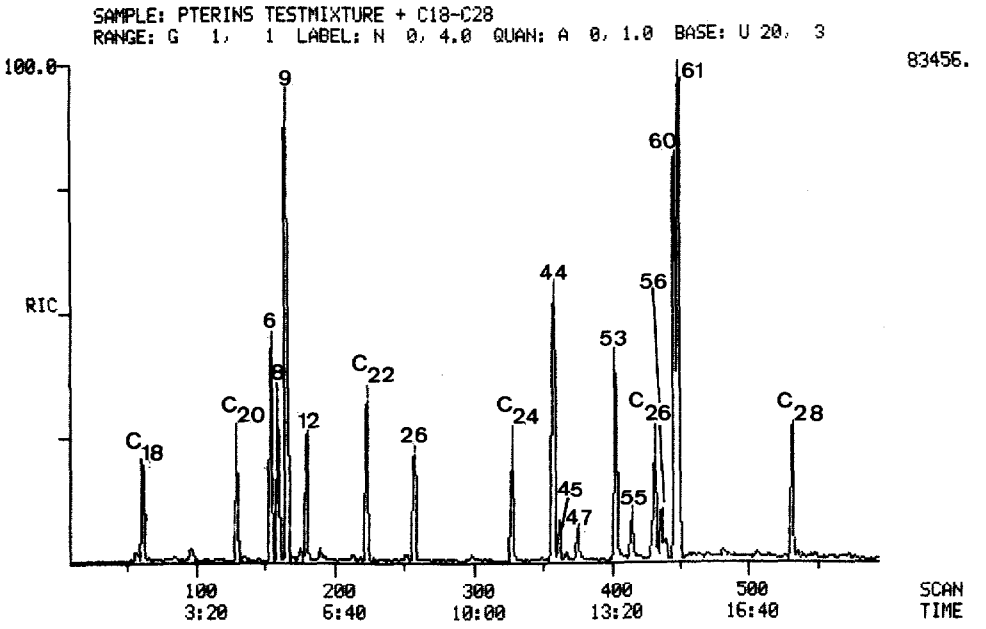


Fig. 2. Total ion current chromatogram of a pterin test mixture (different amounts of each compound) together with the even-numbered straight-chain alkanes $C_{18}$—$C_{28}$ (10 ng each on the column). For peak identification see Table I.

*Mass spectrometry*

The mass spectra of hydroxylated and/or methylated and carboxylated pterins and lumazines are rather simple and need no further explanations. As often reported, the spectra of positional isomers differ in the relative intensities only (e.g. Table I, Nos. 3/4, 15/22, 23/29 and 39/43). In the lumazine series, $M^{\ddagger}$ forms the base peak (an exception is leukolumazine, No. 14), whereas the pterin derivatives often show the $M^{\ddagger}-15$ ion as the most intensive peak. When the pyrazine ring is partially or fully hydrated, the tendency for the molecular ion to become the base peak increases. Because of the particular biological interest in pteridines substituted in position 6 by a hydroxylated carbon chain, the fragmentation of such derivatives is discussed in more detail.

Fig. 3 shows the electron-impact mass spectra of *erythro*-neopterin (No. 60), 7,8-dihydro-*erythro*-neopterin (No. 52) and (6*R*)-5,6,7,8-tetrahydro-*erythro*-neopterin (No. 62), and the *m/z* (relative intensity) values of the five most prominent ions for the investigated compounds are given in Table I.

A parallel fragmentation pattern is found in the aromatic biopterins, biolumazines, neopterins and neolumazines (Nos. 30, 44, 47, 48, 49, 56 and 60) which all have a vicinal 1′,2′-glycol residue $R_1$ in position 6. The dominating fragmentation yielding the base peak involves this substituent and is accompanied by a hydrogen rearrangement. Pictorially, this reaction may be formulated by a six-membered transition state as outlined in Fig. 4 for neopterin. The primary ionization involves the double bond in position 6,7. The prerequisite is the aromatic ring system and the availability of an —OR ether function in position 2′. So 2′-deoxybiopterin (No. 31) does not show

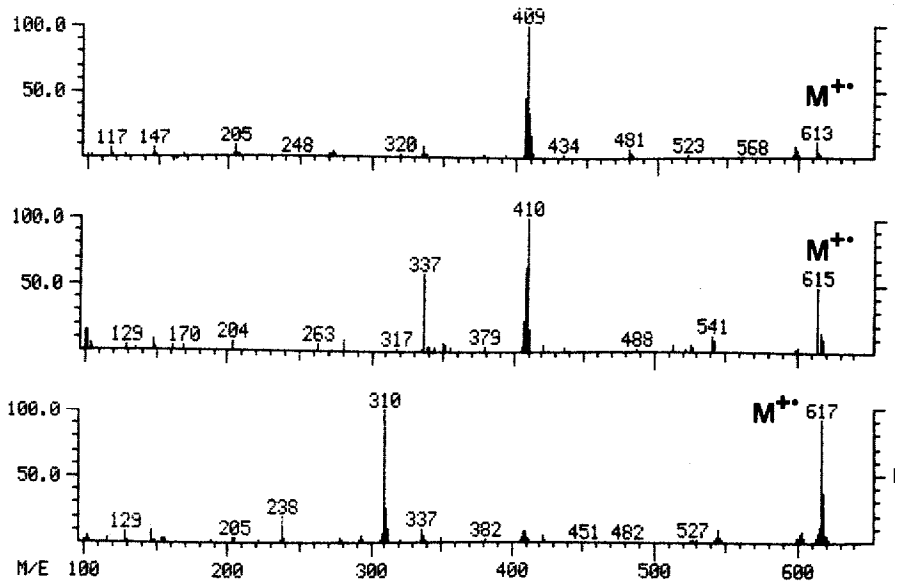


Fig. 3. Electron-impact (30 eV) mass spectra of D-*erythro*-neopterin (above), 7,8-dihydro-D-*erythro*-neopterin (centre) and (6*R*)-5,6,7,8-tetrahydro-D-*erythro*-neopterin (below).

an ion at *m/z* 409, but has its base peak at *m/z* 408 which arises by simple 1′—2′ bond cleavage. 1′,2′-Diacetyl-L-*erythro*-biopterin (No. 57) shows how sensitive the mass spectrum can be towards changes in functional groups. Dominating reactions here are acetic acid elimination (*m/z* 465→405) followed by ketene elimination yielding *m/z* 363. The rearrangement mentioned in Fig. 4 plays a minor role only: *m/z* 379, equivalent to *m/z* 409 in biopterin, has a relative intensity of 6% but the acetyl elimination (43 mass units) following yields one of the most prominent ions at *m/z* 336.

In the 7,8-dihydro compounds Nos. 37, 46, 52 and 59, the hydrogen rearrangement of Fig. 4 is suppressed. Instead, pushed by the 5,6-double bond, intense ions arise by cleavage of the 1′,2′-bond to yield *m/z* 410 and 482, respectively (Fig. 5). The same cleavage process also occurs in the 7,8-dihydro compound sepiapterin (Nos. 38 and 50) yielding the ions at *m/z* 336 and 408,

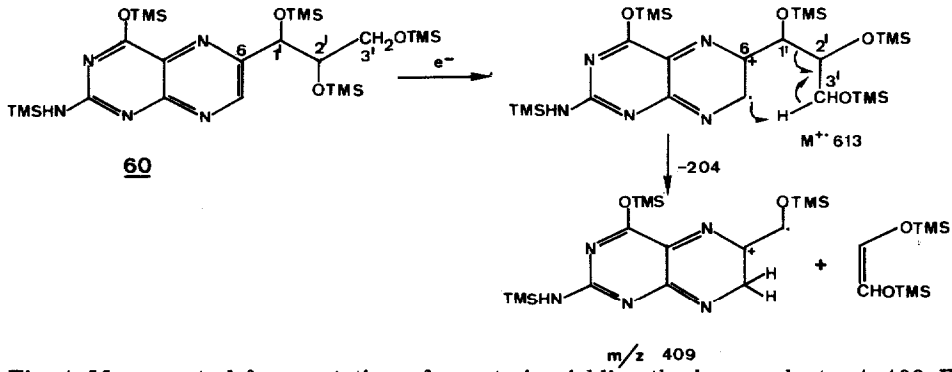


Fig. 4. Mass spectral fragmentation of neopterin yielding the base peak at *m/z* 409. Erroneously, 60 is shown in L-*threo* configuration; OTMS at C-2′ should be drawn upwards.

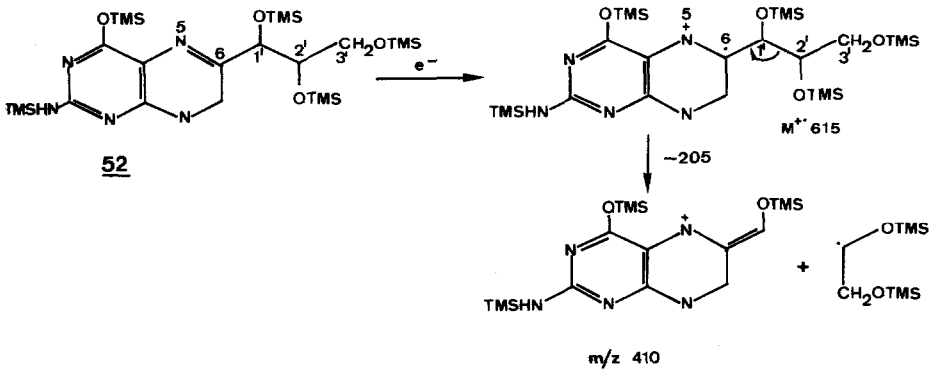


Fig. 5. Mass spectral fragmentation of 7,8-dihydroneopterin yielding the base peak at *m/z* 410. Erroneously, 52 is shown in L-*threo* configuration; OTMS at C-2′ should be drawn upwards.

respectively. The dominating fragmentation in 2′-deoxy-3′-hydroxysepiapterin (Nos. 58 and 67) is trimethylsilanol elimination ($M^{\ddagger}$—90) to yield *m/z* 435 and 507, a process which cannot be observed in the other compounds and thus might be an indication for an isolated primary alcohol function.

In the 5,6,7,8-tetrahydro compounds (Nos. 45, 53, 54, 55, 61, 62, 64, 65 and 66), due to the lack of a second double bond in ring B, the ionization can be formulated to occur primarily at the N-5 followed by the 6,1′-α-cleavage yielding very intense ions at *m/z* 238, 310 and 382 (Fig. 6).

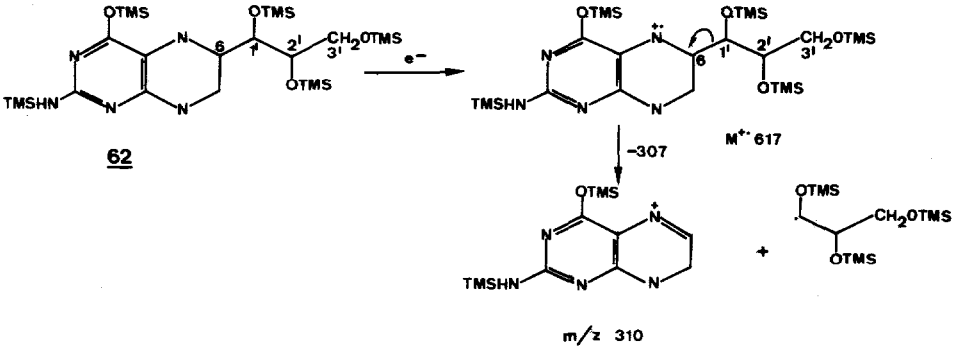


Fig. 6. Mass spectral fragmentation of (6*R*)-5,6,7,8-tetrahydroneopterin yielding the base peak at *m/z* 310. Erroneously, 62 is shown in L-*threo* configuration; OTMS at C-2′ should be drawn upwards.

It must be added that, due to the poor and non-specific fragmentation, the mass spectra of the TMS derivatives alone are not very suitable for structure elucidation. Often there remain several possibilities of interpretation and the position of the substitution cannot be determined. In combination with the GC methylene units, however, the number of possible structures can be reduced in many cases to a few or even to one only. Table II is an attempt to generalize the data from Table I, in order to have guidelines for the interpretation of unknown pteridines.

TABLE II

GUIDELINES FOR INTERPRETATION OF PTERIDINE MASS SPECTRA

| Finding | Possible interpretation |
|---|---|
| Methylene unit $\leq$ 23 | Simple substituted pteridine |
| Methylene unit $\geq$ 23 | More complex substituted pteridine |
| $M^{\ddagger}$: | |
| Odd-numbered | Pterin |
| Even-numbered | Lumazine |
| Low intensity | Aromatic skeleton |
| Medium intensity | 7,8-Dihydro compound |
| High intensity | 5,6,7,8-Tetrahydro compound |
| Same of two compounds | Compound with lower retention time is the 7-substituted isomer |
| Intense ions at *m/z*: | |
| 238, 310 or 382 | 5,6,7,8-Tetrahydropterin structure |
| 239, 311 or 383 | 5,6,7,8-Tetrahydrolumazine structure |
| 409 | Aromatic pterin with vicinal glycol moiety in position 6 or 7 |
| 410 | Aromatic lumazine with vicinal glycol moiety in position 6 or 7; or 7,8-dihydropterin with a 6-(1′-hydroxy) or 6-(1′-oxo) group |
| $M^{\ddagger}$—90 | Isolated hydroxyl group in side-chain |
| Similar fragmentation, MW difference 72 methylene units | Same compound with an additional TMS group |

ACKNOWLEDGEMENTS

We wish to express our thanks to Prof. W. Pfleiderer, Prof. M. Viscontini and Dr. B. Schircks for their generous gifts of reference compounds. We thank Dr. W.L.F. Armarego, Australian National University, Canberra, Australia, for a gift of 6,6-dimethyl-5,6,7,8-tetrahydropterin and we are grateful to Mrs. Ana Matasović for her skilful technical assistance. This work was supported by the Swiss National Science Foundation, project No. 3.266-0.82.

REFERENCES

1 IUPAC—IUB Commission on Biochemical Nomenclature, Biochim. Biophys. Acta, 107 (1965) 11.
2 H. Wachter, H.-Ch. Curtius and W. Pfleiderer (Editors), Biochemical and Clinical Aspects of Pteridines, Vols. I and II, Walter de Gruyter, Berlin, 1982 and 1983.
3 J.A. Blair (Editor), Chemistry and Biology of Pteridines, 7th Int. Symp. St. Andrews, Scotland, 1982, Walter de Gruyter, Berlin, 1983.
4 H.-Ch. Curtius, A. Niederwieser, R.A. Levine, W. Lovenberg, B. Woggon and J. Angst, Lancet, i (1983) 657.
5 T. Fukushima and J.C. Nixon, Anal. Biochem., 102 (1980) 176.
6 A. Niederwieser, W. Staudenmann and E. Wetzel, in H. Wachter, H.-Ch. Curtius and W. Pfleiderer (Editors), Biochemical and Clinical Aspects of Pteridines, Vol. I, Walter de Gruyter, Berlin, 1982, p. 81.

7 K. Kobayashi and M. Goto, in K. Iwai, M. Akino, M. Goto and Y. Iwanami (Editors), Chemistry and Biology of Pteridines Int. Acad. Printing Co., Tokyo, 1970, p. 57.
8 T. Lloyd, S. Markey and N. Weiner, Anal. Biochem., 42 (1971) 108.
9 F. Röthler and M. Karobath, Clin. Chim. Acta, 69 (1976) 457.
10 A. Niederwieser, A. Matasović, H.-Ch. Curtius, W. Endres and J. Schaub, FEBS Lett., 118 (1980) 299.
11 E. Wetzel, Th. Kuster and H.-Ch. Curtius, J. Chromatogr., 239 (1982) 107.
12 Th. Kuster and E. Wetzel, Int. J. Mass Spectrom. Ion Phys., 46 (1983) 173.
13 B. Schircks, J.H. Bieri and M. Viscontini, Helv. Chim. Acta, 61 (1978) 2731.