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# A NEW TRITERPENIC ACID FROM THE WOOD ROTTING FUNGI

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**Key Word Index**—Basidiomycetes; Polyporaceae; white wood rot fungi; triterpene acid; polyporenic acid D; 3α-hydroxy-4,4,14α-trimethyl-5α-ergosta-8,24(28)-dien-26-oic acid.

**Abstract**—A new triterpenic acid, assigned the trivial name polyporenic acid D, has been isolated from the wood rotting fungus *Polyporus officinalis*. It has been shown to have the structure 3α-hydroxy-4,4,14α-trimethyl-5α-ergosta-8,24(28)-dien-26-oic acid.

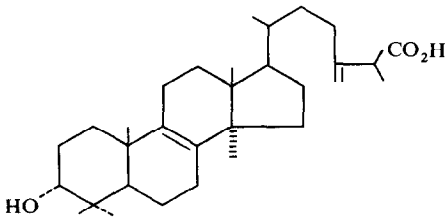
## INTRODUCTION

Wood rotting fungi belonging to the Polyporaceae (Basidiomycetes) are classified into two types; white-rot fungi (lignin decomposers) and brown-rot fungi (cellulose decomposers). These fungi are a rich source of lanostane-type tetracyclic triterpenes and more than 30 compounds, especially $C_{30}$ or $C_{31}$ carboxylic acids, have been isolated so far. The triterpenic acids isolated so far belong to the trametenolic, eburicoic and polyporenic acid series [1–3]. A new acid isolated in this study in large amounts from the white-rot fungus *Polyporus officinalis* belongs to the polyporenic acid series but does not contain the C-12 hydroxyl and hence is designated polyporenic acid D. This acid forms a missing link in the triterpenic acids of the lanostane group.

## RESULTS AND DISCUSSION

On extraction with *n*-hexane *Polyporus officinalis* gave a white amorphous solid, mp 268–270°, (polyporenic acid D). It gave $M^{+\cdot}$ at *m/z* 470 and analysed for $C_{31}H_{50}O_3$. The presence of a $C_9H_{15}O_2$ side chain was clearly indicated by the loss of a unit of mass 155 from the parent peak in the mass spectrum, giving a fragment at *m/z* 315. The compound was therefore a tetracyclic triterpene with a $C_9$ side-chain containing the carboxyl group and one double bond (as a methylene group as indicated by the $^1H$ NMR spectrum). The carboxyl group was at either C-20 or C-25. The possibility of the carboxyl group being at C-20 was ruled out because the 100 MHz $^1H$ NMR spectrum showed a downfield doublet centred at $\delta$ 1.23 ($J$ = 2 Hz). This is only possible if C-25 is carrying a carboxylic function, the C-27 methyl group appeared therefore as a doublet at a lower field. Moreover, a compound with a carboxyl group at C-20 is eburicoic acid, $C_{31}H_{50}O_3$, mp 292° [4, 5]. Polyporenic acid D was different from eburicoic acid in its physical and spectral data. The presence of the carboxylic group in polyporenic acid D was further confirmed by the formation of a monohydroxy monomethyl ester, mp 125°, $M^{+\cdot}$ at *m/z* 484, which analysed for $C_{32}H_{52}O_3$. The presence of a carboxyl group attached to C-25 was further confirmed by various chemical methods. Polyporenic acid D did not undergo cyclization with polyphosphoric acid; if the carboxylic group had been at C-20, it would have cyclized with the C-24 methylene to give a $\delta$-lactone. Oxymercuration–demercuration of the $\Delta^{24(28)}$ double bond was also attempted to produce such a $\delta$-lactone but it also did not succeed. Therefore, the carboxylic group in polyporenic acid D is placed at C-25 and not at C-20. That the new acid belongs to the lanostane series was further confirmed by comparing the $^1H$ NMR signals of the five nuclear methyls of polyporenic acid D acetate with those of the lanostanes reported in the literature [6, 7]. The five methyls appeared at 0.77 (H-18), 0.98 (H-19) 0.87 (H-30 and H-31), 1.04 (H-32) and 0.96 (*d*, $J$ = 6 Hz, H-21). Moreover, the presence of the fragments in the mass spectrum at *m/z* 301 and 241 confirmed the presence of the 14α-methyl group thus establishing the lanostane nucleus for the new acid [8].

The presence of a hydroxyl group was indicated by the IR spectrum and as usual this group should be at C-3. The $^1H$ NMR spectrum showed a triplet at 3.27 assigned to H-3 which further moved down to 4.48 on acetylation. A strong band in the IR at 898 $cm^{-1}$ indicated the presence of a tetrasubstituted double bond which could be placed between C-8 and C-9 because this double bond was inert to hydrogenation, even under vigorous conditions. The presence of the tetrasubstituted double bond between C-8 and C-9 was also confirmed by the $^{13}C$ NMR spectrum where the two carbon resonances appeared at 137.04 and 136.86. The $^{13}C$ NMR spectrum of its acetate agreed with the proposed polyporenic acid D acetate structure where the signals for carboxylic carbon appeared at 185.61, and the acetyl carbon at 173.52. The tetrasubstituted C-24 appeared at 157.54 and C-28 at 109.66. The carbon resonances of C-24 and C-28 were shifted on hydrogenation while those of C-8 and C-9 were not affected. On the basis of the above data, polyporenic acid D has been assigned the structure 3α-hydroxy-4,4,14α-trimethyl-5α-ergosta-8,24(28)-dien-26-oic acid (**1**) where the carboxylic group is placed at C-25. The mass fragmentation pattern also supported the proposed structure where the fragments appeared at *m/z* 470 ($M^{+\cdot}$), 452 ($M^{+\cdot} - H_2O$), 408 ($M^{+\cdot} - H_2O - CO_2$), 355 ($M^{+\cdot} - C_6H_9O_2$), 315 ($M^{+\cdot} - SC$), 273 ($M^{+\cdot}$ – cleavage




1 Polyporenic acid D

across ring D with loss of one hydrogen) and 219 ($M^{+\cdot}$ − cleavage across ring C with loss of one hydrogen).

## EXPERIMENTAL

$^1H$ NMR spectra were recorded at 60 and 100 MHz. The $^{13}C$ NMR spectrum was obtained on a 25.2 MHz Varian XL-100 machine using TMS as an internal standard and $CDCl_3$ as the solvent, taking 3000 scans. Assignments on $^{13}C$ NMR were made by noise-decoupled and off-resonance decoupled spectra.

The *Polyporus officinalis* used for this work was available in the Indian market under the local name 'Garikhoon' and it is being used in the Ayurvedic medicine system. The species was purchased from the market and identified by the mycologists of this laboratory.

*Isolation.* Soxhlet extraction of the powdered fungus with *n*-hexane gave an extract which deposited a white crystalline material on chilling. Recrystallization from MeOH gave a white crystalline solid, mp 268–270°, $[\alpha]_D$ ($C_5H_5N$) + 40°. $M^+$ at *m/z* 470. Analysis $C_{31}H_{50}O_3$. IR $\nu_{max}$ $cm^{-1}$ 3525 (OH), 2700–2500, 1710 and 1698 (C=O), 1640 (>C=CH$_2$) and 898 (>C=C<). $^1H$ NMR (60 MHz, $C_5D_5N$): $\delta$ 0.90–1.02 (6 methyls), 1.23 (*d*, $J$ = 2 Hz, H-27), 4.81 (2 H, *d*, H-28) and 3.27 (1 H, *t*, H-3). MS *m/z*: 470, 468, 455, 437, 409, 354, 279, 273, 247, 234, 220, 219, 211, 210, 203, 201, 202, 186, 185, 144, 116 and 115.

*Acetate.* Polyporenic acid D (25 mg), 1 ml $Ac_2O$ and 1 ml pyridine at room temp. were kept for 12 hr and work-up gave a white solid, mp 235°, $M^+$ at *m/z* 512, $[\alpha]_D$ +23.2° (MeOH). Analysis $C_{33}H_{52}O_4$. $^1H$ NMR: $\delta$ 2.0 (3 H, *s*, acetate), 4.48 (1 H, *m*, 3-H).

*Methyl ester.* On treatment with $CH_2N_2$, polyporenic acid D gave the methyl ester, mp 125°, $M^+$ at *m/z* 484. Analysis $C_{32}H_{52}O_3$. $^1H$ NMR: $\delta$ 3.64 (3 H, *s*, $-CO_2Me$), 2.85 (1 H, *m*, 25-H).

*Acetate methyl ester.* The acetate of polypoporenic acid D was treated with $CH_2N_2$ to give the acetate methyl ester, mp 137–139°, $M^+$ at *m/z* 526. Analysis $C_{34}H_{54}O_4$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.10 (3 H, *s*, acetate), 3.75 (3 H, *s*, $-CO_2Me$).

*Hydrogenation.* The acid (25 mg) in MeOH (50 ml) over Pd/C (10 mg) absorbed only 1 mol of $H_2$ and work-up gave a white solid, crystallized from MeOH, mp 245°, $M^+$ at *m/z* 472. Analysis $C_{31}H_{52}O_3$. $^1H$ NMR: no signal at $\delta$ 4.81; the position of the other signals remained the same.

*Attempted cyclization.* The acid (20 mg) in polyphosphoric acid (5 ml) was heated on the water bath for 12 hr. The mixture was poured over ice and the white solid was filtered out. This was identified as unchanged polyporenic acid D.

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