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DROSOPHILIN, A METHYL ETHER FROM *MYCENA MEGASPORA*

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Key Word Index—*Mycena megaspora*; Basidiomycetes; Agaricales; fungi; drosophilin A methyl ether; 1,4-dimethoxy-2,3,5,6-tetrachlorobenzene.

Plant. Mycena megaspora Kauffman = *Mycena permixta* (Britzelm) Sacc. CBS 363·50. *Previous work.* Drosophilin A (4-methoxy-2,3,5,6-tetrachlorophenol) from *Psathyrella subatrata* (Batsch ex Fr.) Gill. = *Drosophila subatrata* (Batsch ex Fr.) Quél[1]. Drosophilin A *O*-methyl ether from *Fomes fastuosus* (Lév.) Cooke[2] and from *Phellinus robinae* (Murrill) A. Ames = *Fomes robinae* (Murrill) Sacc. et D. Sacc.[3, 4].

Present work. The presence of long white crystalline needles was observed in 6–10 months old cultures of *Mycena megaspora* grown on malt agar slants. About 1 mg of crystals was collected and purified by recrystallisation from acetone: mp 162–163°. The compound in EtOH showed aromatic absorptions in UV: λ_{max} 209, 225*sh*, 236*sh* 286 and 294 nm. The MS revealed molecular ion peaks characteristic for a substance with 4 Cl atoms: MW found; 273·91529. Calc. for $C_8H_6Cl_4O_2$: 273·91219 for ^{35}Cl. Fragmentation pattern identical with that of drosophilin A *O*-methyl ether[5]. The identity of the two compounds was confirmed by comparison of the natural sample (mmp, UV, IR[4]) with the synthetic one, prepared by methylation of tetrachlorohydroquinone with MeI and NaOMe in MeOH according to the method described[6] for the preparation of catenarin-6-methyl ether.

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SULPHATE ESTERS OF CAFFEYL- AND *p*-COUMARYLGLUCOSE IN FERNS

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Key Word Index—*Adiantum* spp.; Adiantaceae; *Pteridium aquilinum*; Dennstaedtiaceae; sulphate esters of caffeyl- and *p*-coumarylglucose.

Plants and sources. Adiantum species (listed below) from the living or herbarium collections of the Royal Botanic Gardens, Kew, and *Pteridium aquilinum* L. (Kuhn) from 17 locations throughout the world. Voucher specimens are held in the Herbarium at Kew. *Previous work.* Many ferns, including *Adiantum* species and *P. aquilinum*, have been shown to contain hydroxycinnamic acids [1,2] and related flavonoid compounds [1–5]. *Plant part examined.* Fronds (pinnae) from both fresh and herbarium material were examined. However, both compounds described were actually isolated from freshly harvested fronds. *Present work.* The fronds were extracted with 80% MeOH (*ca* 10 ml/g) and the concentrated extracts examined by 2-dimensional PC in *n*-BuOH–HOAc–H_2O (6:1:2) and 15% HOAc.

In 10 of the 58 species of *Adiantum* examined (*A. brasiliense* Raddi, *A. chiliense* Klf., *A. concinnum* H.B.Willd, *A. jordani* K., *A. lucidum* (Cav.)

Sw., *A. macrophyllum* Sw., *A. pulverulentum* L., *A. tenerum* Sw., *A. terminatum* Kze. and *A. villosum* L.) and in all samples of *Pteridium aquilinum*, chromatograms showed the presence, in UV light, of a green–blue fluorescent arrow-shaped spot (**A**) with relatively low mobility in organic solvents ($R_f \times 100$ in BAW 20, Phenol 6) and high mobility in aq solvents (15% HOAc 68,81). All species listed above (except *A. concinnum*) and *A. tetraphyllum* H.B.Willd. showed the presence of a 2nd spot *B*), similar in shape to (*A*), absorbing in UV but appearing bright-blue after fuming with ammonia. Again, R_f values were lower in organic solvents (BAW 43, Phenol 23) than in aq solvents (15% HOAc 73).

The 2 compounds were isolated (**A** from *P. aquilinum* and **B** from *A. pulverulentum*) and purified by conventional thick PC and TLC [6,7]. Both **A** and **B** were found to be highly mobile on Whatman No. 3 paper electrophoretically at pH 2·2, using HCOOH–HOAc buffer [8] (mobility of *A* and *B* relative to tartaric acid 4·8 and 5·2 respectively), suggesting the possible presence of sulphate or phosphate ester groups. On acid hydrolysis, **A** gave caffeic acid (PC, TLC, UV), glucose (PC, TLC and aniline phosphate spray reagent), and sulphate (ppte with $BaCl_2$; no colour with molybdate): the presence of sulphate was confirmed, following either base (N KOH) or acid (2N HCl) hydrolysis of **A** and TLC of the products on cellulose in 20% 0·1N HCl in EtOH using aq $Na_3CO(NO_3)_6$ as a spray [8], by the appearance of a white spot at R_f 80. Potassium was also detected as a yellow spot at R_f 17. Under the same conditions, **B** gave *p*-coumaric acid, glucose and sulphate. To show that the sulphate was attached to the glucose moiety, both **A** and **B** were incubated with commercial sulphatase (extracted from *Helix pomata*) [9] at pH 5·0, 37° for 3 hr, and both gave the corresponding 1-glucose ester (**A**: 1-caffeyl glucose $R_f \times 100$ BAW 52, Phenol 24, 15% HOAc 65,80; **B**: 1-*p*-coumarylglucose BAW 60, Phenol 37, 15% HOAc, 70,77), together with caffeic or *p*-coumaric acids presumably as the result of the presence of esterases in the enzyme preparation [9].

UV spectra of **A** at various pH's and in the presence of $AlCl_3$ and Na molybdate [10] showed that it was a caffeyl ester with both phenolic hydroxy groups free. By the same criteria **B** was identified as a *p*-coumaryl ester with the phenolic hydroxy group unsubstituted. [λ(nm) max. **A**: *Acid* 245,300*sh*,330; *Neutral* 245,300*sh*,330; *Base* 269,315*sh*,373; $AlCl_3$ 245,300*sh*,338; *Molyb.* positive. **B**: *Acid* 314; *Neutral* 314; *Base* 362; $AlCl_3$ 314; *molyb.* negative].

From the above criteria **A** is obviously a sulphate ester of caffeylglucose, the sulphate probably being attached to the 6-OH group of the sugar. Similarly **B** is presumably the 6′-*O*-sulphate ester of *p*-coumarylglucose.

Sulphate esters of flavone and flavonol glycosides (mainly with SO_4'' attached to phenolic hydroxyl groups) have recently been shown to be widespread in angiosperms [8,9,11] and, while they appear to be restricted to families which are herbaceous or are advanced morphologically, it is interesting to note that they are found mainly in plants which, like ferns, have association with aquatic habitats [9]. None of these compounds has been detected so far in ferns. This is the first time that naturally occurring sulphate derivatives of hydroxycinnamic acid glucose esters have been isolated. Preliminary reports of these findings have been given elsewhere [2,12] and their taxonomic significance will be discussed in a future paper.

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