Tetrahedron Letters No.26, pp. 3035-3039, 1966. Pergamon Press Ltd.
Printed in Great Britain.

THE BIOSYNTHESIS OF COCHLIOBOLINS A AND B

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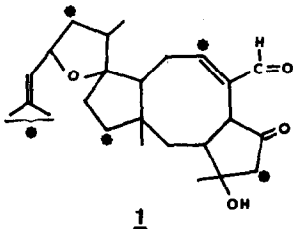
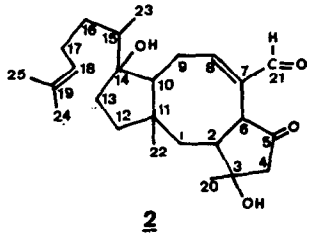
(Received 25 April 1966)

The elucidation of the structures of the cochliobolins A 1 and B 2 has recently been reported[1,2].

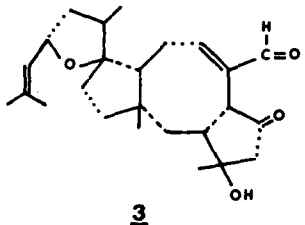


1



2

Inspection suggests that these substances are constructed from five isoprene units linked together head to tail, as shown in 3 for cochliobolin A.



3

It has proved possible to test this by degrading labelled cochliobolin A obtained from $[2\text{-}^{14}C]$ mevalonic acid lactone as substrate.

The labelling pattern expected from the usual mode of incorporation of labelled precursor is as shown in 1 (^{14}C asterisked).

Cochliobolus miyabeanus was cultured in a synthetic medium, DL [2-^{14}C] mevalonic acid lactone (50 µC) being added after two days growth. Cochliobolin B 2 was isolated after three days and a half (after chromatography and several crystallizations 15 mg obtained, 2.83 10^{-3}µC/mg, incorporation 0.42% [a]); cochliobolin A 1 was isolated after six days growth (after chromatography and several crystallyzations 218 mg obtained, 4.55 10^{-3}µC/mg, incorporation 2.48%).

By ozonolysis or oxidation with CrO_3 in acetic acid the labelled[b] cochliobolin A 1 yielded acetone isolated as 2,4-dinitrophenylhydrazone[c] (18.9% of the total activity). This result shows that the C19, C24, C25 fragment is labelled.

By ozonolysis of 1[d] and removal of acetone we were able to obtain a product which yielded 1.35 moles of acetic acid per mole of 1 (67% of two CH_3-C) by Kuhn-Roth oxidation. From this value, it seems that acetic acid comes from C3-CH_3 and C15-CH_3 only and it does not derive from C11-CH_3: in fact the hypothetic degradation product containing the C11-CH_3, i.e. 2-methyl-1,2,4-butanetricarboxylic acid, did not yield acetic acid by Kuhn-Roth oxidation. Acetic acid was converted into p-bromophenacylacetate which had 1.7% of the original activity of 1. Therefore we think that C3,

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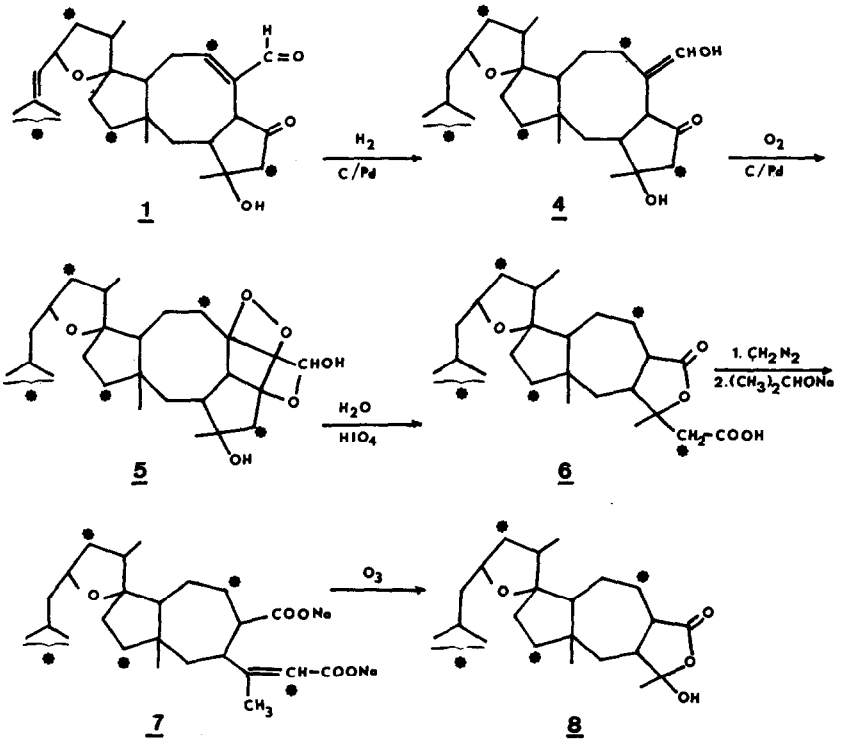
a) Radio-assays were made by measurement in liquid scintillation spectrometer.
b) Cochliobolin A was diluted with inactive material to 5.86 10^{-4} µC/mg.
c) Radio-assay of this derivative was made by combustion to CO_2 followed by measurement in proportional counter with anticoincidence circuit.
d) Cochliobolin A was diluted with inactive material to 4.01 10^{-4} µC/mg.

C15, C20 and C23 are not directly derivable from C2 of mevalonic acid.

As previously described[1], on hydrogenation 1 yielded 4 (after crystallization this product had 100% of the original activity of 1). 4 was converted into 5. By reaction with HIO_4 and H_2O 5 yielded formic acid, isolated as p-bromophenacylformate (0% of label), and the acid lactone 6 deriving from contraction of the eight-membered ring (107% of the original activity of 1).

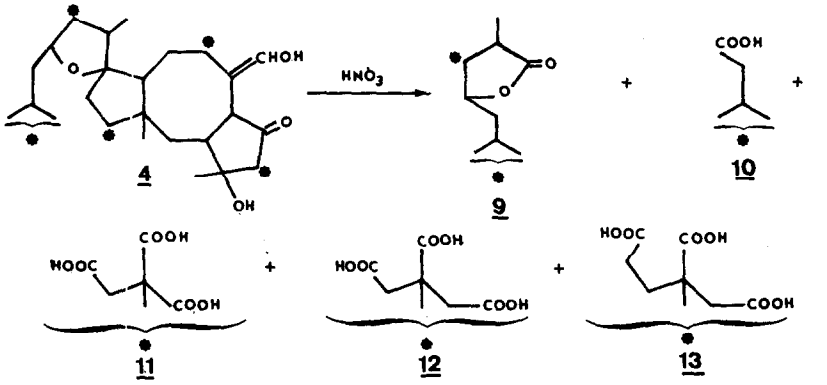


Methyl ester of 6 was converted into the α,β-unsaturated carboxylic acid 7 which was directly ozonised to 8, $C_{22}H_{36}O_4$, m.p. 135°C; its mass spectrum showed the expected parent peak at

m/e = 364; its I.R. spectrum showed bands at ν_{max} 3340, 1760 cm^{-1}. This product had 83% of the original activity of 1.

In agreement with this result we notice that C5 or more probably C4 are derived from the 2-position of mevalonic lactone.

By nitric acid oxidation[1] 4 gave as volatile products 2,6-dimethyl-4-hydroxyheptanoic acid lactone 9 and iso-butyric and iso-valeric acids 10. p-Bromophenacylisovalerate was isolated by chromatography (19.9% of the original activity of 1). 9 contained 40.2% of the label.



Comparison of the activities of acetone, 9, acetic and iso-valeric acids indicated that only C14 and C16 of 1 could derive from the labelled position of mevalonic acid.

Nitric oxidation of 4 gave also as non volatile products polycarboxylic acids whose methyl esters were isolated by preparative gas-chromatography. We had so obtained trimethyl esters of 1,2,3-propanetricarboxylic 11 (22.7% of the total activity of 1), 2-methyl-1,2,3-propanetricarboxylic 12 (21.7% of the total activity of 1), and 2-methyl-1,2,4-butanetricarboxylic 13 (20.5% of the total activity of 1) acids.

Comparing the activities of these three acids we recognised that C14 and C2 or C13, or probably both, were not labelled.

In this way we have demonstrated the presence of a labelled carbon

atom in the isopropylidene group (C19,C24,C25), of another one at C16 and lack of label at C3, C14, C15, C17, C18, C20, C21, C23 and probably at C2 and C13. These and the other results we obtained are conclusive in showing that 1 derives from mevalonic acid and in confirming that byosinthesis follows the expected pattern 3.

We have also proved that cochliobolin B 2 is a biosynthetic precursor of 1. Cochliobolus miyabeanus was cultured, 2 (10 mg, 2.83 10^{-3} μC/mg, in ethanol) being added after two days growth. 1 was isolated after seven days (after chromatography and several crystallizations 135 mg obtained, 2.1 10^{-5} μC/mg, incorporation 9.9%) together with 128 mg of 2 (2.3 10^{-5} μC/mg, 10.3% of the original activity).

Our results give the first experimental evidence of direct biosynthesis of a terpenoid substance constructed from five isoprene units linearly linked head to tail.

Acknowledgements.- Grateful acknowledgement is made to Prof. E. Grossi Paoletti of Institute of Pharmacology of University of Milan for measurements with scintillation spectrometer. We are also grateful to Dr. P. Duranti Valentini of Laboratori Riuniti SNAM for measurements with proportional counter.

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