Communications to the editor

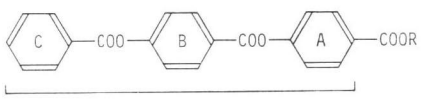
THE STRUCTURES OF THIELAVINS A, B AND C. PROSTAGLANDIN SYNTHETASE INHIBITORS FROM FUNGI

Sir:

As reported in the previous paper,[1] thielavins A (**1**) and B (**2**) produced by *Thielavia terricola* SANK 10475 are potent inhibitors of prostaglandin (PG) synthetase. Thielavins A and B were shown to have *O*-substituted salicylic acid as evidenced by UV absorption at 310~315 nm in basic MeOH and 275~278 nm in acidic MeOH and by IR absorption at 1760, 1610 and 795 cm^{-1}. Thielavins A and B possessed molecular compositions of $C_{29}H_{30}O_{10}$ and $C_{31}H_{34}O_{10}$, respectively, and gave the same ester (**3**), mp 208~210°C, $C_{33}H_{38}O_{10}$ (*m/z* 594), by reaction with diazomethane. The ester (**3**) gave a monoacetate, ($C_{35}H_{40}O_{11}$, *m/z* 636) by acetylation with acetic anhydride in pyridine. The structures of thielavins A and B were thus concluded to have the same tridepside skelton with a difference in substitution at the same position, *i.e.* hydroxyl or methoxyl group in A or B, respectively. Here we report further structure elucidation of thielavins A and B as well as thielavin C, a recently isolated minor component of the same group also produced by the said microorganism.

Hydrolysis of **3** with $NaOCH_3$ in MeOH gave phenolic compound (**4**), mp 91~92°C, $C_{11}H_{14}O_4$, *m/z* 210, and didepside (**5**), mp 168~169°C, $C_{23}H_{28}O_7$, *m/z* 416. The ^1H NMR spectrum of **4** showed two methyl groups at 2.08 and 2.51, methoxyl at 3.85, ester methyl at 3.94, aromatic proton at 6.29 and phenolic hydroxyl at 11.8 ppm. Also, in the ^{13}C NMR spectrum the aromatic carbons in **4** indicated signals at 162.2(s), 161.4(s), 140.1(s), 110.9(s), 105.8(d), and 105.5(s) ppm. It is well known that the signals of carbons with

Fig. 1.



1: $-CH_3\times8$, $-OH\times4$, $-H\times1$, R=H
2: $-CH_3\times8$, $-OH\times2$, $-OCH_3\times2$, $-H\times1$, R=H
3: $-CH_3\times8$, $-OH\times1$, $-OCH_3\times3$, $-H\times1$, $R=CH_3$
7: $-CH_3\times8$, $-OH\times4$, $-H\times1$, $R=CH_3$
8: $-CH_3\times9$, $-OH\times2$, $-OCH_3\times2$, R=H

phenolic OH at *ortho* and *para* positions shifted to upfield, 12.8 ppm and 7.1 ppm, respectively, and that of *meta* shifted to downfield, 1.6 ppm.[2] Two carbons at 105.8 and 105.5 ppm in the high field were assigned *ortho* and/or *para* substituted phenol. From the above results and in consideration of biogenetic pathway, the structure of **4** was assumed to be 2,4-dihydroxy-3,6-dimethylbenzoic acid derivative, which was also verified in comparison with the hydrolysis product of diffractic acid in alkali solution. Hydrolysis of thielavin A methyl ester (**7**), obtained by treatment of thielavin A with one mole diazomethane, with $NaOCH_3$ in MeOH gave phenolic compound (**6**), mp 141~143°C, $C_{10}H_{12}O_4$, *m/z* 196. This compound (**6**) was identical with the hydrolysis product of diffractic acid methyl ester and monomethoxy derivative of **6** was also identical with **4**. This phenolic compound (**4**) originated from C-ring, not A-ring in thielavin A or B, because deuterated methyl ester ($C_{11}H_{11}D_3O_4$, *m/z* 213) of **4** was obtained by reaction of **3** with $NaOCD_3$ in deuterated methanol. The structure of didepside (**5**) was determined by X-ray analysis. The crystals obtained from hexane-acetone mixture are triclinic, with $a=9.491(3)$, $b=13.000(5)$, $c=9.673(5)$Å, $\alpha=79.36(3)$, $\beta=68.70(4)$, $\gamma=81.03(4)°$. $Z=2$, $D_{calc}=1.271$ g cm^{-3}. Space group $P\bar{1}$ was assigned during the structure

Fig 2.

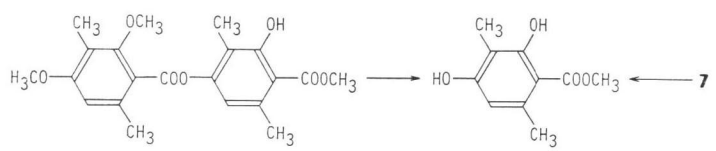
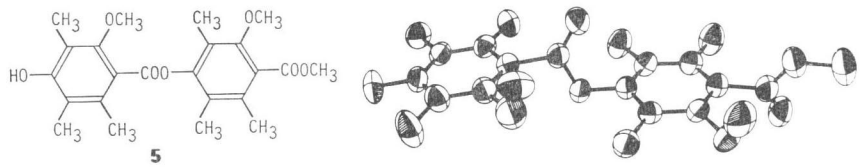


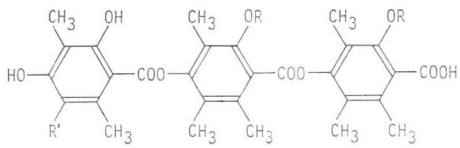
Fig. 3.



refinement. Intensity data were collected on a Rigaku four-circle diffractometer with monochromatic Cu-Kα radiation (λ=1.5418Å) with the $\omega-2\theta$ scan method. The structure was solved by direct methods using MULTAN 78[3] and refined by the method of block-diagonal least-squares. The final R value was 0.085 for 2492 observed reflections with intensities greater than 3 e.s.d.'s. Fig. 3 shows the stereoscopic drawing of **5**. Dihedral angle between the two benzene rings is 6.2° and each ring is planar with a maximum deviation of 0.017Å. The atoms directly attached to each ring are almost on a plane. The bond lengths and the angles are within the values of usually observed. From the above results and X-ray analysis, the structures of thielavins A and B were assumed to be structures **1** and **2**.

Thielavin C (**8**), mp 107~109°C, $C_{32}H_{36}O_{10}$, was isolated from rechromatography of the fractions containing thielavin B on silica gel. From ^{1}H NMR and ^{13}C NMR spectra, thielavin C (**8**) was determined to have the partial structures. Treatment of **8** with diazomethane gave monomethoxy ester (**9**), $C_{34}H_{40}O_{10}$, *m/z* 608. Hydrolysis of **8** with $NaOCH_3$ in MeOH gave phenolic compound (**10**, mp 93~94°C, $C_{11}H_{14}O_4$, *m/z* 210) and didepside (**5**). Therefore A-B ring system in **8** was identical to that in thielavin A. Compound (**10**) originated from C-ring and three methyl groups (2.14 ppm×2, and 2.23 ppm) and two phenolic protons (5.10 ppm and 11.4 ppm) were observed in ^{1}H NMR spectrum. The structure of compound (**10**) was assumed to be 2, 4 - dihydroxy - 3, 5, 6 - trimethylbenzoic acid methyl ester from the above results together with biogenetic considerations. Therefore, the structure of thielavin C was deduced to be **8**.

Fig. 4.



A(**1**) R=H, R′=H
B(**2**) R=CH_3, R′=H
C(**8**) R=CH_3, R′=CH_3

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