Tetrahedron Letters No.47, pp. 4843-4846, 1968. Pergamon Press. Printed in Great Britain.

MICROBIOLOGICAL TRANSFORMATIONS OF FUSIDANE-TYPE ANTIBIOTICS

A CORRELATION BETWEEN FUSIDIC ACID AND HELVOLIC ACID

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(Received in UK 10 August 1968; accepted for publication 16 August 1968)

The structural work on the antibiotic helvolic acid culminated recently when Okuda et al.[1] proposed formula I on the basis of convincing chemical and spectroscopic evidence. According to this proposal the compound contains the same framework as fusidic acid (II)[2a,b] and thus belongs to the fusidane class of antibiotics. The structure of fusidic acid has been verified by X-ray analysis[3], and a correlation between this compound and helvolic acid would therefore be desirable. Attempts to achieve this by chemical means have been unsuccessful so far, but we now wish to report an interrelation accomplished by means of a combination of chemical and microbiological transformations*1. This approach became possible when we found that the fungus Acrocylindrium oryzae, Saw. Kominami produces helvolic acid when grown aerobically in deep culture. Thanks to this observation, we now had at our disposal organisms capable of introducing oxygen functions in the fusidane framework at C-11 (Fusidium coccineum) as well as at C-6 and C-7 (Acrocylindrium oryzae).

When 7-deacetoxy-1,2-dihydrohelvolic acid (III)[5] (100 µg/ml) was added to an actively growing culture of a mutant of Fusidium coccineum, K. Tubaki, it was rapidly transformed into a more polar compound as indicated by thin layer chromatography (tlc)*2. No trace of this compound could be detected in parallel fermentations without added III. Isolation of the metabolite was accomplished by preparative layer chromatography (plc)*2 of an ethyl acetate extract of the acidified culture fluid, Treatment of the amorphous acid (IVa) thus obtained with ethereal diazomethane gave a methyl ester $C_{32}H_{48}O_7$*3 (M^+ = 544), m.p. 155-156°C, assigned structure IVb on the

*1 An elegant interrelation between the two antibiotics has independently been achieved by Okuda et al.[4]

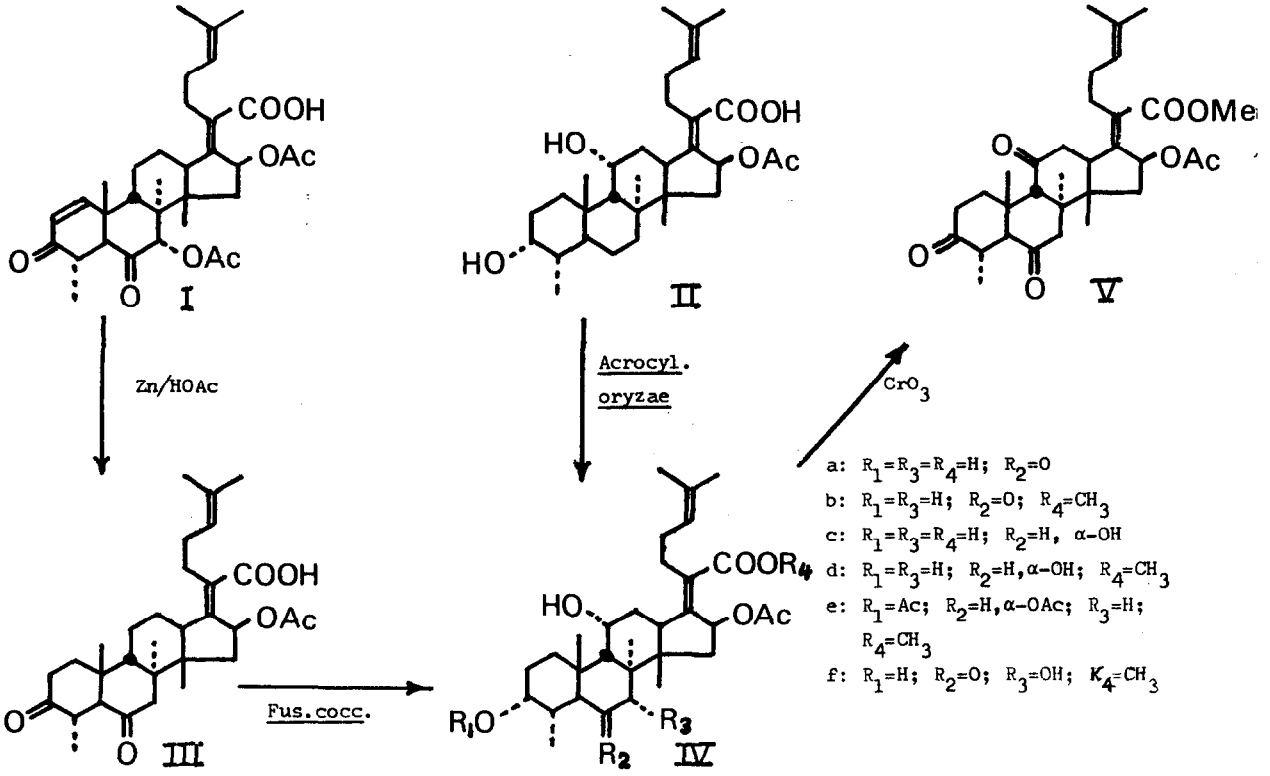
*2 For tlc and plc, silica gel HF_{254} was used as adsorbent. The solvent system was 10% methanol in methylene chloride.

*3 All compounds for which empirical formulae are given, gave satisfactory microanalyses.

TABLE 1 (n.m.r. data)*

Compound	CH-16	CH-24	CH-11	CH-7	CH-6	CH-3	CH_3-O-C(=O)-	CH_3-C(=O)-O-
Methyl fusidate	5.86/d J = 8.5	5.12/b J = 7	4.36/m			3.75/m	3.63/s	1.98/s
IVb	5.84/d J = 8.5	5.06/b	4.42/m			3.82/m	3.61/s	1.95/s
IVd	5.78/d J = 8.5	5.05/b	4.35/m		3.89/m J~8	3.70/m	3.60/s	1.94/s
IVe	5.81/d J = 8.5	5.08/b	4.38/m		ca.4.92/m	ca.4.9/m	3.65/s	1.97/s 2.01/s 2.07/s
IVf	5.83/d J = 8.5	5.06/t	4.47/m	3.90		3.90/m	3.62/s	1.97/s
V	5.88/d J = 8.5	5.05/b					3.63/s	1.97/s

* The spectra were obtained with a Varian HA-100 (100 Mc) instrument, $CDCl_3$ being used as solvent. The line positions are given in δ-values and with TMS as internal reference. For characterization of the signals, the following abbreviations are used: s (singlet), d (doublet), t (triplet), m (multiplet), b (broad, ill-defined signal).



basis of the following evidence. The UV spectrum (λ_{Sh}^{EtOH} 22o mμ (ε = 1o,15o)) indicates the presence of the same chromophoric system as in III, and a band at 17o5 cm^{-1} in the infrared suggests the presence of a six ring ketone. That the latter is at C-6 was inferred from the CD-curve (dioxane), which shows a strong negative Cotton effect ($\Delta\varepsilon_{31o}$ = -3.42 and $\Delta\varepsilon_{3o2}$ = -3.54 with respect to the keto group) similar to that reported for 6-keto derivatives in the helvolic acid series[1], but incompatible with the curves reported for 3- or 11-monoketoderivatives in the fusidic acid series[2b]. The presence of α-orientated hydroxyl groups at C-3 and C-11 is consistent with the fact that the chemical shift and splitting of the two HO-C(-H)- signals in the n.m.r. spectrum (cf. Table 1) correspond closely to the signals due to the protons at C-3 and C-11 in the spectrum of methyl fusidate[*4]. IVb was further characterized by oxidation with Jones reagent to the triketo ester V, $C_{32}H_{44}O_7$, m.p. 187-188°C. The fact that IVb and V could also be obtained from fusidic acid (vide infra) afforded clear-cut evidence of the location of the oxygen functions in these compounds.

Introduction of oxygen functions at C-6 and C-7 in fusidic acid was accomplished by means of Acrocylindrium oryzae. When a culture of this organism was incubated with II (15o μg/ml) three transformation products were formed as indicated by tlc. None of these compounds, which all are more polar than the parent compound, could be detected in parallel fermentations without added II. Isolation of the metabolites was accomplished by plc of an ethyl acetate extract of the acidified culture fluid. This procedure gave three carboxylic acids as amorphous but homogeneous powders, designated A, B, and C in order of decreasing polarity.

The main metabolite A gave with diazomethane a methyl ester, $C_{32}H_{5o}O_7$ (M^+ = 546), m.p. 165-166°C, showing ultraviolet absorption at 22o mμ (ε = 9,o5o). This compound was assigned structure IVd for the following reasons. The n.m.r. spectrum (cf. Table 1) contains HO-C(-H)- signals indicating the presence of three secondary hydroxyl groups. The chemical shift and splitting of two of these signals correspond to those due to the C-3 and C-11 protons in methyl fusidate. That the third hydroxyl group is at C-6 follows from the fact that oxidation with Jones reagent afforded a triketo ester $C_{32}H_{44}O_7$, m.p. 187-188°C, identical in every respect with V. The equatorial conformation of the C-6 hydroxyl group in IVd follows from the width of the n.m.r. signal due to the proton at C-6, which indicates a diaxial coupling with a neighbouring proton. The formation of a 3,6,16-triacetate (IVe) on acetylation of IVd under mild conditions (acetic anhydride/pyridine; room temp.) is also consistent with the equatorial and therefore unhindered nature of the

*4 The C-3 proton in 3-epi-fusidic acid resonates at δ = 3.58, and the C-11 proton in 11-epi-fusidic acid at δ = 3.8o.

C-6 hydroxyl group[*5].

Metabolite C was assigned structure IVa since treatment with diazomethane gave a methyl ester, $C_{32}H_{48}O_7$, m.p. 155-156°C, identical in all respects with IVb obtained via the sequence I→III → IVa →IVb.

Metabolite B gave with diazomethane an amorphous methyl ester, $C_{32}H_{48}O_8$ (M^+ = 560), tentatively assigned structure IVf on the basis of biogenetic considerations and the following observations. The n.m.r. spectrum (cf. Table 1) reveals the presence of three secondary hydroxyl groups. One of the HO-C-H signals is similar to that due to the C-11 proton in methyl fusidate. The remaining two are overlapping, but have the same chemical shift as the CH-3 signal in methyl fusidate. Finally, the CD-curve ($\Delta\varepsilon_{336}$ = - 1.90 and $\Delta\varepsilon_{326}$ = -2.56) corresponds closely to the curves reported for 6-keto-7α-hydroxyl compounds in the helvolic acid series.

The formation of IVb and V from fusidic acid as well as from helvolic acid indicates that these antibiotics have a common framework and - in view of the good arguments previously set forth[1,7] for the location and stereochemistry of the ring B substituents in helvolic acid - supports the correctness of formula I. That I also represents the absolute configuration of helvolic acid is a consequence of the fact that II represents the absolute stereochemistry of fusidic acid[2b,3].

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*5 It is known that the C-11 hydroxyl group in the fusidic acid series is not acetylated under these conditions.[2b]

Acknowledgement. We wish to thank Professor G. Ourisson, University of Strassbourg, for the CD-curves, Dr. B.C. Das, Gif-sur-Yvette, for the mass-spectra, and Dr. U. Scheidegger, Varian AG, Zürich, for the 100 MHz n.m.r. spectra.

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