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SHORT COMMUNICATION

ATROMENTIC ACID FROM *CLITOCYBE ILLUDENS**

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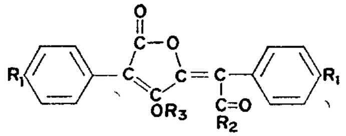
Abstract—Atromentic acid was isolated from culture liquids of *Clitocybe illudens*. Identification, based on analyses and spectra of the compound and a number of new derivatives, was confirmed by direct comparison of its tetramethylated derivative with a synthetic sample. Products, giving a blueing reaction have been detected in the same culture liquids, and an as yet unidentified compound has been isolated in crystalline form as its methylation product, $C_{42}H_{36}O_{12}$. The presence of pulvinic acid derivatives in Basidiomycetes other than the Boletaceae has been reported in only one other instance.

A number of metabolites have been isolated from culture liquids of the basidiomycete *Clitocybe illudens*. All of these are sesquiterpenoids and appear to be closely related biogenetically.[1] We have now obtained a quite unrelated type of compound, the pulvinic acid derivative atromentic acid (Ia). Identification of this metabolite was based on analyses and spectral characteristics of the compound and several derivatives: a partial methylation product (Ib) the diacetate of this product (Ic), an anilide (Id), the trimethylether of the anilide (Ie), and a tetramethyl derivative (If).



	R1	R2	R3
I a	OH	OH	H
I b	OH	OCH_3	CH_3
I c	CH_3CO-	OCH_3	CH_3
I d	OH	C_6H_5NH-	H
I e	OCH_3	C_6H_5NH-	CH_3
I f	OCH_3	OCH_3	CH_3
I g	H	OH	H

The location of the methyl groups in Ib was revealed by comparison of the mass spectra of Ib, Ic, and If with that reported for atromentic acid.[2a,b] The latter shows two fragments, 133 and 105, formed from the aromatic rings. Our dimethylated product Ib shows the same fragments. The diacetate (Ic), as expected,[3] also shows these fragments, while the completely methylated product shows the corresponding fragments: 147 (133 + 14) and 119

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* Part VIII in the series "Metabolic Products of *Clitocybe illudens*". For Part VII see T. C. McMorris, M. S. R. Nair, P. Singh and M. Anchel, *Phytochem*, **10**, 1611 (1971).

[1] M. S. R. Nair, H. Takeshita, T. C. McMorris and M. Anchel, *J. Org. Chem.* **34**, 240 (1969).

[2a] W. Steglich, W. Furtner and A. Prox, *Z. Naturforsch.* **23b**, 1044 (1968).

[2b] M. C. Gaylord, R. G. Benedict, G. M. Hatfield and L. R. Brady, *J. Pharm. Sci.* **59**, 1420 (1970).

[3] D. R. Buckley and E. S. Waight, *Org. Mass Spec.* **2**, 367 (1969).

(105 + 14). These last fragments were obtained as well from methoxy-pulvinic acid dilactone.[4] In further agreement with the assigned position of the two methyl groups in (Ib), the mass spectrum shows fragments at 281 (249 + 32) and 309 (277 + 32) corresponding to fragments of 249 and 277 in the mass spectrum of methyl vulpinate.[4]

Of the derivatives prepared, the tetramethyl compound (If) was the only one previously described.[5] Direct comparison of If with a synthetic sample confirmed the identity of the two.

Besides atromentic acid, another compound has been isolated from the culture liquids as a fully methylated derivative. The mass spectrum of this compound suggests that it is related to the pulvinic acid group.[4] It has mol. wt. 732 (M.S.). Analytical values indicate a molecular formula $C_{42}H_{36}O_{12}$ and 7 methoxyl groups. On this basis, the formula $C_{35}H_{22}O_{12}$ is tentatively suggested for the parent compound, which may be a dimer of a pulvinic acid derivative. The fraction from which this compound was isolated gives a 'blueing' reaction with alkali, pointing to the presence of pulvinic acid derivatives with a catechol nucleus.[6,7] However, since only a methylated derivative has been obtained and not the parent compound, we do not know as yet whether this unidentified compound is responsible for the blueing reaction.

Pigments of the pulvinic acid group are of widespread occurrence in the lichens,[8] and have been isolated recently from non-lichen forming fungi.[9] They are biogenetically derived from the corresponding terphenyl quinones,[10] a number of which have been isolated from Basidiomycetes.[11] Atromentic acid itself has long been known as a synthetic product,[5,12,13] but its natural occurrence was reported only recently.[2a,b] One mg of the compound was isolated from the bolete *Xerocomus chrysentenon*, and identified by comparison of its high resolution mass spectrum with those of other pulvinic acid derivatives.[2a] It has now been isolated also from *Paxillus atrotomentosus*[2b] to which *Clitocybe* is more closely related.

The isolation of atromentic acid from culture liquids of *Clitocybe illudens* gains added interest from the reported occurrence of atromentin in the fruiting bodies of this species.[14]

EXPERIMENTAL

Isolation of atromentic acid (Ia). A single spore isolate from a fruiting body of *Clitocybe illudens* collected in the wild,* when grown in surface culture (cornsteep liquid, 25°, glass wool support) was observed to produce strikingly more than the usual amount of pigment. Culture liquid from 60 Fernbachs (1 l. in each) was harvested after 6 weeks, and extracted with EtOAc three times, using a total of 40% of the vol. The organic extract was concentrated to a small volume and extracted with 1% $NaHCO_3$. The bicarbonate solution,

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* This fruiting body was collected in Mamaroneck, N.Y. by DR. SUSAN CANHAM, who brought it into culture and made the single spore isolates.

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[5] F. KÖGL, H. BECKER, G. DE VOSS and E. WIRTH, *Ann.* **465**, 243 (1928).

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after extraction with EtOAc to remove neutral material, was acidified with HCl and reextracted with EtOAc. The residue of this extract, a brown semi-solid, was fractionated by countercurrent distribution between $CHCl_3$ and 25% EtOH in a 50 tube train. The distribution was continued until 100 portions of the upper phase were used. All tubes showed yellow colour and all had UV max at 250 nm. The emergent 50 fractions of upper layer, and tubes 39–49, showed a major spot (yellow fluorescence under a 356 nm UV lamp) with R_f 0·4 on a Brinkman pre-coated Si gel G TLC plate developed with a 30% EtOH in $CHCl_3$ solution. These fractions were combined, the organic solvent removed, and the resulting aq. solution extracted with EtOAc. The material in this extract was further purified by countercurrent distribution between benzene–EtOAc (3:1) against H_2O–EtOH (3:1). The purest fractions from this distribution (23–44) were combined and subjected to a third countercurrent distribution between benzene–EtOAc (19:1) and H_2O–EtOH (19:1). The upper phases of tubes 28–32 were combined, and the residue from these was recrystallized from $CHCl_3$–petrol., then from benzene–petrol. Yield, 2–5 mg/l. culture fluid. Yellow needles, m > 300° (less pure samples melted *ca.* 220°) $EtOH_{max}$ 258, 387 nm (log ϵ 4·12, 3·81), on addition of NaOH, 260, 362 nm (log ϵ 4·07, 4·12), on addition of dilute H_2SO_4, 260, 400 nm (log ϵ 4·03, 3·87) ν_{max} (KBr) 1739 cm^{-1} δ (acetone D6) 6·73, 6·77, 6·87. 6·90, 7·12, 7·27 (apparent singlets representing two overlapping AA^1 XX^1 systems, 8H, JAX–9 c/s) 7·93 (2H, singlet), 8·05 (1H, singlet) 8·2 (1H, singlet). In D_2O the last three disappear. (Found: C, 64·35; H, 3·99; O, 31·41. Calc. for $C_{18}H_{12}O_7$: C, 63·53; H, 3·55; O, 32 91.) The compound was difficult to purify.

p,p′-*Dihydroxy methyl vulpinate* (Ib). A solution of Ia (100 mg) in a small amount of ethanol or EtOAc was treated with excess ethereal CH_2N_2. After 5 min most of the solvent was blown off in N_2. The residue showed one major spot on TLC, and a less polar, minor one. It was separated on a Si gel column. After removing the less polar component by elution with $CHCl_3$, the major product was eluted with $CHCl_3$–MeOH (19:1). Yellow crystals from EtOAc–petrol., m.p. 223–225°. $EtOH_{max}$ 240, 366 (log ϵ 4·38, 4·52), shifted on treatment with NaOH to 260 and 453 nm (log ϵ 4·46, 4·62) ν_{max} (KBr) 1739 cm^{-1} δ (acetone D6) 3·81 (3H, singlet), 3·89 (3H, singlet) 6·8–7·71 (8H, multiplet) 8·57 (1H, singlet) and 8·73 (1H, singlet). In D_2O the last two disappear. Mass spectrum *m/e* 369 (p + 1, 16) 368 (p, 80), 310 (15) 309 (65) 281 (35), 253 (52) 235 (15) 134 (15), 133 (41) 121 (25) 105 (62) 45 (15) 43 (100) 29 (24). (Found: C, 65·28; H, 4·35; O, 30·29; OCH_3, 17·15. $C_{20}H_{16}O_7$ requires: C, 65·21; H, 4·38; O, 30·41; 2-OCH_3, 16·85.)

p,p′-*Diacetoxy methyl vulpinate* (Ic). A solution of Ib (50 mg) in Ac_2O (2 ml) and pyridine (2 ml) was allowed to stand at room temp. overnight. The solution was poured on ice and the yellow solid obtained was purified by preparative TLC. Light yellow needles from EtOAc–petrol, m.p. 178–180° $EtOH_{max}$ 230, 331 (log ϵ 4·29, 4·47), shifts on addition of alkali to 258, 450 nm (log ϵ 4·40, 4·42) ν_{max} (KBr) 1764, 1724, 1202 cm^{-1}. Mass spectrum *m/e* 453 (p + 1, 23) 452 (p, 80) 411 (51) 410 (52), 369 (83), 368 (99), 351 (19), 310 (42), 309 (51) 253 (24) 133 (18) 105 (28) 43 (100). (Found: C, 63·24; H, 4·43; O, 32·17; CH_3CO, 18·02; OCH_3, 14·20. $C_{24}H_{20}O_9$ requires: C, 63·71; H, 4·46; O, 31·83; 2 CH_3CO, 18·5; 2 OCH_3, 13·75.)

Atromentic acid anilide (Id). The anilide was prepared in the usual way ($SOCl_2$, aniline) and recrystallized from EtOAc–petrol. Yellow plates m.p. > 360°, $EtOH_{max}$ 234, 247, 395 (log ϵ 4·36, 4·39, 4·23) on addition of NaOH, 255, 320, 360, 393 nm (sh) (log ϵ 4·67, 4·24, 4·39, 4·27). (Found C, 69·05; H, 4·61; O, 23·69; N, 2·66. $C_{24}H_{17}O_6N$ requires: C, 69·39; H, 4·13; O, 23·11; N, 3·37.)

Atromentic acid anilide trimethylether (Ie). A solution of Id in EtOH–EtOAc was treated with excess CH_2N_2 in ether and allowed to stand overnight. The ether was obtained as yellow needles, m.p. 197–199°. Mass spectrum *m/e* 458 (p + 1, 21), 457 (p, 47), 340 (18), 339 (20) 338 (51), 337 (100), 324 (31), 323 (77), 309 (30), 295 (35), 281 (37), 147 (20), 119 (40) 57 (15). (Found: C, 69·77; H, 4·87; N, 3·09; OCH_3, 20·10. $C_{27}H_{23}O_6N$ requires C, 70·88; H, 5·07; N, 3·06; 3-OCH_3, 20·30.)

p,p′-*Dimethoxy methyl vulpinate* (If). This was prepared from Ia using CH_2N_2 overnight. It was worked up in the same way as the partial ether (Ib). The major portion of the product was eluted from the column with $CHCl_3$, and gave a single spot on TLC (R_f 0·6 with EtOAc–petrol, 1:1, and R_f 0·57 with benzene–$CHCl_3$, 1:1). Yellow needles from benzene–petrol, or EtOAc–petrol, m.p. 170–171°. $EtOH_{max}$ 237, 360 nm (log ϵ 4·52, 4·63) shifted on addition of alkali to 276, 360 nm (log ϵ 4·47, 4·24) ν_{max} (KBr) 1773, 1770, 1247 cm^{-1}. δ ($CDCl_3$) 3·77 (3H, singlet) 3·84 (6H, singlet) 3·9 (3H, singlet) 6·85–7·77 (8H, multiplet) (acetone D6) 3·75 (3H, singlet) 3·80 (6H, singlet) 3·84 (3H, singlet) 6·80–7·62 (8H, multiplet). Mixed m.p. with synthetic p,p′-dimethoxy methyl vulpinate[5] gave no depression; the UV and the IR spectra of the two samples were superimposable. Mass spectrum *m/e* 397 (p + 1, 27) 396 (p, 100) 338 (80), 337 (90), 309 (42) 282 (20), 281 (95) 238 (16) 148 (21) 147 (52), 135 (45), 120 (17), 119 (99), 76 (15) 65 (15). (Found: C, 66·98; H, 5·05; O, 27·94; OCH_3, 30·64. Calc. for $C_{22}H_{20}O_7$; C, 66·66, H, 5·09, O, 28·25, 4-OCH_3, 31·31.)

Isolation of high molecular weight pigment. This material was isolated from the fractions of the countercurrent distribution immediately on the less polar side of the atromentic acid fraction. After several redistributions a non-crystalline, material was obtained (*ca.* 1 mg/l. culture liquid) which was treated with ethereal CH_2N_2 overnight. The methylated product was purified by preparative TLC and crystallized from aq. EtOH. Yellow crystals m.p. 122–125°. $EtOH_{max}$ 254 (sh), 356 nm (log ϵ 3·86, 3·74). ν_{max} (KBr) 1761, 1724, 1686, 1600, 1504, 1247 cm^{-1}. M. W. (M.S.) 732. (Found: C, 69·00; H, 4·82; O, 25·98; OCH_3 29·31. $C_{42}H_{36}O_{12}$ requires: C, 68·84, H, 4·95; O, 26·20; 7·00 OCH_3, 29·70.) Mass spectrum *m/e* 734 (13), 733 (52), 732 (p, 100), 336 (16), 147 (41), 135 (47), 120 (23), 119 (35), 44 (43).

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