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A NAPHTHALENOID PULVINIC ACID DERIVATIVE FROM THE FUNGUS *PISOLITHUS TINCTORIUS*

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Key Word Index—*Pisolithus tinctorius*; Sclerodermataceae; basidiomycete; pigment; naphthalenoid pulvinic acid derivative; norbadion-A.

Abstract—Fruitbodies of the ectomycorrhizal fungus *Pisolithus tinctorius* contain a high concentration of a naphthalenoid pulvinic acid derivative, which was identified by chemical and spectroscopic methods.

INTRODUCTION

The fungus *Pisolithus tinctorius* (Pers.) Coker et Couch is a gasteromycete of worldwide distribution [1]. The organism grows in mycorrhizal association with a wide variety of tree genera [1] and since these mycorrhizae are well adapted to adverse soil and climatic conditions the fungus has high potential in forestation and reclamation efforts [2, 3]. The benefits to health and growth rate of the host plant upon association with *P. tinctorius* are superior to those with other mycorrhizal fungi [4] and methods for inoculating vegetative mycelium into nursery soils have recently been developed [5].

The dense, globular fruitbodies of this fungus are common in S.E. Australia during May–August and are noted [6] and exploited [7] for their intense pigmentation. The prospect that the compound(s) responsible for this colour might play a role in the physiology and ecology of this important organism has led us to study the constituents of *P. tinctorius*. We describe here the isolation from fruitbodies of a predominant yellow metabolite to which we assign the novel naphthalenoid pulvinic acid structure 1†. This is the first reported occurrence of a pulvinic acid derivative in the Sclerodermataceae and is only the second report of naphthalenoid pulvinic acids in nature.

RESULTS AND DISCUSSION

Extraction of fresh sporophores of *P. tinctorius* with acetone afforded **1** on concentration as yellow crystals of the dipotassium salt. $C_{35}H_{16}O_{15}K_2 \cdot 4H_2O$, in a yield corresponding to *ca* 9% of the dry wt of the fungus. The pigment (**1**) itself, $C_{35}H_{18}O_{15}$, was obtained upon acidification of the potassium salt and crystallized as a red hexahydrate from aqueous acetone. The ^{1}H NMR spectrum of **1** shows, in addition to doublets ($J = 8.8$ Hz) centred at δ7.37, 7.32, 6.92 and 6.90 characteristic of two *para*-hydroxylated phenyl rings, an aromatic proton singlet at δ7.56 and a pair of *meta*-coupled aromatic proton doublets at δ9.08 and 8.92. In contrast to the simplicity of the proton spectrum, the ^{13}C NMR spectrum of **1** at 25 MHz is complex and reveals the presence of at least 34 carbon atoms in the molecule (*vide infra*).

The recognition of **1** as a pulvinic acid derivative followed acetylation to a yellow crystalline acetate, $C_{41}H_{20}O_{16}$, which exhibited characteristic [9] dilactone carbonyl absorption at 1830 and 1800 cm^{-1} in the IR spectrum which closely resembles that of the triacetate derivative (**4**) of xerocomic acid (**5**) [10]. The ^{1}H NMR spectrum of this lactone triacetate (**3**) (Table 1) distinguishes two near coincident OAc groups from the third and reveals significant deshielding of the isolated aromatic proton in **1** upon acetylation.

Neither **1** nor its potassium salt or acetate derivatives gave satisfactory mass spectra. However, methylation of **1** [8] gave the yellow heptamethylated derivative (**2**), $C_{42}H_{32}O_{15}$, which exhibited an abundant ion at m/z 777 ($[M+1]^+$) in the mass spectrum. In the ^{1}H NMR spectrum of **2** (Table 1) the protons of each of the seven methoxyl groups resonate as discrete singlets while the *meta*-coupled aromatic protons appear shielded relative to their counterparts in **1** [10].

All of the data discussed so far are consistent with a partial structure for **1** involving two *para*-hydroxylated pulvinic acid side-chains appended to a nucleus $[C_{11}H_3O_2]OH$. The presence of two such pulvinic acid residues finds support in the ^{13}C NMR spectrum which reveals signals at δ166.7 and 166.8, 103.7, 162.6 and 163.2, 154.0 and 154.4, 118.6, 173.3 and 174.2, 124.8, 132.6, 115.5 and 158.5 which are assigned to the carbons C-1′,1″ to C-10′,10″, consecutively, in **1** by comparison with the shifts of the corresponding carbons in the spectrum of atromentic acid (**6**) [8].

Further refinement of the structure of **1** became possible when methylation of the pigment under reflux gave the nonamethylated derivative (**7**). A molecular

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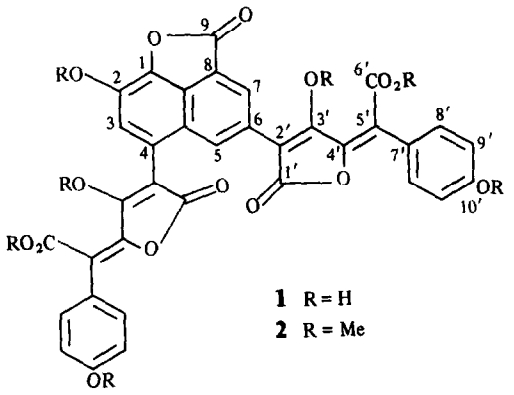
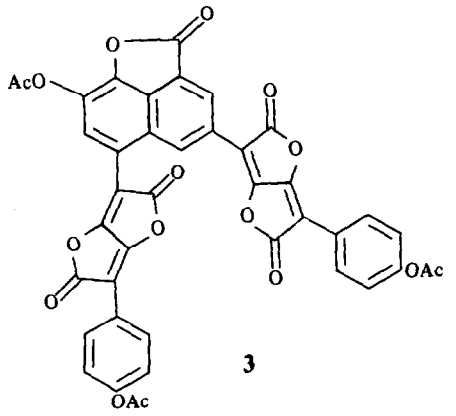
†During the course of this work we learned (Steglich, W., Plenary Lecture, Royal Australian Chemical Institute, Division of Organic Chemistry, 8th National Conference, University of Western Australia, May, 1984) of the isolation of a minor constituent of the capskin of *Xerocomus badius* which has been assigned structure **1** on the basis of ^{13}C NMR data [8]. We are grateful to Professor Steglich for helpful discussions and for access to ref. [8] prior to publication.



1 R = H
2 R = Me



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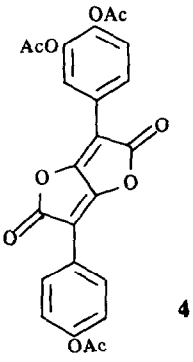

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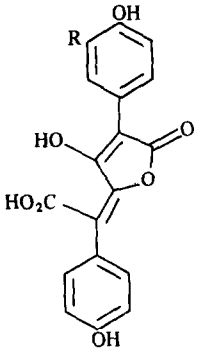
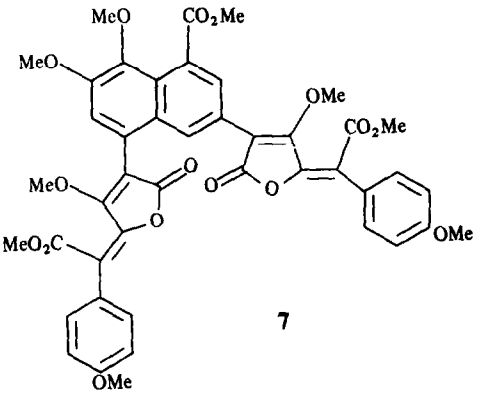


5 R = OH
6 R = H



7

formula $C_{44}H_{38}O_{16}$ for **7** was indicated by an abundant molecular ion in the MS at *m/z* 822 and was supported by the ^{1}H NMR spectrum (Table 1) which shows nine discrete methoxyl proton signals. The difference C_2H_6O in the molecular composition of the methyl derivatives **2** and **7** indicates the presence of a third lactone moiety in the central nucleus in **1**. The composition of the nucleus is thus resolved to $C_{10}H_4O$ and since this must accommodate one hydroxyl group and three aromatic protons the identity of the carbon skeleton as a naphthalene nucleus (C_{10}) becomes apparent.

Disposition of substituents in the naphthalene ring of **1** follows two considerations, firstly, that the lactone must bridge the *peri* positions and, secondly, that a (possible) biogenetic link between **1** and xerocomic acid (**5**) would place two hydroxyl groups and one pulvinic acid residue in the mutual 1,2,4- arrangement shown. Placement of the second pulvinic acid chain at C-6 then follows consideration of the ^{1}H NMR spectrum of **1**.

Structure **1** is supported by the ^{13}C NMR spectrum of the pigment at 25 MHz. Thus, in the fully proton-coupled spectrum both C-9 (δ167.2) and C-1 (δ123.8) appear as doublets due to 3-bond coupling to H-7 (J = 3 Hz) and H-3 (J = 9 Hz), respectively. C-2 is the most deshielded

Table 1. ^{1}H NMR data for **1** and its derivatives **2**, **3** and **7** [^{1}H chemical shift, δ_H (coupling constant, J Hz)]

Compound	H-3 (*s*)	H-5 (*d*)	H-7 (*d*)	H-8′8″ (*d*)	H-9′9″ (*d*)	Other protons
1*	7.56	9.08 (1)	8.92 (1)	7.37 (8.8) 7.32 (8.8)	6.92 (8.8) 6.90 (8.8)	
3	8.09	9.26 (1)	8.93 (1)	8.16 (9.0) 8.11 (8.8)	7.30 (9.0) 7.27 (8.8)	2.47, 2.36, and 2.35 (each *s*, 3H, OAc)
2	7.35	8.36 (1)	8.16 (1)	7.70 (9.0) 7.67 (9.0)	6.95 (9.0) 6.94 (9.0)	4.30, 3.92, 3.91, 3.85, 3.84, 3.81 and 3.74 (each *s*, 3H, OMe)
7	7.40	7.89 (1.5)	7.67 (1.5)	7.70 (9.0) 7.65 (9.0)	6.96 (9.0) 6.93 (9.0)	4.02, 3.98, 3.94, 3.91, 3.88, 3.86, 3.84, 3.78 and 3.62 (each *s*, 3H, OMe)

*Solvent Me_2CO-d_6 + D_2O.

carbon of the naphthalene ring (δ138.1) and exhibits coupling through only two bonds (J = 3 Hz) to H-3. Significantly, the alternative substitution pattern involving a pulvinic acid residue at C-2 and a hydroxyl group at C-4 should show the hydroxylated carbon coupled both through 2 and through 3 bonds [8]. Of the other naphthalene ring carbons only signals due to C-3 (δ126.6, *d*, J = 162 Hz), C-5 (δ131.9, *dd*, J = 166, 7 Hz) and C-7 (δ126.6, *dd*, J = 166, 7 Hz) are unequivocally assigned at the low field strength employed.

Final confirmation of the identity of **1** was obtained by direct chromatographic and spectroscopic comparison* with norbadion-A, isolated from the capskin of *Xerocomus badius* and assigned structure **1** after extensive analysis of the high field ^{13}C NMR spectra both of norbadion-A itself and of the closely related metabolite badion-A [8].

The pulvinic acid derivative **1** comprises *ca* 20% of the dry wt of *P. tinctorius* fruitbodies where it is present almost entirely as the potassium salt. Spectroscopic examination of the total acetone extractives from *P. tinctorius* reveals **1** in an almost pure state, signals due to lipids, etc., constituting only a minor component of the ^{1}H NMR spectrum. With such high concentrations present in the fruitbodies and possibly in the mycelium and mycorrhizae of *P. tinctorius* [Marx, D., personal communication] it is tempting to implicate this metabolite in the physiology and possibly in the ecology of the fungus.

Naphthalenoid pulvinic acids, of which this is but the second report, constitute the most recent addition to the group of biosynthetically related fungal and lichen secondary metabolites which includes the terphenyl quinones, pulvinones, grevillins and the pulvinic acids themselves [11]. The occurrence of hydroxylated pulvinic acids in higher fungi is restricted to the order Boletales [12] and close allies, and thus the isolation of norbadion-A (**1**) from *P. tinctorius* and its presence in the yellow gasteromycete *Scleroderma flavidum* [Gill, M. and Lally, D. A., unpublished] provides strong chemotaxonomic evidence for a close relationship between the Sclerodermataceae and the Boletales.

*This comparison was kindly performed by Professor W. Steglich, University of Bonn.

†Typical fruitbodies of *P. tinctorius* on freeze-drying lose, on average, 72% of their fr. wt.

‡Tentative assignment.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded at 99.55 MHz (^{1}H) and 25.00 MHz (^{13}C) in $CDCl_3$ (unless stated otherwise) with TMS as int. standard. Mps are uncorr. Voucher specimens of *Pisolithus tinctorius* (Pers.) Coker et Couch are deposited in the herbariums of the Royal Botanic Gardens, Edinburgh, and the New South Wales Department of Agriculture Biological and Chemical Research Institute, Rydalmere, N.S.W., under collection numbers WAT 16916 and DAR 49163, respectively. Combustion analyses were performed by the Australian Microanalytical Service, Melbourne.

Extraction and isolation of **1**. Whole fresh sporophores (280 g) of *Pisolithus tinctorius* collected at Eltham, Victoria, Australia, were chopped and immersed overnight in Me_2CO (800 ml) at room temp. After filtration and re-extraction (2 × 800 ml) the combined extracts were evaporated to low vol. under red. pres. The yellow crystalline solid (6.7 g: 2.4% fr. wt 8.5% dry wt† of fungus) was filtered off and recryst. from MeOH to give the *dipotassium salt of norbadion-A*, mp > 300° (Found: C, 50.65; H, 3.05; K, 8.7. $C_{35}H_{16}O_{15}K_2 \cdot 4H_2O$ requires C, 50.85; H, 2.95; K, 9.5%). To a suspension of this K salt (100 mg) in Me_2CO (5 ml) was added dropwise aq. HCl (2 M) and the suspension was heated on the steam bath until the pigment had dissolved. KCl was filtered off and the filtrate was further diluted with aq. HCl to afford *norbadion-A* (**1**; 85 mg) as clusters of red needles, mp > 300° (Found: C, 53.25; H, 3.9. $C_{35}H_{18}O_{15} \cdot 6H_2O$ requires C, 53.45; H, 3.85%). IR ν_{max}^{KBr} cm^{-1}: 3700–2100 (OH), 1770 and 1750 (C=O), 1600 br, 1510, 1260 br, 1050 and 960. UV λ_{max}^{EtOH} (log ε): 268 (4.87), 368 (4.39), and 416 sh (4.26); + 2 M NaOH: 252 (4.62), 379 (4.67), and 495 (4.02). ^{13}C NMR (Me_2CO-d_6): δ103.7 (*m*, C-2′,2″), 115.5 (*dd*, J = 160 and 3 Hz, C-9′,9″), 118.6 (*m*, C-5′,5″), 120.5 (*s*, C-8‡), 123.8 (*d*, J = 9 Hz, C-1), 124.3 (*m*, C-4‡), 124.8 (*t*, J = 8 Hz, C-7′,7″), 126.6 (*dd*, J = 166 and 7 Hz, C-7), 126.6 (*d*, J = 162 Hz, C-3), 129.3 (*s*, C-6‡), 131.0 (*m*, C-4a‡), 131.9 (*dd*, J = 166 and 7 Hz, C-5). 132.6 (*dd*, J = 160 and 8 Hz, C-8′,8″), 133.6 (*m*, C-8a‡), 138.1 (*d*, J = 3 Hz, C-2), 154.0 and 154.4 (each *s*, C-4′,4″), 158.5 (*br t*, J = 8 Hz, C-10′,10″), 162.6 and 163.2 (each *s*, C-3′,3″), 166.7 and 166.8 (each *s*, C-1′,1″), 167.2 (*d*, J = 3 Hz, C-9), 173.3 and 174.2 (each *s*, C-6′,6″).

Acetylation of **1**. Compound **1** (200 mg) in Ac_2O (2 ml) containing conc H_2SO_4 (1 drop) was heated on the steam bath overnight. The mixture was cooled and filtered to give the *lactone triacetate* (**3**) (175 mg) as lemon yellow tablets, mp 283–286° (Found: C, 63.9; H, 2.85. $C_{41}H_{20}O_{16}$ requires C, 64.05; H, 2.6%). IR ν_{max}^{KBr} cm^{-1}: 1830, 1800 sh and 1770 (C=O), 1660, 1605, 1505, 1370, 1350, 1200, 1170 and 1040.

Heptamethyl derivative (**2**). A suspension of **1** (50 mg) and

K_2CO_3 (excess) in dry Me_2CO (3 ml) containing Me_2SO_4 (12 drops) was stirred at room temp. overnight. The mixture was diluted with H_2O (10 ml) and extracted with $CHCl_3$ (2 × 5 ml). Evaporation of the washed (H_2O) and dried ($MgSO_4$) organic phase followed by addition of EtOH to the oily residue gave **2** (35 mg) as yellow needles (from MeOH–$CHCl_3$), mp 248–250° (lit. mp 247–249° [8]). IR ν_{max}^{KBr} cm^{-1}: 3000, 2960 and 2840 (C–H), 1780, 1770 sh and 1730 (C=O), 1640, 1600 and 1515. EIMS (probe) 10 eV, *m/z* (rel. int.): 777 $[M+1]^+$ (76), 731 (11), 730 (18), 149 (11), 126 (10), 96 (100), 66 (24), 32 (86), 31 (69).

Nonamethyl derivative (**7**). A suspension of **1** (50 mg) and K_2CO_3 (excess) in dry Me_2CO (3 ml) containing Me_2SO_4 (20 drops) was heated under reflux for 24 hr. After cooling to room temp. the mixture was worked up as described above for **2** to yield the nonamethyl derivative (**7**) (25 mg) as bright lemon–yellow microcrystals, mp 147–148° (Found: M^+, *m/z* 822. $C_{44}H_{38}O_{16}$ requires M^+, *m/z* 822). IR ν_{max}^{KBr} cm^{-1}: 3000, 2950 and 2840 (C–H), 1775 sh, 1765, 1735 and 1730 (C=O), 1630, 1600 and 1510. EIMS (probe) 70 eV, *m/z* (rel. int.): 822 $[M]^+$ (23), 69 (22), 64 (24), 50 (18), 44 (100), 32 (15), 31 (21), 29 (21), 28 (39).

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