Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 7, pp. 1911-1914, 1996
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0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00228-5

Fungal Metabolites 40. The Structure and Absolute Configuration of Two Novel Triterpene Depsipeptides from the Fruiting Bodies of *Hebeloma senescens*

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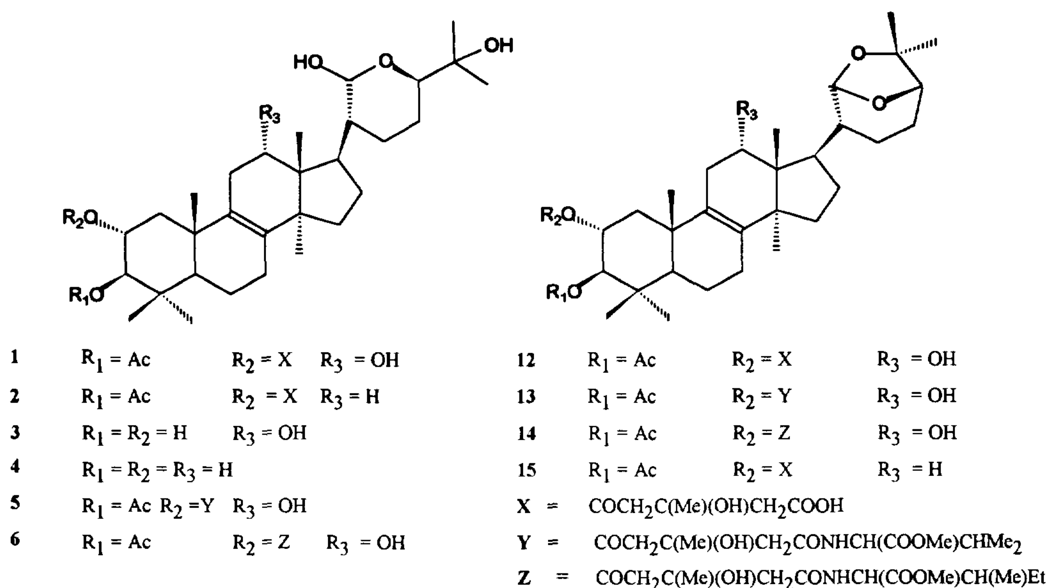
Abstract - Hebelomic acids H **5** and I **6** were isolated from *Hebeloma senescens*, and identified as the amides of hebelomic acid A **1** with (S)-valine and (2S,3S)-isoleucine, respectively, by chemical and chiral chromatograpic methods. Copyright © 1996 Elsevier Science Ltd

Recently, a few new polyhydroxy lanostane triterpenes, e.g. hebelomic acids A **1** and E **2**, have been isolated from the fruiting bodies of inedible *Hebeloma* species (Basidiomycetes, family Cortinariaceae).[1] All of them are diesters either of crustulinol **3** or senescensol **4** whose C-3 and C-2 OH groups are linked, respectively, to an acetyl group and a (*S*)-3-hydroxy-3-methylglutaryl (HMG) moiety.[1c] Continuing our studies on an EtOAc extract of *H. senescens* (Fr.) Berk. ex Br, herein we report the structures of two new minor triterpenes, named hebelomic acids H **5** and I **6**. They were isolated, as an inseparable 4:1 mixture (29 mg from 15.6 Kg of fresh fruiting bodies), by multiple chromatographic separations on Si gel columns of a few fractions collected from the previous chromatographic isolation of compound **2**.[1c]

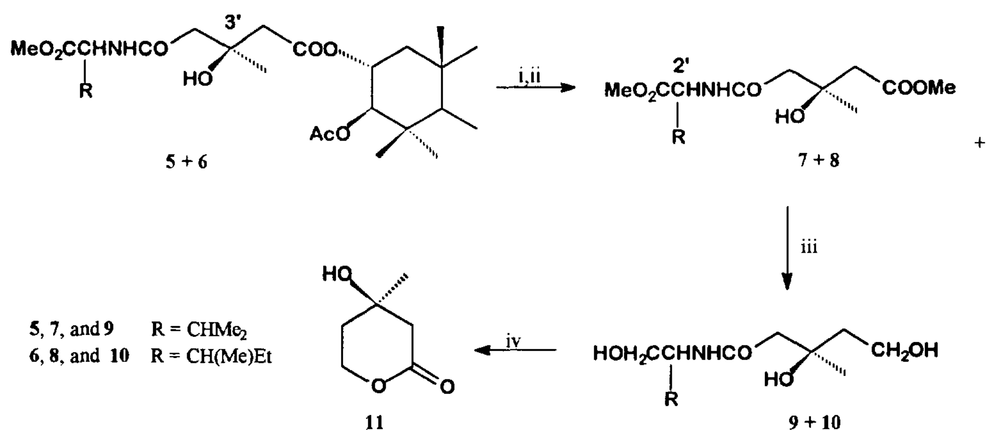
Comparison of the ^{1}H-NMR and ^{13}C-NMR spectra[2] of **5** and **6** with those of hebelomic acid A **1**,[1a] and ^{1}H-^{13}C NMR decoupling and correlation experiments clearly indicated that both compounds were derivatives of **1**, the structural variations being in the glutaryl ester appendage. This was confirmed by the two molecular ion peaks at *m/z* 805 and 819 in the CIMS (NH_3) spectrum which corresponded to the molecular ion of **1** plus $C_6H_{11}NO$ and $C_7H_{13}NO$, respectively. Eventually, the formulas **5** and **6** were assigned to these compounds on the basis of the IR amide bands and the remaining ^{1}H-NMR and ^{13}C-NMR signals.[2] As expected, hydrolysis of a sample of **5** and **6** gave crustulinol **3**,[1] 3-hydroxy-3-methylglutaric acid (HMGA), valine and isoleucine (relative configuration at C-2 and C-3 to be determined).

These new metabolites are rare compounds in nature;[3] indeed, to our knowledge, they are the first triterpene depsipeptides isolated from living organisms which contain valine and isoleucine, respectively.[3] Given the singularity of the structures **5** and **6**, it was therefore important to establish the relative and absolute configuration of the amino acid and HMG residues. Indeed, confirmation that the two amino acids belong to the (L)-family had to be proven since D-amino acids are more common in nature than normally thought.[4]

Moreover, although **5** and **6** formally are derivatives of **1**, in principle amide bond formation between the amino acid and HMGA might precede the esterification of the 2-OH group. In this case, enantiodifferentiation of the carboxylic groups of HMGA would be controlled by the stereospecificity of the



enzyme(s) assisting the amide and not the ester bond formation. Therefore, the configuration at C-3' in **5** and **6** is not necessarily the same as in **1**.[1c] The stereochemistry at C-3' in **5** and **6** was established by correlation with mevalonolactone as shown in Scheme 1. Alkaline hydrolysis of a sample of **5** and **6**, followed by reaction with CH_2N_2, afforded crustulinol **3**[1] and a mixture of ester-amides **7** and **8** in which the two CO groups of the HMG moiety remained chemically differentiated while only the C-2' stereocenter had suffered extensive racemisation.[5]



Scheme 1 - (i) (a) 1 M NaOH, MeOH, r.t. (b) 1 N HCl, 90 %. (ii) CH_2N_2, $MeOH\text{-}Et_2O$, 100 %. (iii) $LiBH_4$, THF, 65°C, 70 %. (iv) (a) 6 M NaOH, reflux. (b) 6 M HCl, 70 %.

Chemoselective reduction of the ester groups in **7** and **8** with $LiBH_4$ in THF, followed by hydrolysis of triols **9** and **10**, gave (R)-(-)-mevalonolactone **11**, $[\alpha]^{20}_D$ -17.6 (*c* 0.08, EtOH) [Lit.[6] $[\alpha]^{20}_D$ -21.8 (*c* 1.1, EtOH)] verifying the *S* configuration at C-3' in **5** and **6**. The absolute configuration of the amino acids was determined by chromatographic and chemical methods. At first, we relied upon a chiral ligand exchange HPLC method devised in our laboratories for the analysis of unmodified amino acids.[4] This consists of two interconnected chromatographic systems, the former for the ionic exchange analysis of the amino acids, the latter for their chiral discrimination.[7] Fig.1 shows the almost base-line separation of the two enantiomers of valine and the four stereoisomers of isoleucine and allo-isoleucine. A few mg of **5** and **6** were then hydrolysed with 6M HCl at 100 °C for 6 h in a sealed test tube. Under these conditions negligible racemisation was observed;[4] moreover, L-valine and L-isoleucine were identified by peak enrichment.

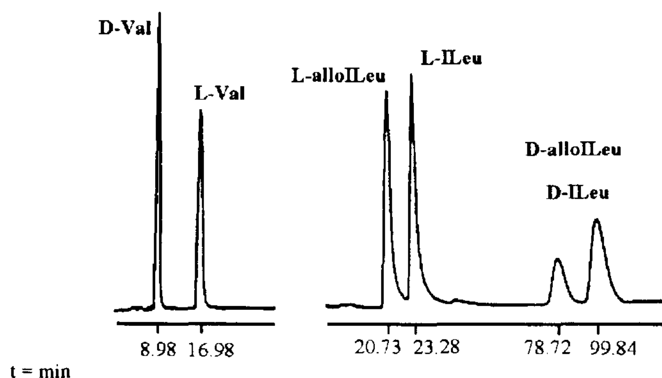


Fig. 1. Enantiomeric discrimination of D, L-valine, D, L-isoleucine, and D,L-allo-isoleucine (see Reference 7 for chromatographic conditions)

Later, we found that the absolute configuration of the two amino acids in **5** and **6** could be established straightforwardly also by ^{1}H-NMR spectroscopy utilizing anhydro-hebelomic acid A **12**[1a,b] as a chiral selector. To this aim, the free carboxylic group of **12** was separately condensed, on exposure to 1-methylpiperidine and BOP reagent[8] in anhydrous CH_2Cl_2,[1c] with each methyl ester hydrochloride[9] of the following amino acids: L-valine, D,L-valine, L-isoleucine, D,L-isoleucine, L-allo-isoleucine, D,L-isoleucine in mixture with D,L-allo-isoleucine. The expected triterpene amides of general formula **13** and **14** were obtained stereochemically pure in satisfactory 68-75% yields. Moreover, to our satisfaction, the ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of each compound showed a significantly distinct double-doublet signal for the methine linked to the acylamino group,[10] allowing an unambiguous stereodifferentiation of the enantiomers of valine and the stereoisomers of iso- and allo-isoleucine. Thus, the two double doublets occurring at δ 4.51 and 4.56 ppm in the ^{1}H-NMR spectrum of the 21,24-anhydro-derivatives of 5 and 6 [11] could safely be assigned to the amides of L-valine and L-isoleucine, respectively. In conclusion, the configuration of the valine and isoleucine residues in amides **5** and **6** is *S* and 2*S*,3*S*, respectively We believe that the methods described in this paper will be of general applicability for assigning the absolute configuration to an increasing number of triterpene depsipeptides, related to **5** and **6**, which are being isolated from Basidiomycetes in our laboratories.

Acknowledgments - This work was supported by grants from the Italian MURST and CNR.

References and Notes

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2. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.58, 0.90, 0.93, 1.03, 1.09, 1.13, 1.15, 1.26 (24H overall, 8xs, H_3-18,-30,-29,-19,-28,-26,-27,-3'), 0.91 (3H, t, J=7.5 Hz, *Me*CH_2),[a] 0.94 (3H, d, J=7.0 Hz, *Me*CH),[a] 0.945 and 0.975 (6H, 2xd, J=6.3 Hz, Me_2CH),[b] 2.09 (3H, s, *Me*CO_2-), 2.44, 2.52, 2.57, 2.62, (4H overall, 2 AB_q, J=14.5 Hz, H_2-2' and H_2-4'), 3.695 (1H, brd, J=11.2 Hz, H-24), 3.72 (3H, s, CO_2*Me),* 3.82 (1H, brd, J=8.2 Hz, H-12), 4.485 (1H, dd, J=8.5 and 5.0 Hz, C*H*NH),[b] 4.535 (1H, dd, J=8.5 and 5.0 Hz, C*H*NH),[a] 4.83 (1H, d, J=10.5 Hz, H-3), 5.17 (td, J=10.5 and 4.5 Hz, H-2), 5.45 (1H, brs, H-21), 7.20 (1H, d, J=8.5 Hz, N*H*). [a,b]Signals assigned to the L-isoleucine and L-valine moieties in **6** and **5**, respectively. ^{13}C-NMR (62.9 MHz, $CDCl_3$) δ:172.6(0), 172.0(0), 171.9(0), 171.2(0), 135.5(0), 132.1(0), 93.5(1), 80.4(1), 74.8(1), 72.6(1), 72.3(0), 71.2(1), 70.2(0), 56.7(1), 52.2(3), 50.2(1), 50.1(0), 49.8(0), 46.9(2), 45.9(2), 43.0(1), 41.0(2), 39.5(0), 39.1(1), 38.1(0), 37.5(1), 31.8(2), 29.5(2), 28.4(3), 27.4(2), 26.9(3), 26.4(3), 26.4(2), 25.9(2), 25.3(2), 24.0(3), 23.7(3), 23.6(2), 21.3(3), 19.9(3), 18.2(2), 17.7(3), 17.2(3), 15.9(3), 11.8(3). IR ν 3367, 1737, 1655, 1536, 1455,1436, 1375, 1241, 1033, 999, 901 cm^{-1}.
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5. All new compounds were fully characterized by MS, IR, and NMR spectra determination.
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7. (a) Dossena, A.; Galaverna, G.; Corradini, R., E. ; Marchelli, R. *J. Chromatogr.* **1993**, *653*, 229. (b)The eluent for the ionic exchange analysis was a Li citrate-Li chloride buffer from pH 2.9 to pH 10.5 and the column utilised was an AA Interaction Lithium form (6 μm, 12 cm x 0.46 cm). The enantiomeric separation was obtained using a Spherisorb ODS2 column (3 μm, 15 cm x 0.46 cm) utilizing Cu(L-MePheA)$_2$ or Cu(D-MePheA)$_2$ (NaOAc 1 mM, pH adjusted to 6.8-8.5 with NaOH) as the chiral selector. 3 % MeCN was added to reduce the retention times. Each amino acid was detected by post-column derivatisation with OPA (*ortho*-phtalaldehyde) and fluorimetric detection (λ_{ecc} = 330 nm, λ_{em} = 440 nm) and it was identified by peak-enrichment with an authentic sample (Sigma). See reference 4 for other details on experimental procedure and equipment.
8. Acronym for benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (see Castro, B.; Dormoy, J.R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219).
9. Each methyl ester was prepared from the corresponding commercially available free amino acid (Sigma) according to the procedure described in: Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109.
10. L-Valine: δ 4.513 (J=8.5 and 5.0 Hz); D-valine: δ 4.541 (J=8.5 and 5.0 Hz); L-isoleucine: δ 4.560 (J=8.5 and 5.0 Hz); D-isoleucine: δ 4.575 (J=8.5 and 5.0 Hz); L-allo-isoleucine: δ 4.682 (J=9.0 and 4.0 Hz); D-allo-isoleucine: δ 4.693 (J=9.0 and 4.0 Hz).
11. They were obtained from **5** and **6** according to the procedure described for the preparation of compound **15**. [1c]

(*Received in UK* 7 *May* 1996)