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AN ANTIBACTERIAL AND ANTIFUNGAL COMPOUND FROM CALVATIA LILACINA.

A.Gasco, A.Serafino, V.Mortarini and E.Menziani
Istituto di Chimica Farmaceutica e Tossicologica
della Università di Torino, Italy.

M.A.Bianco and J.Ceruti Scurti
Istituto Botanico della Università di Torino, Italy.

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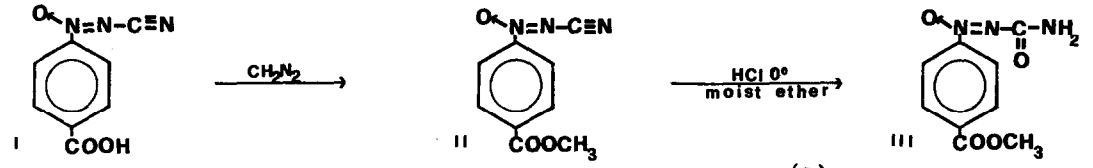
We wish to report the isolation and identification of the substance responsible for the antibacterial and antifungal activity of the culture broth of Calvatia lilacina (Berk.) Henn. P.[1].

To isolate the active substance the ethyl acetate extract was evaporated under vacuum. The residue, dissolved in a mixture of chloroform, ethyl acetate, acetic acid (80, 20, 5) was filtered on a silica gel column. The eluate was evaporated under reduced pressure at 40°C and the residue recrystallized from warm DMSO; yellow crystals were obtained. These, after being desiccated under vacuum at 60°C, lost a molecule of DMSO and gave a light yellow powder (m.p. 198-9°C with dec.; 50.11%C, 2.84%H, 21.68%N). A low resolution mass spectrum of this material showed the parent ion at m/e 191 and the principal peaks at m/e 175, 174, 151 (base peak), 121, 119.4*, 103, 97*, 92, 76, 75, 65, 51, 50, 39. Thus the molecular formula $C_8H_5N_3O_3$ was deduced.

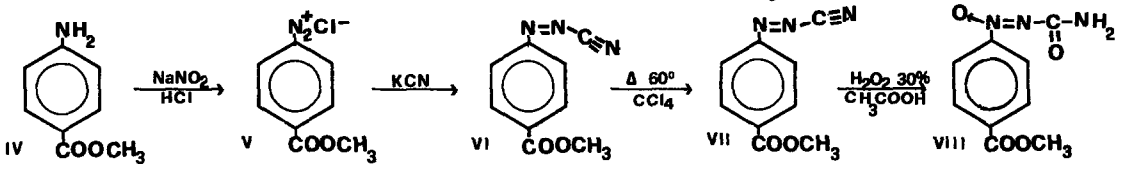
The appropriate metastable ions, in combination of high mass resolution data, (191.0334=$C_8H_5N_3O_3$, 151.0268=$C_7H_5NO_3$, 121.0283=$C_7H_5O_2$) show that an important fragmentation pathway involves initial loss of $CN_2^{\cdot}$ from the parent ion followed by sequential loss of NO molecule. NMR spectrum in CD_3COCD_3 showed an absorption typical of the p-disubstituted benzene derivatives centered at δ8.40,4H; and an absorption at δ11.46,1H, which disappears when a few drops of D_2O is added to the solution. IR spectrum (KBr) showed the presence of the following moieties: -COOH (3000-2400, 1695 cm.$^{-1}$), -C≡N strongly conjugated (2205 cm.$^{-1}$), p-disubstituted benzene ring (3120, 3085, 3068, 1605, 1495sh, 880 cm.$^{-1}$). The presence in the IR spectrum of two absorptions at 1478 and 1325 cm.$^{-1}$ which can be tentatively assigned to an azoxy group[2], in combination of partial results above described sug-

gested the structure I (p-carboxyphenylazoxycyanide) for the metabolite. The structure I was proved by following chemical transformations.



I II III

Treatment of I with diazomethane gave the methyl ester II[3] (m.p. 111-2°C from isopropyl ether), which was transformed into III[3] (m.p. 202-4°C with dec., from methanol), by action of gaseous HCl in ether solution. From p-aminobenzoic acid methyl ester IV the following transformations were easily obtained.



IV V VI VII VIII

The unstable syn-diazocyanide VI was not characterized but immediately isomerized into the stable anti-isomer VII[3] (m.p. 97-8°C from carbon tetrachloride-ligh petroleum). By Pieroni's method[4] VII was transformed into VIII[3][4]. This latter compound was identical to III (IR,UV, mixed m.p.). These results support the proposed structure I for the metabolite; in particular, according to references (4) and (5), confirm the position of the oxygen atom in the azoxyamide group and consequently in the azoxycyano group, and suggest some considerations about the stereoisomerism of I. In fact it is well known that in the stable forms of the benzenediazocyanides the benzene ring and the cyano group have an anti-orientation[6]: it is probable that this configuration is retained in VIII identical to III and thus in I. Further work about this point is in progress.

References.

1) M.A.Bianco, J.Ceruti Scurti, Allionia 18, 79 (1972).

2) N.B.Colthup, L.H.Daly and S.E.Wiberley, Introduction to Infrared and Raman Spectroscopy, Academic Press, New York and London (1964),p. 288.

3) All new compounds had analytical and spectroscopic properties consistent with the assigned structures. All melting points were taken on a capillary melting point apparatus and are uncorrected.

4) A.Pieroni, Gazz. Chim. Ital., 52, II, 32 (1922). C.A.,17, 1439 (1923).

5) A.Angeli, Gazz. Chim. Ital., 47, I, 213 (1917) and other works of the series.

6) Cf. Houben-Weyl, Methoden der organishen Chemie, Georg Thieme Verlag, Stuttgart, 10/3, 593 (1965).

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