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AUSTAMIDE, A NEW TOXIC METABOLITE FROM ASPERGILLUS USTUS

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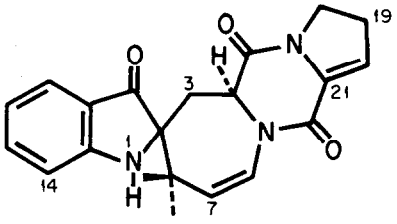
The extraction and fractionation of toxic maize-meal cultures of Aspergillus ustus CSIR 1128, guided by bio-assay in ducklings, led to the isolation of austamide (I) as one of the toxic metabolites. The structural elucidation of I is reported herein.

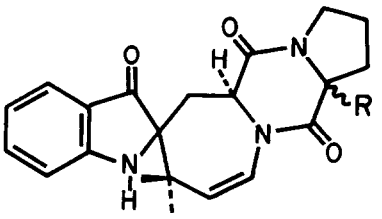
Austamide, $[\alpha]_D^{20^o}$ + 152° (c 1, EtOH) is a yellow amorphous compound with an intense green fluorescence and has the molecular composition $C_{21}H_{21}N_3O_3$.[1] It showed λ_{max}^{EtOH} 234, 256, 268 (sh), 282 and 392 nm (log ε 4.42, 3.07, 3.04, 3.94 and 3.43, respectively) which was unchanged upon the addition of acid or base. The absorption at 234, 256 and 392 nm is typical of the ψ-indoxyl chromophore.[2] Only the absorption at 268 and 282 nm is absent in tetrahydroaustamide (IIIa and IIIb), and is therefore associated with the enamide chromophore which is not conjugated to the ψ-indoxyl moiety.

In the IR spectrum of I the NH group was inferred from the sharp band at 3420 cm^{-1}, while the CO groups gave multiple absorption at 1700 (ψ-indoxyl CO), 1680 and 1650 cm^{-1} (diketopiperazine CO groups). The latter absorption together with the absence of the amide II band near 1550 cm^{-1} strongly supported the presence of the diketopiperazine unit.[3] Mass spectroscopy established the presence of one exchangeable proton in I. Furthermore, the secondary nature of the ψ-indoxyl nitrogen was proved by preparation of N-nitrosotetrahydroaustamide. It showed λ_{max}^{EtOH} 206, 237, 252, 283 and 328 nm (log ε 4.20, 4.08, 4.10, 3.79 and 3.67, respectively) (similar values were reported[3] for N-nitrosobrevianamide A) and ν_{max} 1715 (ψ-indoxyl CO), 1660 (diketopiperazine CO groups), and 1455 cm^{-1} (N-NO).

The NMR spectrum of austamide had the following characteristic features. The two non-equivalent geminal methyl groups resonated as singlets at τ 8.62 and τ 9.12, and the cis-olefinic protons comprising the other part of the isoprene unit appeared as an AB pattern at



I



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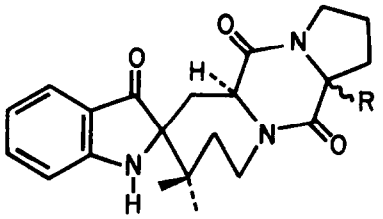
IIb R = ◄ H



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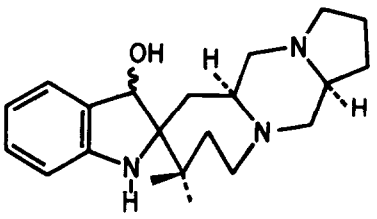

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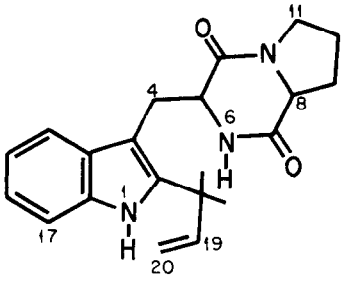

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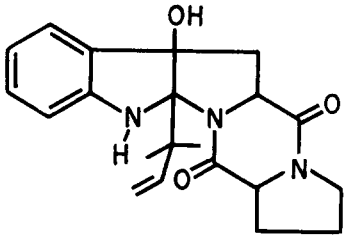

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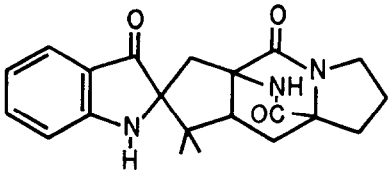

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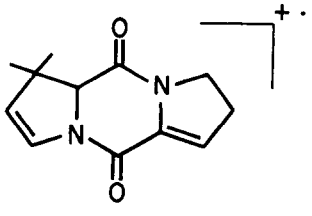

a

τ 5.11 and τ 3.18 (J_{AB} 10 Hz). The methylene protons at C_3 resonated as the AB part of an ABX system (a pair of quartets). The equatorial proton resonated at τ 6.94 (J_{AB} 14, J_{AX} 5 Hz) and the axial proton at τ 7.90 (J_{AB} 14, J_{BX} 12 Hz). The X-part of this pattern was represented by the methine proton on the diketopiperazine which appeared as a pair of doublets at τ 5.01 (J_{AX} 5, J_{BX} 12 Hz). A J_{BX} 12 Hz is consistent with a dihedral angle of close to 180° between the axial proton and the methine proton, while J_{AX} 5 Hz represents a dihedral angle of close to 50° between the equatorial proton and the methine proton.[4] The protons comprising the unsaturated proline part of I appeared as an A_2M_2X system. Two overlapping triplets[5] at τ 6.15 (J 9, 9 Hz) were assigned to the methylene protons adjacent to the nitrogen of the proline ring. The two allylic protons at C_{19} resonated as a distinct sextet at τ 7.60 (J 3, 9, 9 Hz) and the adjacent olefinic proton at C_{20} as a triplet at τ 3.94 (J 9, 9 Hz). The four protons which were arranged in a 1,2,3,4 pattern on the aromatic ring absorbed between τ 2.3 and τ 3.4. The broad N-H signal at τ 5.27 was eliminated upon the addition of D_2O.

Upon catalytic hydrogenation of I over Pd/C in EtOH 1.2 mole equivalents of H_2 were taken up within 12 minutes. Two pairs of diastereoisomers, each in the ratio of 3:1, were obtained, the main pair being the dihydroderivatives $C_{21}H_{23}N_3O_3$ (IIa and IIb) and the minor products, the tetrahydroderivatives $C_{21}H_{25}N_3O_3$ (IIIa and IIIb). Each of the four compounds was obtained chromatographically homogenous. The four compounds had essentially identical UV spectra, e.g. IIa showed λ_{max}^{EtOH} 234, 256 and 392 nm (log ε 4.40, 4.05 and 3.46, respectively), and they exhibited the same main IR features. The absence of the olefinic triplet at τ 3.94 and the appearance of the newly created proton on the diketopiperazine ring at τ 5.84 in the NMR spectra of IIa and IIb indicated that reduction of the unsaturated proline had occurred. The remaining cis-olefinic protons were still distinctly displayed, e.g. at τ 5.26 and τ 3.27 (J 10 Hz) in IIa. The mass spectra of the dihydroderivatives and of the tetrahydroderivatives showed a very prominent peak at m/e 70 C_4H_8N, which originated from the saturated proline ring. The NMR spectra of IIIa and IIIb showed no olefinic absorption. The four methylene protons comprising the saturated isoprene unit appeared in IIIa as broadened quartets at τ 5.70 and τ 6.75 for the methylene protons at C_6 while the adjacent protons at C_7 appeared as a one-proton quartet at τ 8.50 (J 9, 15 Hz) and at τ 7.70. The fine structure of the proton at τ 7.70 was obscured by resonance of the protons at C_3 and C_{20}.

Acid hydrolysis of the major tetrahydroderivative IIIa gave approximately one mole

equivalent of proline $[\phi]^{0.1N\ HCl}_{224\ nm} + 460^{o}$.[6] In the major derivatives, viz. IIa and IIIa, the newly formed chiral centre at C_{21}, therefore had the S-configuration. From a study of the Dreiding models of I, IIa, IIb, IIIa and IIIb it appears that the most favourable conformation for the seven-membered spiran ring would require the proton at C_4 to have the configuration as shown in the formulae of these compounds. This assignment is strongly supported by the coupling constants of the protons comprising this ring. Upon LAH reduction of IIIa a pair of diastereoisomeric hydroxyindolines $C_{21}H_{31}N_3O$ (IV) was obtained.

The most important mass spectral fragmentation product of (I) arose from cleavage of the spiran ring to lead to the fragment m/e 218 $C_{12}H_{14}N_2O_2$ (60%)(a) which lost a methyl group to give m/e 203 $C_{11}H_{11}N_2O_2$ (100%). Fragments representing this alicyclic part (a) of the molecule appeared at m/e 220 $C_{12}H_{16}N_2O_2$ in IIa and at m/e 222 $C_{12}H_{18}N_2O_2$ in IIIa.

A related metabolite (V) was isolated from the culture of A. ustus. It was homogenous by TLC and showed λ^{EtOH}_{max} 225, 275 (sh), 283 and 291 nm (log ε 4.51, 3.85, 3.91 and 3.85, respectively); $\nu^{CHCl_3}_{max}$ 1685 and 1670 cm^{-1} (CO groups); τ ($CDCl_3$) 1.25 (1H, s, 1-NH), 4.28 (1H, s, 6-NH), 2.48-3.05 (4H, m, aromatic protons), 3.90 (1H, X part of AA'X system, J_{AX} 18.2, $J_{A'X}$ 9 Hz, 19-CH),4.92 (2H, AA' part of AA'X system, J_{AX} 18.2, J_{AX} 9 Hz, $H_2C{=}CH_2$), 5.56 (1H, X part of ABX system, J_{AX} 4, J_{BX} 11 Hz, 5-CH), 6.25 and 6.83 [2H, AB part of ABX system, H_A (τ 6.25) J_{AB} 15.5, J_{AX} 4 Hz and H_B (τ 6.83) J_{AB} 15.5, J_{BX} 11 Hz, $4\text{-}CH_2$], 5.95 (1H, t, 8-CH), 6.34 (2H, broadened t, $11\text{-}CH_2$), 7.6-8.20 (4H, m, $-CH.CH_2.CH_2.CH_2\text{-}N-$), 8.52 (6H, s, $C(CH_3)_2$); mass spectrum 351 (M^+, $C_{21}H_{25}N_3O_2$)(9%), 198 ($C_{14}H_{16}N$)(100%), 183 (14%), 182 (10%), 168 (6%), 167 (5%). This metabolite is clearly identical to desoxybrevianamide E,[3] a compound obtained by Birch upon treatment of brevianamide E with Zn in HOAc. It is probably the biogenetic precursor of I, VI and of brevianamide A (VII).[3]

REFERENCES

(1) The elemental composition of all the new compounds as well as of mass spectral fragments was established by high-resolution mass spectroscopy.

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