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BRASSILEXIN,A NOVEL SULPHUR-CONTAINING PHYTOALEXIN FROM BRASSICA JUNCEA L. , (CRUCIFERAE)

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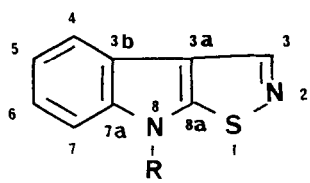
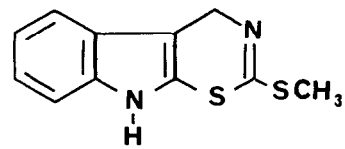
Summary : Structure 1 is established for brassilexin, a new sulphur-containing phytoalexin isolated from the leaves of Brassica juncea (Cruciferae), on the basis of spectrographical data (UV, IR, high resolution MS, ^{13}C and ^{1}H NMR).

Brassica juncea (Cruciferae) is naturally resistant to the blackleg disease caused by the fungus Leptosphaeria maculans (Desm.) Ces. et de Not., [Phoma lingam (Tode ex Schw.) Desm.]. Contaminations by L.maculans, Alternaria brassicae, or abiotic elicitations by sprays with $AgNO_3$ or $CuCl_2$ in dilute solutions, initiate the accumulation of a phytoalexin in B.juncea[1]. A relationship between the accumulation of this compound and the resistance of the plant to the fungus L.maculans was furthermore established[1]. We describe now the isolation and structure determination of this phytoalexin. Brassilexin was extracted from elicited leaves of B.juncea by hot 95% EtOH, and isolated by column chromatographies on SiO_2 (hexane and then AcOEt), on silanized SiO_2 (MeOH-H_2O 1:1) and finally by HPLC, on C_{18}-grafted SiO_2 (Partisil ODS_2), reversed phase (MeOH-H_2O 7:3), UV detection. The isolated phytoalexin (30 to 50 mg per kg leaves, fresh weight), showed in vitro antifungal properties similar to those previously noticed with the raw extract.

Brassilexin 1 shows in UV[2a] the presence of a highly conjugated system. IR[2b] indicates





Ac_2O, Py, 20h, 20°C

1 R = H

2 R = $COCH_3$

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indole and isothiazole substructures, MS[2c] leads for this product to a molecular ion at m/z 174, high resolution carried out on the acetate 2 being in agreement[2d] with the structure 1. ^{1}H NMR[2e] and ^{13}C NMR[2f] brought final arguments in favour of the skeleton, four quaternary and five CH atoms being present. Assignements were made by comparing with reported values[3] for indole and 2 or 3-methyl indoles[2f]. NOe ^{1}H NMR experiments performed on the C-3 olefinic proton produced a slight enhancement of the signal of the proton at C-4, this effect being confirmed in a 2D study.

Brassilexin can be biogenetically derived from indole 3-carboxaldehyde, a previously reported metabolite of tryptophan of plant origin[4]. Several sulphur-containing indoles were recently reported from cruciferae species[5,6] as phytoalexins, among which cyclobrassinin 3 shows a somewhat analogous structure. Brassilexin 1 remains however of particular interest due to its originality.

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2a- Brassilexin 1: m.p.164-167°C, colourless microcrystals; UV (MeOH nm, ε): 218 ($5x10^4$), 245 ($1.4x10^4$), 264 ($1.2x10^4$), highly conjugated system.

2b- IR ($CHCl_3$ cm^{-1}), 3460, sharp, NH, 3250, 2980, 2920, 2850, isothiazole substructure[7], confirmed by sharp bands at 860 and 740 (KBr).

2c- MS m/z 174, M^+ ,(100%), 147,(5%), 146,(6%), 142,(12%).

2d- Acetate 2 (AC_2O/Py, 20h, 20°C), m.p. 172-178°C, MS, m/z, 216, M^+, (24%), 174, M-42$^+$, (100%). High resolution MS of 2, calcd. for $C_{11}H_8N_2OS$ 216.03573, fd. 216.0357; calcd. for $C_9H_6N_2S$ 174.02517, fd. 174.0252. The existence of the S atom is corroborated by comparing the P+2 peak at 176 which contains this atom (3.9% of $^{34}S/^{32}S$) with the relative intensity at 144 (1.5%) which does not contain sulphur ($C_9H_6N_2$, calcd. 142.05310, fd. 142.0530, that is, M-42-32$^+$). The ion at m/z 147 corresponds to a loss of HCN from 174 (calcd. for C_8H_5NS, 147.01427, fd. 147.0144).

2e- ^{1}H NMR of 1 (Bruker 400MHz , CD_3OD, ppm from TMS): 7.90, d, 1H, H-C-4, $J_{4,5}$ = 8Hz; 7.48, d, 1H, H-C-7, $J_{7,6}$ = 8Hz; 7.32, t, 1H, H-C-5, $J_{5,4} = J_{5,6}$ = 8Hz; 7.20, t, 1H, H-C-6, $J_{6,7} = J_{6,5}$ = 8Hz, relative positions of protons confirmed by irradiation; 8.70, s, 1H, this signal integrates for 2H in $CDCl_3$ and thus corresponds to the exchangeable NH plus the proton at position 3.

2f- ^{13}C-NMR (CD_3OD) 120.8 (C-4), 124.8 (C-5), 121.5 (C-6), 112.8 (C-7), 145.9 (7a), 160.5 (8a), 148.6 (C-3), 121.3 (3a), 128.4 (3b).

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