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THE STRUCTURE OF ZIZANIN-A AND -B [1], C_{25}-TERPENOIDS ISOLATED FROM HELMINTHOSPORIUM ZIZANIAE

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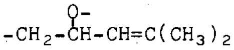
In our previous communication [2], the complete structure and the absolute stereochemistry of ophiobolin, fungal metabolite from Ophiobolus miyabeanus, was established as III by three dimensional X-ray crystallography of its bromo-methoxy derivative. We also reported the isolation of three new crystalline congeners, zizanin-A and -B, and the compound having m.p. 136° [3], from the cultured filtrate of Helminthosporium zizaniae or Ophiobolus heterostrophus. These compounds were structurally closely related and particularly noteworthy, from biogenetic standpoint, was their unusual carbon number, which had not been encountered hitherto among the terpenoid compounds. We wish to present herewith the structure and stereochemistry of zizanin-A and -B, additional examples of C_{25}-terpenoids.

Zizanin-B (I), m.p. 174° , $[\alpha]_D$ 273° (c, 0.55, $CHCl_3$), showed the infrared absorption at 3500, 1745, 1675, and 1630 cm^{-1}, which were very similar to those of ophiobolin (III), the ultraviolet spectrum was practically identical with that of III, (λmax 239 mμ, ε ,14,300). The mass spectrum confirmed the empirical formula $C_{25}H_{38}O_4$ (molecular ion at 402, and the intense peak at 366 = $M-2H_2O$). The n.m.r. spectrum * showed the presence of five methyl groups at 0.85 (d, J=6.5), 0.91 (s), 1.36 (s), 1.60 (s), 1.69 (s); α-substituted αβ-unsaturated aldehyde system at 7.19 (t, J=7.7, 1H) and at 9.20 (s, 1H); C_4-methylene at 2.8 and 2.4 (AB q, J=20, 2H), C_6-methine at 3.25 (d, J=10,1H) and an olefinic proton at 5.07 (t, J=6, 1H), thus exhibiting considerable similarity (except the signals due to the grouping  $-CH_2-\overset{O-}{C}H-CH=C(CH_3)_2$) to that of III. Mild acid treatment of I led to anhydrozizanin-B (VII), m.p. 180° , $C_{25}H_{36}O_3$, λ max 228 mμ (ε, 27,800), ν_{max}^{Nujol} 3510, 2670, 1705, 1685 and 1630 cm^{-1}; n.m.r. spectrum of which showed new signals at 2.03 (s, 3H) and at 6.00 (s, 1H) due to the β-methyl group and the α-proton of the αβ-unsaturated ketone system. The similar spectral changes were observed in the case of III to anhydro derivative$^{4)}$, however, the infrared spectrum of VII clearly showed the presence of the hydroxyl group. The results presented above provide the indication that zizanin-B contains an additional hydroxyl group instead of the tetrahydrofuran ring in III as in structure I.

To confirm this structure, direct correlation of III to I was carried out. Lithium aluminum hydride reduction of III gave a separable mixture of the isomeric triols VIa and VIb$^{4)}$. Further reduction of triol VIa, m.p. 180-183°, $C_{25}H_{40}O_4$, with lithium in liquid ammonia to hydrogenolyze the allylic ether linkage in the side chain afforded the tetraol IVa, m.p.109-113°,

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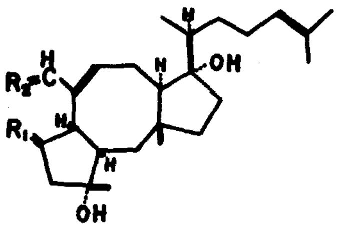
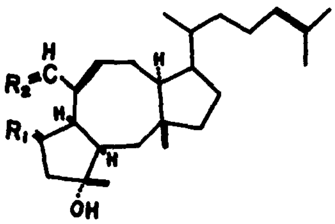
* N.m.r. spectra were determined on a Varian-100 Mc Spectrometer in $CDCl_3$. Chemical shift were expressed in δ-value from tetramethylsilane, and coupling constant in c.p.s..

$C_{25}H_{42}O_4$. Mild oxidation of tetraol IVa with chromium trioxide in pyridine gave I. Reduction of I with lithium aluminum hydride led to a mixture of two isomeric tetraols IVa and IVb (Ca. 1 : 1), from which could be isolated tetraol IVa, 109-113°, identical with the sample derived from III. The n.m.r. spectrum of diacetate of IVa showed the signals at 2.04, 2.06 (s, 6H), acetyl methyls, 3.49 (t, J=7.7, 1H), C_6-methine, 4.85 (s, 2H), C_{21}-methylene, 5.11 (m, 2H), C_{18}-H and C_5-H, 5.96 (t, J=7.6,1H), C_8-H. The other isomer IVb was isolated in pure form as a diacetate (glass); n.m.r. signals at 2.00, 2.05 (s, 6H), 3.12 (d, J=8.2, 1H), 4.63 (AB q, 2H), 5.08(bt, J=6.6, 1H), 5.41 (m, 1H) and 5.92 (t, J=7.8, 1H), which could be assigned analogously. The comparison of the n.m.r. spectra of the epimeric diacetates suggested the secondary hydroxyl group to have α-configuration in IVa and β-configuration in IVb. The above interrelation reveals that the structure of zizanin-B, including the absolute configuration, is as expressed by I.

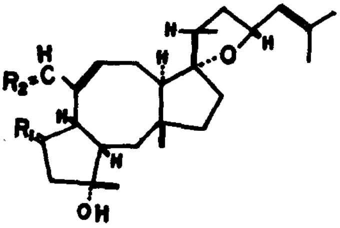
The second less-polar compound, zizanin-A (II) , (m.p. 121°, $[\alpha]_D$ 363° (c,0.6, $CHCl_3$), $\nu_{max}^{CHCl_3}$ 3500, 1742, 1670, 1630 cm^{-1}) showed an ultraviolet spectrum almost identical with those of I and III (λ max 240 mμ (ε, 12,700). The mass spectrum indicated the formula $C_{25}H_{38}O_3$ (molecular ion at 386). In the n.m.r. spectrum of II, the assignable signals are almost identical with those of I, except that secondary methyl group slightly shift to higher field. The n.m.r. peaks are at 0.78 (d, J=6.4) , 0.89 (s), 1.35 (s), 1.61 (s), 1.78 (s) due to the five methyls; 7.20 (t, J=7.2, 1H), 9.26 (s, 1H) due to α-substituted αβ-unsat-



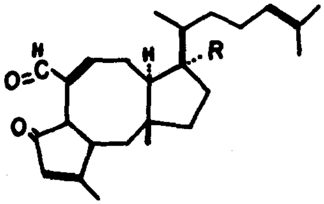
I $R_1=R_2=O$
IVa (a; $R_1=\alpha$-OH,β-H, R_2=H,OH)
IVb (b; $R_1=\beta$-OH,α-H, R_2=H,OH)



II $R_1=R_2=O$
Va
Vb



III $R_1=R_2=O$
VIa
VIb

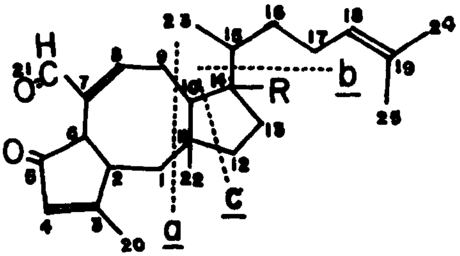


VII R=OH
VIII R=H

urated aldehyde system, and at 3.26 (d, J=10, 1H), 2.46, 2.82 (AB q, J=20, 2H) due to a methylene and a methine adjacent to the carbonyl group, and at 5.11 (t, J=6, 1H) due to an olefinic proton in the side chain. Dehydration of II with acid gave anhydrozizanin-A (VIII)(glass), λ max 229 mu (ε, 28,000); $\nu_{max}^{CHCl_3}$ 1702, 1642, 1621 cm ; n.m.r. peaks at 2.03 (s, 3H), 6.00 (s,1H), 3.44 (d, J=4.5, 1H) and 6.81 (dd, 1H). These spectral changes are very similar to those of VII. Lithium aluminum hydride reduction of II afforded a mixture of two isomeric triols, from which could be isolated pure triol Va, m.p. 153-155°, $C_{25}H_{42}O_3$. The n.m.r. spectrum of the diacetate of Va showed the signals at

2.03, 2.08 (s, 6H), 3.50 (t, J=7, 1H), 4.90 (s, 2H), 5.16 (m,2H) and 5.96 (t, J=7.5, 1H), which are essentially the same with those of diacetate IVa. These spectral properties and the chemical behaviours suggested that the structure of zizanin-A could be represented by II5).

The mass spectra of these compounds provide further support for the proposed structure I and II, i.e., II and III both exhibited intense peak at m/e 176=$C_{11}H_{12}O_2$, corresponding to fission-a in the formula IX. I also showed this peak in medium intensity. On the other hand, I gave intense peak at m/e 255 = $C_{17}H_{19}O_2$ ($M-C_8H_{15}-2H_2O$), while II showed intense peak at m/e 257 = $C_{17}H_{21}O_2$ ($M-C_8H_{15}-H_2O$), both of which correspond to fission-b. III gave another characteristic peaks at m/e 273 = $C_{17}H_{21}O_3$6) (fission-b) and at m/e 1656)= $C_{11}H_{17}O$(fission-c)



IX

Very recently, L. Canonica et al. reported7) the structure of chochliobolin-A and -B without any stereochemical assignment. We consider that these compounds are undoubtedly identical with our ophiobolin and zizanin-B respectively.

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REFERENCES

1) We proposed the structure I and II for zizanin-B and -A at the 9th Symposium on the Chemistry of Natural Products at Osaka in Oct. 1965; Abstract p.30; and at the Meeting of the Pharm. Soc. of Japan at Tokyo in Nov. 1965;Abstract.p.31.

2) S. Nozoe, M. Morisaki, K.Tsuda, Y. Iitaka, N. Takahashi, S. Tamura, K. Ishibashi, M. Shirasaka; J. Am. Chem. Soc. 87 4968 (1965)

3) This compound was proved to be anhydroophiobolin.

4) Chemical reaction of III will be published in future.

5) The stereochemistry at C_{14} and C_{15} are tentative.

6) Hydrogen shift occurs. The detailed interpretation of the fragmentation of mass spectra will be published elsewhere.

7) L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala; Tetrahedron Letters, 1211, 1329 (1966)