*Phytochemistry*, Vol 23, No 11, pp 2693–2694, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00


# PYRENOCINE C, A PHYTOTOXIN-RELATED METABOLITE PRODUCED BY ONION PINK ROOT FUNGUS, *PYRENOCHAETA TERRESTRIS*

SALVATORE A SPARACE, J BRIAN MUDD, BASIL A BURKE and ARNE J AASEN

ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568, U S A

(*Revised received* 27 *March* 1984)

**Key Word Index**—*Pyrenochaeta terrestris*, onion pink root fungus, pyrenocine C, (±)-(2′*E*)-5-(1′-hydroxybut-2′-enyl)-4-methoxy-6-methyl-2-pyrone

**Abstract**—The structure of pyrenocine C, a new metabolite isolated from onion pink root fungus, *Pyrenochaeta terrestris* (Hansen) has been elucidated as (±)-(2′*E*)-5-(1′-hydroxybut-2′-enyl)-4-methoxy-6-methyl-2-pyrone by spectroscopic methods and chemical correlation with pyrenocine A

Previous reports on phytotoxins produced by the onion pink root fungus, *Pyrenochaeta terrestris*, have described the structures of pyrenochaetic acids A, B and C, and pyrenocine A and B [1–3] The structure of pyrenocine A (3) has been established by X-ray diffraction analysis [2] Pyrenocine A is identical to citreopyrone from *Penicillium citreo-viride* [4] The structure determination of a new metabolite from *P terrestris*, pyrenocine C (1), constitutes the subject of the present communication Details of the biological activity and the role of this metabolite will be provided elsewhere However, preliminary experiments suggest the pyrenocine C is only weakly phytotoxic

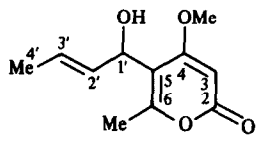
The $^1H$ and $^{13}C$ NMR spectra of pyrenocine C (1) [$C_{11}H_{14}O_4$(MS)] indicated close structural resemblance with pyrenocine A (3) except for having a (2′*E*)-1′-hydroxybut-2′-enyl substituent rather than a (2′*E*)-but-2′-enoyl group attached to the 2-pyrone moiety The spectra did not, however, unambiguously distinguish between two possible substitution patterns, 5-(1′-hydroxybut-2′-enyl)-6-methyl or 5-methyl-6-(1′-hydroxybut-2′-enyl) This ambiguity was resolved by selective hydrogenation of the butenyl double bond in pyrenocine C to a dihydro derivative which was indistinguishable from 2′,3′-dihydropyrenocine C (2) prepared from pyrenocine A by sodium borohydride reduction in ethyl acetate Addition of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [5, 6] to the NMR solutions induced spectral nonequivalence of the diastereoisomeric solvates revealing that both reduction products were racemic This, together with a negligible optical rotation ($[\alpha]_D^{20}$ +0 3°), suggests that pyrenocine C is racemic and, thus, its structure is (±)-(2′*E*)-5-(1′-hydroxybut-2′-enyl)-4-methoxy-6-methyl-2-pyrone

Attempts to convert pyrenocine A to pyrenocine C, or *vice versa*, employing various oxidative [7, 8] and reductive [9, 10] methods failed

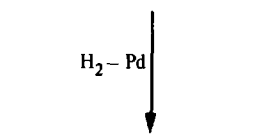
## EXPERIMENTAL

Mps uncorr, TLC and prep TLC silica gel ('Baker' Si250F) using $Et_2O$ as solvent, unless otherwise specified, GLC glass column (1 m × 3 mm) packed with 10% SE-30, column temp 175°, $^1H$ NMR (300 MHz) and $^{13}C$ NMR (75 MHz): $CDCl_3$, TMS as int standard

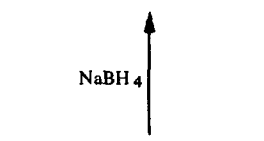
*Isolation* Pyrenocines A (3) and C (1) (30 and 9 mg respectively per liter) were isolated from filtrates of *P terrestris* grown for 10 days at 28° and shaken at 175 rpm in potato-dextrose broth consisting of glucose (20 g), $KNO_3$ (2 5 g), $KH_2PO_4$ (1 0 g), and the broth of potato (30 g per liter of growth medium) Culture filtrates were extracted with EtOAc and the extract dried *in vacuo* The residue was redissolved in $C_6H_6$ and applied to a silica gel




**1** Pyrenocine C




**2** 2′,3′– Dihydropyrenocine C




**3** Pyrenocine A

column which was eluted stepwise with increasing proportions of EtOAc in $C_6H_6$ and monitored by TLC Pyrenocine A was recrystallized from $Et_2O$ Fractions containing pyrenocine C were subjected to prep TLC twice, first with $Et_2O$–EtOH (50 1) and then with EtOAc–hexane (17 3) Recrystallization from $Et_2O$ yielded pyrenocine C as colourless needles

*Pyrenocine C* (**1**) Mp 81 5–82 0°, $[\alpha]^{20}$ +0 3° (589 nm), +0 5° (578 nm), +0 8° (546 nm), +2 8° (436 nm), +4 0° (365 nm) ($CHCl_3$, *c* 0 37), $R_f$ 0 3, UV $\lambda_{max}^{MeOH}$ nm (log ε) 284 (3 9), IR $\nu_{max}^{film}$ $cm^{-1}$ 3410, 1700–1720 (broad absorption band), 1640, 1563, 1456, 1405, 1250, 1078, EIMS 70 eV *m/z* (rel int ) 210 0893 [calc for $C_{11}H_{14}O_4$ 210 0892] (21), 195 (20), 177 (20), 169 (21), 167 (100), 69 (32), 59 (23), $^{13}C$ NMR δ17 6 (*q*, C-4′), 17 8 (*q*, C-6 Me), 56 2 (*q*, OMe), 68 6 (*d*, C-1′), 88 7 (*d*, C-3), 112 5 (*s*, C-5), 127 6 (*d*, C-2′ or C-3′), 131 1 (*d*, C-2′ or C-3′), 159 5 (*s*, C-6), 163 8 (*s*, C-2), 170 0 (*s*, C-4), assignments were based on the $^1H$ coupled spectrum, selective $^1H$ decoupling experiments, and values given in ref [2] for pyrenocine A and B $^1H$ NMR δ1 71 (3H, *d*, *J* = 5 5 Hz), 2 31 (3H, *s*), 2 64 (OH, *d*, *J* = 8 2 Hz), 3 86 (3H, *s*), 5 15 (1H, *t*, *J* = *ca* 7 Hz), 5 50 (1H, *s*), 5 6–5 8 (2H, *m*), the multiplet at δ5 6–5 8 constituted the AB-moiety of an $ABMX_3$ spin system as judged from spin decoupling experiments Irradiation at δ1 71 and 5 15, respectively, resolved the AB-pattern at δ5 66 (H-3′) and 5 74 (H-2′) with $J_{AB}$ = 15 4 Hz. On irradiation at δ5 68 the doublet at δ1 71 collapsed to a singlet

*2′,3′-Dihydropyrenocine C* (**2**) Initial microscale experiments revealed that hydrogenation had to be performed for a short period of time only to avoid saturation of the 5,6 double bond Pyrenocine C (3 5 mg) in EtOAc (2 5 ml) was hydrogenated for 55 sec at atmospheric pres and ambient temp in the presence of 5% Pd–C (1 3 mg) The mixture was filtered, and the residue obtained after evaporation of the solvent examined by $^1H$ NMR which revealed the presence of 2′,3′-dihydropyrenocine C and pyrenocine C in a 1 2 ratio The mixture was subjected to two more hydrogenations [a EtOAc (2 ml), 5% Pd–C (1 8 mg), 90 sec, b EtOAc (2 ml), 5% Pd–C (1 9 mg), 60 sec] which gave 2′,3′-dihydropyrenocine C and pyrenocine C in the ratios 4 1 and 94 6, respectively The final product mixture was subjected to prep TLC furnishing 2 8 mg (80%) 2′,3′-dihydropyrenocine C $R_f$ 0 3, UV $\lambda_{max}^{MeOH}$ nm 283, EIMS 70 eV *m/z* (rel int ) 212 $[M]^+$ (8), 169, (73), 151 (8), 127 (57), 95 (12), 69 (14), 55 (10), 43 (100), 41 (23), $^{13}C$ NMR δ13 8, 17 8, 19 4, 39 2, 56 2, 68 6, 89 0, 113 0, 158 9, 163 8, 170 4, $^1H$ NMR δ0 95 (3H, *t*, *J* = 7 3 Hz), 1 2–1 5 (2H, *m*), 1 55–1 95 (2H, *m*), 2 30 (3H, *s*), 2 45 (OH), 3 87 (3H, *s*), 4 65 (1H, *t*, *J* = *ca* 7 Hz), 5 51 (1H, *s*)

*2′,3′-Dihydropyrenocine C from pyrenocine A* Initial reductions of pyrenocine A with $NaBH_4$ in MeOH led to mixtures of products including 2′,3′-dihydropyrenocine C and diastereoisomers of 2′,3′,5,6-tetrahydropyrenocine C $NaBH_4$ (12 mg) was added to a soln of pyrenocine A (10 9 mg) in EtOAc (3 ml) at room temp The reduction was monitored by TLC Additional $NaBH_4$ (25 and 30 mg) was added after 30 and 65 min After one hr most of the pyrenocine A ($R_f$ 0 5) had been converted to an intermediate ($R_f$ 0 55, presumably 2′,3′-dihydropyrenocine A) which during the following 2 hr was reduced to 2′,3′-dihydropyrenocine C $H_2O$ (1 ml) was added to the reaction mixture followed by extraction with EtOAc (30 ml) The extract was dried ($Na_2SO_4$) and the solvent removed *in vacuo* The product was purified by prep TLC giving 5 mg (45%) 2′,3′-dihydropyrenocine C which cochromatographed [TLC, GC ($R_t$ 8 3 min)] with 2′,3′-dihydropyrenocine C prepared from pyrenocine C The UV, mass, $^1H$ NMR and $^{13}C$ NMR spectra agreed with those of 2′,3′-dihydropyrenocine C derived from pyrenocine C Niwa *et al* [4] has previously reduced citreopyrone to 2′,3′-dihydropyrenocine C employing $NaBH_4$ in THF

*Chiral purity of 2′,3′-dihydropyrenocine C* Portions of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol were successively added to the NMR solns of 2′,3′-dihydropyrenocine C derived from pyrenocine A and C, respectively, until complete nonequivalence of the signals representing C-6 Me, OMe and H-3 were obtained (6–15 1 chiral solvating agent substrate ratio) Both compounds revealed double sets of signals which were of equal intensities

*Acknowledgements*—The authors wish to acknowledge David S Hirano and Pat W Bethel for the mass spectroscopy of the pyrenocines and their derivatives

## REFERENCES

1 Sato, H , Konoma, K and Sakamura, S (1979) *Agric Biol Chem* **43**, 2409
2 Sato, H , Konoma, K , Sakamura, S , Furusaki, A , Matsumoto, T and Matsuzaki, T (1981) *Agric Biol Chem* **45**, 795
3 Sato, H , Konoma, K and Sakamura, S (1981) *Agric Biol Chem* **45**, 1675
4 Niwa, M , Ogiso, S , Endo, T and Furukawa, H (1980) *Tetrahedron Letters* **21**, 4481
5 Pirkle, W H , Sikkenga, D L and Pavlin, M S (1977) *J Org Chem* **42**, 348
6 Pirkle, W H and Boeder, C W (1977) *J Org Chem.* **42**, 3697
7 Bowers, A , Halsall, T G , Jones, E R H and Lemin, A J (1953) *J Chem. Soc* 2548
8 Corey, E J and Schmidt, G (1979) *Tetrahedron Letters* 399
9 Brown, H C and McFarlin, R F (1958) *J Am Chem Soc* **80**, 5372
10 Marshall, J A and Johnson, W S (1963) *J Org Chem* **28**, 595