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# Pneumocysterol [(24*Z*)-ethylidenelanost-8-en-3$\beta$-ol], a rare sterol detected in the opportunistic pathogen *Pneumocystis carinii hominis*: Structural identity and chemical synthesis

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**ABSTRACT** ***Pneumocystis carinii* pneumonia (PcP) remains among the most prevalent opportunistic infections among AIDS patients. Currently, drugs used clinically for deep mycosis act by binding ergosterol or disrupting its biosynthesis. Although classified as a fungus, *P. carinii* lacks ergosterol. Instead, the pathogen synthesizes a number of distinct $\Delta^7$, 24-alkylsterols, despite the abundance of cholesterol, which it can scavenge from the lung alveolus. Thus, the pathogen-specific sterols appear vital for organism survival and proliferation. In the present study, high concentrations of a $C_{32}$ sterol were found in human-derived *P. carinii hominis*. The definitive structural identities of two C-24 alkylated lanosterol compounds, previously not reported for rat-derived *P. carinii carinii*, were determined by using GLC, MS, and NMR spectroscopy together with the chemical syntheses of authentic standards. The $C_{31}$ and $C_{32}$ sterols were identified as euphorbol (24-methylenelanost-8-en-3$\beta$-ol) and pneumocysterol [(24*Z*)-ethylidenelanost-8-en-3$\beta$-ol], respectively. The identification of these and other 24-alkylsterols in *P. carinii hominis* suggests that (*i*) sterol C-24 methyltransferase activities are extraordinarily high in this organism, (*ii*) 24-alkylsterols are important components of the pathogen's membranes, because the addition of these side groups onto the sterol side chain requires substantial ATP equivalents, and (*iii*) the inefficacy of azole drugs against *P. carinii* can be explained by the ability of this organism to form 24-alkysterols before demethylation of the lanosterol nucleus. Because mammals cannot form 24-alkylsterols, their biosyntheses in *P. carinii* are attractive targets for the development of chemotherapeutic strategies against this opportunistic infection.**

Sterols and their biosyntheses are excellent targets for chemotherapeutic attack against infectious microbes, especially the fungi. Polyene antibiotics such as amphotericin B bind avidly to ergosterol in fungal cell membranes. After the sterol–drug complexes aggregate, large pores in the membranes are formed and other cell functions. If host sterols do not fulfill the precise stereochemical requirements of the parasite sterol, the pathogen synthesizes at least low levels of its own sterol for these vital functions. The parasite-specific sterols have been described as "metabolic" sterols, and represent attractive targets for drug development (5). Beside representing putative metabolic sterols, the rare occurrence of these molecules make these good markers—or signature lipids—of microorganisms. Improved diagnostic procedures for *P. carinii* pneumonia (PcP) could be developed based on the detection of *P. carinii*-specific sterols.

In the present study, two sterols that have not been reported for *P. carinii carinii* were detected in a *P. carinii hominis*-infected lungs, in human bronchoalveolar lavage fluid (BALF), and in organisms isolated from human lungs with PcP. The structural identities of $C_{31}$ euphorbol and a rare $C_{32}$ sterol, for which the trivial name pneumocysterol was proposed (6), are herein described.

## MATERIALS AND METHODS

**Biological Specimens.** A whole formalin-fixed human lung from an AIDS patient who did not receive treatment for, and died of, PcP was generously provided by Miercio Perreira, (Tufts New England Medical Center, Boston, MA). Pieces ($\approx$100 g) were removed and homogenized with distilled water in a 125-ml stainless steel blender cup (Baxter Scientific Products, McGaw Park, IL). Alternatively, samples were homogenized with organic solvents for simultaneous extraction of lipids. Most structural analyses were performed on pneumocysterol purified from this large specimen. Formalin-fixed autopsied lung specimens from individuals with no histological evidence of *P. carinii* infection served as controls.

To examine whether formalin fixation destroyed or altered the *P. carinii*-specific sterols, PcP-containing rat lungs were fixed in 2% formalin and left at room temperature for 1–18 weeks. The sterols of these infected rat lungs were compared with those of