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LL-Z1271β, AN ADDITONAL C_{16} TERPENOID METABOLITE FROM AN ACROSTALAGMUS SPECIES

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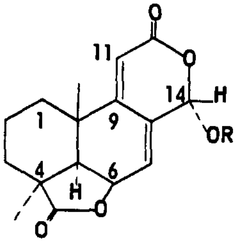
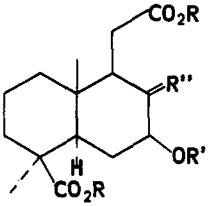
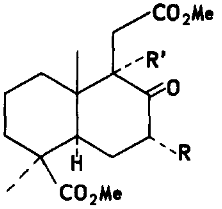
We have reported previously the isolation and structure elucidation of two terpenoid mold metabolites I and II, called LL-Z1271α and γ, obtained from the fermentations of an Acrostalagmus species.[1] During isolation studies on this culture, a third acidic metabolite was isolated which we labelled LL-Z1271β and have now characterized as III.







I, R = Me
II, R = H

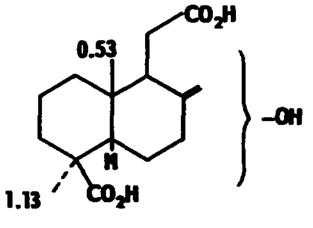
III, $R = R' = H$, $R'' = CH_2$
IV, $R = Me$, $R' = Ac$, $R'' = CH_2$
V, $R = Me$, $R' = Ac$, $R'' = O$
VI, $R = Me$, $R' = H$, $R'' = O$

VII, $R = R' = H$
VIII, $R = OAc$, $R' = H$
IX, $R = H$, $R' = OAc$

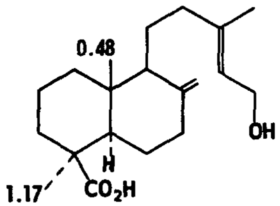
Elemental and mass spectral analyses of III (mp 200° dec; $[\alpha]_D$ +22.5° in MeOH) showed the molecular formula to be $C_{16}H_{24}O_5$. Its infrared absorption spectrum exhibited bands characteristic of hydroxyl (3500 cm^{-1}), carboxyl (2750 and 1710 cm^{-1}) and exocyclic methylene (1650 and 900 cm^{-1}) functional groups. Treatment of III with acetic anhydride and pyridine followed by esterification with diazomethane gave the diester acetate IV ($[\alpha]_D$ +43.6° in $CHCl_3$).[2]

The nmr spectrum[3] of III showed the signals of two tertiary C-methyl groups at δ0.53 and 1.15 and characteristic broadened doublet of an exocyclic methylene group at δ4.44 and 5.08. Also present was a broad one-proton signal at δ3.75 assigned to an axial carbinol proton ($W_{h/2}$ 20 Hz).[4a] Ozonolysis of IV at -70° in methanol with dimethyl sulfide work-up[5] gave the ketone V (mp 107-108°; $[\alpha]_D$ -33.1° in MeOH) the nmr spectrum of which showed the carbinol proton signal at δ5.27 as a one-proton quartet with apparent couplings [4b] of $J_{6a,7a}$=10.5 Hz and $J_{6e,7a}$=7.5 Hz. The hydroxyl group in III is, therefore, secondary, equatorial and adjacent to a methylene group.

In conjunction with the functional groups noted and the molecular formula the co-occurrence of III with I and II suggested, on biogenetic grounds, the partial structure X for this metabolite. The high field C-methyl signal at δ0.53 is assigned to the C-10 methyl group, as its chemical shift necessitates not only a <u>cis</u> 1,3 diaxial relationship with the C-4 carboxyl group but the additional shielding of the C-8 exocyclic methylene group as shown by Dreiding models. This relationship at the same time dictates the A/B <u>trans</u> ring juncture as indicated. An almost identical situation is observed for isocupressic acid (XI).[6] The equatorial nature of the acetic acid side chain at C-9 can be inferred from steric and biogenetic considerations. Consistent with this was the fact that ketone V was unaffected by refluxing in methanolic sulfuric acid, conditions which would be expected to epimerize a less stable axial side chain to the equatorial orientation through the enol of the C-8 carboxyl group.

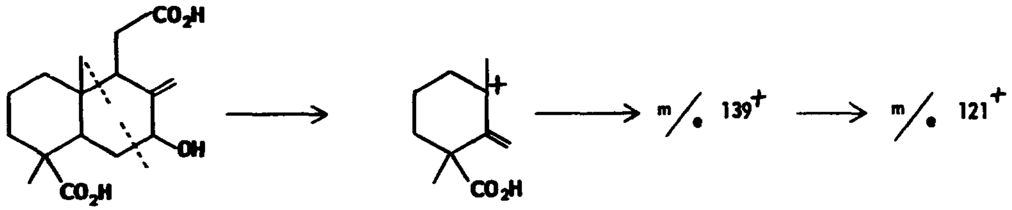


X

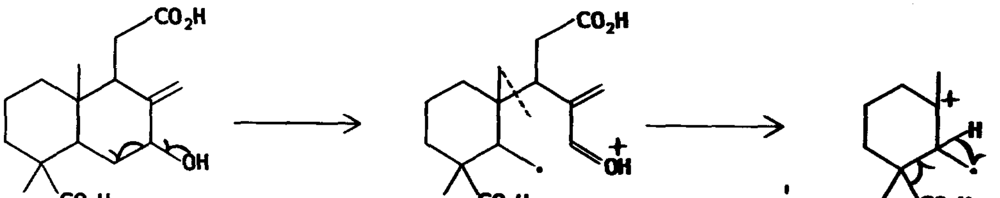


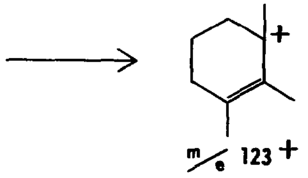
XI

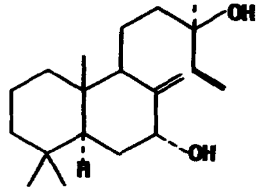
The mass spectra of III and its derivatives were most helpful in confirming these assignments and, in addition, gave strong evidence for placing the secondary hydroxyl group at C-7. In common with the fragmentation patterns of diterpenes with C-4 carboxyl and C-8 exocyclic methylene groups,[7] peaks at m/e 167^+, 139^+, and 121^+ were observed in the fragmentation of III, a result of cleavage of the allylic bonds at C_6 and C_9 as shown. However, the predominant



fragmentation in III seems to be controlled by the hydroxyl group and results in the base peak at m/e 123^+ analogous to that observed in the mass spectrum of 7α-hydroxymanool XII.[8] Thus, the hydroxyl is at C-7 in III. This breakdown can be depicted as shown after Cambie *et al.*[8]







XII

Chemical evidence for the existence of the α-acetoxy ketone grouping in V was obtained by mild basic hydrolysis[9a] of V to the α-ketol VI (mp 124-125°; $[\alpha]_D$ -5.2° in MeOH) which gave a positive acyloin test with bismuth oxide.[9]

Further degradation of V to the known 7-deoxy derivative VII obtained from communic acid[10] and podocarpic acid[11], was precluded by the scarcity of material. A brief attempt to synthesize V from VII by treatment of the latter with lead tetraacetate[12] gave in small yield the epimeric 7α-acetate VIII (mp 129-132°; $[\alpha]_D$ -10.2° in MeOH) and the 9α-acetate IX (mp 148-151°; $[\alpha]_D$ +39.9° in MeOH) in approximately equal amounts. However, equilibration of VIII with hydrobromic acid in acetic acid[13] gave no identifiable product. As expected, the equatorial carbinol proton signal in the nmr spectrum of VIII was found upfield as a triplet at δ5.01 ($W_{h/2}$ of 6 Hz) from the corresponding axial proton signal (δ5.27) in V.[4,14]

The circular dichroism curve of V showed a strong negative Cotton effect at 283 nm ($\theta = -47 \times 10^3$) in methanol which, from the octant rule,[15] suggests the absolute configuration of III as shown.

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2. We were unable to crystallize this compound although tlc, along with ir, nmr and mass spectral data gave no indication of a mixture.
3. Melting points were determined on a Fisher-Johns melting point block and are uncorrected. Nmr spectra were recorded with a varian A60D. The nmr spectrum of III was measured in d_6-DMSO. All other compounds were run in $CDCl_3$; shifts are expressed in δ values (parts per million) from tetramethylsilane as an internal standard and coupling constants (J) are expressed in cycles per second (Hz). Infrared spectra were recorded in KBr discs or $CHCl_3$ on a Perkin-Elmer 137 Infracord. Satisfactory analyses (elemental or mass spectral or both) were obtained for all compounds reported. The circular dichroism curve was obtained on a Cary-60 spectropolarimeter.
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