Studies on Translocation XI:

Structure-Function Relationships of the Fusidane-Type Antibiotics[1]

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SUMMARY: A group of compounds possessing the cyclopentanoperhydrophenanthrene framework common to the fusidane-type antibiotics, fusidic acid and helvolic acid, were tested for their capacity to stabilize the ribosome-translocation factor-GDP complex. The presence of a carboxyl group at C-20 and a 17,20-double bond as well as the geometry of the latter (16,21-cis) seem to be the structural features most essential to this unique activity. The nature and stereochemistry of the other functional groups are - although contributary - less critical.

The structure-function relationships of the antibiotic fusidic acid are of interest for three reasons. First of all, the antibiotic is widely used in molecular biology as a probe in investigating the mechanism of protein synthesis for it is a specific inhibitor of the translocation step of protein

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chain elongation (1-3). Secondly, fusidic acid has an unusual and perhaps unique mode of action. It indirectly inhibits the translocation-associated hydrolysis of GTP by specifically preventing the dissociation of a product-containing complex, the ribosome-translocation factor-GDP complex (4,5). Finally, fusidic acid and related compounds are the only known examples of antibiotics possessing the cyclopentanoperhydrophenanthrene ring system in common with steroids. These antibiotics are characterized by an unusual stereochemistry of their basic ring system (the fusidane ring system). In this structure the four rings are fused in a trans-syn-trans-anti-trans

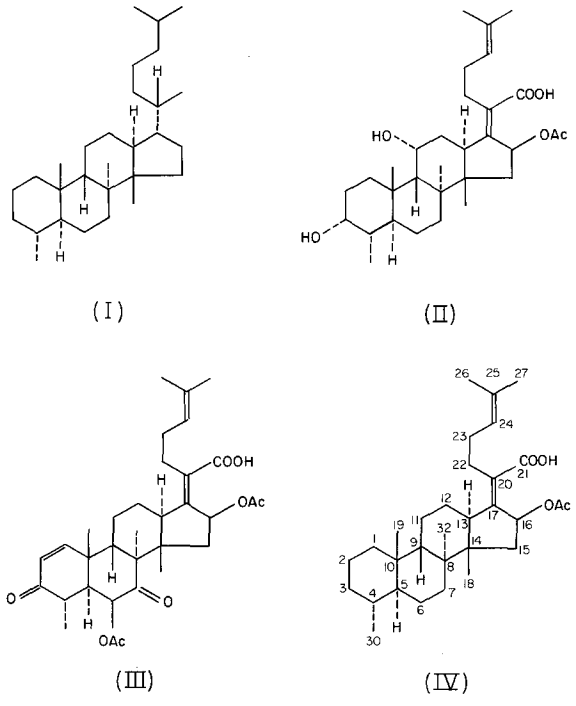

Fig. 1. The chemical structures of the fusidane-type antibiotics. The hypothetical hydrocarbon fusidane (I), fusidic acid (II), helvolic acid (III), and the structural elements common to all known fusidane-type antibiotics (IV).

manner (cf. Fig. 1, Formula I) which forces ring B into a boat conformation (6).

Eight chemically related fusidane-type antibiotics of defined structure have thus far been isolated from natural sources (6). The structures of two of these, fusidic acid (the most widely known) and helvolic acid (the most

structurally distinct from fusidic acid), are shown in Fig. 1 (Formulae II and III, respectively). The structural features common to all of the known fusidane antibiotics are also indicated in this figure (IV). These include the fusidane skeleton, a β-orientated acetoxyl group at C-16, a carboxyl group at C-20 and two double bonds ($\Delta^{17(20)}$ and Δ^{24}).

Functional activity of chemical analogues of these fusidane-type antibiotics can be readily assessed by the degree to which they promote isolation of the ribosome-G factor-^{3}H-GDP complex (7). Use of this assay is crucial to these studies because, unlike other assays which could be chosen, it requires a positive rather than a negative response and thus removes the difficulties in interpretation which might arise from secondary or unrelated inhibitory effects at high concentrations of otherwise inactive analogues. In the present report, we have analyzed a selected list of chemical analogues of fusidic acid for their ability to stabilize the ribosome-G factor-^{3}H-GDP complex and compared this with their ability to inhibit in vitro protein synthesis and bacterial growth.

RESULTS

In Table I, it will be seen that the analogues which we have examined can, on the basis of complex stabilization, be divided into three categories. Those which are approximately as active as fusidic acid or as in the case of helvolic acid, slightly more so. Secondly, those compounds which are clearly less active than fusidic acid but which exhibit significant complex stabilization at least at a high concentration. Fianlly, those analogues which, even at the highest concentrations, exhibit negligible complex stabilization (<5%). The most active compounds in terms of complex stabilization were also the most effective at low concentrations in inhibiting in vitro amino acid polymerization. However, at high concentrations, all of the analogues, even those which were clearly inactive in stabilizing complex, demonstrated significant inhibition of polymerization. In view of the concentrations involved and the detergent-like character of these compounds, this is not surprising but it is clear that this inhibitory activity does

not meaningfully discriminate among these compounds and it was, therefore,

Table I

Activities of Analogues of the Fusidane-Type Antibiotics

Compound	^{3}H-GDP Binding* Percent		Polymerization† Percent Inhibition		IC_{50}‡	
	500 µg/ml	50 µg/ml	625 µg/ml	62 µg/ml	Sensitive µg/ml	Resista
lvolic Acid	178	75	--	--	1.5	93
sidic Acid (FA)	100*	51	93	74	0.06	49
-hydroxy-FA	85	59	93	83	0.5	>100
,25-dihydro-FA (DHFA)	91	51	88	77	0.07	18
epi-DHFA	30	2	--	--	5	25
-keto-FA	24	12	71	40	0.18	--
,26,27-tris-nor-FA	19	12	61	35	--	--
keto-4-dehydro-DHFA	16	3	--	--	16	500
acetyl-FA	15	2	56	8	25	200
-epi-FA	14	-1	--	--	25	600
-epi-desacetyl-FA	11	5	46	10	13	450
-desacetyl-FA	5	5	49	0	20	56
11-diketo-FA	4	2	28	11	4	>1000
ni-DHFA	3	-1	--	--	250	280
trahydro-FA	1	3	53	4	79	130

The preparation of these compounds, which were employed in the form of their sodium salts, is described elsewhere (6,8-11).

*The formation of the ribosome-G factor-^{3}H-GDP complex and its detection by Millipore filtration was conducted as previously described (7) except that the fusidic acid derivatives were present in the reaction only. The 50 µl reaction mixture contained 25 µg E. coli A-19 ribosomes, 6.9 units G factor (12), and 13.4 pmoles ^{3}H-GTP (5,860 cpm/pmole). All of the data are expressed as percent of the enhancement of binding seen with 500 µg/ml fusidic acid. In a typical experiment, 0.21 pmoles ^{3}H-GDP were retained by Millipore filters in the absence of fusidic acid and in the presence of this antibiotic, 2.20 pmoles were retained.

†The polymerization of ^{14}C-phenylalanine in response to poly U was conducted as described elsewhere (12). The reaction was performed for 30 min at 37° in a final volume of 40 µl. The incorporation in the absence of inhibitor was corrected for an appropriate blank and corresponded to 88 pmoles phenylalanine.

‡Concentration of the compounds which reduce the growth by 50% of a fusidic acid sensitive and resistant strain of S. aureus, respectively. These determinations were performed as described elsewhere (6) and a portion of these results were presented in this report.

not used in assigning function.

Of the fifteen compounds tested, only four fell in the inactive category. The 3,11 diketo analogue showed no activity but a variety of individual modifications to the ring hydroxyls produced active analogues. Perhaps more importantly, the pattern of substitution in helvolic acid is quite different from that in fusidic acid and since both antibiotics exhibit complex stabilizing activity, it would appear that no single ring substitution, either keto or hydroxyl, is essential to this activity. A decision with respect to the functional essentiallity of the 16-acetoxyl group was more difficult. We were unable to detect any activity associated with 16-desacetyl fusidic acid but the 16-epi-desacetyl analogue consistently exhibited small but significant activity. Therefore, the 16-acetyl group per se does not appear essential to activity.

Two other compounds, both of which involved alterations in the 17,20 double bond, were inactive; lumi-24,25-dihydrofusidic acid (the 17,20 geometric isomer of 24,25-dihydrofusidic acid) and 17,20,24,25-tetrahydrofusidic acid.[2] The functional essentiallity of this region of the molecule is further indicated by the fact that a fusidic acid resistant bacterium lacked significant resistance to these compounds (Table I). One other class of compounds exhibited a complete lack of activity. Nine compounds in which the carboxyl group was altered, either by esterification or lactonization with the 16-hydroxyl group, were inactive in both complex stabilization and inhibition of polymerization when tested at saturating concentrations (data not shown). Although these compounds are very insoluble in water, when compared to fusidic acid, this result demonstrates the essentiallity of a free carboxyl group,

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[2]That this is a specific reflection of alterations in the 17,20 double bond is shown by the fact that 24,25-dihydrofusidic acid is indistinguishable from the parent molecule, both in terms of in vitro (Table I) and in vivo (Table I and Godtfredsen, 1967) activities.

even if only for the possibly trivial reason of their insolubility.

The activity exhibited by 24,25-dihydrofusidic acid and 25,26,27-trisnor-fusidic acid (lacking the three terminal carbons of the 8-carbon side chain) indicates that the terminal portion of the side chain is relatively unimportant with respect to function. Similarly, the data in Table I indicate that considerable modification of the A and B rings is also possible without complete loss of activity.

DISCUSSION

The present results indicate that no single substitution on the fusidane framework, including the 16-acetoxyl group is responsible for the unusual activity of the fusidane-type antibiotics. Similarly, the 24,25 double bond and the terminal 3-carbon atoms of the side chain are dispensible. However, the occurrence and stereochemistry of the 17,20 double bond and a free carboxyl group at C-20 appear to be essential to activity. This latter observation makes it seem unlikely that any of the commonly occurring steroids (e.g., hormones) will be found to possess "fusidic acid-like" activity.

Additionally, two practical considerations emerge from the present study. Inhibition of a process by fusidic acid is frequently taken to imply an involvement of G factor in that process. However, the high levels of the antibiotic which are frequently employed might give rise to nonspecific inhibitory effects due to the "detergent-like" character of the compound. We, therefore, suggest that under questionable circumstances, the effect of fusidic acid be compared with that of the inactive analogues tetrahydro- or lumi-fusidic acid. Secondly, 24,25-dihydrofusidic acid can be readily prepared (7) from fusidic acid by reductive hydrogenation (tritiation). Furthermore, 24,25-dihydrofusidic acid appears to be indistinguishable from the parent compound in its in vitro activity and therefore offers a convenient means of examining its interaction with G factor and the ribosome.

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