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TWO NEW PHENOLIC REDUCTONES FROM ASPERGILLUS TERREUS

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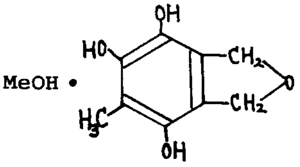
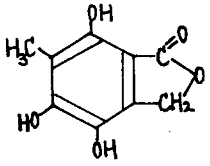
In our screening program to find new reductone compounds like Vitamin C among fungal metabolites, we found that 2,6-dichlorophenol-indophenol sodium (2,6-D.P.I.) was remarkably reduced by substances which are produced by Asp. terreus strain No. 82-A. We have succeeded in the isolation of three new phenolic reductones, one of which was previously shown to be 3,5-dihydroxy-1,2-dimethylbenzene[1]. We now report that the structures of the other two compounds were determined as 1,3-dihydro-5-methyl-4,6,7-trihydroxy-isobenzofuran· MeOH (I) and 6-methyl-4,5,7-trihydroxy-phthalide (II), respectively.



(I)



(II)

(I): $C_9H_{10}O_4 \cdot CH_3OH$, m.p. 122-125°C (decomp.); Anal, Found: C,56.75; H,6.21; O,37.04; $-OCH_3$,14.60; Calcd. for $C_{10}H_{14}O_5$: C,56.07; H,6.59; O,37.35; $-OCH_3$,14.49%; Rf: 0.7 on p.p.c. (ethyl acetate: acetic acid: H_2O=2:1:2); $[\alpha]_D^{15}$ 0°; λ_{max}^{MeOH} mμ (ε): 273 (3640), 280 (3240) and 310 (1560), gave positive color reactions for the reagents of 2,6-D.P.I. (decolor), 0.1% $FeCl_3$ (dark purple), ammoniacal $AgNO_3$ (black) and phosphomolybdic acid (blue in neutral and in alkaline solution). According to Brauer$^{2)}$, the last color reaction indicated that (I) might be a 1,2,4-trihydroxybenzene type-phenol. The IR spectrum showed bands at $\nu^{KBr}\mathrm{cm}^{-1}$: 1630 and 1510 (aromatic double bonds), 2920 and 1470 (methyl groups), and 3480 and 3200 (hydroxyl groups). The NMR spectrum of (I) showed signals at δ (ppm) in $CDCl_3$: 2.05 (3H,s,aromatic CH_3), 3.25 (3H,s,$-OCH_3$), 4.50-4.90 (4H,m) and 7.90-8.50 (4H,OH) which disappeared after addition of D_2O. As signals corresponding to the aromatic proton were not detected, a benzene ring must be fully substituted. In order to assure the number of OH group, (I) was acetylated which acetic anhydride -pyridine or -conc. H_2SO_4 to afford the triacetate (III), m.p. 290-291°C; Anal. Found: C,58.69; H,5.10; O,36.02; Calcd. for $C_{15}H_{16}O_7$:C,58.44; H,5.11; O,36.33%,m/e: 308 (M^+);$\nu^{KBr}\mathrm{cm}^{-1}$1770, 1370 and 1200 (acetyl groups). The NMR spectrum showed three acetyl singals at δ 2.18 ppm (3H) and 2.25 ppm (6H). The UV spectrum,$\lambda_{max.}^{MeOH}$ mμ (ε): 263 (730) and 270 (680), was very similar to that of 1,2,4-triacetoxy-3,5,6-trimethylbenzene, thus indicating that the chromophore of (I) should be the benzene ring with three hydroxyl groups at 1,2,4-positions and with one methyl. (I) was converted with diazomethane in MeOH into the trimethyl ether (IV), m.p. 120-121°C; Anal. Found: C,64.62; H,7.01; O.28.03; $-OCH_3$,41.11; Calcd. for $C_{12}H_{16}O_4$: C,64.27; H,7.19; O, 28.54; $-OCH_3$,41.50%; m/e: 224 (M^+). The NMR spectrum showed 3 $-OCH_3$ at δ 3.82, 3.86 and 3.88 ppm (9H) and in the IR spectrum no free hydroxyl absorption was observed. The oxidation of (IV) with alkaline-$KMnO_4$ at 80°C for a day and subsequent decarboxylation and demethylation of the acidic product with HI-red phosphor yielded 1,2,4,-trihydroxybenzene; m.p. 140.5-141°C and its IR spectrum ($\nu^{KBr}\mathrm{cm}^{-1}$: 3200, 1620, 1510, 1385, 830 and 782), which was completely identical with that of authentic specimen. The mild oxidation of (IV) with alkaline-$KMnO_4$ at room temperature for a day yielded 4-methyl-3,5,6-trimethoxy-1,2-benzene-dicarboxylic anhydride (V), m.p. 134-135°C; Anal. Found: C,57.45; H, 4.78; O, 37.54;

Calcd. for $C_{12}H_{12}O_6$:C,57.14; H,4.80; O,38.00%; m/e: 252 (M^+). The IR spectrum showed characteristic bands for carboxylic anhydride atν^{KBr}1830 and 1770 cm^{-1}. (V) was identified by comparing its IR spectrum with that of synthesized specimen derived from oxidation of 1,2,5-trimenthyl-3,4,6-trimethoxy-benzene with alkaline -$KMnO_4$. Based upon above data, (I) was determined as 1,3-dihydro -5-methyl-4,6,7-trihydroxy-isobenzofuran·MeOH.

(II): $C_9H_8O_5$, m.p. 179-180°C (decomp.); <u>Anal</u>. Found: C,54.98; H,3.86; O,40.77: Calcd. for $C_9H_8O_5$: C,55.10: H,4.11; O,40.78%; Rf: 0.85 on p.p.c. (ethyl acetate:acetic acid:H_2O=2:1:2); $[\alpha]_D^{15}$ 0^o; $\lambda_{max.}^{MeOH}$ mμ (ε): 217 (13500), 268 (6850) and 300 (2130); m/e: 196 (M^+), gave positive color reactions for the reagents of 2,6-D.P.I. (decolor), 0.1% $FeCl_3$ (dark green) and phosphomolybdic acid (yellowish blue in neutral and blue in alkaline solution). The IR spectrum showed bands atν^{KBr} 1630 and 1520 cm^{-1} (aromatic double bonds) andν^{KBr} 1740 cm^{-1} (a lactone ring like phthalide) which was absent in that of (I). The NMR spectrum showed the presence of following groups: aromatic cH_3 atδ2.10 ppm (3H,s), $-CH_2-O-$ atδ5.10 (2H,s) and three free OH atδ7.95-8.15 ppm (3H) in $CDCl_3$ which disappeared after addition of D_2O. Methylation of (II) with diazomethane in MeOH and then demethyl sulfate-K_2CO_3 in acetone afforded the trimethyl ether, which was oxidized in mild condition with alkaline-$KMnO_4$ to afford(V). By this result, the existence of phthalide ring in (II) was assured. In order to confirm wether (II) is 5- or 6-methyl substituted phthalide, the calculated value of E.T. band, that is, $\lambda_{max.}$ 264 mμ for 5-methyl substituted and 275 mμ for 6-methyl substituted case, were applied based on Scott rule[3]. As 5-membered lactone in the phthalide system generally has a tendency to lower the calculated value of E.T. band, this information indicated that the observed value ($\lambda_{max.}$ 263 mμ) must be assigned to that of 6-methyl substituted phthalide. From these evidence (II) was concluded to have the structure, 6-methyl-4,5,7-trihydroxy-phthalide.

(I) and (II) isolated from the culture filtrate of *Asp. terreus* Strain No. 82-A are clarified to be new phenolic reductones first found naturally.

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