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FUNGAL METABOLITES XIII[1]:NEW CYTOTOXIC TRITERPENE FROM HEBELOMA SPECIES (BASIDIOMYCETES)*

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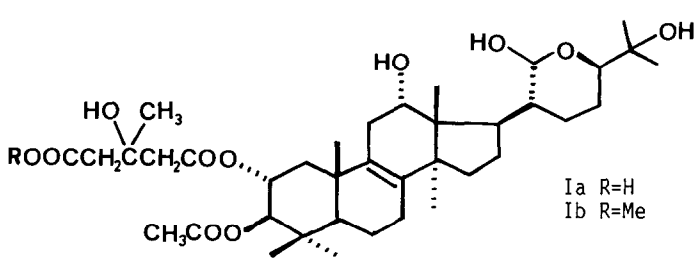
A new cytotoxic lanostane triterpene, named 3-β-acetyl-2-α-(3'-hydroxy-3'-methyl)glutarylcrustulinol, was isolated from Hebeloma crustuliniforme and H. sinapizans. The structure has been elucidated by chemical correlations and spectroscopic evidences.

During a screening program on cytotoxic activity of crude extracts of Basidiomycetes, it was found that two Hebeloma species exhibited high activity in the HL-60 and P-388 leukemia tests[2]. These results prompted us to investigate these mushrooms in order to identify the active compounds Hebeloma crustuliniforme (Bull.ex Fr.) Quélet and Hebeloma sinapizans (Fr.) (order Agaricales, family Cortinariaceae) considered not edible for their unpleasant taste, can be collected in large amounts in Italy in fall[3]. From the acetone and ethanolic extracts of both species a white solid compound (Ia) was isolated. Ia, $[\alpha]_D^{20}$ -10.83° (MeOH, c=1), m.p. 204-205°C, was largely the main metabolite of H.crustuliniforme (0.22% on fresh mushrooms) and precipitated directly during the concentration of the initial acetone extract.

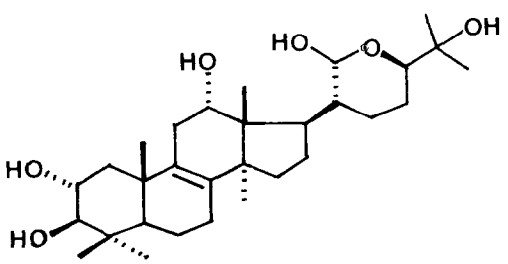
From the PMR and ^{13}C-NMR spectra it was possible to deduce the molecular formula $C_{38}H_{60}O_{11}$[4] and to establish the presence of three acid or ester carbonyls, one of which attributable to an acetyl group (three singlets at ca. 170-174 ppm in ^{13}C-NMR), two tertiary and four secondary alcoholic functions, a methine linked to two oxygens (δ 5.48 and 93.2 ppm) and a tetrasubstituted double bond (singlets at 135.8 and 132.8). By treatment of Ia with CH_2N_2 a monomethyl ester (Ib) was obtained (δ 3.71). Hydrolysis of Ia in MeOH with solid K_2CO_3 gave II and 3-hydroxy-3-methylglutaric acid, identified as dimethyl ester (GC, IR).



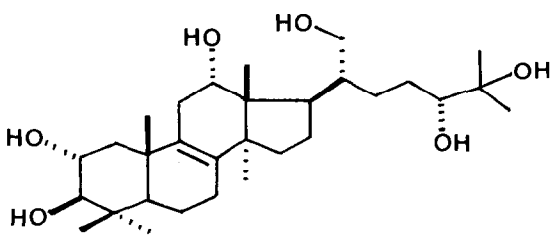
Ia R=H
Ib R=Me

II (M^+= 506, corresponding to $C_{30}H_{50}O_6$), m.p. 238-240°C, named crustulinol, showed in the NMR spectra the presence of seven methyl singlets and six oxygenated carbon atoms but no CO groups indicating that, by hydrolysis, also the acetyl group has been cleaved. The molecular formula and the spectroscopic features suggested for II a pentacyclic unsaturated triterpene structure, with a 2α,3β-dihydroxy substituted ring A, as was demonstrated by decoupling experiments and coupling constants in the PMR spectrum. In I the corresponding signals of these two CH-O protons are moved downfield, thus indicating that the hydroxyls at C-2 and C-3 were esterified with the acetyl and glutaryl residues. The similarity of behaviour of II and I to the fasciculols and their depsipeptides, previously isolated by us from Nematoloma sublateritium [5], led us to try a chemical correlation between these products. Indeed fasciculol C (III) was obtained in quantitative yields by $NaBH_4$ reduction of II showing that crustulinol has a lanostane skeleton and contains, further five oxygenated groups at C-2, C-3, C-12, C-24 and C-25, a hemiacetal at C-21.

By oxidation of Ib with the Fetizon reagent the hemiacetal was converted to a δ-lactone showing that the hemiacetal ring was formed between the aldeyde at C-21 and the OH at C-24 : H-24 δ4.32. The conformation of the tetrahydropyran ring could be established from the PMR spectrum of Ia, where the C-21 proton appeared as a broad singlet at δ5.48 thus forming an angle of 90° with H-20, while H-24 occurred as a broad doublet at δ3.71, being coupled (J= 10.0 Hz) with only one of the C-23 protons. Examination of the Dreiding models revealed that one can account for these NMR data when the ring assumes a boat conformation with the anomeric OH and the isopropyl group in a pseudo-axial configuration. Because of the proximity of the C-21 and C-25 hydroxyls in this conformation, an internal acetal could be easily obtained by intramolecular dehydration. In fact the reaction of II in THF with a catalytic amount of conc. H_2SO_4 gave anhydrocrustulinol (IVa) (M^+= 488), m.p. 271-272°C. In a similar way when II was treated with Me_2CO and p.TsOH two products both containing the acetal system were obtained: besides traces of IVa, the corresponding 2α-3β-acetonide V, m.p. 268-269°C, was formed in good yields. Acetylation of IVa with Ac_2O/py at room temperature yielded the 2α,3β-diacetyl derivative (IVb). As expected, no changes in the chemical shifts of H-21 (br s at δ5.6) and of H-24 (br s at δ3.60) were observed, while C-12 OH is not acetylable in these conditions because of the severe 1,3-diaxial interaction with the αC-14 methyl. However the free hydroxyl in V was nicely oxidized by PCC to the corresponding 12-keto derivative (1710 cm^{-1}), m.p. 278-280°C.



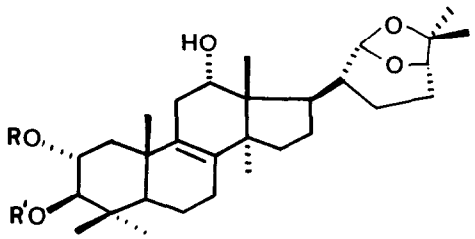
II



III

Established the structure, including the absolute configurations of all chiral centres, of II, we finally determined the relative positions of the acyl groups at C-2 and C-3 in Ia by a two steps selective acylation of anhydrocrustulinol (IVa): the more bulky glutaryl group was expected to react mainly on C-2 and then the more hindered position 3 could be acetylated. In fact when IVa was treated with 3 equivalents of 3-hydroxy-3-methylglutaric anhydride[6] in pyridine containing catalytic amount of DMAP, after methylation with CH_2N_2 two monoacyl derivatives in a ratio of 7:1 were obtained. To the main product (IVc),m.p. 134-136°C, was assigned the structure with the acyl group at C-2, while the minor isomer (IVd) had the glutaryl residue at C-3, as was immediately established by the downfield shift of 2β-H (from δ3.7 to 5.0) and 3α-H (from δ3.02 to 4.6) respectively. Each compound was then acetylated with Ac_2O/py:as expected IVe, m.p. 126-128°C,$[\alpha]_D^{20}$ -8.34°(MeOH), was obtained from IVc and IVf from IVd. The two isomers have very similar IR spectra and only minor, but significant, differences in the PMR spectra. IVe was identical (m.p,m.m. p., IR,PMR,R_f) to the intramolecular acetal derived from Ib (THF/p.TsOH). Therefore to Ia was assigned the structure 2α-(3'-hydroxy-3'-methyl)glutaryl-3β-acetylcrustulinol.

Cytotoxic tests showed that Ia was the most active compound of the two Hebeloma extracts. Studies on the minor metabolites and on the correlation between structure and cytotoxic activity of these triterpenes will be reported in due time.



IVa R=R'=H

IVb R=R'=MeCO

IVc R=MeOOCCH$_2$C(OH)(Me)CH$_2$CO R'=H

IVd R=H R'=MeOOCCH$_2$C(OH)(Me)CH$_2$CO

IVe R=MeOOCCH$_2$C(OH)(Me)CH$_2$CO R'=MeCO

IVf R=MeCO R'=MeOOCCH$_2$C(OH)(Me)CH$_2$CO

V R=R'=$(Me)_2C<$

Ia: IR(KBr, cm^{-1}):3470, 3370 (OH),1735,1720 (C=O). PMR(100 MHz,Me_2CO-d_6,TMS=0): δ0.66, 0.92, 0.96, 1.08 (s,3H each,Me), 1.10 (s,6H,Me), 1.15, 1.37 (s, 3H each, Me), 0.9-2.4 (m,19H), 2.03 (s,3H, MeCO), 2.67 (ABq, 4H, H-2', H-4'), 3.71 (br d,J 10.0 Hz,H-24), 3.88 (br d,J 8.0 Hz, 1H,H-12), 4.77 (d,J 10.5 Hz, 1H,H-3), 5.18 (txd, J_{2-3} J_{1-2} 11.0 Hz, $J_{1'-2}$ 4.5 Hz, 1H, H-2),5.48 (br s, 1H, H-21). ^{13}C-NMR(25.2 MHz,py-d_5, TMS=0,ppm): 174.3(s,C-6'), 171.2,170.6 (s, C-1' and MeCO), 135.8, 132.8 (s, C-8 and C-9),93.2 (d,C-21), 80.2(d,C-3), 74.9(d,C-24), 72.6(d,C-12), 71.2 , 69.8(s,C-25 and C-3'), 70.2(d,C-2), 50.4, 50.0(s,C-13 and C-14), 50.2(d,C-5), 46.5, 46.3(t,C-2' and C-5'), 43.9(d,C-20), 40.9(t,C-1), 39.8(d,C-17), 39.5, 38.2(s,C-4 and C-10), 32.2, 32.0, 27.6, 26.4, 26.1, 24.4(t,CH_2), 28.3(q,C-29 and C-4'), 26.6, 26.1, 24.2(q,CH_3), 21.0(q,MeCO), 19.8(q,C-19), 18.3(t,C-6), 17.6, 17.2(q,C-18 and C-30).

II: IR(KBr, cm^{-1}):3540, 3360 (OH),. PMR(100 MHz,CD_3OD,TMS=0): δ0.65, 0.83, 1.02(s, 3H each, Me), 1.05 (s, 6H, Me), 1.14 (s, 6H,Me), 1.1-2.3(m, 18H),2.64(m, 1H, H-11), 2.94 (d,J 10.0 Hz,1H,

H-3), 3.4-3.8 (m,2H,H-2 and H-24), 3.88 (br d, J 8.5 Hz,H-12), 5.43 (br s, 1H, H-21). ^{13}C-NMR (25.2 MHz,CD_3OD,TMS=0,ppm): 136.1, 134.2(s, C-8 and C-9), 94.2(d,C-21), 84.2(d,C-3), 75.5(d,C-24), 73.9(d,C-12), 72.6(s,C-25), 69.8(d,C-2), 51.9(d,C-5), 51.2, 50.9(s,C-13 and C-14), 44.8(t,C-1), 44.5(d,C-20), 40.5(d,C-17), 40.3, 39.2(s,C-4 and C-10), 32.8, 32.4, 28.3, 27.5, 26.5, 24.8(t,CH_2), 29.1(q,C-29), 25.8(q,C-26 and C-27), 24.4(q,C-28), 20.4(q,C-19), 19.4(t,C-6), 17.6, 17.3 (q,C-18 and C-30). EI-MS(70 eV,DIS)m/z(%): 506(M^+,2), 488(8), 473(16), 471(15), 470(44), 456(14), 455(44), 452(13), 437(33), 419(12), 341(13), 313(13), 295(12), 185 (11), 173(13), 171(13), 169(11), 168(25), 161(11), 159(20), 157(16), 147(13), 145(21), 143(15), 135(19), 133(19), 131(13), 129(11), 125(13), 124(10), 123(29), 122(65), 121(26), 119(27),109 (22), 107(34), 105(26), 95(35), 93(23), 91(27), 83(15), 81(34), 79(21), 77(12), 71(20), 69(46), 67(23), 59(34), 57(17), 55(53), 53(13), 43(100), 41(61).

IVc: IR(KBr,cm^{-1}):3360(OH),1735,1725(C=O). PMR(80 MHz,$CDCl_3$,TMS=0): δ0.6,0.93(s,3H each,Me), 1.12(s,9H,Me),1.28(s,6H,Me),1.43(s,3H,Me),2.65(br s,4H,H-2',H-4'), 3.22 (d,J 10.0 Hz,1H,H-3), 3.72(s,3H,MeO), 3.7-3.95(m,2H,H-12 and H-24), 5.03(txd,J 10.0 Hz, J 4.0 Hz,1H,H-2), 5.58(br s, 1H,H-21).

IVd: IR(KBr,cm^{-1}):3480(OH),1735,1725(C=O). PMR(80 MHz,$CDCl_3$,TMS=0): δ0.70, 1.01, 1.02, 1.15, 1.20 (s,3H each, Me), 1.37(s,6H,Me), 1.52(s,3H,Me), 2.75(AB system,4H,H-2',H-4'), 3.80(s,3H,MeO), 3.82-4.05(m,3H,H-2,H-12,H-24), 4.67(d,J 10.0 Hz,1H,H-3), 5.67(br s,1H,H-21).

IVe: IR(KBr,cm^{-1}):3510,3485(OH), 1737(C=O).PMR(80 MHz,$CDCl_3$,TMS=0): δ0.57(s,3H,Me),0.93,1.10(s,6H each,Me), 1.25, 1.33,1.42(s,3H each,Me), 2.03(s,3H,MeCO), 2.6(AB system,4H,H-2',H-4'), 3.68 (s,3H,MeO),3.7-3.97(m,2H,H-12,H-24), 4.75(d,J 10.5 Hz,1H,H-3), 5.13(txd,J 10.5 Hz,J 4.5 Hz,1H, H-2), 5.56(br s, 1H, H-21). FAB-MS:m/z 727(M^++K), 711(M^++Na), 671(M^++H-H_2O)

IVf: IR(KBr,cm^{-1}):3520(OH),1740(C=O). PMR(80 MHz,$CDCl_3$,TMS=0): δ0.56(s,3H,Me),0.91(s,6H,Me),1.07 (s,6H,Me),1.25, 1.33, 1.40(s,3H each,Me),1.96(s,3H,MeCO), 2.61(br s,4H,H-2',H-4'), 3.65(s,3H, MeO),3.67-3.95(m,2H,H-12,H-24), 4.75(d,J 10.5 Hz,1H,H-3),5.05(txd,J 10.5 Hz, J 4.5 Hz,H-2) 5.52(br s, 1H,H-21).

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* Dedicated to Prof. Ulrich Weiss on the occasion of his 75th birthday.
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