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PUBESCINE A, A CYCLOPEPTIDE ALKALOID FROM *DISCARIA PUBESCENS**

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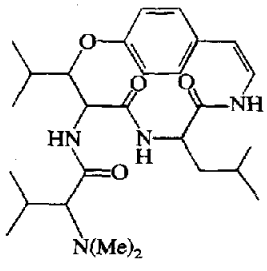
Key Word Index—*Discaria pubescens*; Rhamnaceae; peptide alkaloid; pubescine A; fungicide effect.

Abstract—From *Discaria pubescens*, a new peptide alkaloid, pubescine A (**1**), belonging to the frangulanine type has been isolated and its structure elucidated. Pubescine A seems to be isomeric to melonovine A. For the first time, D-leucine has been identified in a cyclopeptide alkaloid.

In continuing our studies on cyclopeptide alkaloids, we succeeded in isolating and identifying from *Discaria pubescens* pubescine A [2], which is a frangulanine-type alkaloid [3]. Plant material of *D. pubescens* was extracted and worked up in the usual way [4]. The crude alkaloids so obtained were separated by column chromatography and TLC on silica gel. The molecular formula of the isolated compound **1** ascertained by high resolution MS was $C_{27}H_{42}N_4O_4$.



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Based on comparison by chromatography and MS **1** was found to be identical with melonovine A [5], but the melting points and optical rotation are obviously different. Our studies have shown that **1** and melonovine A are therefore diastereomeric, differing only in the configuration of the attached amino acids, similar to the scutianines –D, –E [6] and –G [7]. For determination of its configuration **1** was ozonized and subsequently hydrolysed. By PC, *erythro*-β-hydroxyleucine, leucine and *N,N*-dimethylvaline could be identified in the hydrolysate, as well as traces of *threo*-β-hydroxyleucine originating from racemization under these conditions. Subsequently, the hydrolysate was treated with D- and L-amino acid oxidase. Only *erythro*-β-hydroxyleucine reacted with L-amino acid oxidase, whereas leucine did not respond. Control experiments with authentic D- and L-leucine with either enzyme demonstrated a complete oxidation of L-leucine by L-amino acid oxidase, whereas D-leucine did not react with the corresponding D-enzyme. Greenstein and Winitz [8] have reported similar results obtained by treatment of D-leucine with D-amino acid oxidase (isolated from hog kidney) and of the L-amino acid with the corresponding L-enzyme (from *Crotalus adamanteus*). By relating these experiments with the L-enzyme originating from *C. atrox* it is essential, in order to get comparable results, to compare the activities of both oxidases. Singer and Kearny [9] have published results on the oxidation of L-leucine by L-amino acid oxidases derived from snake venoms. In this connection, the authors have ascertained an O_2 consumption of 743 μl/hr per mg dried venom from *C. adamanteus* and a leucine concentration of 7×10^{-3} mol. In the case of the oxidation of L-leucine by the corresponding enzyme derived from *C. atrox*, an O_2 absorption of 416 has been determined. Consequently, an enzymatic determination of L-leucine with L-amino acid oxidase from different venoms is practicable whereas it seems to be impossible to identify D-leucine with the corresponding D-enzyme from hog kidney by qualitative methods. So the only possibility for the determination of the configuration of leucine by the specific enzymatic methods is an indirect one. If no reaction with L-amino acid oxidase can be achieved, it is evident that leucine must have D-configuration. Thus, these results have demonstrated that pubescine A contains L-*erythro*-β-hydroxyleucine, D-leucine, and *N,N*-dimethylvaline. Consequently, the 14-membered peptide alkaloid pubescine A has the following structural elements: styrylamine, L-*erythro*-β-leucine, D-leucine, and *N,N*-dimethylvaline. Besides D-β-phenylserine [6, 7, 10], an additional amino acid of the D-configuration, namely D-leucine, has been identified in a cyclopeptide alkaloid.

The retardation of growth of pubescine A against bacterial cultures of *Escherichia coli* and *Bacillus subtilis* as well as against lower fungi, such as *Pythium debaryanum* and *Trichoderma viride*, has been tested.

* Part 32 in the series "Alkaloids from Rhamnaceae". For Part 31 see ref. [1].

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Whereas no inhibition of the bacterial cultures was found, an inhibition zone of 18 mm was observed in the case of *T. viride* and one of 20 mm for *P. debaryanum*.

EXPERIMENTAL

MS were recorded at 70 eV with an ion source temp. of *ca* 220°. TLC was carried out on Si gel HF_{254}, PC on 204 b (Schleicher + Schüll) and No. 1 (Whatman), and column chromatography on Si gel (Hermann).

Pubescine A (**1**) (29 mg from 2 kg plant material) was obtained in the usual way by repeated TLC in CH_2Cl_2–MeOH (18:1). It crystallized in colourless needles from MeOH–petrol with mp 247–250°. $[\alpha]_D^{20}$ −230° (*c* 0.076, MeOH). IR (KBr) cm^{-1}: 3280 (NH), 2795 (N—Me), 1670 (amide), 1625 (C=C), 1235 and 985 (phenol ether). UV (MeOH): aromatic end absorption. ^1H NMR ($CDCl_3$/TMS): δ 0.64–1.11, 1.15–2.17 (*m*, CH_3 of leucine and hydroxyleucine), 2.44 (*s*, N–CH_3); after D_2O-exchange: 6.39 and 6.63 (AB-system, $J_{AB} = 8$ Hz, 2 olefine-H), 6.33–6.83 and 6.96–7.33 ppm (*m*, aromatic and 2 exchangable H). ($C_{27}H_{42}N_4O_4$, Calc.: 486.3206. Found: 486.3188 (MS)).

Ozonolysis. **1** (5 mg) was dissolved in 50 ml HCO_2H and ozonolysed. After evapn of the solvent the residue was heated with HBr (48%) under N_2 atmosphere. HBr was then evapd and the residue dried over KOH. The residue was then dissolved in 3 ml H_2O and chromatographed in the following systems: (1) MeCOEt–*n*-BuOH–H_2O–NH_3 (3:5:1:1) for the identification of *erythro*- and *threo*-β-hydroxyleucine, (2) *n*-BuOH–HOAc–H_2O (4:1:5) for the detection of leucine, (3) MeCOEt–Py–H_2O–HOAc (70:15:15:2) for determination of *N,N*-dimethylvaline.

Determination of configuration with L-*amino acid oxidase.* L-Amino acid oxidase (5 mg) from *C. atrox* (Serva) was dissolved in 2 ml 0.1 M Tris–HCl buffer pH 7.2 and 1 ml authentic aq. amino acid soln Hydrolysate (1 ml) was added and incubated in an O_2 atmosphere. The soln was then reduced to 1 ml vol. and chromatographed on paper with authentic samples and untreated hydrolysate.

Determination of configuration with D-*amino acid oxidase.* To an aq. D-amino acid oxidase soln (3 mg in 2 ml 0.1 M pyrophosphate buffer of pH 8.2) 1 ml authentic amino acid soln, and 1 ml hydrolysate was added. The mixture was incubated in an O_2 atmosphere for 72 hr, and then analysed by PC.

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