ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. A NEW ROTENOID, 11-HYDROXYTEPHROSIN, FROM AMORPHA FRUTICOSA

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The isolation, structure determination and bioactivity of 11-hydroxytephrosin (1) from Amorpha fruticosa is reported.

In a screening program for antimicrobial agents from higher plants,[1] ethanolic extracts of the powdered fruits, stems and leaves of Amorpha fruticosa L. (fam. Leguminosae), false indigo, showed reproducible activity in vitro against Mycobacterium smegmatis (ATCC 607) and Staphylococcus aureus (ATCC 13709). Bioassay-directed fractionation, including extensive silica gel chromatography, produced numerous active fractions from extracts of the fruits. One of the fractions contained an active substance which was homogeneous by chromatographic and spectroscopic analysis but could not be induced to crystallize. Structural analysis allows the assignment of this new antimicrobial agent to be 11-hydroxytephrosin (1). A closely related rotenoid, 6a,12a-dehydro-α-toxicarol (2), has recently been isolated from the same species although no biological data was reported.[2]

11-Hydroxytephrosin, $C_{23}H_{22}O_8$ (M^+ 426, anal. C,H), gives a blue-green phenolic test with $FeCl_3$-EtOH and a positive Durham test,[3] characteristic of rotenoids, which was confirmed by the similarity of its uv spectrum [λ_{max}^{MeOH} 228 nm (log ε 4.43), 234 (4.43), 263 (4.54), 272 (4.59), 294 (4.24) and 311 (4.16)] to that of tephrosin (3).[4] The mass spectrum of 1 shows the typical retro Diels-Alder fragmentations of 6a,12a-saturated rotenoids and fragments at m/e 218 (4) and 208 (5) were particularly helpful.[5] IR bands (KBr) at 3560 and 1670 cm^{-1} confirmed the presence of a chelated α-ketol and bands at 1645, 1380 and 1360 were characteristic of the 2,2-dimethylchromene moiety[4] as were pmr bands ($CDCl_3$) at δ1.33 (3H, s, CH_3), 1.40 (3H, s, CH_3), 5.36 (1H, d, J 10 Hz) and 6.45 (1H, d, J 10 Hz).[6,7] Other useful features in the pmr spectrum were bands at δ3.70 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 5.87 (1H, br.s., ArH_{10}),[8] 6.40 (1H, s, ArH_4) and 6.68 (1H, s, ArH_1)

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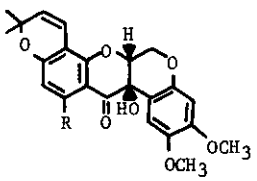
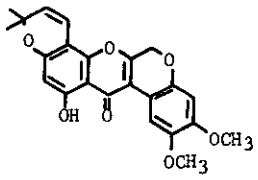
(6a,12a-cis),[9,10] 11.67 (1H, s, exchangeable with D_2O, ArOH••O=C), and 4.50 (3H, br.s., $OCH(CH_2O)$-COH). The general features of the structure were confirmed by treatment with diazomethane to give a monomethyl ether derivative of 1 (M^+ 440, RDA=m/e 232 and 208) and dehydration with 10% H_2SO_4-MeOH to give the crystalline dehydro derivative (M^+ 408) whose uv and ms data are identical with that reported for 6a,12a-dehydro-α-toxicarol (2).[2]

Base was avoided in the isolation of 11-hydroxytephrosin and the substance is optically active (ORD (C=0.000025, MeOH): $[\phi]_{380}$ 0, $[\phi]_{340}$ -23850, $[\phi]_{311}$ 0, $[\phi]_{303}$ +4100, $[\phi]_{298}$ 0, $[\phi]_{280}$ 0, $[\phi]_{270}$ -10250, $[\phi]_{260}$ 0, $[\phi]_{240}$ -34100, $[\phi]_{233}$ 0 and $[\phi]_{225}$ +20450) indicating that it most probably is a true natural product.[4] The ORD spectrum is consistent with the absolute configuration depicted in formula 1.[4]

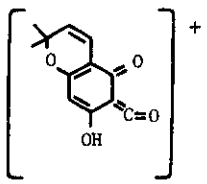
The relatively weak antibacterial potency of 1 (only active against Mycobacterium smegmatis, ATCC 607, at 100 mcg/ml)[1] precludes clinical interest in this substance. Present work involves the separation and structural characterization of the remaining active principles of Amorpha fruticosa.



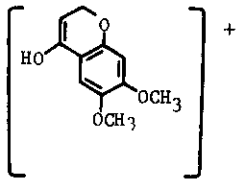
(1) R = OH
(3) R = H



(2)



(4)



(5)

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