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ISOLATION AND CONSTITUTION OF COCHLIOBOLIN B

L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala
Istituto di Chimica Organica dell'Università
Centro Naz. Chim. Sost. Org. Nat. del C.N.R. - Milano (Italy)

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From seven days old cultures of Helminthosporium orizae we have isolated the already described[1] cochliobolin A 2 [a]; while from younger cultures of the same strain we have succeeded in isolating another crystalline product, i.e. cochliobolin B 1, $C_{25}H_{38}O_4$, m.p. 175°, $(\alpha)_D^{20} = 300$ ($CHCl_3$), which differs from cochliobolin A only for a tertiary hydroxyl in C14 instead of the oxide bridge between C14 and C17. U.V. (λ_{max} 237 mμ, ε 10000, in CH_3OH) and I.R. (ν_{max} 3436, 1739, 1669, 1629 cm^{-1}, in $CHCl_3$) spectra of 1, are similar to those of 2. N.M.R.[b] spectrum in $CDCl_3$ displays the following signals: 0.84 (doublet, J=6cps, 3H, C15-CH_3), 0.87 (singlet, 3H, C11-CH_3), 1.38 (singlet, 3H, C3-CH_3), 1.40 (OH), 1.72 (broad singlet, 6H, two C19-CH_3), 2.62 (AB quartet, J=20cps, 2H, C4-H_2), 3.25 (doublet, J=10cps, 2H, C6-H, OH), 5.14 (multiplet, 1H, C18-H), 7.22 (triplet, 1H, C8-H), 9.27 (singlet, 1H, C21-H). This spectrum is very similar to that

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a) In this paper the cochliobolin A is the previously described cochliobolin[1].
b) N.M.R. spectra were determined at 60 Mc with TMS as internal reference, chemical shifts as δ = ppm; all the centesimal analyses of the mentioned products are in agreement with the reported formulae; microanalyses are carried out in the Microanalysis Laboratory of our Institute.

of 2, the main differences being observed for signals at 0.84 (in 2 at 1.12), at 1.40 (absent in 2) and at 5.14 (multiplet in 1 and doublet at 5.18, J=7cps in 2). Moreover 2 shows a signal at 4.45 (multiplet, 1H), assigned to its C17-H and absent in 1.

Mass spectrum of 1 shows a peak m/e = 366 (P - $2H_2O$) and m/e = = 255 (P - $2H_2O$ - C_8H_{15}) which suggests a C_8 side chain, while the peak m/e = 165 is absent. The last one is particularly showed by 2 and by its unhydrogenated derivatives, having an oxide bridge between C14 and C17.

By gentle treatment with alkali 1 is dehydrated to 3-anhydrocochliobolin B 3, $C_{25}H_{36}O_3$, m.p. 176°, U.V. spectrum: λ_{max} 231 mμ, ε 20000; its I.R. spectrum shows bands at ν_{max} 3450 (OH), 1700, 1670, 1635, 1630 cm^{-1}. Its N.M.R. spectrum in $CDCl_3$ shows disappearance of the signals at 1.38, 2.62, 3.25 present in 1, while new signals at 2.08 (broad singlet, 3H, C3-CH_3), 3.57 (multiplet, 1H, C6-H), 6.05 (broad singlet, 1H, C4-H) are observed. The signal at 1.4 (1H, C14-OH) is present too.

These results confirm the presence in 1 of C3-OH β-to C5=O.

As previously described[1], in the same conditions 2 is converted to 3-anhydrocochliobolin A, which has no hydroxyl group.

By ozonolysis 1 yields acetone. This result confirms the presence of isopropylidene group.

On catalytic hydrogenation 1 is converted to a tetrahydroderivative 4, $C_{25}H_{42}O_4$, m.p. 90°; its I.R. spectrum in nujol has bands at ν_{max} 3500, 3400 (broad), 1735, 1660 cm^{-1}. Its N.M.R. spectrum in dimethyl sulfoxide-d_6 shows signals at 0.77 (doublet, J=6cps, 3H, C15-CH_3), 0.87 (doublet, J= =6cps, 6H, two C19-CH_3), 0.90 (singlet, 3H, C11-CH_3), 1.30 (singlet, 3H, C3-CH_3), 4.02 (singlet, 1H, OH), 5.06 (sin-

glet, 1H, OH), 6.13 (doublet, J=7cps, 1H, C21-H), 8.86 (doublet, J=7cps, 1H, C21-OH). These results show that in 1 the α-β unsaturated aldehyde group is in the same position than in 2. In fact in tetrahydrocochliobolin B 4 too, the aldehyde group is stabilized in enol form by steric influences; the spectroscopic properties of 4 suggest that the C3-OH is involved in this stabilization through a hydrogen bond, as it happens in the tetrahydrocochliobolin A[1].

Nitric oxidation of 4 gives 3-methylbutanoic, 4-methylpentanoic, 5-methylhexanoic, 2,6-dimethylheptanoic, 5, acids, and among non-volatile acids, 1,2,2-propanetricarboxylic, 1,3,3-butanetricarboxylic, 2-methyl-1,2,3-propanetricarboxylic and 2-methyl-1,2,4-butanetricarboxylic, 6, acids. Their methyl esters have been separated by gas-liquid chromatography on 3% SE 30 on Gaschrom P packed glass column[c] and identified by their mass spectra by direct comparison with synthetic specimens. Nitric oxidation of 4 does not give (+)methylsuccinic acid and the lactone of 2,6-dimethyl-4-hydroxyheptanoic acid, which are the typical products obtained from tetrahydrocochliobolin A[1].

These results prove 1 has a C_8 side chain and the skeletons of 1 and 2 are very similar.

The final correlation between cochliobolin A 2 and cochliobolin B 1, was obtained by lithium aluminium hydride reduction of 1. In this manner we have isolated 7, $C_{25}H_{42}O_4$, m.p. 110-15°, which was hydrogenated on palladium on carbon catalyst to give 8, $C_{25}H_{44}O_3$, m.p. 160-3° undepressed in mixture with the already described hydrogenolysis product of 9[1]. Infrared, N.M.R. and mass spectra of the two products are

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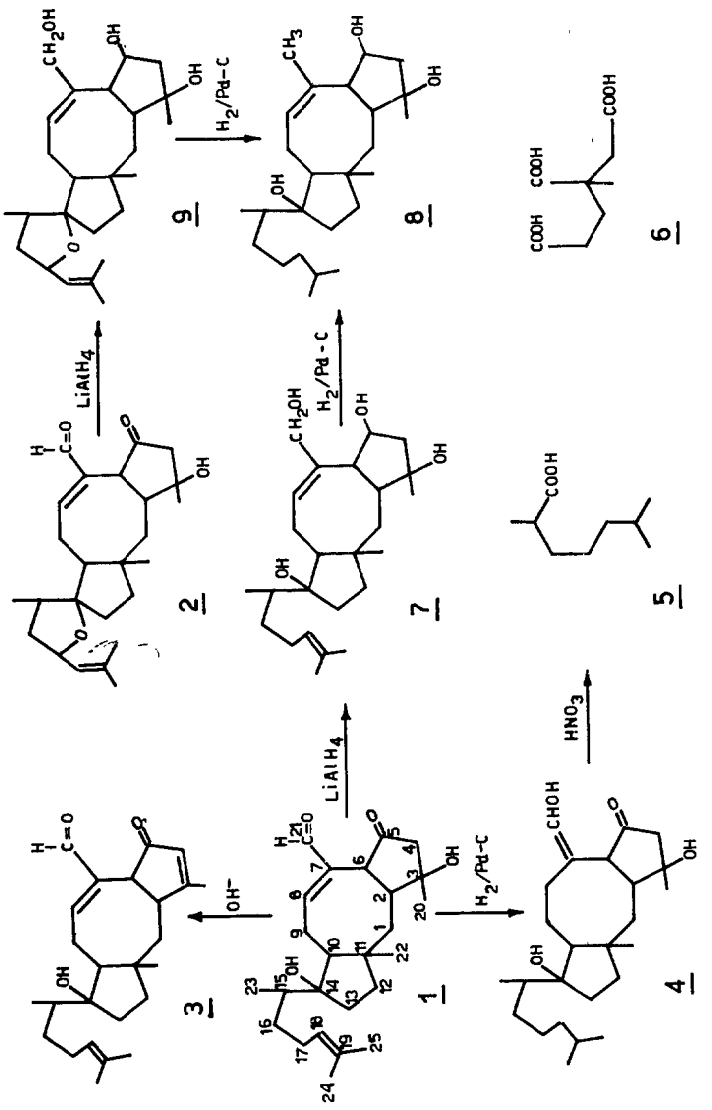
c) A L.K.B. 9000 A gas-chromatograph mass-spectrometer has been used.


LiAlH4
H2/Pd-C
OH⁻
HNO3
1
2
3
4
5
6
7
8
9

identical.

Nozoe et al. call zizanin B, $C_{25}H_{38}O_4$, m.p. 173°, a product having unknown structure, they isolated from Helminthosporium zizaniae[2]. Its physico-chemical properties[3], except the U.V. spectrum (λ_{max} 237 mμ, ε 19600) are very similar to those of cochliobolin B. We suspect two substances are identical. Findings of cochliobolin B only in young cultures of Helminthosporium orizae and its almost complete disappearance in older ones, strongly suggest that it is a biogenetic precursor of cochliobolin A.

For this reason, it is possible that K. Ishibashi did not succeed in isolating it from cultured broth of Cochliobolus miyabeanus[4], perfect form of Helminthosporium orizae.

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