NOTES

Sch 420789: A Novel Fungal Metabolite with Phospholipase D Inhibitory Activity

M. S. PUAR*, E. BARRABEE, M. HALLADE and M. PATEL

Schering-Plough Research Institute,
2015 Galloping Hill Road,
Kenilworth, New Jersey 07033-0539 USA

(Received for publication March 8, 2000)

In the course of screening for novel natural products, a fermentation culture broth (SCF0953) of an unidentified fungus showed strong activity in the phospholipase D (PLD) inhibitor assay. Phospholipase D is a lipolytic enzyme involved in the hydrolysis of phosphate bond of the phospholipid substrate generating phosphatidic acid. PLD activation is implicated in a wide range of biological activities such as inflammation, antitumor, and antimicrobial[1]. The culture sample was described as a fungal with sterile, dematiaceous mycelium with low, dry, small blastos[2]. The fermentation broth was extracted with EtOAc at harvest pH (6.5). The combined extract was purified on column chromatography followed by reverse phase HPLC. The purified solid was a white powder. We

Fig. 1. The structure of **1** with relative stereochemistry.

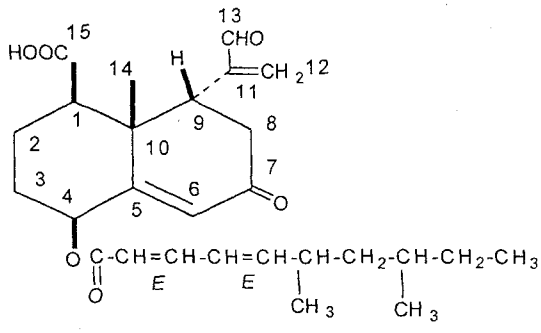


Fig. 2. Partial structures of **1**.

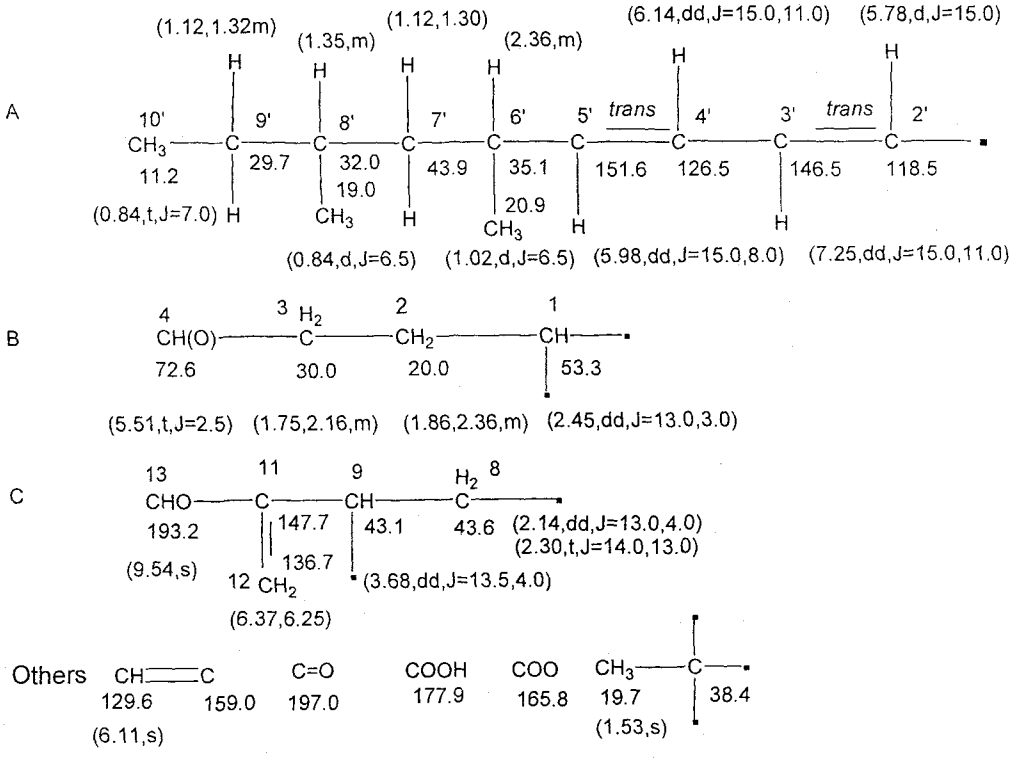
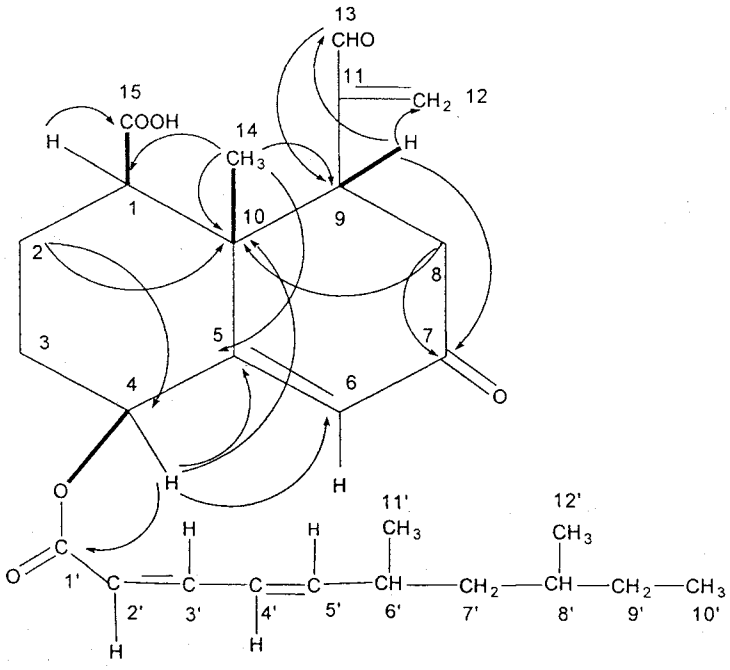


Fig. 3. Selective INEPT analysis.



report herein the structure determination of this novel PLD active compound, Sch 420789 (**1**).

Compound **1** has the molecular formula $C_{27}H_{36}O_6$ [*m/z* 457.2 $(M+H)^+$] by MS, ^1H and ^{13}C NMR data for a total of ten degrees of unsaturation and UV (MeOH) λ_{max} 266 nm. The ^1H (400 MHz, $CDCl_3$, values in parenthesis) and ^{13}C (75.5 and 100 MHz, $CDCl_3$) NMR spectra and their extensive analysis using APT, DEPT, ^1H-^1H and ^1H-^{13}C COSY led to the assignment of following partial structures shown in Fig. 2.

Extensive selective INEPT[3] studies allowed the construction of the plain structure shown in Fig. 3. The mass spectrum (SIMS) indicated major ions at *m/z* 479.2 $(M+Na)^+$, 457.2 $(M+H)^+$, 261.1 (−196; loss of fatty acid, $C_{12}H_{20}O_2$), 179 (−196 and −82; $C_{12}H_{20}O_2$ and C_5H_6O) consistent with assigned structure.

The relative stereochemistry of **1** was determined on the basis of ^1H-^1H coupling constants and NOESY experiments. Thus, the 2′-*trans*, 4′-*trans* conjugated dienoate system of the fatty acid side chain was established on the basis of large coupling constants of $J_{2',3'}$=15.0 Hz, $J_{3',4'}$=11.0 Hz, and $J_{4',5'}$=15.0 Hz. In the NOESY spectrum, the presence of a cross peak between H_{14} and H_9 suggested that the methyl and the methine protons are *cis* to each other. H_9 was assigned β-axial because of its large J values with H_8 (J=14.0, 4.0 Hz). The lack of cross peak between H_{14} and H_1 suggested *trans* relationship. The H_{14} protons also showed cross peaks to β-axial protons of H_2 through 1,3 interactions. The protons H_4 and H_6 showed allylic connectivity *via* W-coupling in the COSY spectrum and a cross peak observed in the NOESY spectrum between H_4 and H_6 provided justification for the hydroxy group being β-axial at C_4. On the basis of above data the structure of **1** was assigned.

Compound **1** showed *in vitro* inhibitory activity in the PMA- and *f*MLP-stimulated phospholipase (PLD) assay[4]. The IC_{50} values of **1** were ~20 and ~8 μM, respectively. Compound **1** lacked selectivity.

References

1) BILLAH, M. M.: Phospholipase D and cell signalling. Curr. Opin. Immunol. 5: 114~123, 1993
2) The microorganism was supplied by Dr. B. KATZ of MYCOsearch Lab (MYCO 0929)
3) For a recent application of this technique towards structure elucidation see; PETTIT, G. R., SINGH, S. B., GOSWAMI, A., NIEMAN, R. A. Tetrahedron 44: 3349, 1988
4) PAI, J.-K.; E. A. FRANK, C. BLOOD & M. CHU: Novel ketoepoxides block phospholipase D activation and Tumor cell invasion. Anti-Cancer Drug Design 9: 363~372, 1994