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THE STEREOCHEMISTRY OF FUSIDIC ACID

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Abstract—Evidence is presented which shows that the antibiotic fusidic acid possesses the relative and absolute stereochemistry depicted in 2. The biogenetic implications of this result are briefly discussed.

PREVIOUS chemical evidence with fusidic acid, an antibiotic formed by a strain of Fusidium coccineum,[1] has been summarized in terms of formula 1.[2*] In the original paper[2] the geometry of the 17–20-double bond was established and it could be shown that the A/B ring junction corresponds stereochemically to a normal *trans*-fused steroid. The hydroxyl group at C-3 as well as the secondary methyl group at C-4 were assigned the α-configuration and arguments were advanced for the axial orientation of the hydroxyl group in ring C. Finally, an α-orientation was proposed for the acetoxyl group at C-16, whereas the stereochemistry at the remaining centers (C-8, C-9, C-13 and C-14) was left unsettled.

Fusidic acid is characterized by an unusual substitution pattern of the fundamental ring system and this raises some intriguing stereochemical problems in the light of current theories on the biogenesis of tetracyclic triterpenes and related compounds.[4,5] This accentuated our inherent interest in elucidating the full stereochemistry of the antibiotic and in the following our approach to the problem will be described. During this work reactions were encountered which made it necessary to change the previous location of the hydroxyl group in ring C from C-12 to C-11 and to invert the orientation tentatively assigned to the 16-acetoxyl group. Our experiments have led to the establishment of the complete structure as depicted in formula 2. Some of the results reported in this paper have been the subject of preliminary communications.[6a,b,c]

Since the location of the hydroxyl group in ring C is essential for the derivation of the stereochemistry of fusidic acid it seems convenient to discuss this point in the first place.

The original assignment of a hydroxyl group at C-12 was based upon the formation of an ene-dione on base-catalysed elimination of acetic acid from the compound

* Lynch *et al.*[3] later independently confirmed the composition of the compound by mass spectrometry.

[1] W. O. Godtfredsen, S. Jahnsen, H. Lorck, K. Roholt and L. Tybring, *Nature, Lond.* **193**, 987 (1962).
[2] W. O. Godtfredsen and S. Vangedal, *Tetrahedron* **18**, 1029 (1962).
[3] J. F. Lynch, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Experientia* **19**, 211 (1963).
[4] A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta* **38**, 1890 (1955).
[5] L. Ruzicka, *Pure and Applied Chem.* **6**, 493 (1963).
[6a] D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Marquet and A. Melera, *Experientia* **19**, 521 (1963). [b] D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Melera and S. Vangedal, *Experientia* **20**, 344 (1964); [c] D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Melera and S. Vangedal, *Int. Symposium on the Chemistry of Natural Products.* Kyoto, April (1964).

obtained through ozonolysis of dihydrofusidic acid methyl ester, a process supposed to proceed according to the scheme **3** → **5**. A first suspicion that this interpretation might be wrong came from NMR studies. Thus it could be shown by the double irradiation technique[7] that in the NMR spectrum of dihydrofusidic acid methyl ester (**8**) there is no evidence for spin-spin interaction between the proton at C-13 ($\delta = 3{\cdot}02$) and the proton at the carbon atom in ring C bearing the hydroxyl group ($\delta = 4{\cdot}40$), as should have been the case if the hydroxyl group has been indeed located at C-12. Even more compelling evidence against the presence of a hydroxyl group at C-12 was obtained on dehydration of the known dihydrolactone (**6**)[2] with thionyl chloride in pyridine, when a compound **7** resulted, which contained a new trisubstituted double bond (NMR: 1 olefinic proton, $\delta = 5{\cdot}5$) while retaining the chromophore of the starting material.*

These experiments made a reconsideration of the ene-dione formation mandatory, and a new interpretation based upon the assumption of an 11-hydroxyl group and thus consistent with the new observations was formulated (cf. scheme **8** → **10**). Clear-cut proof of the correctness of the new location of the hydroxyl group was eventually provided by selenium dioxide dehydrogenation of the ketone **11**, available from the chromium (VI) oxide oxidation of **6**,[2] to a dehydroderivative, the UV-spectrum of which ($\lambda_{max} = 280$ mμ, $\log \varepsilon = 4{\cdot}24$) is consistent with the presence of the extended chromophore shown in **12**. A compound with a very similar chromophore was also obtained from the 3-O-acetylderivative of **8** in a parallel sequence.

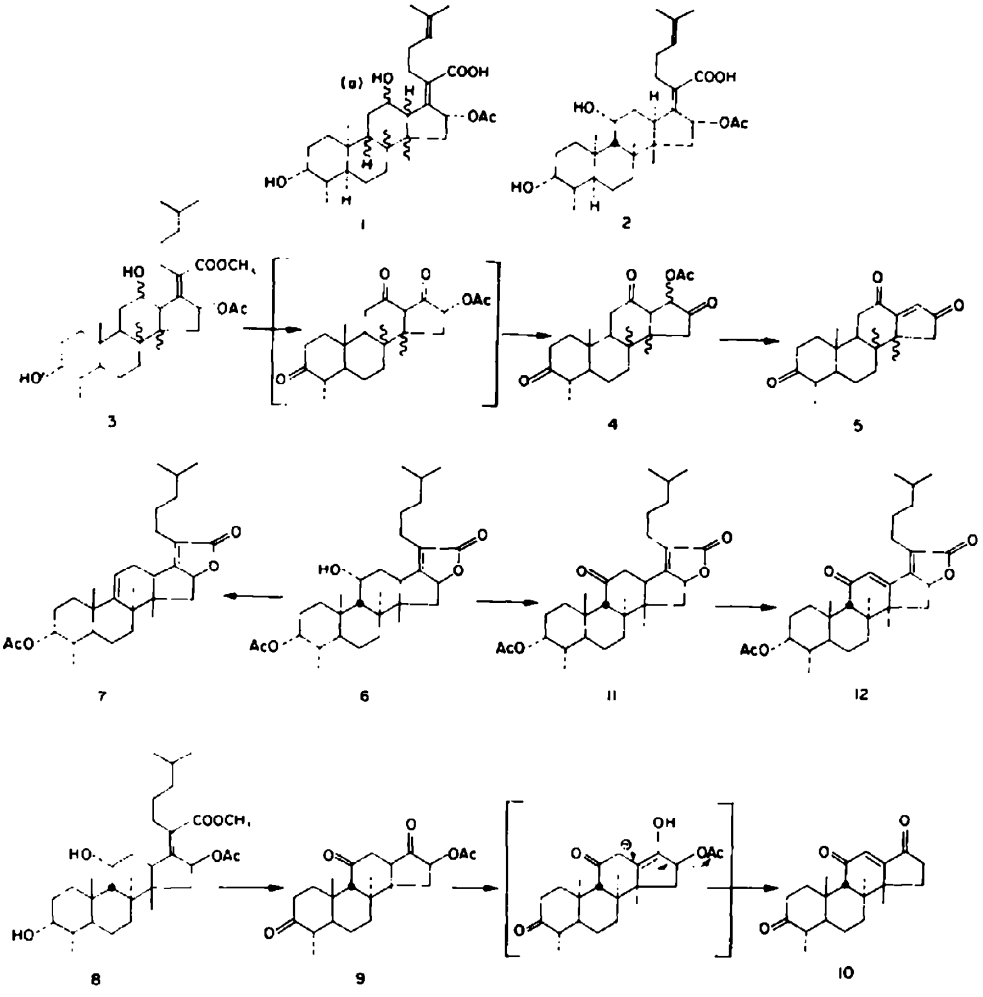
The new location of the hydroxyl group invalidates the argument previously[2] set forth for the absence of a methyl group at C-9, since this was based upon the formation of a supposed $\Delta^{9,11}$-12-ketone, which in view of the new information clearly must have structure **13**. However, the presence of a hydrogen atom at C-9 is *now* required by the formation of the $\Delta^{9,11}$-unsaturated compound **7** as well as by the previously reported[2] base catalyzed epimerizations of derivatives of fusidic acid having a carbonyl group in ring C. The revision described above opened up new possibilities for attacking the problem of the stereochemistry at C-8, C-9, and C-11, and in the following these problems will be discussed beginning with C-9 and C-11.

That the stereochemistry at C-9 is "abnormal" was first suggested by the unexpected epimerization of fusidic acid derivatives bearing a keto group at C-11. To study this effect in greater detail a set of C-9-epimeric 11-ketones was prepared as follows: 3-O-acetyl-tetrahydrofusidic acid[2] (**14**) was oxidized to the corresponding 11-ketone **15** which upon drastic alkaline hydrolysis afforded a mixture of the two 9-epimeric ketones **16a** and **17a**. The former was reconverted to **15** on acetylation and has therefore the same stereochemistry at all centers. Epimerization at centers other that C-9 (e.g. C-3, C-16 and C-20) in the formation of **17a** is ruled out by the fact that oxydation of **16a** and **17a** afforded two different ketones, **18** resp. **19**, together with the observation that alkaline hydrolysis of **14** under identical experimental conditions gave as the sole product **20**, which on oxydation was transformed into **18**. As judged by thin layer chromatography, the isomer with the unnatural configuration at C-9 is largely predominating at equilibrium. In a first approximation the establishment of such an

* In a following paper[8] proof will be presented that no rearrangement is involved during the dehydration of **6** to **7**.

[7] R. Freeman and D. Whiffen, *Mol. phys.* **4**, 321 (1961).

[8] D. Arigoni, W. O. Godtfredsen, A. Melera and S. Vangedal, *Tetrahedron*, to be published.



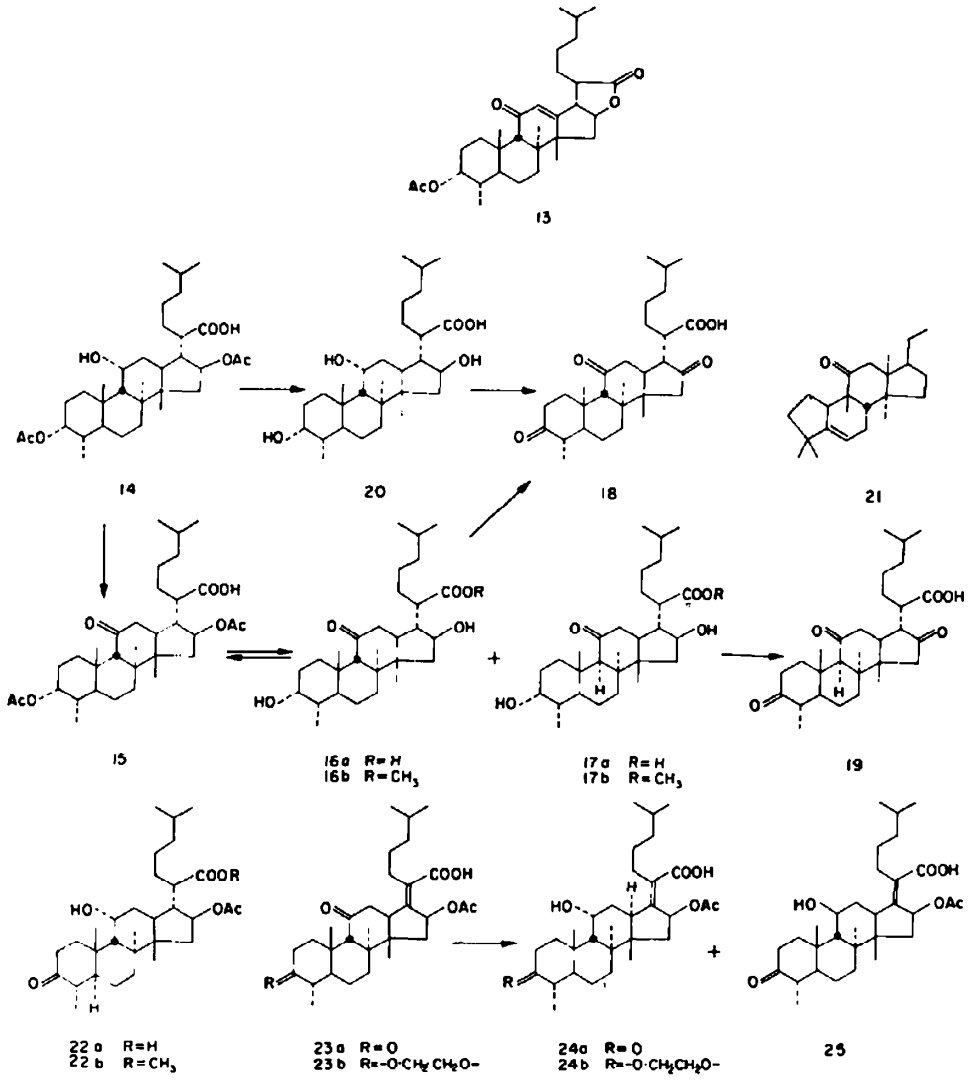
equilibrium can be taken to imply a *syn*-relationship between the methyl group at C-10 and the hydrogen atom at C-9 in fusidic acid; the actual configuration of the methyl group at C-8 is irrelevant to the argument, since both a *trans-syn-cis* and a *trans-syn-trans* arrangement of the first three rings would be expected to be thermodynamically less stable than their *trans-anti-trans* resp. *trans-anti-cis* counterparts.[9]

Corroborative information was provided by the CD-curves of the methyl esters **16b** and **17b**, which are shown in Fig. 1. It will be noted that inversion at C-9 involves a rather drastic change in the amplitude and that both curves differ in sign from those reported for steroidal 9α,11-ketones.[10] On the other hand the CD-curve of the 9-epi-compound **17b** with its moderately strong amplitude is reminiscent of those reported[11]

[9] W. S. Johnson, *Experientia* 7, 315 (1951).

[10] L. Velluz, M. Legrand, and M. Grosjean, *Optical Circular Dichroism*. Verlag Chemie GMBH, Weinheim (1965).

[11] G. Biglino, J. M. Lehn, and G. Ourisson, *Tetrahedron Letters*, 1651 (1963).



for 11-keto-cucurbitacin derivatives (e.g. **21**), to which, however, it bears an antipodal relationship.

Since the bulk of the available evidence thus strongly favoured a β-orientation of the hydrogen atom at C-9 the next step was to provide a chemical proof of this. The problem can be approached in the following manner: Inspection of Dreiding models shows that in a compound in which rings A and B are *trans*-fused and which contains an axial hydroxyl group at C-11 a ring closure between C-1 and this hydroxyl group with formation of an unstrained 1,11-ether is possible only if the hydrogen atom at C-9 is on the same side (and the axial 11-hydroxyl group consequently on the opposite side) as the angular methyl group at C-10. Inversely, the formation of such an 1,11-ether would unambiguously prove the orientation of the hydrogen atom at C-9, provided that the two requirements mentioned (A/B *trans*; 11-OH axial) are fulfilled.

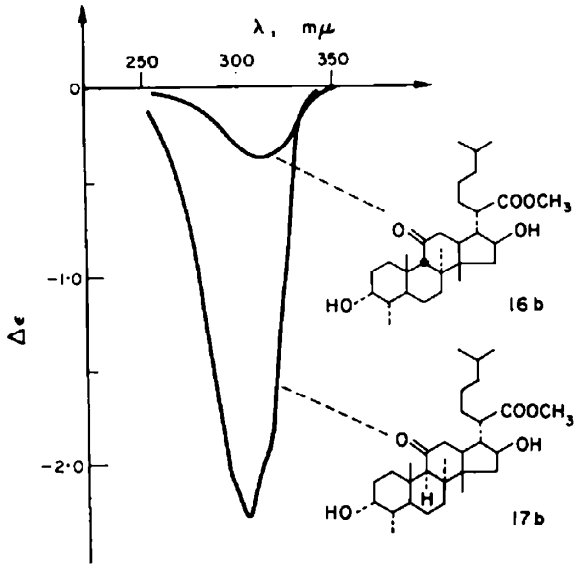


FIG. 1

The relative and absolute stereochemistry of the A/B junction in fusidic acid were originally deduced from the ORD-curve of the 3-monoketone **22a**, which shows a positive Cotton effect,[2] and has now been confirmed by the CD-curve of the related methyl ester **22b** (Fig. 2), which corresponds in sign and shape to those of 3-keto-A/B-*trans*-steroids.[10] Finally, as shown below, bromination of the 3-keto derivative **29** leads exclusively to the 2-bromo derivative **30** and this again is consistent with a *trans* A/B-junction only.

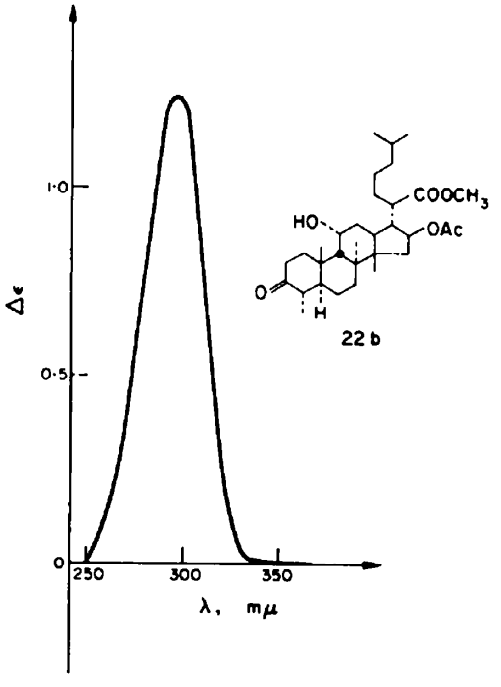


FIG. 2

Several lines of evidence support the axial orientation of the hydroxyl group at C-11: (a) the original assignment[2] was based on the observation that sodium borohydride reduction of 11-keto derivatives of fusidic acid, in which the carbonyl group is known to be hindered, leads mainly to compounds with a natural orientation of the newly produced hydroxyl group; (b) in the 11-*epi* compound **25**, available in ca. 3% yield as a byproduct of the reduction of **23b** with sodium borohydride, the OH-group is readily acetylated and therefore less hindered than in the main product of the reduction, **24a**, which has a natural configuration at C-11 and is known to resist acetylation under normal conditions*; (c) the width of the NMR signal corresponding to the proton at C-11 in many derivatives of fusidic acid averages 7 c/s; this rules out the operation of the axial-axial spin-spin interaction which would be required by the presence of an equatorially orientated hydroxyl group; (d) a 1,3 diaxial relationship between the 11-hydroxyl group and one of the t-methyl groups is strongly suggested by the fact that the NMR signal of the latter is consistently shifted to higher fields on oxydation of the former, the average shift amounting to $\Delta\delta = 0{\cdot}15$. In addition, unambiguous chemical proof of the axial orientation of this hydroxyl group will be provided in the sequel.

Having thus verified that the desired requirements are in fact fulfilled the problem of establishing an 1,11-ether could now be attacked. The 3-keto-11-hydroxy derivative **29**, easily available from the known[2] tetrahydrolactone **26** by the sequence **26** → **27** → **28** → **29** served as the key intermediate. To check that the 11-hydroxyl group of **29** still has the natural orientation a pure sample was catalytically hydrogenated to **26**. Bromination of **29** with phenyltrimethylammonium tribromide[12] gave the 2-α-bromo derivative **30**. The location of the bromine atom follows from the NMR-spectrum (signal at $\delta = 4{\cdot}9$ corresponding to the proton at C-2) and its orientation from the IR-spectrum, which showed a displacement of the keto band to higher frequencies ($\Delta\nu = 20\ cm^{-1}$), when compared with the starting material, and contained a band at 750 cm^{-1} not present in the latter. Both of these features are characteristic for equatorial bromoketones.[13a,b] Dehydrobromination of **30** with collidine afforded smoothly the Δ^1-unsaturated compound **31**.† The chemical shifts of the two doublets due to the protons at C-1 and C-2 in the NMR spectrum of **31** ($\delta = 7{\cdot}66$ and $5{\cdot}88$ resp.) as well as the wave length of the UV-maximum (238 mμ) match very well with the values reported for Δ^1-unsaturated 3-keto steroids containing a hydroxyl group at C-11.[15]

The critical experiment could now be carried out. Under the influence of strong

* Both epimers **24a** and **25** gave the same diketone, **23a**, on oxidation.

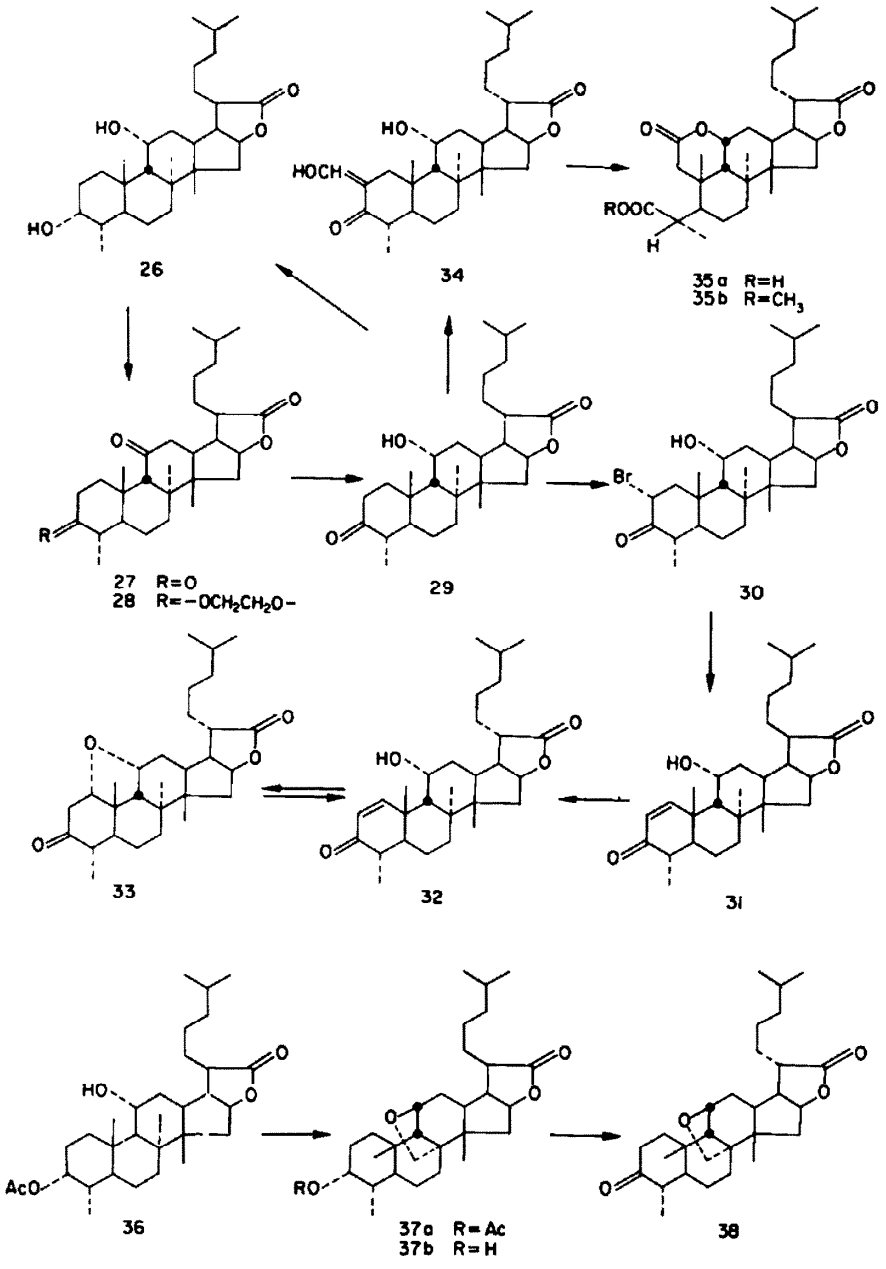
† If the dehydrobromination of **30** is performed with LiCl in dimethylformamide a Δ^1-unsaturated isomer (λ_{max} 256 mμ; no olefinic protons in the NMR spectrum) is obtained instead. The outcome of both types of dehydrobrominations has a close parallel in the chemistry of many 2-bromo-3-keto-A/B-*trans* steroids[14] and can be taken as additional evidence for the relative configurations at C-5 and C-10.

[13a] A. Marquet, M. Dvolaitzky, H. B. Kagan, L. Mamlok, C. Ouannes, and J. Jacques, *Bull. Soc. Chim. Fr.* 1822 (1961); [b] A. Marquet and J. Jacques, *Ibid.* 90 (1962).

[13a] R. N. Jones, D. A. Ramsay, F. Herling, and K. Dobriner, *J. Amer. Chem. Soc.* **74**, 2828 (1952); [b] E. G. Cummings and J. E. Page, *J. Chem. Soc.* 3847 (1957).

[14] B. J. Magerlein, *J. Org. Chem.* **24**, 1564 (1959).

[15] J. Kalvoda, G. Anner, D. Arigoni, K. Heusler, H. Immer, O. Jeger, M. Lj. Mihailovic, K. Schaffner, and A. Wettstein, *Helv. Chim. Acta* **44**, 186 (1961).



base **31** was isomerized to its C-20-epimer **32*** simultaneously with the establishment of an equilibrium between **32** and the 1α,11α-ether **33**. The ring closure was reflected in the UV spectrum as a drop in extinction and in the thin layer chromatogram as the

* Isomerizations at C-20 under the same conditions were observed with many other derivatives of fusidic acid containing the 16β,17β-lactone ring (*vide infra*). Similar isomerizations in the steroid field have been reported by Mazur *et al.*[16]

[16] Y. Mazur, N. Danieli, and F. Sondheimer, *J. Amer. Chem. Soc.* **82**, 5889 (1960).

appearance of a new, less polar spot. No traces of **31** could be detected in the mixture. On acidification the equilibrium was frozen and **32** and **33** could now be separated by chromatography. The structure of **33** is well substantiated by its spectroscopic properties: the UV spectrum lacks the characteristic absorption of the starting material, the IR spectrum shows the disappearance of the hydroxyl group and the presence of a saturated six-membered ring ketone ($\nu_{max} = 1715\ cm^{-1}$); finally, the NMR-spectrum of **33** contains triplets at $\delta = 3{\cdot}49$ and $4{\cdot}00$ which can be assigned to the protons at C-11 and C-1 respectively.

On the basis of UV measurements the amount of **33** in the equilibrium (EtOH) was estimated to 60%. It is noteworthy in this connection that in steroids with the normal *anti*-arrangement between C-9 and C-10 an equilibrium between 11-hydroxy-Δ^1-3-ketones and the corresponding 1α,11α-ethers has not been detected, such ethers being quantitatively isomerized to the Δ^1-unsaturated compounds on treatment with base.[15] A significant factor in the deviating behaviour of the two series may lie in the fact that in the steroid case the equatorial hydroxyl group at C-11 is nearly in the plane of the Δ^1-double bond, whereas in the fusidic acid derivatives **31** and **32** the critical hydroxyl group is located below the plane of the double bond.

A second set of experiments, which independently disclosed the configuration at C-9 was the preparation of the lactone **35a**. Here again the starting material was the 3-ketone **29**, which was converted in the usual way to the hydroxymethylene derivative **34** with simultaneous epimerization at C-20. Ozonolysis of **34** followed by oxydative cleavage of the ozonide with hydrogen peroxide according to Weisenborn *et al.*[17] afforded a diacid, which spontaneously lactonized to **35a**, further characterized as the methyl ester **35b**. On the basis of the established axial orientation of the 11-hydroxyl group this spontaneous lactonization requires a *syn*-arrangement of the methyl group at C-10 and the hydrogen atom at C-9, as visualized by inspection of Dreiding models. The α-configuration of the hydroxyl group follows then directly from its axial character and from the known β-configuration of the angular methyl group at C-10.

Having thus established the stereochemistry at C-9 and C-11 we next directed our attention against the methyl group at C-8. As pointed out above, NMR-studies suggested a 1,3-diaxial relationship between the 11-hydroxyl group and a tertiary methyl group, since the signal of one of these was consistently shifted to higher fields on oxydation of the hydroxyl group. In view of the now established α-orientation of the latter, the β-orientated methyl group at C-10 can be left out of consideration and the shifted signal attributed to the C-8 methyl group, which therefore must also be α-orientated. As a consequence of its location this methyl group should be susceptible to attack by means of the lead (IV)-acetate–iodine reagent.[18] Actually, treatment of **36**, $C_{31}H_{50}O_5$, with this reagent afforded a mixture from which an ether $C_{31}H_{48}O_5$ (IR spectrum: no hydroxyl band, no new carbonyl band) could be isolated in ca. 30% yield.* In the NMR spectrum of this material the signal at $\delta = 1{\cdot}34$ corresponding to the C-8 methyl group of the starting material is lacking, and an AB-system with two

* Chromatography of the mother liquors afforded a second compound, which according to the NMR spectrum ($\delta = 9{\cdot}55$, 1 H) is an aldehyde. The exact structure of this compound has not been determined; however, it is clear that its formation must involve the irreversible fission of the C-9–C-11 bond.

[17] F. L. Weisenborn and H. E. Applegate, *J. Amer. Chem. Soc.* **81**, 1960 (1959).

[18] K. Heusler, J. Kalvoda, Ch. Meystre, G. Anner and A. Wettstein, *Helv. Chim. Acta* **45**, 2161 (1962).

doublets at $\delta = 3{\cdot}68$ and $\delta = 3{\cdot}76$ ($J_{AB} = 10$ c/s) indicates the presence of the grouping —O—CH_2—C(—)—, the magnitude of the coupling constant being consistent with the expected formation of a five-membered ether ring.[19] For these reasons the new compound can be assigned formula **37a**. The ether was further characterized by alkaline hydrolysis to **37b**, followed by chromium (VI)-oxide oxydation to **38**. The spectroscopic properties of these two compounds are also consistent with the given structures.

It is essential to our argumentation that epimerizations have never been observed 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 reaction mixture.[20] The formation of the ether **37a** therefore vindicates both the axial orientation of the hydroxyl group at C-11 and its cis-relationship to the angular methyl group at C-8. Incidentally it may be noted that in the hypothetic iodohydrin intermediate generated in the oxydation of **36** the 11α-oxygen atom, the carbon atom at C-8 and the iodine atom can easily attain the linear orientation which is a prerequisite for ether formation;[20] accordingly, no products of a double substitution at the C-8 methyl group (e.g. hemiacetals or lactones) could be detected.

We next turned to the problem of the stereochemistry at the C/D ring junction. Our first approach involved the preparation of a pair of C-13 epimeric 17-monoketones carrying no other substituents in ring D. To this purpose the known diketone **23a**[2] was converted by the dioxolane method[21] into the corresponding 3-ethylene ketal **23b** and the crude product reduced with sodium borohydride to **24b**, in which C-11 has the natural configuration.

Acid catalysed cleavage of the ketal group gave **24a** which on hydrolysis with sodium bicarbonate in aqueous ethanol afforded the expected 16-*epi* deacetylated compound **39a**.* The corresponding methyl ester **39b** could be mesylated selectively at C-16 and the mesylate **40** on treatment with boiling collidine converted into the doubly unsaturated ester **41**, the UV-spectrum of which ($\lambda_{max} = 272$ mμ, $\log \varepsilon = 4{\cdot}28$) is consistent with the structure shown. The disubstituted double bond in **41** was then selectively hydrogenated in the presence of a Pd catalyst and the 3-keto group in the compound **42** thus obtained reduced with sodium borohydride with formation of the 3β-hydroxy compound **43a**. This on acetylation with an acetic acid–acetic anhydride–*p*-toluenesulfonic acid mixture afforded the corresponding 3,11-diacetate **43b**. Ozonolysis of the latter followed by cleavage of the ozonide with zinc in acetic acid gave a single product formulated as **44**. On passing an ethereal solution of this compound through a column of basic alumina an equilibrium mixture was produced from which a pure sample of the C-13 epimer **45** could be secured by fractional crystallization. A similar equilibration was observed under acidic conditions. The fact that no trace of the epimer **45** was present in the crude ozonolysis mixture (as evidenced by thin layer chromatography) can therefore be taken as evidence for the natural configuration at C-13 in **44**.

* For a similar epimerization at C-16 cf.[2] That the hydrolysis of **24a** indeed involved inversion at C-16 was shown by the fact that acetylation of **39a** led to an isomer of **24a**.

[19] J. F. Bagli, P. F. Morand and R. Gaudry, *J. Org. Chem.* **28**, 1207 (1963).
[20] K. Heusler and J. Kalvoda, *Angew. Chem.* **76**, 518 (1964).
[21] G. Rosenkranz, M. Velasco and F. Sondheimer, *J. Amer. Chem. Soc.* **76**, 5024 (1954).

The CD-curves of **44** and **45** are shown in Fig. 3. The curve of **44** is antipodal to those recorded for C/D-*trans*-17-keto steroids,[10] whereas the curve of its C-13 epimer **45** displays the typical fine structure of a *cis*-hydrindan-1-one and corresponds in sign to the curves of 3-keto, 5β, A-*nor*-steroids.[22] This strongly indicates a 13α,14β-configuration in **44** and therefore in fusidic acid itself. The equilibrium between **44** and **45**,

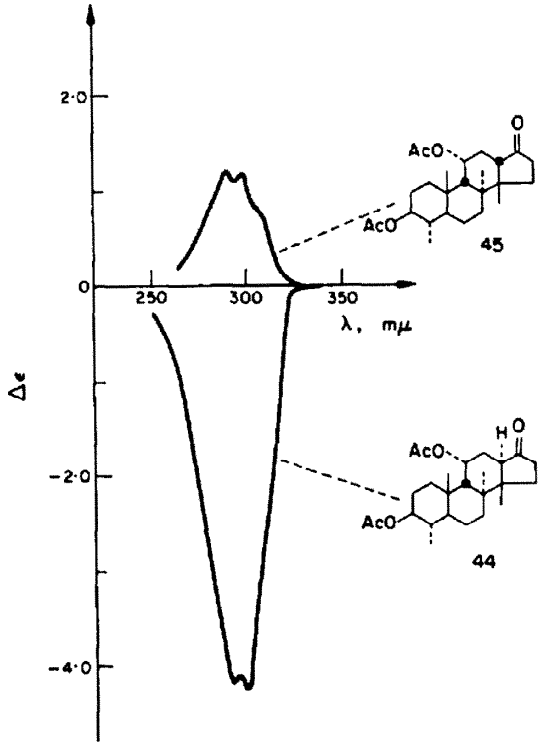


FIG. 3

which was studied optically in acetic acid containing a trace of hydrochloric acid, deserves a special comment. It was found that under these conditions the *trans*-isomer **44** is slightly predominating the ratio between the *trans*- and *cis*-isomer being **55**:**45**. This is in contrast to the large *cis*-predominance (>97%) usually observed with steroidal hydrindan-1-ones[23,24] as well as to the moderate *cis*-predominance (59%) detected for similar compounds in the dammarane series.[24] In the latter case the relative destabilization of the *cis*-isomer has been ascribed to interactions between C-17 resp. C-16 and the angular methyl group at C-8 (cf. Fig. 4a). Similar interactions can be recognized in the fusidic acid series in the *cis*-isomer **45** (cf. Fig. 4b); however, the additional interaction with C-17 introduced in this case by the presence of the axial C-11 acetoxyl group causes a further shift of the equilibrium toward the *trans*-form, which now in fact becomes predominating. On this basis predominance of the *cis*-form would be expected again for an analogous pair of compounds having a

[23] Unpublished data by P. Witz, H. Hermann and G. Ourisson.
[23] N. L. Allinger, R. B. Hermann and C. Djerassi, *J. Org. Chem.* **25**, 922 (1960).
[24] J. F. Biellmann, D. Francetic and G. Ourisson, *Tetrahedron Letters* No. 18, 4 (1960).

trigonal arrangement of C-11, a prediction which has been verified with the C-13 epimeric triketones **47** and **48** mentioned below.

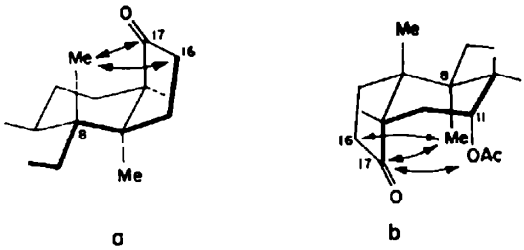


FIG. 4

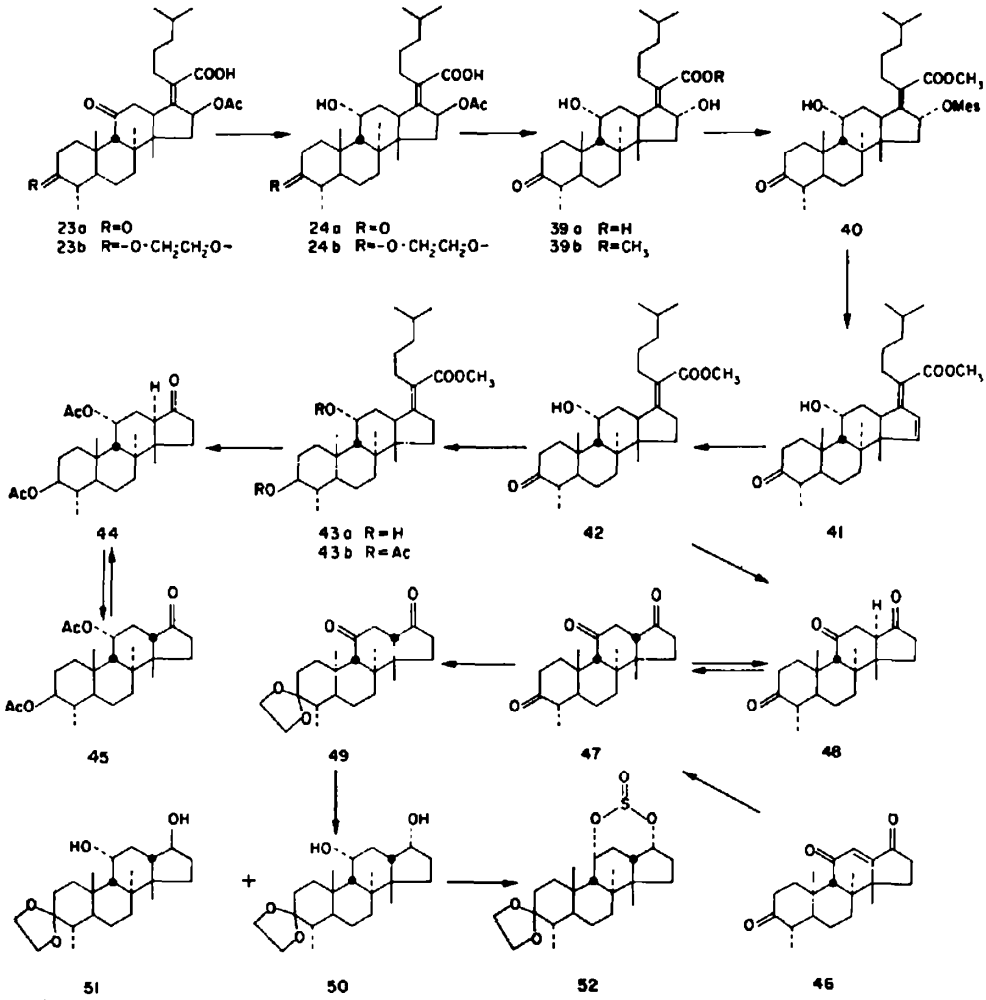
Chemical proof of the correctness of the stereochemistry implied by these findings could subsequently be secured as follows: The known ene-dione **46**[2] was reduced with zinc and acetic acid to a saturated triketone, **47**. On the other hand, ozonolysis of **42** gave a different triketone, **48**, efficiently (>90%) converted into **47** upon very mild treatment with base. Since the mild conditions applied are sufficient for equilibration at C-13 (cf. **44** ⇄ **45**) whereas equilibration at C-9 occurs only under rather drastic conditions (cf. **15** → **16a** + **17a**) we infer that **48** and **47** differ in the configuration at C-13 only. The former isomer belongs to the natural series, hence the unnatural C/D-*cis* ring junction is established for the latter.* Conversion of **47** to the corresponding 3-ethylene ketal **49** followed by reduction with sodium borohydride gave a mixture of the diol **50** and a second compound which is best represented as the C-17 epimer **51**; the overall change occurs with retention of the configurations at C-9 and C-13, as evidenced by the fact that **47** was regenerated from **50** as well as from **51** on acid catalysed hydrolysis followed by oxydation with chromium (VI)-oxide. Finally, the diol **50** on treatment with thionylchloride–pyridine at −20° was smoothly transformed into the cyclosulfite **52**, the intramolecular nature of the sulfite bridge being certified by mass-spectrometric as well as by vaporometric measurements of the mol. wt.

The formation of such an 11-17 bridge is possible only with an axially orientated hydroxyl group at C-11 and provides therefore unambiguous evidence for the stereochemistry indicated in the formula. It follows that fusidic acid itself has a 13α,14β-*trans* junction of rings C and D.

The stereochemistry now revealed made a reconsideration of the previously[2] proposed α-orientation of the 16-acetoxyl group mandatory, since the arguments advanced for this orientation were based upon the assumption of a "normal", steroid-like C/D-*trans* junction.

The first of these arguments involved a comparison of ΔM_D-values of steroidal models with the one observed in going from tetrahydrofusidic acid to the corresponding deacetylated derivative. Such an argument might be (and in fact is) vitiated both by the nature of the solvents used in the determination and by the "abnormal" stereochemistry of the neighbouring asymmetric centers in the fusidic acid series. The second argument was based upon the observation that deacetyl-fusidic acid lactonizes much more easily than its 16-epimer. Inspection of models shows that in the case of a normal 13β,14α ring junction the torsion angle defined by α-O-C_{16}-C_{17}-C_{20} is considerably smaller than the β-O-C_{16}-C_{17}-C_{20} angle, whereas the reverse is true for a 13α,

* The reasons allowing for the predominance of the *cis*-isomer in this particular case have already been discussed above.



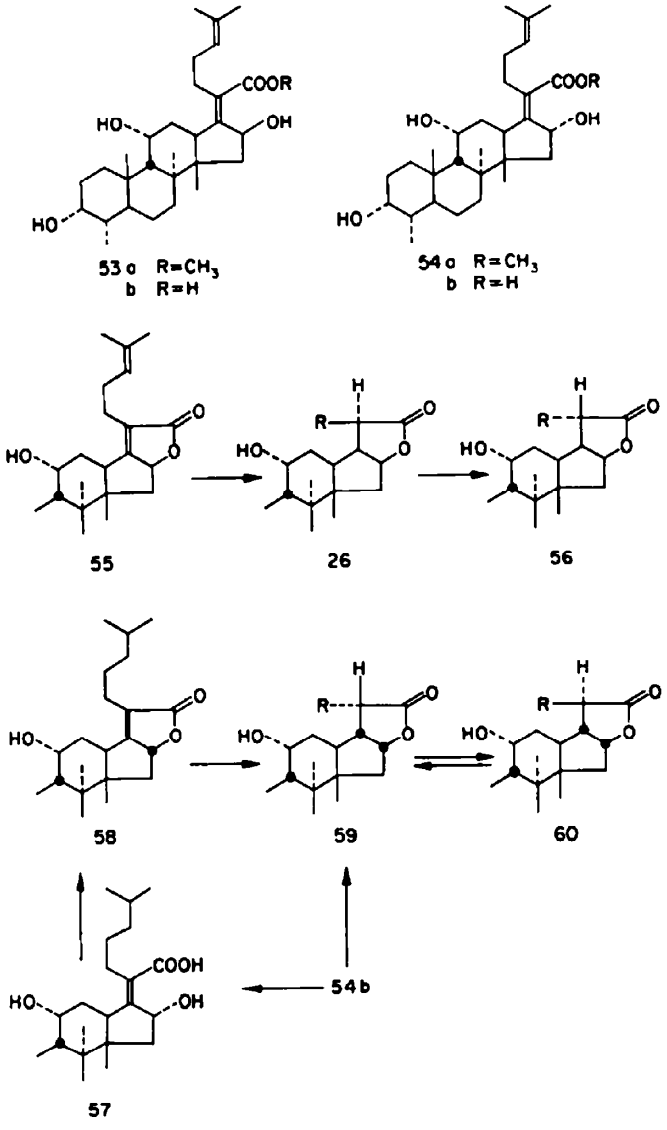
14β-compound. Since fusidic acid belongs to this latter type and since it must be assumed that a small angle facilitates lactonization these observations now favour a β-orientation of the 16-acetoxyl group.

TABLE 1. NMR VALUES (IN δ) OF FUSIDIC ACID DERIVATIVES

Compound	14β-CH_3	13α-H
16-deacetyl-fusidic acid methyl ester (**53a**)	1·12	3·16
16-epi-deacetyl fusidic acid methyl ester (**54a**)	0·74	3·40

This assignment has been supported by an analysis of the NMR spectra of deacetyl-fusidic acid methyl ester, **53a**, and its 16-epimer **54a** (cf. Table 1). It will be noted that in accordance with expectations the methyl group at C-14 is less shielded in **53a** than in **54a** ($\Delta\delta = 0{\cdot}38$) and that at the same time the reverse is true for the α-hydrogen atom at C-13 ($\Delta\delta = 0{\cdot}24$).

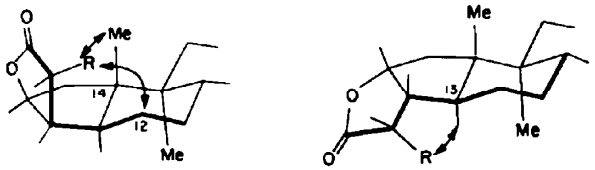
The behaviour of the four possible *cis*-fused tetrahydrolactones **26**, **56**, **59** and **60** deserves mentioning in this connection, since it provides additional evidence for the assignment just met. The first of these lactones, **26**, was obtained on catalytic hydrogenation of deacetyl-fusidic acid lactone (**55**).[3]



The stereochemistry of **26** stems from the fact that only a *cis*-fusion of the lactone ring with the cyclopentane ring is possible and from the usual *cis* mode of hydrogen addition to the double bond.* On treatment with methanolic sodium hydroxide **26**

* A similar case in the steroid field has been discussed in detail by Mazur *et al.*[16] It is also relevant in this respect that deacetyltetrahydrofusidic acid (**20**) does not lactonize, in contrast to 16-*epi*-deacetyltetrahydrofusidic acid which lactonizes spontaneously to **59** subsequent to its formation on catalytic hydrogenation of 16-epi-deacetylfusidic acid (**54b**).

was smoothly converted to **56**, the driving force for the isomerization being steric repulsions between the C-22 methylene group and the β-hydrogen at C-12 as well as between the former and the methyl group at C-14 (cf. Fig. 5a). By means of thin layer chromatography which allows a ready separation of the two isomers, it could be shown that the isomerization is quantitative. A third tetrahydrolactone, **59**, was prepared by catalytic hydrogenation of the 16-*epi*-dihydrolactone **58**, and this in turn was formed by lactonization of the 24,25-dihydro-compound **57** available from 16-epi-deacetyl-fusidic acid (**54b**). The stereochemistry of **59** is dictated by factors similar to those operating in the formation of **26**. A model of **59** (cf. Fig. 5b) shows a steric interference between the C-22 methylene group and the α-hydrogen atom at C-13, which, however, is less severe than the steric interactions in **26**. Consequently **59** on treatment with methanolic sodium hydroxide is isomerized only in part to **60**. At a rough estimate (thin layer chromatography) 5–10% of **59** are still present in the equilibrium.



a b

FIG. 5

With the elucidation of this last point the stereochemistry of fusidic acid is completely defined as in **2**. The choice of the absolute stereochemistry used throughout this work is dictated by the CD-curves of different derivatives of fusidic acid; these have already been discussed in the text and need no further comment. A perspective representation of the molecule is shown in Fig. 6. It will be noted, that ring B is forced into a boat conformation by the *trans-syn-trans* arrangement of the polycyclic system. The implications of this unusual feature on the stability of the ring system and on the unexpected reactivity of certain derivatives of fusidic acid will be dealt with explicitly in a following paper.[8]

Since the appearance of our preliminary communication an X-ray analysis of the 3-p-bromobenzoate of fusidic acid methyl ester has independently established the structure of the antibiotic as in **2** (cf. addendum).

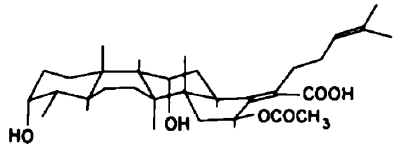


FIG. 6

The structural problem of fusidic acid has also been approached by a French group. In the first paper Bucourt *et al.*[25] independently recognized the correct location of the hydroxyl group in ring C and advanced a proposal for the B/C and C/D ring junctions which was based essentially on the interpretation of several CD-curves. This proposal

[25] R. Bucourt, M. Legrand, M. Vignau, J. Tessier and V. Delaroff, *C.R. Acad. Sci., Paris* **257**, 2679 (1963).

was later[26] abandoned because of discrepancies with NMR data; a stereoformula differing from **2** only in the configuration at C-16 was subsequently considered among other possibilities, with the explicit statement that, although satisfactory in some respects, it also failed to account for alleged anomalies of spectroscopic nature.

A possible close relationship between fusidic acid and two other antibiotics, cephalosporin P_1[27] and helvolic acid,[28] has been suggested on several occasions.[2,29] This view is strengthened by the fact that bacteria which have acquired resistance to one of the antibiotics are also resistant to the others.[30] Although no correlation between any of the three substances has been reported so far, the bulk of available evidence is in best agreement with the assumption of a common framework.*

From the biogenetic point of view it can now be recognized that fusidic acid is closely connected to the lanostane group of tetracyclic triterpenes and therefore to steroids. In the light of current theories[4] it can be postulated that biosynthesis of fusidic acid proceeds from *all-trans* squalene **61** through the tetracyclic carbonium ion **62** which is then stabilized by extrusion of a proton from C-17. The intermediate **63** thus obtained is subsequently converted to fusidic acid via a series of oxydative transformations involving among other things the removal of one of the methyl groups at C-4.† A most interesting point in this biogenetic sequence lies in the fact that the required ionic intermediate **62** is identical in every respect with the tetracyclic ion postulated some time ago[4] as an obligatory intermediate in the biosynthesis of lanosterol, **64**, from a *chair-boat-chair-boat* folded squalene precursor. Whereas the formation of lanosterol from **62** involves a series of stereospecific 1,2-shifts of hydrogen atoms and methyl groups,[33a,b] no such rearrangement occurs in the production of the hypothetic tetracyclic C_{30} precursor (**63**) of fusidic acid.‡ The isolation of fusidic acid thus fills an important gap in the systematic of tetracyclic triterpenes and can be taken as a vindication for the correctness of the proposed mechanism of lanosterol formation

* Evidence supporting this view has in the meantime been obtained both for helvolic acid in Professor Tsuda's group (private communication of Dr. Ishikawa and Professor Okuda, Tokyo) and for cephalosporin P_1 in Oxford (private communication of Professor Sir Ewart. R. H. Jones).

† Fermentation of *Fusidium coccineum* in the presence of DL-2-C^{14}-mevalolactone led to the production of C^{14}-labeled fusidic acid in a radioactive yield of 6%.[31] Degradation of this material revealed that removal of the methyl group at C-4 occurs with retention of configuration.[32]

‡ A search for this intermediate in the unsaponifiable lipid fraction of F. coccineum has not yet met with success. However, the isolation of ergosterol from this fraction demonstrates the ability of the fungus to impart to the squalene precursor the folding required by the theory.

[26] R. Bucourt and M. Legrand, *C.R. Acad. Sci., Paris* **258**, 3491 (1964).

[27] H. S. Burton and E. P. Abraham, *Biochem. J.* **50**, 168 (1951).

[28a] S. A. Waksmann, E. S. Horning, and E. L. Spencer, *J. Bacter.* **45**, 233 (1943); [b] E. Chain, H. W. Florey, M. A. Jennings, and T. I. Williams, *Brit. J. Exp. Path.* **24**, 108 (1943); [c] A. E. O. Menzel, O. Wintersteiner and J. C. Hoogerheide, *J. Biol. Chem.* **152**, 419 (1944).

[29a] T. G. Halsall, E. R. H. Jones, and G. Lowe, *Proc. Chem. Soc.* 16 (1963); [b] A. Melera, *Experientia* **19**, 565 (1963); [c] S. Okuda, S. Iwasaki, K. Tsuda, Y. Sano, T. Hata, S. Udagawa, Y. Nakayama, and H. Yamaguchi, *Chem. Pharm. Bull.* **12**, 121 (1964).

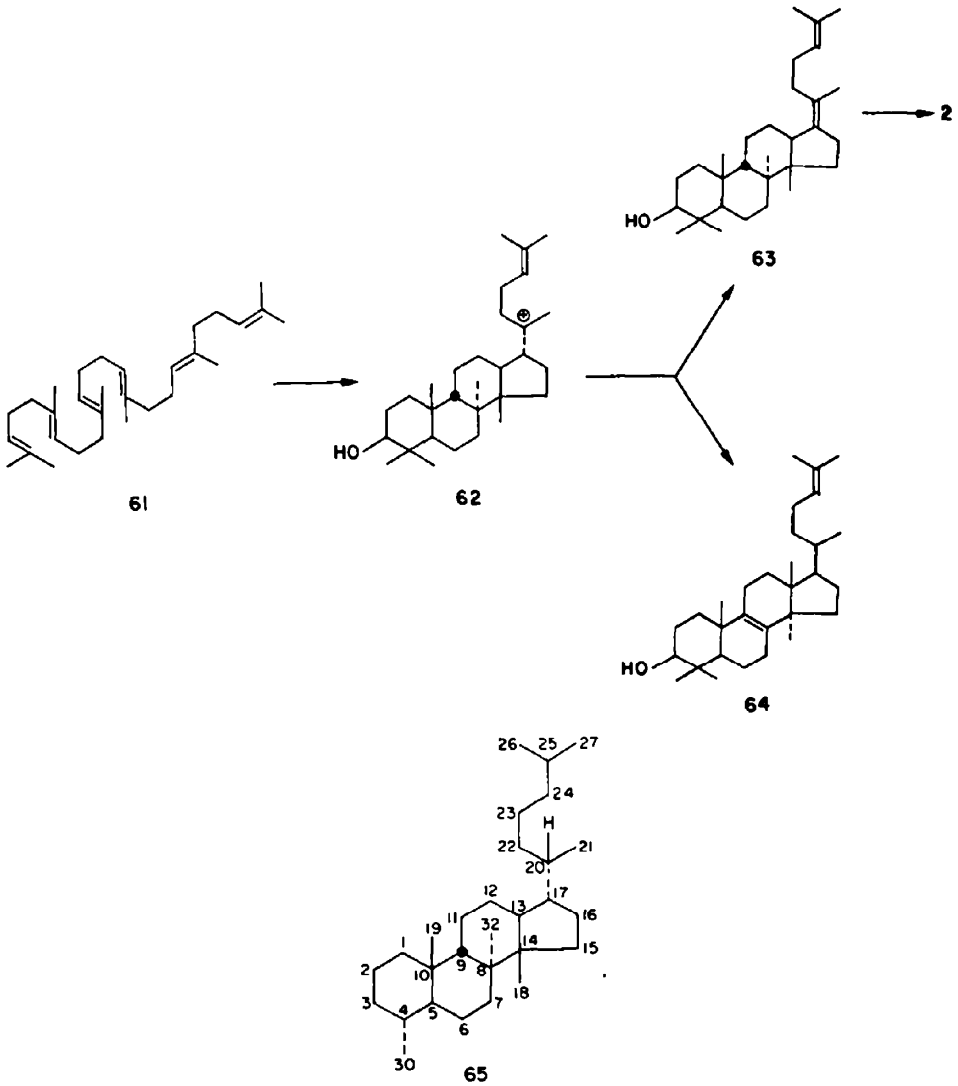
[30a] W. O. Godtfredsen, K. Roholt, and L. Tybring, *The Lancet* 928 (1962); [b] M. Barber and P. M. Waterworth, *Ibid.* 931 (1962).

[31] Unpublished results of H. Lorck and W. O. Godtfredsen.

[32] Unpublished results of A. Marquet and D. Arigoni; cf. D. Arigoni in *Biogenesi delle Sostanze Naturali Roma Academia Nazionale dei Lincei* p. 1 ff. (1964).

[33a] J. W. Cornforth, R. H. Cornforth, R. H. Pelter, M. G. Horning and G. Popják, *Tetrahedron* **5**, 311 (1959); [b] R. K. Maugdal, T. T. Tchen and K. Bloch, *J. Amer. Chem. Soc.* **80**, 2589 (1958).

with regard to two details (boat-shaped ring B and β-orientation of the hydrogen atom at C-9 in the ionic intermediate) for which so far direct experimental evidence has not been available.



Nomenclature. For the purpose of a rational nomenclature we propose that the parent hydrocarbon **65** be named fusidane and numbered as indicated. The choice of an α-orientation for the C-17-side chain was dictated by biogenetic considerations (see above), whereas the choice of the (S)-configuration at C-20 is arbitrary. Accordingly the rational name of fusidic acid will be 3α,11α-dihydroxy-16β-acetoxy-fusida-[17,20 (16-21*cis*); 24]-diene-21-oic acid.

EXPERIMENTAL

All m.ps are corrected. Optical rotations, unless otherwise stated, were measured in $CHCl_3$ (c = 1) and UV spectra in 96% EtOH solution. The IR spectra were obtained with a Perkin–Elmer

Model 21 spectro-photometer with a NaCl prism and the NMR spectra with Varian HR-100 (100 Mc) and Varian A-60 (60 Mc) high resolution spectrometers, $CDCl_3$ being used as solvent. The line positions are given in δ-values and with tetramethylsilane 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).

Microanalyses were performed in the microanalytical laboratory of Leo Pharmaceutical Products by Mr. G. Cornali and Mr. W. Egger. For thin layer chromatography (TLC) silica gel G was used as adsorbent.

Dehydration of 16*-deacetyl-*24,25*-dihydrofusidic acid lactone* 3*-acetate* (**6**) *to* **7**

16-Deacetyl-24,25-dihydrofusidic acid lactone 3-acetate[2] (**6**; 15 g) was dissolved in dry pyridine (150 ml). The solution was cooled to −30°, and thionylchloride (18 ml) added over 30 min. After standing at −20° for 1 hr, the cooling bath was removed and the mixture allowed to warm to 0°. After 30 min at 0° the mixture was poured into ice-water, forming an oil, which shortly afterwards solidified. The crude product, thus obtained, was recrystallized from EtOH–water to yield 13·6 g of **7**, m.p. 143·5–144°. $[\alpha]_D^{20}$ +26°. UV: λ_{max} 221 mμ (ε 15500). (Found: C, 76·98; H, 9·45. $C_{31}H_{46}O_4$ requires: C, 77·13; H, 9·61%.)

Oxidation of **6** *to the corresponding* 11*-ketone* (**11**)

To a solution of **6** (4·96 g) in acetic acid (20 ml) 10% chromium (VI)-oxide in 95% acetic acid (6·5 ml) was added with stirring. After standing for 15 min, water (10 ml) was added slowly. Upon scratching the reaction product crystallized, yield of crude **11**: 4·5 g, m.p. 150–152°. Recrystallization from ether–pentane raised the m.p. to 153–154°. $[\alpha]_D^{20}$ +113°. UV: λ_{max} 222 mμ (ε 13800). (Found: C, 74·56; H, 9·36. $C_{31}H_{44}O_5$ requires: C, 74·66; H, 9·30%.)

Selenium dioxide dehydrogenation of **11** *to* **12**

To a solution of **11** (2·0 g) in t-butanol–acetic acid (99:1) (20 ml) SeO_2 (440 mg), moistened with water, was added, and the mixture refluxed for 20 hr. After cooling, the solvent was removed *in vacuo*, and the residue treated with ether. Insoluble material (360 mg) was filtered off, and the filtrate evaporated to dryness. Treatment of the residue with MeOH gave 730 mg of **12**, m.p. 176–179°. Several recrystallizations from MeOH raised the m.p. to 188–189°. $[\alpha]_D^{20}$ −358°. UV: λ_{max} 280 mμ (ε 17500). (Found: C, 74·81; H, 8·93. $C_{31}H_{44}O_5$ requires: C, 74·96; H, 8·93%.)

Conversion of tetrahydrofusidic acid via **14** *to* **15**

Tetrahydrofusidic acid[3] (13 g) was dissolved in a mixture of acetic anhydride (40 ml) and pyridine (40 ml). After standing for 16 hr at room temp water was added, and the amorphous precipitate (**14**) hereby obtained collected, washed with water and, after drying, dissolved in acetic acid (50 ml). To the resulting solution 10% chromium (VI)-oxide in 95% acetic acid (25 ml) was added and, after standing for 30 min, the product was precipitated with water. The crude material was recrystallized from MeOH–water to yield 10·5 g of the 11-ketone **15**, m.p. 149–151°. Two further recrystallizations from acetone–hexane gave the analytical specimen, m.p. 155–156°. $[\alpha]_D^{20}$ +3·3°. (Found: C, 70·46; H, 9·39. $C_{33}H_{52}O_7$ requires: C, 70·68; H, 9·35%.)

Saponification of **15** *to* **16a**

A solution of **15** (2·0 g) in a mixture of EtOH (30 ml) and 2N NaOH (10 ml) was refluxed for 1 hr. After cooling, the solution was acidified with acetic acid and diluted with water. Upon scratching the product crystallized to yield 1·45 g of **16a**, m.p. 238–240°. Recrystallization from acetone raised the m.p. to 241–242°. $[\alpha]_D^{20}$ +15° (pyridine). (Found: C, 73·01; H, 10·12. $C_{29}H_{44}O_6$ requires: C, 73·07; H, 10·15%.)

The corresponding methyl ester (**16b**) was obtained on esterification with ethereal diazomethane in the usual way, m.p. 164·5–165°. $[\alpha]_D^{20}$ −3°. (Found: C, 73·47; H, 10·24. $C_{30}H_{46}O_6$ requires: C, 73·43; H, 10·27%.) CD-curve: λ^{dioxan} 314 mμ ($\Delta\varepsilon$ = −0·35).

Epimerization of **16a** *to* **17a**

Compound **16a** (1·0 g) in a mixture of EtOH (45 ml) and 33% NaOHaq (5 ml) was refluxed for 2 hr. After cooling, the solution was acidified with acetic acid and diluted with water. Upon

scratching the product crystallized to yield 850 mg of **17a**, m.p. 224·5–225·5°. Recrystallization from acetone gave the analytical sample, m.p. 225–225·5°. $[\alpha]_D^{20}$ −111° (pyridine). (Found: C, 73·19; H, 10·12. $C_{28}H_{44}O_5$ requires: C, 73·07; H, 10·15%.)

The corresponding methyl ester (**17b**) was obtained on esterification with ethereal diazomethane in the usual way, m.p. 157·5–158°. $[\alpha]_D^{20}$ −150°. (Found: C, 73·49; H, 10·32. $C_{29}H_{46}O_5$ requires: C, 73·43; H, 10·27%.) CD-curve: λ^{dioxan} 302 mμ ($\Delta\varepsilon = -2{\cdot}25$).

Acetylation of **16a** *to* **15**

Compound **16a** (200 mg) was acetylated in the usual way (acetic anhydride–pyridine). The product crystallized on addition of water. Recrystallization from ether–hexane afforded 160 mg, m.p. 152–154° alone and in admixture with **15**. The IR spectra were identical.

Triketo-acid **18**

(*a*) *From* **20**. To a suspension of **20**[8] (390 mg) in acetic acid (10 ml) 10% chromium (VI)-oxide in 95% acetic acid (1·6 ml) was added. After standing for 1 hr water (40 ml) was added and the mixture extracted with ether. The combined extracts were washed with water, $NaHCO_3$aq and finally with water, dried and evaporated to dryness. The residue crystallized from ether–hexane to yield 294 mg of **18**, m.p. 190–190·5°. Recrystallization from acetone–hexane raised the m.p. to 191·5–192°. $[\alpha]_D^{20}$ +136°. (Found: C, 73·51; H, 9·43. $C_{28}H_{44}O_5$ requires: C, 73·69; H, 9·38%.)

(*b*) *From* **16a**. Compound **16a** (500 mg) in acetic acid (10 ml) was oxidized with 10% chromium (VI)-oxide in 95% acetic acid (2·0 ml) in the usual way. The crude product (260 mg, m.p. 186–188°) was recrystallized from acetone–hexane to yield a product with m.p. 189·5–190·5° undepressed on admixture with the compound from (a) above. The IR spectra of the two products were identical.

Triketo-acid **19**

Compound **17a** (270 mg) in acetic acid (5 ml) was oxidized with 10% chromium (VI)-oxide in 95% acetic acid (1·0 ml) in the usual way. The crude **19** (220 mg, m.p. 222–226°) was recrystallized from ether to yield material of m.p. 224·5–226°. $[\alpha]_D^{20}$ −89°. (Found: C, 73·58; H, 9·46. $C_{28}H_{44}O_5$ requires: C, 73·69; H, 9·38%.)

Keto-ester **22b**

This compound was obtained on esterification of the corresponding acid (**22a**)[9] with ethereal diazomethane in the usual way, m.p. 67–70°. $[\alpha]_D^{20}$ +6·1°. (Found: C, 71·70; H, 9·80. $C_{32}H_{52}O_6$ requires: C, 72·14; H, 9·84%.) CD-curve: λ^{dioxan} 293 mμ ($\Delta\varepsilon = +1{\cdot}24$).

3-*Monoketal* **23b**

A solution of the diketo-acid **23a**[8] (78·8 g) and *p*-toluenesulfonic acid (3·0 g) in butanone ethyleneketal (580 ml) was refluxed for 20 min. After cooling, pyridine (6 ml) and ether (290 ml) were added, and the resulting solution was washed with water, dried, and evaporated to dryness *in vacuo*. The 3-monoketal **23b** could not be isolated in a crystalline form, but a crystalline sodium salt was obtained as follows: The residue was dissolved in MeOH (200 ml), neutralized with 2N NaOH, and the solvent removed *in vacuo*. On addition of acetone 68 g of the sodium salt crystallized as a dihydrate, m.p. 213–214° (dec). For analysis a sample was recrystallized from MeOH–acetone. M.p. 215–216° (dec). IR (KBr): 1565 and 1715 cm^{-1}. (Found: C, 64·23; H, 8·54. $C_{32}H_{49}O_7Na$, 2 H_2O requires: C, 64·26; H, 8·66%.)

Reduction and hydrolysis of **23b**

To a solution of the sodium salt of **23b** (68 g) in MeOH (270 ml) 20% $NaBH_4$aq (68 ml) was added with stirring and the mixture allowed to stand for 45 min. After neutralization with acetic acid, water (1·35 l) was added, and the oily precipitate, which formed, extracted with ether. The extract was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was chromatographed on florisil (1·2 kg). Elution with benzene–EtOH, 96:4, gave 51·2 g of material, which was crystallized from ether–hexane to afford 46·4 g of **24b**, m.p. 186–187°. Recrystallization from ether–hexane did not raise the m.p. $[\alpha]_D^{20}$ +8°. UV: λ_{max} 221 mμ (ε 8350). IR (KBr): 1100, 1250, 1715,17 30, and 3440 cm^{-1}. (Found: C, 70·59; H, 9·40. $C_{32}H_{52}O_7$ requires: C, 70·68; H, 9·35%.)

A solution of **24b** (45·4 g) in methanol (440 ml) was acidified with dil HClaq and heated for 20 min on the steam-bath. After cooling, water (2·2 l) was added and the oily precipitate, which formed, extracted with ether. The extract was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was crystallized from ether–pentane to yield 36·8 g of **24a**, m.p. 183–184°. Further recrystallizations from ether raised the m.p. to 189–190°. $[\alpha]_D^{20}$ +39°. UV: λ_{max} 220 mμ (ε 8150). IR (KBr): 1260, 1700, 1715, 1720 and 3420 cm^{-1}. (Found: C, 72·06; H, 9·28. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%.)

The mother liquor from **24b** was evaporated to dryness *in vacuo* to afford 4·2 g of an amorphous residue. A solution of this material in methanol (42 ml) was acidified with dil. HClaq and heated on the steam bath for 20 min. After cooling, the mixture was poured into water, the oily precipitate extracted with ether, the ethereal layer washed with water, dried, and evaporated to dryness *in vacuo*. Crystallization of the residue from ether–pentane gave 1·46 g of material with m.p. 162–165°. On recrystallization from ether 0·58 g of **25**, m.p. 184–185°, was obtained. Further recrystallizations from ether raised the m.p. to 190–191°, $[\alpha]_D^{20}$ +81°, UV: λ_{max} 218 mμ (ε 7900). IR (KBr): 1260, 1690. 1705, 1735 and 3440 cm^{-1}. (Found: C, 71·82; H, 9·35. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%).

Oxidation of **24a**

Compound **24a** (100 mg), dissolved in acetic acid (5 ml) was oxidized with 10% chromium (VI)-oxide in 95% acetic acid (0·4 ml) in the usual way. The residue was crystallized from ether–hexane to yield 52 mg of a product with m.p. 196–198°. Two recrystallizations from ether raised the m.p. to 205–206°, undepressed on admixture with an authentic sample of **23a**. The IR spectra of the two products were identical.

Oxidation of **25**

Compound **25** (100 mg) was oxidized in the same manner as described above to afford 46 mg of a product with m.p. 192–196°. Recrystallization from ether raised the m.p. to 204–205°, undepressed on admixture with a sample of **23a**. The IR spectra were identical.

Acetylation of **25**

To a solution of **25** (100 mg) in pyridine (4 ml), acetic anhydride (1 ml) was added, and the mixture allowed to stand at room temp for 48 hr. Work up in the usual way afforded 42 mg of the 11-acetate of **25**, m.p. 190–191·5°. Recrystallization from ether raised the m.p. to 193–194·5°, UV: λ_{max} 218 mμ (ε 8300). IR (KBr): 1235, 1705 and 1730 cm^{-1}. (Found: C, 70·95; H, 9·05. $C_{33}H_{50}O_7$ requires: C, 70·93; H, 9·03%.)

Attempted acetylation of **24a**

Compound **24a** (100 mg) was treated with acetic anhydride (1 ml) in pyridine (4 ml) in the same manner as described for compound **25**. TLC of the crude product revealed that only unchanged starting material was present. Several recrystallizations from ether gave 36 mg with m.p. 187·5–189°, alone and in admixture with **24a**. The IR spectra were identical.

Oxidation of 16*-deacetyltetrahydrofusidic acid lactone* (**26**) *to the diketolactone* **27**

To the tetrahydrolactone **26**[3] (38·3 g) suspended in acetic acid (200 ml), 10% chromium (VI)-oxide in 95% acetic acid (120 ml) was added with stirring. After standing for 30 min water (1·2 l.) was added, and the precipitate, which formed. extracted with ether. The organic layer was washed with $NaHCO_3$aq and water, dried, and concentrated *in vacuo* to about 200 ml. On standing 31·5 g of **27**, m.p. 206–208° crystallized. Recrystallization from ether gave the analytical sample, m.p. 208–210°. $[\alpha]_D^{20}$ +90°. (Found: C, 76·06; H, 9·59. $C_{29}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

Ketalization of **27** *to the* 3*-monoketal* (**28**)

A solution of **27** (22·75 g) and *p*-toluenesulfonic acid (1·15 g) in butanone ethyleneketal (175 ml) was refluxed for 10 min. After cooling, pyridine (4·6 ml) and ether (100 ml) were added, and the resulting solution washed with water, dried, and evaporated to dryness *in vacuo*. The residue was crystallized from acetone to yield 17·2 g of **28**, m.p. 212–214°. Two recrystallizations from ether raised the m.p. to 223–225°. $[\alpha]_D^{20}$ +20·5°. IR (KBr): 1105, 1712 and 1770 cm^{-1}. (Found: C, 74·52; H, 9·80. $C_{31}H_{48}O_6$ requires: C, 74·36; H, 9·66%.)

Reduction of **28** *to* **29**

To a solution of **28** (18·3 g) in methylcellosolve (500 ml) 10% methanolic $NaBH_4$ (43 ml) was added with stirring. After standing for 1 hr the mixture was neutralized with dil acetic acid, water (2 l.) was added, and the precipitate extracted with ether. The organic layer was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH (100 ml), acidified with HCl, and heated on the steam-bath for 20 min. After cooling, water (400 ml) was added, and the oily precipitate, which formed, extracted with ether. The extract was washed with water, dried, and evaporated to dryness. The residue was crystallized from ether to yield 13·0 g of **29**, m.p. 191–192°. Recrystallization from the same solvent raised the m.p. to 192–193°. $[\alpha]_D^{20}$ +7°. IR (KBr): 1705, 1765, and 3470 cm^{-1}. (Found: C, 75·86; H, 10·12. $C_{29}H_{44}O_4$ requires: C, 75·94; H, 10·11%.)

Hydrogenation of **29** *to* **26**

A solution of **29** (250 mg) in acetic acid (5 ml) containing 2 drops of conc. HCl was shaken at room temp under 1 atm. H_2 in the presence of prereduced PtO_2 (50 mg). In 25 min 12·9 ml H_2 was absorbed and the consumption ceased. After removal of the catalyst the filtrate was diluted with water and extracted with ether. The extract was washed with $NaHCO_3$aq and water, dried, and evaporated to dryness. The product crystallized from acetone–hexane to yield 168 mg of **26**, m.p. 135–140°. Several recrystallizations from ether raised the m.p. to 146–147° alone and in admixture with an authentic sample of **26**.[9] The IR-spectra of the two products were identical.

Bromination of **29**

To a solution of **29** (4·58 g) in dry tetrahydrofurane (50 ml) phenyl trimethyl ammonium bromide perbromide[12a,b] (3·76 g) was added. After standing for 5 min the mixture was diluted with ether (100 ml) and washed successively with $NaHCO_3$aq and water. After drying and removal of the solvent the residue crystallized from MeOH to yield compound **30** (4·24 g), m.p. 147° (dec), raised by recrystallization from MeOH to 156°. $[\alpha]_D^{20}$ +9°. IR (KBr): 750, 1725, and 1760 cm^{-1}. (Found: C, 64·54; H, 8·51. $C_{29}H_{45}BrO_4$ requires: C, 64·77; H, 8·44%.)

Dehydrobromination of **30** *with collidine*

To freshly distilled, boiling 2,4,6-collidine (40 ml) compound **30** (4·2 g) was added, and the mixture refluxed for 20 hr. After cooling, collidine hydrobromide (1·45 g) was filtered off. The filtrate was diluted with ether (100 ml) and washed with dil. HClaq followed by water. After drying and removal of the solvent the oily residue crystallized from ether to afford 1·49 g of **31**, m.p. 203–206°. A second crop (420 mg, m.p. 201–204°) was obtained from the mother liquor upon evaporation to dryness and crystallization of the residue from benzene. Two recrystallizations from ether and benzene, respectively, raised the m.p. to 211·5–214°. $[\alpha]_D^{20}$ −35°, UV: λ_{max} 238 mμ (ε 9200), IR (KBr): 1670 and 1763 cm^{-1}, NMR: δ = 7·66/d CH-1, 5·88/d CH-2 (J_{AB} = 10 c/s). (Found: C, 76·22; H, 9·73. $C_{29}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

Dehydrobromination of **30** *with* LiCl *in dimethylformamide*

A solution of **30** (1·5 g) and anhydrous LiCl (1·5 g) in dimethylformamide (25 ml) was heated under N_2 to 100° for 5 hr. After cooling, the solution was poured into water. The amorphous precipitate, which formed, was collected, washed with water, and dried to yield 950 mg of a product (UV: λ_{max} 255 mμ (ε 10500)) which was chromatographed on florisil (50 g). Elution with benzene–ethyl acetate, 95:5, afforded 250 mg of crystals, m.p. 180–200°. Several recrystallizations from ether raised the m.p. to 211–213°, UV: λ_{max} 256 mμ (ε 14500), IR (KBr): 1595 (shoulder), 1620, 1655, 1765, and 3480 cm^{-1}, NMR: δ = 5·03/m CH-16, 4·17/b CH-11, 1·79/s CH_3-30. (Found: C, 76·06; H, 9·81. $C_{29}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

Isomerization of **31** *into* **32** *and formation of the* 1,11-*ether* (**33**)

A solution of **31** (500 mg) in a mixture of EtOH (30 ml) and 33% NaOHaq (0·5 ml) was left standing for 16 hr at room temp. After neutralization with HClaq and concentration *in vacuo* ether was added, and the solution washed with water, dried, and evaporated to dryness. The residue crystallized from ether to yield 200 mg of **32**, m.p. 200–204°. Two recrystallizations from acetone

raised the m.p. to 209·5–212·5°. $[\alpha]_D^{20}$ −58·6°, UV: λ_{max} 238 mμ (ε 6850), IR (KBr): 1660 and 1761 cm^{-1}, NMR: δ = 7·66/d CH-1, 5·88/d CH-2 (J_{AB} = 10 c/s), 5·03/t CH-16, 4·52/b CH-11. (Found: C, 76·09; H, 9·81. $C_{33}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

The mother liquor from **32**, which according to thin layer chromatograms (solvent system: ethyl acetate–cyclohexane, 1:1. Spray-reagent: 50% phosphoric acid) contained a mixture of **32** and **33**, was chromatographed on silica gel (20 g). Elution with benzene–ethyl acetate (9:1) afforded 113 mg of compound **33**, m.p. 191–194°, raised by two recrystallizations from ether–pentane to 192·5–194°, $[\alpha]_D^{20}$ +56·5°, IR (KBr): 1715 and 1760 cm^{-1}, NMR spectrum: δ = 5·08/t CH-16, 4·00/t + 3·49/t CH-1 + CH-11. (Found: C, 76·26; H, 9·75. $C_{33}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

Demonstration of an equilibrium between **32** *and* **33**

To demonstrate the equilibrium between **32** and **33** the following experiment was performed: To 1% ethanolic solutions of **32** and **33** (200 μl), which according to TLC (solvent system: ethyl acetate–cyclohexane, 1:1. Spray-reagent: 50% phosphoric acid) were pure, was added 8N NaOH (5 μl). After standing for 2 hr at room temp the solutions were neutralized with 4N HCl (10 μl) and subjected to thin layer chromatography. Both solutions turned out to contain **32** and **33** in approx. equal amounts.

Conversion of **29** *to the 2-hydroxymethylene derivative* **34**

A suspension of **29** (3·0 g) in ether (60 ml) was added to a mixture of MeONa in MeOH (20 ml) (prepared from 2·0 g Na and 20 ml MeOH) and ethyl formate (30 ml). After stirring for 16 hr at room temp the mixture was hydrolysed with phosphate buffer (p_H 8), washed with water, dried, and evaporated to dryness *in vacuo*. The residue crystallized from ether–pentane to yield 2·15 g of compound **34**, m.p. 158–160°. The analytical sample, obtained after one recrystallization from MeOH, had m.p. 160–161°, $[\alpha]_D^{20}$ +13°, UV: λ_{max} 292 mμ (ε 7600), IR (KBr): 1585, 1635, and 1745 cm^{-1}. (Found: C, 74·02; H, 9·54. $C_{30}H_{46}O_6$ requires: C, 74·03; H, 9·53%.)

Ozonolysis of **34** *with formation of the lactone* **35a**

Ozonized oxygen was bubbled through a solution of **34** (1·1 g) in dry CH_2Cl_2 (20 ml) containing dry pyridine (0·2 ml) at −75° until a blue coloration persisted. The solution was diluted with a mixture of acetic acid (15 ml), water (10 ml), and ethyl acetate (10 ml), whereafter 33% H_2O_2 (3 ml) was added. The resulting mixture was concentrated in a rotatory evaporator until a one-phase system was obtained and then left standing overnight. After addition of ether (100 ml) the solution was washed repeatedly with water, dried, and evaporated to dryness. The residue crystallized from ether to yield 840 mg of **35a**, m.p. 223–226°. Recrystallization from acetone–pentane raised the m.p. to 225–226°. $[\alpha]_D^{20}$ +38°. (Found: C, 71·13; H, 9·13. $C_{29}H_{44}O_6$ requires: C, 71·28; H, 9·08%.)

The corresponding methyl ester **35b** was prepared by adding an ethereal solution of diazomethane to a suspension of **35a** (180 mg) in ether (5 ml) until the yellow colour persisted. The resulting solution was evaporated to dryness under red. press., and the residue crystallized from ether–pentane to yield 150 mg of **35b**, m.p. 130–130·5°. Recrystallization did not raise the m.p., NMR: δ = 5·06/t CH-16, 4·76/m CH-11, 3·71/s $COOCH_3$. (Found: C, 71·60; H, 9·20. $C_{30}H_{46}O_6$ requires: C, 71·68; H, 9·22%.)

Oxidation of **36** *with lead* (IV)*acetate–iodine to* **37a**

Calcium carbonate (dried over P_2O_5; 7·0 g) and $Pb(OAc)_4$ (dried over KOH *in vacuo*; 21·0 g) were refluxed for 15 min in dry cyclohexane (375 ml). After cooling, I_2 (5·6 g) and 16-deacetyltetrahydrofusidic acid lactone 3-acetate (**36**;[2] 3·5 g) were added, and the mixture refluxed under irradiation with a 250 W photolamp until the I_2 colour disappeared (2 hr). After cooling, the mixture was filtered through Celite, and the precipitate washed with ether. The combined filtrate and washings were washed with 10% $Na_2S_2O_3$ (2 × 150 ml) followed by water (3 × 150 ml). After drying and evaporation to dryness *in vacuo*, the residue was crystallized from MeOH to yield 1200 mg of **37a** m.p. 156·5–157°. Recrystallization from MeOH raised the m.p. to 157·5–158°, $[\alpha]_D^{20}$ −44°, IR: No OH bands, NMR: δ = 5·11/t CH-16, 4·92/b CH-3, 4·43/d CH-11, 3·76/d and 3·68/d CH_2-8 (J_{AB} = 10 c/s). (Found: C, 74·19; H, 9·69. $C_{31}H_{48}O_8$ requires: C, 74·36; H, 9·66%.)

The mother liquor from **37a** was evaporated to yield 2·17 g of an amorphous residue, which according to TLC (solvent system: ethyl acetate–cyclohexane, 1:1) consisted of at least four components, one of which (component B) gave a strong blue colour on spraying with $SbCl_3$ in $CHCl_3$. 1·9 g of the mixture was dissolved in benzene and chromatographed on florisil (100 g). Elution with benzene–ethyl acetate (97:3) gave fractions which according to TLC, contained component B only. These were combined and evaporated to yield 180 mg of this compound, which although amorphous, gave only one spot in TLC. NMR: δ = 9·55/s (1); 5·62/t (1); 4·92/t (1); 4·68/b (1). (Found: C, 73·17; H, 9·65%.)

Saponification of **37a** *to* **37b**

Compound **37a** (100 mg) was dissolved in hot EtOH (1·5 ml), 2N NaOH (0·5 ml) was added, and the mixture refluxed for 1 hr. After cooling and acidification with dil HCl water was added, and the mixture extracted with ether. The extract was washed with water, dried, and evaporated to dryness. The residue (96 mg) crystallized from ether to yield 62 mg of **37b**, m.p. 200–201°. Recrystallization from MeOH–water raised the m.p. to 202–202·5°, $[\alpha]_D^{20}$ −50°, IR (KBr): 1750 and 3440 cm^{-1}, NMR: δ = 5·08/t CH-16, 4·52/d CH-11, 3·82/d and 3·61/d CH_2-8 (J_{AB} = 10 c/s). (Found: C, 75·91; H, 10·04. $C_{29}H_{46}O_4$ requires: C, 75·94; H, 10·11%.)

Oxidation of **37b** *to the corresponding* 3-*ketone* (**38**)

To a solution of **37b** (100 mg) in acetic acid (1·0 ml) 2% chromium (VI)oxide in 95% acetic acid (0·8 ml) was added during 5 min. After standing for 15 min water was added to precipitate 44 mg of **38**, m.p. 193–195°. Recrystallization from MeOH raised the m.p. to 196–198°. IR (KBr): 1707 and 1755 cm^{-1}. (Found: C, 76·34; H, 9·67. $C_{29}H_{44}O_4$ requires: C, 76·27; H, 9·71%.)

Saponification of **24a** *to* **39a**

A solution of **24a** (6·0 g) in MeOH (60 ml) was neutralized with 2N NaOH. Water (540 ml) and a few drops of phenolphthalein were added and the mixture refluxed, while $NaHCO_3$aq was added drop by drop at such a rate that the red colour was just maintained. When the colour was permanent, the refluxing was continued for a further 60 min. After cooling, the solution was acidified with HCl and extracted with ether. The ethereal extract was washed with water, dried, and evaporated to dryness. Crystallization from acetonitrile gave 30 g of **39a**, m.p. 177–178°. Two recrystallizations from acetonitrile raised the m.p. to 183–184°, $[\alpha]_D^{20}$ −14°. UV: λ_{max} 228 mμ (ε 10750), IR (KBr): 1700, 1730 and 3420 cm^{-1}. (Found: C, 73·12; H, 9·97. $C_{29}H_{46}O_5$ requires: C, 73·38; H, 9·77%.)

The corresponding methyl ester (**39b**) was obtained on esterification of **39a** with ethereal diazomethane in the usual way. After removal of the solvent the residue crystallized from ether–hexane to yield material with m.p. 173–174°. Recrystallization from ether–hexane gave the analytical sample, m.p. 178–179°, $[\alpha]_D^{20}$ −9°, UV: λ_{max} 231 mμ (ε 10650). (Found: C, 73·67; H, 9·92. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%.)

Acetylation of **39a**

To a solution of **39a** (400 mg) in pyridine (10 ml), acetic anhydride (2 ml) was added, and the mixture allowed to stand at room temp for 48 hr. Then it was poured into water, the oily precipitate, which formed, extracted with ether, and the ethereal layer washed with dil HClaq, $NaHCO_3$aq and water. After drying, the solvent was removed, and the residue crystallized from ether to afford a product with m.p. 132–133°. Two recrystallizations from ether–hexane gave 120 mg of the 16-acetate of **39a** m.p. 134–134·5°, UV: λ_{max} 226 mμ (ε 9000), IR (KBr): 1240, 1705, 1720, 1735, and 3460 cm^{-1}. (Found: C, 72·39; H 9·61. $C_{32}H_{50}O_6$ requires: C, 72·41; H, 9·50%.)

Conversion of **39b** *via* **40** *to* **41**

To **39b** (1·8 g) dissolved in a mixture of dry pyridine (10 ml) and dry CH_2Cl_2 (10 ml), a solution of methane sulfochloride (0·31 ml) in dry CH_2Cl_2 (2 ml) was added drop by drop with stirring at −15°. After stirring for another 2 hr at −15° and standing in the refrigerator for 16 hr, the mixture was poured on ice and extracted with ether. The ethereal extract was washed successively with dil HClaq, $NaHCO_3$aq and water, dried, and evaporated to dryness *in vacuo*. The crude, amorphous 16-mesylate (**40**; 2·0 g) was dissolved in a mixture of dry toluene (20 ml) and freshly distilled collidine

(17·5 ml) and refluxed for 8 hr. After cooling, the precipitate was filtered off and washed with CH_2Cl_2. To the combined filtrate and washings ether (100 ml) was added, and the solution washed with water, dil HCl, $NaHCO_3$aq and water. After drying and removal of the solvent, the residue was chromatographed on florisil. Elution with benzene–EtOH (96:4) gave 1·14 g of an oil, which crystallized from ether–hexane to afford 0·9 g of **41**, m.p. 109–110·5°. Recrystallization from the same solvent raised the m.p. to 111–112° (dec), $[\alpha]_D^{19}$ +155°, UV: λ_{max} 272–273 mμ (ε 18950), IR (KBr): 1620, 1685 and 1710 cm^{-1}. (Found: C, 76·53; H, 9·81; OCH_3, 6·67. $C_{30}H_{46}O_4$ requires: C, 76·55; H, 9·85; OCH_3, 6·59%.)

Reduction of **41** *to* **42** *and* **43a**

A solution of **41** (640 mg) in a mixture of 96% EtOH (12 ml) and dioxan (3 ml) was shaken at room temp under one atm H_2 in the presence of 5% Pd–$CaCO_3$ (100 mg). In 15 min 33 ml H_2 were adsorbed and the consumption ceased. The catalyst was removed, the filtrate evaporated *in vacuo* and the resulting oily residue purified by chromatography on neutral alumina. Elution with hexane–ether (7:3 and 6:4) gave 612 mg of **42** in a solvated form (m.p. ~40°), from which the solvent in spite of intensive drying could not be removed. A satisfactory analysis was therefore not obtained, UV: λ_{max} 234 mμ (ε 10800), IR (KBr): 1705 and 3470 cm^{-1}.

To a solution of 600 mg of this product in MeOH (6 ml) $NaBH_4$ (120 mg) was added with stirring at 5°. After standing for 45 min water (30 ml) was added and after acidification the mixture was extracted with ether. The extract was washed with water, dried, and evaporated to yield 570 mg of an amorphous residue which was chromatographed on florisil. Elution with benzene–EtOH (98:2) gave 490 mg of **43a**, which according to TLC was pure. Crystallization from ether–hexane gave crystals of m.p. 94–98°. $[\alpha]_D^{20}$ −1°, UV: λ_{max} 234 mμ (ε 12150), IR (KBr): 1705 and 3480 cm^{-1}. (Found: C, 73·20; H, 10·58. $C_{30}H_{50}O_4$, H_2O requires: C, 73·12; H, 10·64%.)

Acetylation of **43a**

Compound **43a** (760 mg) was dissolved in 7·6 ml of a mixture of acetic acid (40 ml), acetic anhydride (20 ml), and *p*-toluenesulfonic acid (10 g). After standing for 40 min water was added, and the oily precipitate, which formed, was extracted with ether. The ethereal solution was washed with $NaHCO_3$aq followed by water, dried, and evaporated *in vacuo*. The residue crystallized from MeOH to yield 624 mg of **43b**, m.p. 119–120°. Recrystallization from MeOH gave the analytical sample, m.p. 120–121°, $[\alpha]_D^{20}$ +16°, UV: λ_{max} 234 mμ (ε 12100), IR (KBr): 1250, 1715 and 1735 cm^{-1}. (Found: C, 73·15; H, 9·83. $C_{34}H_{54}O_6$ requires: C, 73·08; H, 9·74%.)

Ozonolysis of **43b**

Ozonized oxygen was bubbled through a solution of **43b** (520 mg) in dry CH_2Cl_2 (25 ml) containing dry pyridine (0·25 ml) at −75° in 30 min. About 6 mmoles of ozone were passed through the solution. Zn dust (1·25 g) and acetic acid (2·5 ml) were added, and after stirring for 40 min at room temp the precipitate was filtered off and washed with CH_2Cl_2. Ether (100 ml) was added to the combined filtrate and washings, and the resulting solution was washed with water, $NaHCO_3$aq and water. After drying and removal of the solvent 540 mg of a yellowish oil was obtained* which was extracted with hexane (3 × 100 ml). The extract was evaporated *in vacuo* and the residue crystallized from ether–hexane to yield 120 mg of compound **44**, m.p. 176–177°. Two recrystallizations from ether–hexane raised the m.p. to 180·5–181·5°, IR (KBr): 1255, 1730, 1740 cm^{-1}. (Found: C, 71·82; H, 9·10. $C_{35}H_{52}O_5$ requires: C, 71·74; H, 9·15%.) CD-curve: λ^{dioxan} 291 mμ ($\Delta\varepsilon$ = −4·19) and 298 mμ ($\Delta\varepsilon$ = −4·27).

Isomerization of **44** *into* **45**

A solution of **44** (116 mg) in ether (1 ml) was passed through a column of basic alumina (10 g) to afford 96 mg of a crystalline mixture, which according to TLC (solvent system: chloroform–acetone, 95:5. Spray reagent: $SbCl_3$ in $CHCl_3$) was an approximately 1:1 mixture of **44** and **45**. Fractionated crystallization from ether gave 28 mg of pure **45**, raised by recrystallization from the same solvent to 181·5–182·5°, IR(KBr): 1255, 1730, and 1740 cm^{-1}. (Found: C, 71·76; H, 9·11. $C_{35}H_{52}O_5$

* No trace of compound **45** could be detected by TLC of this product (solvent system: $CHCl_3$–acetone, 95:5. Spray reagent: saturated solution of $SbCl_3$ in $CHCl_3$).

requires: C, 71·74; H, 9·15%.) CD-curve: λ^{dioxan} 291 mμ ($\Delta\varepsilon = +1{\cdot}22$), 300 mμ ($\Delta\varepsilon = +1{\cdot}17$), and 310 mμ ($\Delta\varepsilon = +0{\cdot}76$).

Reduction of the ene-dione **46** *to* **47**

To a stirred solution of the ene-dione **46**[2,6a] (3·28 g) in 90% acetic acid (64 ml) Zn dust (6·6 g) was added in small portions during 15 min. After stirring for a further 60 min, the precipitate was filtered off, and the filtrate poured into water. The product was extracted with CH_2Cl_2 and the extract washed with water, dried, and evaporated to dryness *in vacuo*. Crystallization from ether afforded 2·92 g of **47**, m.p. 172–174°. Recrystallization from EtOH gave the analytical sample, m.p. 173–175°. $[\alpha]_D^{20}$ +94°, IR (KBr): 1703 and 1735 cm^{-1}. (Found: C, 76·46; H, 9·36. $C_{21}H_{30}O_2$ requires: C, 76·32; H, 9·15%.)

Ketalization of **47** *to* **49**

To a warm solution of **47** (2·92 g) in butanone ethyleneketal (30 ml) *p*-toluenesulfonic acid (150 mg) was added, and the mixture refluxed for 10 min. After cooling, pyridine (0·6 ml) and ether (30 ml) were added, and the resulting solution washed with water, dried, and evaporated to dryness *in vacuo*. The residue crystallized from ether to yield 1·86 g of **49**, m.p. 194–98°. Recrystallization from the same solvent raised the m.p. to 204–206°, $[\alpha]_D^{20}$ +34·4°, IR (KBr): 1096, 1718 and 1732 cm^{-1}. (Found: C, 73·64; H, 9·13. $C_{23}H_{34}O_4$ requires: C, 73·76; H, 9·15%.)

Ozonolysis of **42** *to the triketone* **48**

Ozonized O_2 was bubbled through a solution of **42** (400 mg) in dry CH_2Cl_2 (25 ml) containing dry pyridine (0·25 ml) at −75° in 2 hr. (It was necessary to pass about 6 mole equivs of O_3 through the solution to complete the reaction.) Zn dust (1 g) and acetic acid (2 ml) were added, and after stirring for 60 min at room temp, the precipitate was filtered off and washed with CH_2Cl_2. The combined filtrate and washings were washed successively with water, $NaHCO_3$aq and water, dried, and the solvent removed *in vacuo*. The residue crystallized from ether to afford 145 mg of **48**, m.p. 185–188°. Two further recrystallizations from ether raised the m.p. to 198·5–200°, IR (KBr): 1705 and 1740 cm^{-1}. (Found: C, 76·19; H, 9·37. $C_{21}H_{30}O_3$ requires: C, 76·32; H, 9·15%.)

Isomerization of **48** *into* **47**

A solution of **48** (30 mg) in a mixture of CH_2Cl_2 (1·5 ml), MeOH (1·5 ml), and 0·1N NaOH (0·3 ml) was allowed to stand under N_2 for 4·5 hr at room temp. TLC (solvent system: cyclohexane–ethyl acetate, 1:1; spray reagent: 50% aqueous phosphoric acid) revealed that the pure starting material ($R_F \sim 0{\cdot}47$) was almost completely transformed into a compound with the same R_F-value (~0·40) as that of **47**.

The mixture was neutralized with acetic acid; ether (25 ml) was added, and the solution washed with water, $NaHCO_3$aq and water. After drying and removal of the solvent, the residue crystallized from ether to yield 7 mg of a product with m.p. 170–171° alone or in admixture with a sample of **47**, prepared by reduction of **46**. The IR spectra of the two products were identical.

Reduction of **49** *to* **50** *and* **51**

To a stirred solution of **49** (12·9 g) in methylcellosolve (350 ml) sodium borohydride (12·9 g) was added in small portions during 30 min. During the addition the temp was kept below 25° by cooling. After standing at room temp for 1 hr the mixture was neutralized with acetic acid and poured into water (1·4 l). The precipitate was extracted with ether, and the extract carefully washed with water, and dried. Upon concentration of the ethereal solution 1·89 g of **50**, m.p. 173–177°, separated. Two recrystallizations from ether raised the m.p. to 183–185°. Paper chromatography of this product (solvent system: Light petroleum (b.p. 60–80°)–MeOH–water (10:8:2). Spray reagent: Saturated solution of $SbCl_3$ in $CHCl_3$) gave only one spot ($R_F = 0{\cdot}41$), $[\alpha]_D^{20}$ −124°, IR (KBr): 1100 and 3370 cm^{-1}, no CO-bands. (Found: C, 72·94; H, 10·04. $C_{23}H_{38}O_4$ requires: C, 72·97; H, 10·12%.)

On further concentration of the mother liquor from **50** and cooling of the solution to 0° 8·03 g of a crystalline material with m.p. 158–166° was obtained. According to paper chromatography in the same system this material was an approximately 1:9 mixture of **50** and a more polar substance

(R_F = 0·19). Several recrystallizations from ether afforded pure **51**, m.p. 171–173°, $[\alpha]_D^{20}$ −109°, IR (KBr): 1100 and 3460 cm^{-1}, no CO-bands. (Found: C, 72·84; H, 10·21. $C_{33}H_{56}O_4$ requires: C, 72·97; H, 10·12%.)

Conversion of **50** *to* **47**

A solution of **50** (400 mg) in 90% MeOH (15 ml) was acidified with 4N HCl and heated on the steam-bath for 10 min. After cooling, the mixture was poured into water and the product extracted with ether. The extract was washed with water, dried, and the solvent removed. The residue crystallized from ether–hexane to yield 240 mg of a product, m.p. 154–156°, raised by recrystallization from the same solvents to 158–159°, $[\alpha]_D^{20}$ −113°, IR (KBr): 1695 and 3440 cm^{-1}. (Found: C, 75·60; H, 10·33. $C_{31}H_{54}O_5$ requires: C, 75·40; H, 10·25%.)

This product (100 mg) in acetic acid (5 ml) was oxidized with 10% chromium (VI)-oxide in 95% acetic acid (1·2 ml) in the usual way. The residue crystallized from ether to afford 52 mg of a product with m.p. 171–172° undepressed in admixture with a sample of **47**. The IR spectra of the two products were identical.

Conversion of **51** *to* **47**

Compound **51** (1·0 g) dissolved in 90% MeOH (25 ml) was hydrolysed in the same way as described above for compound **50**. Crystallization from ether gave 660 mg of a product with m.p. 201–204°. Recrystallization from ether raised the m.p. to 204–206°. $[\alpha]_D^{20}$ −95°, IR (KBr): 1695 and 3440 cm^{-1}. (Found: C, 75·33; H, 10·28. $C_{31}H_{54}O_5$ requires: C, 75·40; H, 10·25%.)

This product (100 mg) was oxidized with chromium (VI)-oxide in the usual manner to yield 54 mg of a product with m.p. 170–171° alone or in admixture with a sample of **47**. The IR-spectra were identical.

Conversion of **50** *to the cyclosulfite* **52**

A solution of **50** (400 mg) in pyridine (10 ml) was cooled to −30° and thionyl chloride (2 ml) added over 5 min with stirring. After stirring for a further 40 min at −20° the mixture was allowed to warm to 0° and then poured on ice. The product was extracted with ether and the extract washed with 4N HCl, $NaHCO_3$aq and water. After drying and concentration 190 mg of **52**, m.p. 158–159° crystallized. Two recrystallizations from ether gave the analytical sample, m.p. 163–164°, IR (KBr): 935, 1095, 1105, and 1198 cm^{-1}; no OH-bands. (Found: C, 65·15; H, 8·58; S, 7·50; MW (vaporometric), 423·1. $C_{33}H_{54}O_6S$ requires: C, 65·06; H, 8·55; S, 7·55%; MW 424·6.) Mass-spectrum: peaks at m/e = 360 (M −64) and 342 (M −64 −18), M^+ = 424 absent.

20-Epi-16-deacetyltetrahydrofusidic acid lactone **56**

To a solution of **26**[3] (1·0 g) in MeOH (50 ml) 33% NaOHaq (1·0 ml) was added. After standing for 20 hr the solution was neutralized with HCl and diluted with water (200 ml). The precipitate was extracted with ether (2 × 50 ml) and the extract washed with water, dried, and evaporated to dryness. The residue crystallized from ether to yield 650 mg of **56**, m.p. 162–164°. Further recrystallizations did not raise the m.p., $[\alpha]_D^{20}$ −41°. (Found: C, 75·47; H, 10·48. $C_{29}H_{46}O_4$ requires: C, 75·60; H, 10·50%.)

16-Epi-deacetyl-24,25-dihydrofusidic acid **57**

A solution of 16-*epi*-deacetylfusidic acid[2] (8·0 g) in 96% EtOH (50 ml) was shaken at room temp under one atm H_2 in the presence of 10% Pd-C (1 g). In 25 min, 430 ml H_2 were absorbed and the consumption ceased. The catalyst was removed, and the filtrate concentrated to 50 ml *in vacuo*. Precipitation with water gave 7·2 g of **57**, m.p. 196–197°, unchanged after recrystallization from acetone. $[\alpha]_D^{20}$ −63°, UV: λ_{max} 229 mμ (ε 8950). (Found: C, 72·90; H, 10·05. $C_{29}H_{48}O_4$ requires: C, 73·07; H, 10·15%.)

16-Epi-deacetyl-24,25-dihydrofusidic acid lactone **58**

Compound **57** (1·0 g) in acetic acid (25 ml) was refluxed for 2 hr. After cooling, the mixture was poured into water, and the precipitate, hereby formed, filtered off, washed with water, and finally dissolved in ether (25 ml). The resulting solution was washed with dil. NaOHaq followed by water, dried, and evaporated to dryness. The residue (800 mg) crystallized from MeOH to yield 210 mg of

the 3-acetate of compound **58** m.p. 230–240°. Two recrystallizations from acetonitrile raised the m.p. to 261–266°, $[\alpha]_D^{20}$ −66°, IR (KBr): 1665 and 1735 cm^{-1}. (Found: C, 74·62; H, 9·23. $C_{33}H_{48}O_5$ requires: C 74·36; H, 9·66%.)

The methanolic filtrate was evaporated to dryness, and the residue chromatographed on florisil (20 g). Elution with benzene–ethyl acetate 95:5 gave an oil, which crystallized from ether–pentane to yield compound **58**, 112 mg, m.p. 131–133°. Recrystallization from the same solvents raised the m.p. to 132–133°. $[\alpha]_D^{20}$ −46·7°, UV: λ_{max} 230 mμ (ε 12800). (Found: C, 75·98; H, 10·14. $C_{29}H_{44}O_4$ requires: C, 75·94; H, 10·11%.)

16-Epi-deacetyltetrahydrofusidic acid lactone **59**

(a) A solution of 16-epi-deacetyl-24,25-dihydrofusidic acid lactone (**58**) (230 mg) in acetic acid (5 ml) was added to pre-reduced Adams catalyst (100 mg) in acetic acid (5 ml) and shaken at room temp under one atm H_2. In 5 min, 12·5 ml H_2 were absorbed, and the consumption ceased. The catalyst was removed, and the filtrate evaporated to dryness. Crystallization from MeOH–water gave 155 mg of **59**, which sintered at 155° and melted at 168–169°. Recrystallizations from ether–pentane raised the m.p. to 177·5–179°. $[\alpha]_D^{20}$ −12·5°. (Found: C, 75·58; H, 10·52. $C_{29}H_{46}O_4$ requires: C, 75·60; H, 10·50%.)

(b) A solution of 16-epi-deacetyl-fusidic acid (**54b**; 3·8 g) in acetic acid (30 ml) was shaken at room temp under one atm H_2 in the presence of PtO_2 (1·0 g) until the uptake of H_2 ceased (4 hr). After removal of the catalyst, water (100 ml) was added to precipitate on amorphous product, which was collected, washed with water and dissolved in ether. The ethereal solution was washed with dil NaOHaq followed by water, dried, and evaporated to dryness. The residue (2·52 g) crystallized from MeOH–water to yield 1·30 g of **59** sintering at 155° and melting at 163–166°. Two recrystallizations from ether–pentane raised the m.p. to 177·5–179° alone or in admixture with the product prepared as described under (a) above. The IR spectra were identical.

20-Epi-16-epi-deacetyltetrahydrofusidic acid lactone **60**

To a solution of 16-epi-deacetyltetrahydrofusidic acid lactone (**59**) (200 mg) in MeOH (10 ml) 33% NaOHaq (0·2 ml) was added. After standing overnight the solution was neutralized with HCl and precipitated with water to yield 144 mg of crystals, m.p. 177–178°. Recrystallization from MeOH–water did not raise the m.p. $[\alpha]_D^{20}$ −20·8°. (Found: C, 75·62; H, 10·51. $C_{29}H_{46}O_4$ requires: C, 75·60; H, 10·50%.)

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