AN EPIDITHIAPIPERAZINEDIONE ANTIVIRAL AGENT FROM ASPERGILLUS TERREUS

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Culture filtrates of Aspergillus terreus were found to be active in an antiviral screen designed to detect specific inhibitors of viral RNA synthesis (Trown et al., 1967). One of the active components appeared to be gliotoxin whose mode of action we recently discussed (Miller et al., 1968). We now describe the isolation and preliminary characterization of another active metabolite, designated LL-S88α, which also belongs to the epidithiapiperazinedione class of natural products. The determination of the structure of LL-S88α by x-ray crystallography is described elsewhere (Cosulich et al., 1968)[2].

LL-S88α is produced by many strains of Aspergillus terreus when this organism is grown on a synthetic medium. Isolation of LL-S88α from culture filtrates was monitored using an assay based on inhibition of incorporation of uridine-5-^{3}H into viral RNA, an in vivo test involving a Coxsackie A21 virus infection of mice and by silica gel thin-layer chromatography. LL-S88α was visualized on thin-layer chromatograms by its uv absorption, charring with sulfuric acid or spraying with a sodium azide-iodine reagent (Feigl, 1956);

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[2] After completion of the x-ray studies, Dr. N. Neuss of the Eli Lilly Co. kindly supplied us with samples of acetylaranotin (I) and bisdethio-di-(methylthio)-acetylaranotin (II) (Nagarajan et al., 1968). We find LL-S88α identical to I and LL-S88β identical to II.

the latter has been reported to be very sensitive in detecting the epidithiapiperazinedione class of compounds (Taylor, 1966).

Culture filtrate of Aspergillus terreus NRRL-3319 was extracted with chloroform and the chloroform extract concentrated to a dark thick syrup which was dried in vacuo. A fraction which did not dissolve in a small volume of dry chloroform was discarded and after evaporation of the solvent, the residual solids were dissolved in hot ethanol. A crystalline product which precipitated from the ethanol was further purified on a silica gel column developed with chloroform. The resulting product was recrystallized from ethanol to give pale yellow tetragonal bipyrimidal crystals, m.p. 215 - 230° dec. Anal. Calcd. for $C_{22}H_{20}N_2O_8S_2$: C, 52.38; H, 4.00; N, 5.56; O, 25.39; S, 12.69. Found: C, 53.13; H, 4.07; N, 5.41; O, 24.11; S, 12.37. The high resolution mass spectrum contained an ion with m/e = 440.1221 which appears to have the composition $C_{22}H_{20}N_2O_8$ (calc. 440.1218) and to be derived from the molecular ion by loss of S_2 (Bose et al., 1968). Uv (CH_3OH) end absorption with shoulders at 270 (ϵ 1800) and 225 mμ (ϵ 10,200); ir (KBr) 5.73, 5.83, 7.32, 7.42, 8.14, 8.76, 9.62, and 9.79 μ; nmr ($CDCl_3$) 2.00, 3.00, 4.00, 4.55, 5.07, 5.60 and 6.25 δ.

A second metabolite, designated LL-S88β, was isolated from the ethanol mother liquors of the LL-S88α crystallization. LL-S88β did not catalyse the reaction between sodium azide and iodine (Feigl, 1956) nor did it inhibit the synthesis of viral RNA. Its isolation was therefore followed by thin-layer chromatography where the compound was detected by its uv absorption and staining with I_2. Silica gel chromatography followed by repeated recrystallization from ethanol yielded pure LL-S88β as white platelets, m.p. 215 - 236° dec. Anal. calcd. for $C_{24}H_{26}N_2O_8S_2$: C, 53.91, H, 4.91; N, 5.24; S, 11.97. Found C, 53.63; H, 4.95; N, 5.21; S, 12.10. The high resolution mass spectrum contained an ion with m/e = 487.1109 which has the composition $C_{23}H_{23}N_2O_8S$ and is presumed to arise from the molecular ion by loss of - SCH_3. A second loss of - SCH_3 would result in an ion with m/e 440 as was observed. The ms of LL-S88α and LL-S88β were similar in the regions below m/e 440. Uv (CH_3OH) end absorption

with shoulders at 255 (ϵ 1900) and 220 mμ (ϵ 14,100); ir 5.74, 5.96, 7.30, 8.21, 8.75, 8.86, 9.72 μ; nmr ($CLCl_3$), 2.06, 2.25, 3.02, 4.68, 5.18, 5.79, 6.29, 6.56 δ.

Analysis of nmr, ms, ir and uv data did not unambiguously suggest a structure for either component. The data did however suggest that LL-S88α is a symmetrically substituted epidithiapiperazinedione and that LL-S88β is its dithiomethyl ether derivative. The interrelationship is supported by the lack of reactivity of LL-S88β with the sodium azide-iodine reagent (Feigl, 1956), and was confirmed by the conversion of LL-S88α to LL-S88β by reduction with sodium borohydride and methylation with methyl iodide in chloroform-methanol. A similar conversion in the sporidesmin series of compounds has been reported (Rahman et al., 1967).

LL-S88α is active in tissue culture against strains of rhino-, Coxsackie, polio- and parainfluenza viruses, and protects mice against lethal infections produced with Coxsackie A21 or influenza B/Md viruses[3]. No antiviral activity has been observed for LL-S88β. LL-S88α completely blocks viral RNA synthesis at levels which are without effect on cellular RNA synthesis[3]. We believe that this specific action is probably the basis for its antiviral activity.

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[3] The biological properties will be described elsewhere.