Pergamon

PII: S0031-9422(96)00367-6

Phytochemistry, Vol. 43, No. 3, pp. 687–690, 1996

0031-9422/96 $15.00 + 0.00

PILIOSTIGMIN, A 2-PHENOXYCHROMONE, AND *C*-METHYLFLAVONOLS FROM *PILIOSTIGMA THONNINGII*

JOSEPH C. IBEWUIKE, ABIODUN O. OGUNDAINI, FRANCIS O. OGUNGBAMILA, MARIE-THÉRESE MARTIN,* JEAN-FRANÇOIS GALLARD,* LARS BOHLIN† and MARY PAÏS*‡

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria; *Institut de Chimie des Substances Naturelles, CNRS, 91198-Gif sur Yvette, France; †Division of Pharmacognosy, Uppsala University, Biomedical Centre, Box 579, S-751 23 Uppsala, Sweden

(*Received in revised form* 30 *April* 1996)

Key Word Index—*Piliostigma thonningii*; Caesalpiniaceae; leaves; 2-phenoxychromone; *C*-methylflavonols.

Abstract—Piliostigmin, a 2-phenoxychromone, and three *C*-methylflavonols, 6,8-di-*C*-methylquercetin 3-methyl ether, 6-*C*-methylquercetin 3,7-dimethyl ether and 6,8-di-*C*-methylquercetin 3,7-dimethyl ether, were isolated from the leaves of *Piliostigma thonningii*, together with the known compounds quercetin, quercitrin, 6-*C*-methylquercetin 3-methyl ether, 6-*C*-methylquercetin 3,7,3′-trimethyl ether, 6,8-di-*C*-methylkaempferol 3-methyl ether and 6,8-di-*C*-methylkaempferol 3,7-dimethyl ether. The structures of the new compounds were established by spectral methods, especially 2D NMR. Complete ^{13}C assignments using HMBC experiments are reported for the four latter *C*-methylflavonols. Copyright © 1996 Elsevier Science Ltd

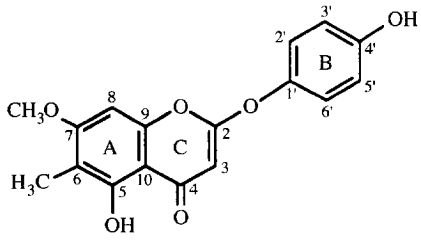
INTRODUCTION

Piliostigma thonningii (Schum.) Milne-Redhead is a savannah species spread throughout tropical Africa. The decoction of the leaves and bark is used for the treatment of wounds and ulcers, gastric and heart pain, gingivitis and as an antipyretic [1, 2]. Previous studies on this plant had resulted in the isolation of quercetin and labdane derivatives and griffonilide [3–6]. In continuation of our studies on the chemical constituents of Nigerian medicinal plants with antibacterial activity [7], we have investigated the leaves of *P. thonningii*. We report here the isolation of a new 2-phenoxychromone, piliostigmin (**1**), and three new *C*-methylflavonols, 6,8-di-*C*-methylquercetin 3-methyl ether (**2**), 6-*C*-methylquercetin 3,7-dimethyl ether (**3**) and 6,8-di-*C*-methylquercetin 3,7-dimethyl ether (**4**). The known quercetin and quercitrin were also isolated, together with four previously reported *C*-methylflavonols, 6-*C*-methylquercetin 3-methyl ether (**5**), 6-*C*-methylquercetin 3,7,3′-trimethyl ether (**6**), 6,8-di-*C*-methylkaempferol 3-methyl ether (**7**) and 6,8-*C*-dimethylkaempferol 3,7-dimethyl ether (**8**). The structures of the latter four methylflavonols were confirmed using HMBC experiments and their ^{13}C NMR data are reported here for the first time.

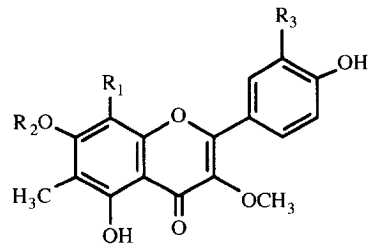
‡Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

The HREI mass spectrum of **1** showed a molecular ion peak at *m/z* 314.0792 (Δ 0.2 mmu), consistent with the molecular formula of $C_{17}H_{14}O_6$. The UV spectrum exhibited two maxima at 232 and 287 nm which were in close agreement with those of capillarisin, a related 2-*O*-(*p*-hydroxyphenyl)chromone previously isolated from *Artemisia capillaris*, thus suggesting the presence of the same chromophore [8]. The ^1H NMR spectrum showed an AA′BB′ system at δ 6.85 and 7.19 (J = 8.9 Hz) and a singlet at δ 5.11 characteristic of the B ring and H-3 of a 2-*O*-(*p*-hydroxyphenyl)chromone, respectively [8–10]. Other signals were a chelated 5-OH at δ 12.95, methyl singlet at δ 2.02, a methoxyl singlet at δ 3.90 and a proton singlet of a pentasubstituted ring A at δ 6.72. In the ^{13}C NHR spectrum, the methine at position 3 appeared at δ 87.3 and the methine on the A ring at δ 90.1, as shown by HMQC data. The observed HBMC correlations depicted in Fig. 1 indicated that this methine was at position 8 (cross peaks H-8/C-4, C-6, C-7, C-9, C-10) whereas the methyl group was located at C-6 (cross peaks OH-5/C-5, C-10 and Me-6/C-5, C-6, C-7) and the methoxyl group at C-7 (cross peak OMe/C-7). No HMBC correlations were observed between rings B and C, thus confirming the presence of the oxygen bridge C_1–O–C_2. From all the above, the structure of piliostigmin was assigned as 2-*O*-(*p*-hydroxyphenyl)-5-hydroxy-6-*C*-methyl-7-methoxychromone (**1**).



1



	R_1	R_2	R_3
2	CH_3	H	OH
3	H	CH_3	OH
4	CH_3	CH_3	OH
5	H	H	OH
6	H	CH_3	OCH_3
7	CH_3	H	H
8	CH_3	CH_3	H

Compound **2** showed a $[MH]^+$ peak in the HRCI mass spectrum at *m/z* 345.0980 (Δ −0.4 mmu) corresponding to the molecular formula of $C_{18}H_{16}O_7$. The UV spectrum exhibited band I and band II absorbances at 356 and 261 nm, respectively. The band II showed a large bathochromic shift in the presence of sodium acetate and an additional band appeared at 340 nm in the methanol + NaOH spectrum. These data suggested a flavonol with a free 7-OH group. The ^{1}H NMR spectrum showed two methyl singlets at δ 2.13 and 233 and a methoxyl singlet at δ 3.78. There were only three aromatic signals at δ 6.92 (1H, *d*), 7.60 (1H, *dd*) and 7.70 (1H, *d*) indicative of a 3′,4′-substituted B ring and a fully substituted A ring with the typical signal of a chelated 5-OH group appearing at δ 12.95. Comparison of the ^{13}C NMR data for **2** with those of known flavonols [11,12] showed that the 3′,4′-substituents were two hydroxyl groups, owing to the chemical shifts of the *ortho* carbons C-5′ and C-2′, both at about δ 115. The methoxyl group was at C-3 (δ 137.4) in agreement with the HMBC correlations OMe-3/C-3. The methyl groups were located at C-6 and C-8, as shown by the HMBC cross peaks OH-5/C-5, C-6, C-10, Me-6/C-5, C-6, C-7 and Me-8/C-7, C-8, C-9. Thus, **2** is 6,8-di-*C*-methyl-3-methoxy-5,7,3′,4′-tetrahydroxylflavone (6,8-di-C-methylquercetin 3-methyl ether).

Compound **3** was an isomer of **2** showing a $[MH]^+$ peak in the HRCI mass spectrum at *m/z* 345.0961 (Δ −0.6 mmu), which corresponded to the molecular formula of $C_{18}H_{16}O_7$. The ^{1}H NMR spectrum exhibited the signal of one methyl group at 2.12 (*s*) and of two methoxyl groups at 3.91 and 4.02. The aromatic region contained the typical signals of a 3′,4′-substituted B ring similar to the one of **2** at δ 7.03 (1H, *d*), 7.61 (1H, *dd*) and 7.73 (1H, *d*), together with a proton singlet located on the A ring at δ 6.91. The signal of a chelated 5-OH group was also present at δ 13.0. The ^{13}C spectrum showed, in the same way as for **2**, that the B ring bore two hydroxyl groups. The HMBC data for ring A were similar to the ones for **1**. The cross peaks OH-5/C-5, C-6, C-10 and Me-6/C-5, C-6, C-7 indicated that the methyl group was located at C-6, and the correlations observed between the proton at δ 6.91 and the carbons C-4, C-6, C-9, C-10 showed that this proton was at C-8. Hence, the two methoxyl groups were at C-7 and C-3, as further confirmed by their HMBC correlations with C-7 and C-3, respectively. Compound **3** is therefore 3,7-dimethoxy-6-*C*-methyl-5,3′,4′-trihydroxyflavone (6-*C*-methylquercetin-3,7-dimethyl ether).

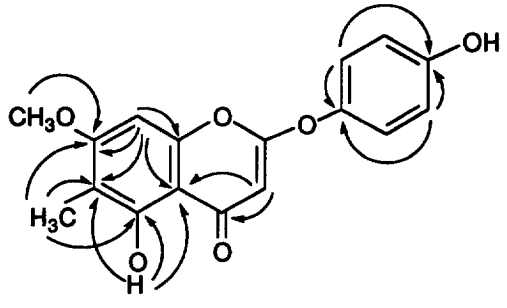


Fig. 1. HMBC correlations for piliostigmin (**1**).

Compound **4** showed a $[MH]^+$ peak in the HRCI mass spectrum at *m/z* 359.1141 (Δ 1.0 mmu) corresponding to the molecular formula of $C_{19}H_{18}O_7$. The ^{1}H and ^{13}C NMR signals of the B ring were similar to those of **1** and **2** (Table 1 and Experimental). In addition the ^{1}H NMR spectrum indicated that ring A was fully substituted and bore a hydroxyl group at C-5, which gave the typical singlet at δ 13.0. One methyl group (δ_H 2.21) was located at C-6, as shown by the HMBC correlations OH-5/C-5, C-6, C-10 and Me-6/C-5, C-6, C-7, while the second one (δ 2.42) was at C-8, since it correlated with C-7, C-8 and C-9. Hence, the methoxyl groups were at C-7 and C-3, giving HMBC cross peaks with C-7 and C-3, respectively. The above results identify **4** as 3,7-dimethoxy-dimethoxy-6,8-di-*C*-methyl-5,3′,4′-trihydroxyflavone (6,8-di-*C*-methylquercetin 3,7-dimethyl ether).

Compounds **5** [13], **6** [14], **7** [15] and **8** [16] have

Table 1. ^{13}C NMR (62.5 MHz) data for compounds **1–8** in DMSO-d_6*

	1	2	3	4	5	6	7	8
2	167.7	155.0	155.7	155.9	155.3	155.3	155.1	156.0†
3	87.3	137.4	137.9	137.8	137.6	137.9	137.4	137.7
4	183.3	178.0	177.9	178.5	177.7	177.8	178.0	178.5
5	157.4	155.5	157.1	155.9	158.1	157.0	155.6	155.9†
6	108.0	106.6	107.0	112.5	106.4	107.0	106.7	112.5
7	162.6	159.7	163.0	162.0	162.2	162.9	159.7	162.0
8	90.1	101.5	89.9	108.4	92.6	90.0	101.6	108.5
9	153.0	151.4	154.4	151.3	153.9	154.3	151.5	151.4
10	102.5	103.9	104.7	107.0	103.7	104.7	104.0	107.0
1′	143.0	121.1	120.7	120.8	120.8	120.7	120.9	120.6
2′	121.7	115.1	115.4†	115.2	115.2	111.9	129.9	130.1
3′	116.4	145.2	145.2	145.3	145.2	147.4	115.7	115.7
4′	156.0	148.5	148.7	148.9	148.6	149.7	160.0	160.3
5′	116.4	115.8	115.6†	115.8	115.7	115.5	115.7	115.7
6′	121.7	120.4	120.5	120.6	120.5	122.1	129.9	130.1
3-OMe		59.5	59.6	59.5	59.6	59.5	59.6	59.6
7-OMe	56.3		55.2	60.3		56.2		60.3
3′-OMe						55.7		
6-Me	7.3	8.0	7.2	8.0	7.3	7.1	8.0	8.0
8-Me		8.2		8.2			8.2	8.2

*Assignments based on HMQC (**1**) or ^1H–^{13}C COSY (**6**) and HMBC (**1–8**) measurements.
†Values in the same column may be interchangeable.

been reported previously. The UV data were in accord with the literature, as well as the ^1H NMR data for **8**. The latter data were not available for the other compounds. The ^{13}C NMR and HMBC spectra entirely supported the structures of these three *C*-methylflavonols in the same way as for the related new compounds **2–4** (Table 1 and Experimental). *C*-Methylflavonols are widespread in various plant genera [13, 17]. In contrast, 2-phenoxychromones are relatively rare [8–10] and this is the first time that a *C*-methyl-2-phenoxychromone is reported.

EXPERIMENTAL

General. Mps.: uncorr.; UV: MeOH; ^1H NMR: 250 or 400 MHz; ^{13}C NMR: 62.5 MHz; 2D experiments: 400 MHz; CC: Merck silica gel 60 (230–400 mesh) and Sephadex LH-20.

Plant material. Leaves of *P. thonningii* (Schum.) Milne-Redhead were collected in August 1994 at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria, and authenticated by G. Adesakin of the Herbarium Section, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, where a voucher specimen is deposited.

Extraction and isolation. Air-dried powdered leaves (1.80 kg) were extracted with aq. EtOH (50%). The extract was concd *in vacuo* to a minimum vol. and partitioned with EtOAc. The EtOAc fr. (59 g) was subjected to accelerated gradient chromatography (AGC) on silica gel [18], using an increasing gradient of EtOAc in toluene, up to 100% and then a gradient of MeOH up to 100% to give 4 frs, P1–P4. Further AGC of P3 (10.7 g) using hexane–toluene (12.5–100%) and EtOAc (0.625–100%), gave 5 frs, fr. I (0.6 g), II (1.7 g), III (2.2 g), IV (2.1 g) and V (3.1 g). CC of fr. III on Sephadex LH-20 using toluene–EtOH (7:3) and recrystallization from EtOH (95%) afforded **6** (10 mg), **8** (14 mg) and **1** (7 mg). CC of fr. IV on Sephadex LH-20 using toluene–EtOH (1:1) gave 4 frs IVa–d. Final purification of fr. IVc (350 mg) on a Lobar® RP-18 column using MeOH–H_2O (7:3) and recrystallization from EtOH–H_2O (1:1) afforded **3** (20 mg), **4** (10 mg) and **7** (20 mg). Purification of fr. IVd (100 mg) using the same method afforded **2** (4 mg), **5** (6 mg) and quercetin (20 mg). AGC of P4 (34 g) with a gradient of EtOAc in toluene (2.5–100%) gave quercitrin (2.5 g).

Piliostigmin (**1**). Needles, mp 212–214°. UV λ_{max} nm (log ε) 231 (4.91), 287 (4.68); +NaOAc: 230, 287; +NaOH: 244, 288; +$AlCl_3$: 234, 252 (sh), 308, 351. EIMS *m/z* (rel. int.): 314 $[M]^+$ (100), 313 (60), 284 (20), 283 (20), 181 (10), 179 (10). ^1H NMR (250 MHz, DMSO-d_6): δ 2.02 (3H, *s*, Me-6), 3.90 (3H, *s*, OMe-7), 5.11 (1H, *s*, H-3), 6.72 (1H, *s*, H-8), 6.85 (2H, *d*, J = 8.9 Hz, H-3′, 5′), 7.19 (2H, *d*, J = 8.9 Hz, H-2′, 6′), 9.78 (1H, *s*, 4′-OH), 12.95 (1H, *s*, 5-OH). ^{13}C NMR: Table 1.

6,8-*Di*-C-*methylquercetin* 3-*methyl ether* (**2**). Yellow powder, mp 253–255° dec. UV λ_{max} nm (log ε): 258 (4.65), 356 (4.61); NaOAc: 271, 364; +NaOH: 274, 340, 410. +$AlCl_3$: 279, 441. CIMS: *m/z* 345 $[MH]^+$. ^1H NMR (250 MHz, DMSO-d_6): δ 2.13 (3H, *s*, Me-6), 2.33 (3H, *s*, Me-8) 3.78 (3H, *s*, OMe-3), 6.92 (1H, *d*, J = 8.6 Hz, H-5′), 7.60 (1H, *dd*, J = 2.3, 8.6 Hz, H-6′), 7.70 (1H, *d*, J = 2.3 Hz, H-2′), 12.95 (1H, *s*, 5-OH). ^{13}C NMR: Table 1.

6-C-*Methylquercetin* 3,7-*dimethyl ether* (**3**). Yellow powder, mp 195–198° dec. UV λ_{max} nm (log ε): 261.0 (4.64), 356 (4.63); +NaOAc: 262, 357; +NaOH: 270, 406; +$AlCl_3$: 278, 436. CIMS: *m/z* 345 $[MH]^+$. ^1H NMR (250 MHz, DMSO-d_6): δ 2.12 (3H, *s*, Me-6), 3.91 (3H, *s*, OMe-3), 4.02 (3H, *s*, OMe-7), 6.91 (1H, *s*,

H-8), 7.03 (1H, *d*, $J = 8.6$ Hz, H-5′), 7.61 (1H, *dd*, $J = 2.3$, 8.6 Hz, H-6′), 7.73 (1H, *d*, $J = 2.3$ Hz, H-2′), 13.00 (1H, *s*, 5-OH). ^{13}C NMR: Table 1.

6,8-*Di*-C-*methylquercetin* 3,7-*dimethyl ether* (**4**). Yellow powder, mp 200–203° dec. UV λ_{max} nm (log ε): 262.0 (4.67), 364 (4.63); +NaOAc: 263, 366; +NaOH: 268, 413; +$AlCl_3$: 280, 446. CIMS: *m/z* 359 $[MH]^+$. ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.21 (3H, *s*, me-6), δ 2.42 (3H, *s*, Me-8), 3.86 (3H, *s*, OMe-3), 3.91 (3H, *s*, OMe-7), 7.03 (1H, *d*, $J = 8.6$ Hz, H-5′), 7.63 (1H, *dd*, $J = 2.3$, 8.6 Hz, H-6′), 7.75 (1H, *d*, $J = 2.3$ Hz, H-2′), 13.00 (1H, *s*, 5-OH). ^{13}C NMR: Table 1.

6-C-*Methylquercetin* 3-*methyl ether* (**5**). Yellow powder, mp 260–262° dec. Identified by spectral comparison (UV, MS) with lit. [13]. ^{1}H MMR (250 MHz, DMSO-d_6): δ 2.17 (3H, *s*, M-6), 3.93 (3H, *s*, OMe-3), 6.67 (1H, *s*, H-8), 7.10 (1H, *d*, $J = 8.6$ Hz, H-5′), 7.64 (1H, *dd*, $J = 2.3$, 8.6 Hz, H-6′), 7.76 (1H, *d*, $J = 2.3$ Hz, H-2′), 13.10 (1H, *s*, 5-OH). ^{13}C NMR: Table 1. HMBC correlations (ring A): OH-5/C-5, C-6, C-10; H-8/C-4, C-6, C-7, C-9, C-10, Me-6; Me-6/C-5, C-6, C7; Me-3/C-3.

6-C-*Methylquercetin* 3,7,3′-*trimethyl ether* (**6**). Yellow needles, mp 185–187°. Identified by spectral comparison (UV, MS) with lit. [14]. ^{1}H NMR (250 MHz, DMSO-d_6), δ: 2.09 (3H, *s*, Me-6); 3.92 (3H, *s*, OMe-3); 3.98 (3H, *s*, OMe-3′); 4.01 (3H, *s*, OMe-7); 6.90 (1H, *s*, H-8); 7.07 (1H, *d*, $J = 8.6$ Hz, H-5′); 7.73 (1H, *dd*, $J = 2.3$, 8.6 Hz, H-6′); 7.78 (1H, *d*, $J = 2.3$ Hz, H-2′). 10.10 (1H, *s*, 4′-OH), 12.95 (1H, *s*, 5-OH). ^{13}C NMR: Table 1. HMBC correlations (ring A): OH-5/C-5, C-6, C-10; H-8/C-4, C-6, C-7, C-9, C-10, Me-6; Me-6/C-5, C-6, C-7; OMe-3/C-3; OMe-7/C-7.

6,8-*Di*-C-*methylkaempferol* 3-*methyl ether* (**7**). Yellow needles, mp 250–253° dec. CIMS: *m/z* 329 $[MH]^+$. Identified by spectral comparisons (UV) with lit. [15]. ^{1}H NMR (250 MHz, DMSO-d_6), δ: 2.15 (3H, *s*, Me-6); 2.35 (3H, *s*, Me-8); 3.88 (3H, *s*, OMe-3); 8.05 (2H, *d*, $J = 9$ Hz, H2′, 6′); 7.10 (2H, *d*, $J = 9$ Hz, H-3′,5′); 13.00 (1H, *s*, 5-OH). ^{13}C NMR: Table 1. HMBC correlations (ring A): OH-5/C-4, C-5, C-6, C-7, C-10; Me-6/C-5, C-6, C-7; Me-8/C-7, C-8, C-9; OMe-3/C-3.

6,8-*Di*-C-*methylkaempferol* 3,7-*dimethyl ether* (**8**). Yellow needles, mp 285–287°. Identified by spectral comparison (UV, MS, ^{1}H NMR) with lit. [16]. ^{13}C NMR: Table 1. HMBC correlations (ring A): OH-5/C-5, C-6, C-10; Me-6/C-5, C-6, C-7; Me-8/C-7, C-8, C-9; OMe-3/C-3; OMe-7/C-7.

Acknowledgements—The authors are grateful to IPICS (International Program in the Chemical Sciences, Uppsala University) for funding the project, and for a six-month Fellowship to J. C. I. The authors also thank Mr G. Adesakin of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, for help with the collection and authentification of the plant material.

REFERENCES

1. Dalziel, J. M. (1937) *The Useful Plants of West Tropical Africa*, p. 174. Crown Agents, London.
2. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, p. 640. E & S. Livingstone, London.
3. Bombardelli, E., Gabetta, B. and Mustich, G. (1973) *Fitoterapia* **44**, 85.
4. Bombardelli, E., Lolla, A., William, R. and Piretti, M. V. (1992) *Planta Med.* **58** (Suppl. 1), A590.
5. Okwute, S. K., Ndukwe, G. I., Watanabe, K. and Ohno, N. (1986) *J. Nat. Prod.* **49**, 716.
6. Snatzke, F. and Wolff, P. (1989) *Bull. Chem. Soc. Ethiopia* **3**, 135.
7. Lamikanra, A., Ogundaini, A. O. and Ogungbamila, F. O. (1990) *Phytother. Res.* **4**, 198.
8. Komiya, T., Tsukui, M. and Oshio, H. (1975) *Chem. Pharm. Bull.* **23**, 1387.
9. Hashidoko, Y., Tahara, S. and Mizutani, J. (1991) *Phytochemistry* **30**, 3837.
10. Huang, Y.-L., Ou, J.-C., Chen, C.-F. and Chen, C.-C. (1993) *J. Nat. Prod.* **56**, 275.
11. Roitman, J. N. and James L. F. (1985) *Phytochemistry* **24**, 835.
12. Breitmeier, E. and Voelter, W. (1990) *Carbon-13 NMR Spectroscopy*, p. 451. VCH, New York.
13. Harborne, J. B., Williams, C. A., Greenham, J. and Eagles, J. (1994) *Phytochemistry* **35**, 1475.
14. Rabesa, Z. and Voirin, B. (1985) *C. R. Acad. Sci. Paris, Ser. III* **301**, 351.
15. Voirin, B. (1983) *Phytochemistry* **22**, 2107.
16. Rasamoelisendra, R., Voirin, B., Favre-Bonvin, J., Andriantsiferana, M. and Rabesa, Z. (1989) *Phytochemistry* **28**, 1996.
17. Wollenweber, E. (1986) in *The Flavonoids Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 285. Chapman and Hall, London.
18. Dunstan, C. A. (1995) *Acta Univ. Upsaliensis* **138**, 8.