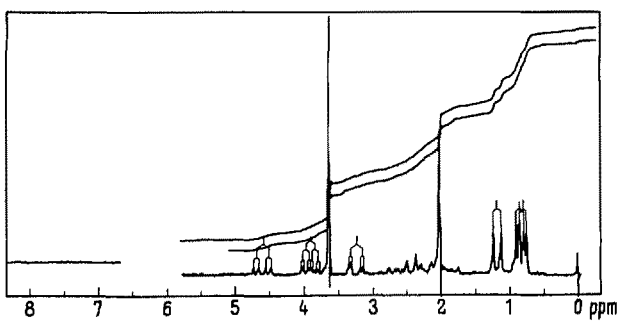
Dem Proton am C-25 entspricht eindeutig die Signalgruppe bei 4,6 ($J_1 = 4{,}5$, $J_2 = 10{,}5$), denn diese Signalgruppe ist nicht anwesend im Spektrum des Oxodicarbonsäure-dimethylesters. Da die grössere Kopplungskonstante J_2 hier wiederum einer Wechselwirkung zweier axialer Protonen entsprechen muss, folgt daraus die axiale Stellung auch dieses Protons. Dies ergibt sich auch aus der chemischen Verschiebung des Singletts der Acetoxygruppe bei 2,03, welche typisch für die äquatoriale Acetoxygruppe bei Cyclohexan- und Tetrahydropyran-Derivaten ist[10]. Die kleinere Kopplungskonstante J_1 muss dann der Wechselwirkung dieses axialen Protons mit einem äquatorialen zukommen. Letzteres muss, nachdem das Proton an C-24 axial ist, das Proton an C-26 sein. Die verschiedene Anordnung an den C-24 und C-26 ist auch aus der Änderung der NMR beim Übergang vom Acetoxy-dimethylester (Figur 1) zum Oxodimethylester (Figur 2) ersichtlich, wobei, abgesehen von der nur gering veränderten Lage des Signals der Methylgruppe an C-22, ein Methylsignal nur schwach verschoben wird, wie es für eine axiale[11], und eines stark in Richtung niederer Feldstärken verschoben wird, wie es für eine äquatoriale Methylgruppe auf Grund der Anisotropie der Carbonylgruppe zu erwarten war. Es lassen sich somit auf diese Weise die relativen Konfigurationen an C-23 bis C-26 bestimmen.



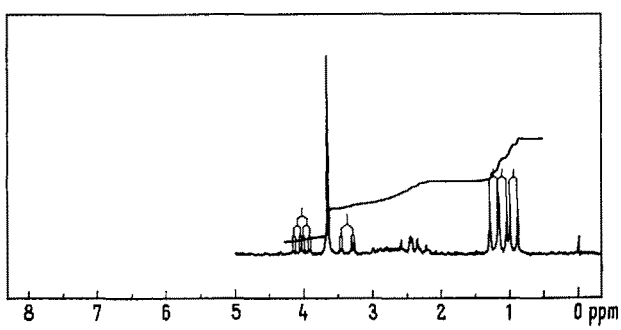


Fig. 1 (oben) und Fig. 2 (unten)

Auf Grund der auf diese Weise abgeleiteten relativen Konfigurationen lässt sich die relative Konfiguration von dreien der vier asymmetrischen Kohlenstoffe in der Tetracarbonsäure $C_{10}H_{14}O_9$ (IV) festlegen. Es bleibt noch die Konfiguration des Kohlenstoffs C-22 zu ermitteln. Die Doublette im NMR des Tetramethylesters dieser Verbindung, die den beiden geminal zum Äthersauerstoff angeordneten Protonen entsprechen, besitzen eine verschiedene Lage, woraus sich schliessen lässt, dass die beiden Hälften der Verbindung nicht die gleiche relative Konfiguration besitzen. Da die Konfigurationen an den Kohlenstoffen C-23 und C-27 gleich sind, muss demnach C-22 die umgekehrte Konfiguration von C-26 aufweisen. Dadurch ist aber auch die relative Konfiguration dieses Kohlenstoffs bestimmt.

Summary. The relative configurations of rifamycin B and related rifamycins have been determined by X-ray analysis and in part by NMR spectroscopy. The absolute configuration has been derived from the configuration of a degradation product, the dextrorotatory α,α'-dimethyl pimelic acid.

J. LEITICH, W. OPPOLZER
und V. PRELOG

Organisch-chemisches Laboratorium der Eidg. Technischen Hochschule, Zürich (Schweiz), 13. März 1964.

[10] F. W. LICHTENTALER, Chem. Ber. *96*, 2047 (1963). – M. NAKAJIMA, A. HASEGAWA und F. W. LICHTENTALER, Liebigs Ann. *669*, 75 (1963).

[11] J. P. KUTNEY, Steroids *2*, 225 (1963).

The Stereochemistry of Fusidic Acid

In a recent note[1] it was shown that fusidic acid, an antibiotic from a Fusidium strain[2], is best represented by formula *1*. Arguments have been previously[3] set forth for the configuration at positions 3, 4, 5, 10 and 16, as well as for the axial orientation of the hydroxyl group in ring C. We now report evidence showing that fusidic acid possesses the novel stereochemistry detailed in *2*.

The known tetrahydrolactone *3*[3] was oxidized with chromium(VI)-oxide to the diketolactone *4*[4] and the latter converted to *6* by reduction of the corresponding monoketal *5* with sodium borohydride. Bromination of *6* with phenyltrimethylammonium tribromide[5] gave a 2-bromo derivative *7*, readily transformed upon treatment with collidine into the Δ^1-unsaturated compound *10*. Under the influence of base, *10* was smoothly isomerized to an epimer, probably the C_{20}-epimer *11*, which was found to be in equilibrium with the saturated 1α,11α-ether *12*. In a second set of experiments, *6* was converted to the 2-hydroxymethylene derivative *8* and then,

[1] D. ARIGONI, W. VON DAEHNE, W. O. GODTFREDSEN, A. MARQUET, and A. MELERA, Exper. *19*, 521 (1963).

[2] W. O. GODTFREDSEN, S. JAHNSEN, H. LORCK, K. ROHOLT, and L. TYBRING, Nature *193*, 987 (1962).

[3] W. O. GODTFREDSEN and S. VANGEDAL, Tetrahedron *18*, 1029 (1962).

[4] Satisfactory analyses and IR spectra consistent with the given structures were obtained for all new compounds. The relevant physical data are collected in the Table.

[5] A. MARQUET, M. DVOLAITZKY, H. B. KAGAN, L. MAMLOK, C. OUANNES, and J. JACQUES, Bull. Soc. chim. France *1961*, 1822. – A. MARQUET and J. JACQUES, Bull. Soc. chim. France *1962*, 90.

through ozonolysis followed by oxidative work-up with hydrogen peroxide in acetic acid, to the δ-lactone *13*. In view of the axial position of the C_{11}-hydroxyl group (see below) and of the trans A/B ring junction in the starting material[3,6], the formation of *12* and *13* is consistent only with the presence of a 9β-hydrogen and an 11α-oxygen function.

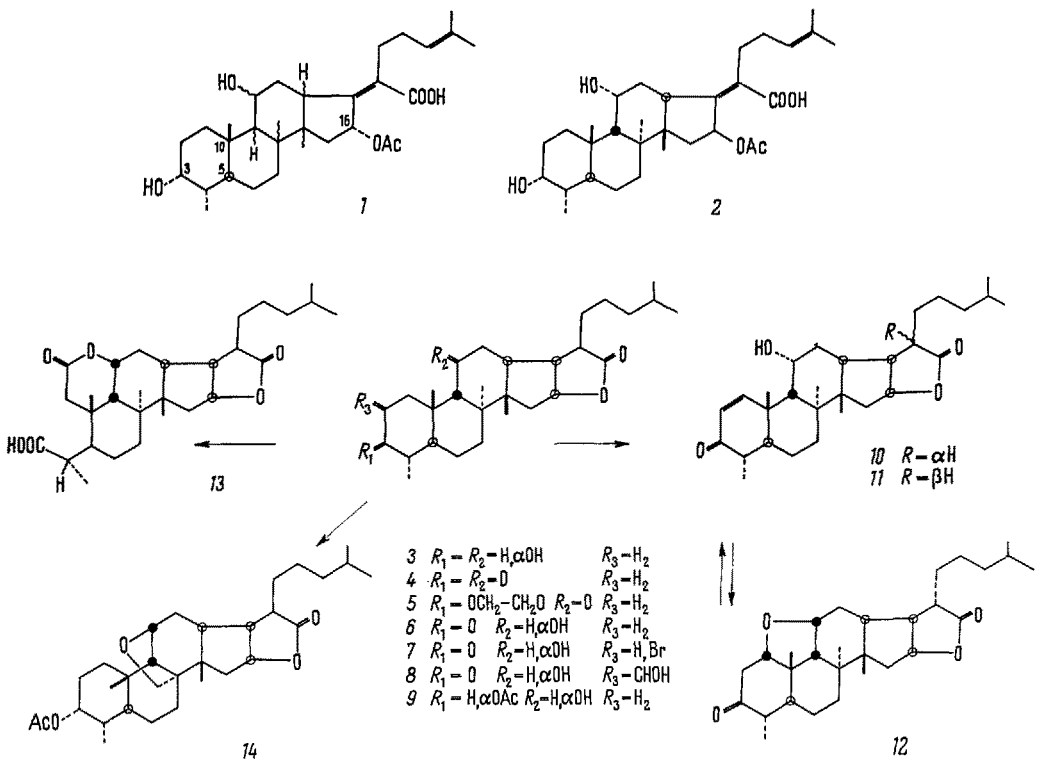
A 1,3-diaxial relationship between the C_{11}-hydroxyl group and the C_8-methyl group was first suspected on the basis of NMR measurements[7], which indicated that the signal of the latter was consistently shifted to higher fields ($\Delta\delta = 0.15$) on removal of the former. Accordingly, treatment of *9*[3] with the lead(IV)-acetate/iodine reagent[8] gave an ether containing the partial structure

$$-O-CH_2-\overset{|}{C}-$$

(NMR: AB-system with two doublets at $\delta = 3.68$ and 3.76; $J_{AB} = 10$ cps) and lacking the angular methyl group responsible for the signal at $\delta = 1.34$ in the starting material. Clearly the new compound must be formulated as in *14*; its formation provides a welcome independent check for the axial position of the C_{11}-hydroxyl group and simultaneously establishes the α position of the C_8-methyl group[9].

In order to investigate the stereochemistry of the C/D ring junction, the 16-epi mesylate *20* was prepared from the known 3,11-diketone *15*[3] via the sequence (15)→(16)→(17)→(18)→(19) and then converted with collidine to the conjugated diene *21*. Partial reduction of the latter with a Pd-catalyst to the 15,16-dihydro derivative *22* followed by treatment with sodium borohydride gave *23*, vigorous acetylation of which furnished the diacetate *24*. Ozonolysis of *24* produced the 17-ketone *25*, which was equilibrated under basic conditions with the C_{13}-epimer *26*. The CD curve of *25*[10] ($\Delta\varepsilon_{298} = -4.26$, $\Delta\varepsilon_{291} = -4.19$) was found to be antipodal to those recorded for C/D-trans 17-keto steroids[11], whereas the CD curve of the epimer *26*[10] ($\Delta\varepsilon_{310} = +0.76$, $\Delta\varepsilon_{300} = +1.17$, $\Delta\varepsilon_{291} = +1.22$) displayed the typical fine structure of a cis-hydrindan-1-one and corresponded in sign to the curve of 3-keto, 5β, A-nor-steroids[11], thus implying a 13α,14β configuration in *25* and a 13β,14β configuration in *26*.

On account of the 'abnormal' stereochemistry disclosed for the ring system of fusidic acid, it was considered mandatory to check the α configuration previously[3] assigned on the basis of molecular rotation differences to the C_{16}-acetoxyl group. A comparative study of the



[6] The steric arrangement at C_5 and C_{10} has been further verified by measurement of the CD-curve of an appropriate 3-keto derivative; cf. P. WITZ, H. HERRMANN, J. M. LEHN, and G. OURISSON, Bull. Soc. chim. France *1963*, 1101.

[7] NMR spectra were recorded on a Varian HR-100 instrument in $CDCl_3$ solutions and calibrated against tetramethylsilane. Chemical shifts are given in δ values.

[8] K. HEUSLER and J. KALVODA, Helv. chim. Acta *46*, 2020 (1963) and previous papers of this series.

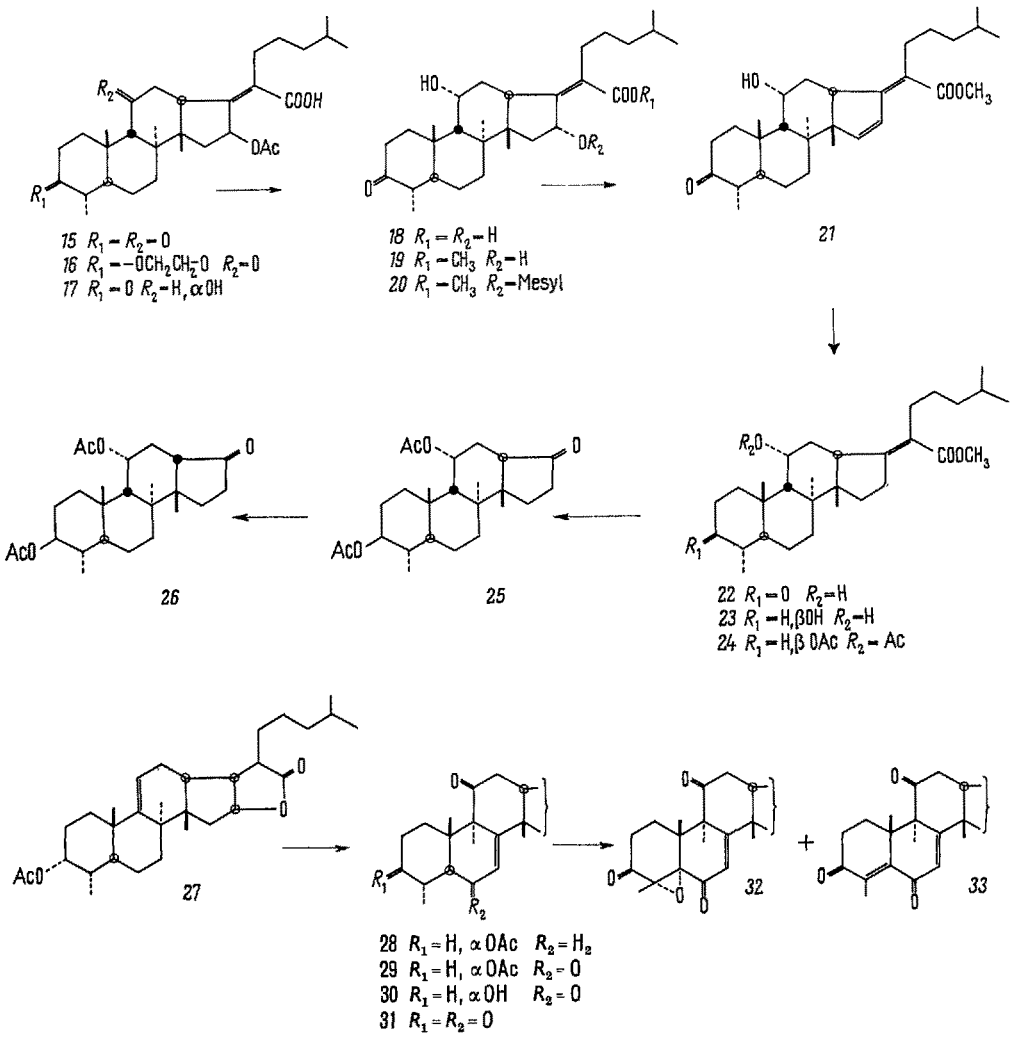
[9] It is relevant to our argument that no epimerizations have been detected so far in reactions involving the lead (IV)-acetate/iodine reagent, even with substrates where such epimerizations are known to occur readily when iodine is omitted from the mixture (private communication of Dr. J. KALVODA).

[10] Recorded in dioxane solution by courtesy of Miss H. HERRMANN and Mr. P. WITZ in the laboratory of Prof. G. OURISSON. We are indebted to Prof. OURISSON for helpful discussions and for providing unpublished reference curves.

[11] Unpublished data by G. OURISSON, P. WITZ, and H. HERRMANN.

NMR spectra of desacetyl-fusidic acid methyl ester[3] and its C_{16}-epimer[3] has now shown that the 14β-methyl group is less shielded in the former than in the latter ($\Delta\delta = 0.38$) whereas the reverse is true for the α-hydrogen at C_{13} ($\Delta\delta = 0.22$). This observation strongly supports the β configuration for the acetoxyl group in fusidic acid. When the findings of MAZUR et al.[12] in the steroid field are taken into account, configurations at C_{17} and C_{20} can be assigned as in *3* for all derivatives obtained on hydrogenation of $\Delta^{17,20}$-16β-lactones in this series.

lated intermediary *28*, to the α,β-unsaturated ketone *29*. The structure of *29* was unambiguously established by deacetylation to *30*, subsequently converted to the corresponding ketone *31* with chromium(VI)-oxide. Air oxidation of the latter in methanolic potassium hydroxide solution yielded the 4,5-epoxide *32* and the yellow dienedione *33*, which displayed the expected characteristic UV absorption. The appearance of a vinylic methyl group in *33* ($\delta = 2.10$) substantiates independently the presence of a methyl group at C_4 in fusidic acid.

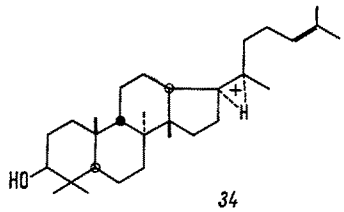


It will be noted that the *trans*-syn-*trans* arrangement of rings A, B and C in fusidic acid forces ring B into a boat conformation and thus affords a basis for understanding the previously[3] observed base-catalyzed isomerization of compounds in which C_{11} is a carbonyl group. The strain imposed on the ring system by the anti arrangement of the methyl groups at C_8 and C_{10} is reflected in the abnormal chemical behaviour of some derivatives of fusidic acid. Thus, the $\Delta^{9,11}$-compound *27*, readily available by thionylchloride/pyridine dehydration of the known *9*[3], underwent methyl group migration upon oxidation with chromium(VI)-oxide in acetic acid, leading, *via* the iso-

Finally we would like to point out the remarkable steric similarity now emerging between fusidic acid (*2*), and the hypothetic carbonium ion *34*, postulated in 1955 on theoretical grounds[13] as a non-rearranged cationic intermediary in the biological formation of lanosterol from

[12] Y. MAZUR, N. DANIELI, and F. SONDHEIMER, J. Amer. chem. Soc. *82*, 5889 (1960).

[13] A. ESCHENMOSER, L. RUZICKA, O. JEGER, and D. ARIGONI, Helv. chim. Acta *38*, 1850 (1955).



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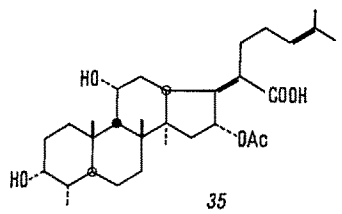

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squalene. The production of fusidic acid may accordingly be considered the result of an aberrant lanosterol biosynthesis. The ability of *Fusidium coccineum* to fold squalene in the manner required by such a scheme is borne out by the identification of ergosterol as one of the components from the mycelial lipid fraction.

Since the appearance of our last note[1], BUCOURT et al.[14] have independently confirmed the location of the hydroxyl group in ring C of fusidic acid and have advanced for the antibiotic an alternative stereochemical expression, *35*, which is incompatible with some of the results reported in the present work. We believe that the data reported by these workers, when viewed in proper perspective, constitute in fact corroborative evidence for the correctness of formula *2*.

Compound		m.p.	UV spectrum	
			λ_{max} (mμ)	ε
4	$C_{29}H_{44}O_4$	208–210°		
5	$C_{31}H_{48}O_5$	223–225°		
6	$C_{29}H_{46}O_4$	192–193°		
7	$C_{29}H_{45}O_4Br$	155–156°		
8	$C_{30}H_{46}O_5$	160–161°	292	7,600
10	$C_{29}H_{44}O_4$	211–214°	238	9,200
11	$C_{29}H_{44}O_4$	209–212°	238	6,850
12	$C_{29}H_{44}O_4$	192–194°		
13	$C_{29}H_{44}O_6$	225–226°		
14	$C_{31}H_{48}O_5$	157-158°		
16	$C_{33}H_{52}O_7$	186–187°	221	8,350
17	$C_{31}H_{48}O_6$	189–190°	220	8,150
18	$C_{29}H_{46}O_5$	183–184°	228	10,750
19	$C_{30}H_{48}O_5$	178–179°	231	10,650
21	$C_{30}H_{46}O_4$	111–112°	272	18,950
22	$C_{30}H_{48}O_4$	amorphous	234	10,650
23	$C_{30}H_{50}O_4H_2O$	94–98°	234	12,150
24	$C_{34}H_{54}O_6$	120–121°	234	12,100
25	$C_{25}H_{38}O_5$	178–179°		
26	$C_{25}H_{38}O_5$	180–181°		
27	$C_{31}H_{48}O_4$	190–191°		
28	$C_{31}H_{46}O_5$	206–207°		
29	$C_{31}H_{44}O_6$	283–286°	238	10,000
30	$C_{29}H_{42}O_5$	257–259°		
31	$C_{29}H_{40}O_5$	204–205°	238	9,600
32	$C_{29}H_{38}O_6$	225–227°	250	11,500
33	$C_{29}H_{38}O_5$	170–174°	289	12,000

Zusammenfassung. Neue chemische Umsetzungen der Fusidinsäure, sowie physikalische Messungen der dabei erhaltenen Abbauprodukte erlauben, zusammen mit früheren Ergebnissen[1,3], dem Antibiotikum die in Formel *2* wiedergegebene Stereochemie zuzuteilen. Ein möglicher Zusammenhang zwischen den Biogenesen von Fusidinsäure und Lanosterin wird kurz diskutiert.

D. ARIGONI*, W. VON DAEHNE**,
W. O. GODTFREDSEN**, A. MELERA***,
and S. VANGEDAL**

Organisch-chemisches Laboratorium der Eidg. Technischen Hochschule, Zürich (Switzerland), Leo Pharmaceutical Products, Copenhagen (Denmark)**, and Varian AG. Research Laboratories, Zürich (Switzerland)***,*
March 23, 1964.

[14] R. BUCOURT, M. LEGRAND, M. VIGNAU, J. TESSIER, and V. DELAROFF, C.R. Acad. Sci. *257*, 2679 (1963). We are indebted to Prof. VELLUZ for sending to us a copy of the manuscript prior to its publication.

Unsaponifiable Constituents in *Spirographis spallanzani*

What we know about the distribution of the Ubiquinones in the animal kingdom can be summarized as follows:

The higher homologues, Ubiquinones 50 and 45, have been demonstrated in mammals[1–3] and fishes[4]. The lower homologues with 6 to 8 isoprenic residues in the lateral chain, are typical of microorganisms[5,6]. Some of these, however, have Ubiquinone 50[7]. Among the insects, the blowfly larva (*Calliphora erythrocephala*) has been found to contain Ubiquinones 50, 45 and 40[8], whereas the housefly (*Musca domestica*) and the cabbage butterfly (*Pieris rapae*) have only Ubiquinone 45[6]. In the earthworm (*Lumbricus terrestris*) a quinone has been tentatively identified as Ubiquinone 50 by LESTER and CRANE[6]. Recently Ubiquinone 50 has been extracted and crystallized from sea urchin sperm[9]. The same homologue is present in the unfertilized eggs of *Paracentrotus lividus*[10].

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[3] N. F. CUNNINGHAM and R. A. MORTON, Biochem. J. *72*, 92 (1959).
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[10] G. CASERTA, Rend. Accad. Naz. Lincei, in press.