TERFEROL, AN INHIBITOR OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE PHOSPHODIESTERASE

II. STRUCTURAL ELUCIDATION

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(Received for publication September 10, 1983)

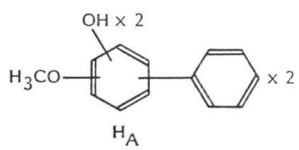
Streptomyces showdoensis SANK 65080 produced terferol, an inhibitor of cyclic adenosine 3′,5′-monophosphate phosphodiesterase (cAMP-PDE). NMR spectrometry and X-ray analysis were used to determine the structure of the compound, a new member of the terphenyl family.

As reported in the previous paper[1], a new inhibitor of cAMP-PDE, terferol was isolated from the cultured broth of *Streptomyces showdoensis* SANK 65080. The inhibition of cAMP-PDE by terferol was non-competitive in regard to cAMP with a *Ki* value of 0.22 μM.

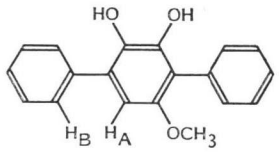
The present paper deals with the structural elucidation of terferol.

Terferol (**1**), mp 184°C, $C_{16}H_{16}O_3$, *m/z* 292, showed characteristic absorption at 264, 315 (sh) nm in methanol or acidic methanol and 232, 273 and 325 (sh) nm in basic methanol. In the NMR spectrum in $(CD_3)_2CO$, methoxy at δ 3.6 and eleven benzene ring protons at δ 6.45 and δ 7.7 suggested the presence of a phenolic compound. Acylation of **1** with acetic anhydride in pyridine (room temperature, overnight) gave diacetate (**2**), $C_{23}H_{20}O_5$, *m/z* 376. The IR spectrum of **2** indicated an acetyl ester bond at 1770 cm^{-1} and disappearance of two hydroxyl groups. Reaction of **1** with excess diazomethane (overnight) gave a methoxy compound (**3**), $C_{21}H_{20}O_3$, *m/z* 320. The NMR spectrum of **3** showed signals for three methoxy groups at δ 3.54, 3.57 and 3.70. From the above results together with the unsaturation number (12), the structure of **1** was assumed to be a terphenyl derivative, having two phenolic hydroxyl groups and one methoxy group. The NMR spectrum of **1** in pyridine-d_5 showed one ring proton (H_A) as a singlet at δ 6.77, which agrees with the presence of a penta-substituted benzene ring as shown in the following partial structure.

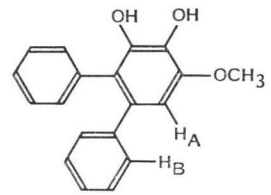
In addition, the NOE between H_A and methoxy increased 25% upon irradiation of methoxy. Therefore H_A and methoxy are *ortho* substituted on the benzene ring. Reaction of **1** with 2,2-dimethoxypropane in absolute benzene in the presence of *p*-TsOH (reflux, 1 hour) yielded an acetonide **4**, $C_{22}H_{20}O_3$, *m/z* 332, whose NMR spectrum showed the presence of two methyl groups at δ 1.63. Therefore, two phenolic hydroxyl groups were *ortho* substituted. From the data described



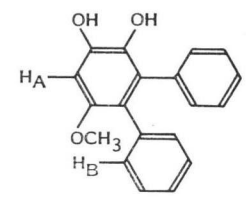
a



1a



1b



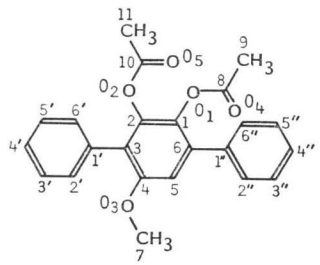
1c

above, the structure of **1** was deduced to be either **1a**, **1b** or **1c**.

The structure **1c** could be eliminated, because ring proton (H_A) at δ 6.73 showed a NOE (increased by 6%) with H_B at δ 7.98, which was *ortho* and *meta* coupling (d-t J_1=6.5 Hz, J_2=1.5 Hz). The structure of **1** was finally determined by X-ray analysis of the diacetate **2** as shown in Fig. 1.

Crystals of diacetylterferol (**2**), grown in a mixture of benzene and ethyl acetate, were white transparent plates. The single crystals of **2** are monoclinic, space group Cc, with a=21.022(4), b=11.656(3), c=8.238(3) Å, β=90.82(6)°, Z=4. Intensity data were measured on a Rigaku four-circle diffractometer (graphitemonochromated Cu-Kα radiation). The structure was solved by direct methods with MULTAN[2], and refined by block-diagonal least-squares methods. The positions of all hydrogen atoms were located from a difference Fourier synthesis. The final discrepancy index R is 0.059 for 1435 observed reflections (Fo$\geq 2\sigma$Fo). Fig. 2. is a stereoscopic drawing of molecule (**2**).

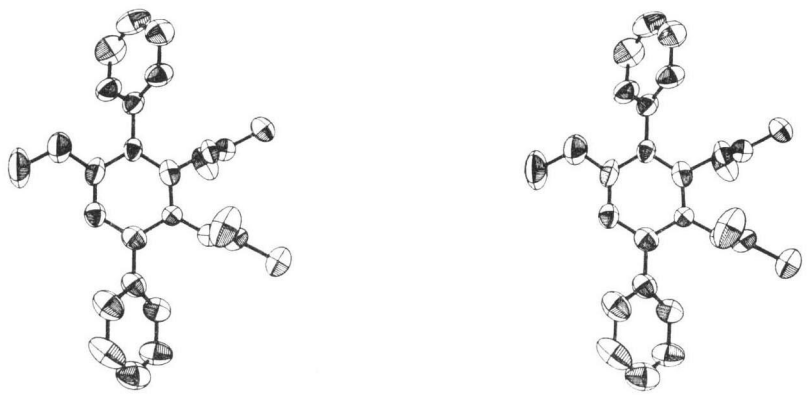
Fig. 1.



2

Recently, TAKAHASHI *et al.* reported a fungal toxic metabolite, terphenyllin, from *Aspergillus candidus*[3]. The concentration required for 50% inhibition (I_{50}) of cAMP-PDE, obtained from rat brain, was found to be 3.1 μg/ml in our experiment.

Fig. 2.



References

1) NAKAGAWA, F.; R. ENOKITA, A. NAITO, Y. IIJIMA & M. YAMAZAKI: Terferol, an inhibitor of cyclic adeno-

sine 3′,5′-monophosphate phosphodiesterase. I. Isolation and characterization. J. Antibiotics 37: 10~12, 1984

2) GERMAIN, G.; P. MAIN & H. W. WOOLFSON: The application of phase relationships to complex structures. III. The optimum use of phase relationships. Acta Cryst. A27: 368~376, 1971

3) TAKAHASHI, C.; K. YOSHIHIRA, S. NATORI & M. UMEDA: The structures of toxic metabolites of *Aspergillus candidus*. I. The compounds A and E, cytotoxic *p*-terphenyls. Chem. Pharm. Bull. 24: 613~620, 1976