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THE STRUCTURE OF FUSIDIC ACID

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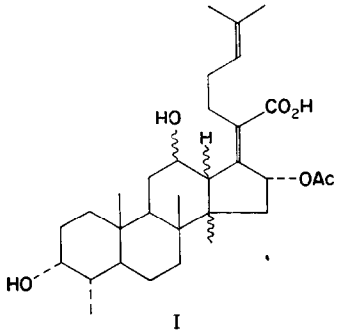
Abstract—Evidence for the assignment of formula I to the antibiotic fusidic acid is presented.

THE isolation of a new antibiotic, fusidic acid, from a strain of *Fusidium* has recently been reported.[1] This substance was characterized as an α,β-unsaturated carboxylic acid, $C_{31}H_{48}O_6$, containing one acetoxyl and two hydroxyl groups. The presence of an isolated trisubstituted double bond was demonstrated, and it was concluded that fusidic acid contains a tetracyclic ring system.[1]

In this paper, arguments for the assignment of formula I to fusidic acid are presented.



I

The elementary analyses of fusidic acid and a considerable number of its derivatives agree well with the formula, $C_{31}H_{48}O_6$, which is further supported by the equivalent weight obtained on electrometric titration.

Functional groups

The IR spectrum of fusidic acid (KBr) has bands at 3420 and 3510 cm^{-1} (OH), 1697 and 1725 cm^{-1} (CO), and 1260 cm^{-1} (ester). The ester band is due to an acetoxyl group as shown by identification of the steam volatile acid, obtained on alkaline hydrolysis, as acetic acid (by IR), and an acetyl determination on fusidic acid agreeing with the presence of one acetoxyl group. Acetylation of fusidic acid and its methyl ester (II) give monoacetates (III and IV), which in the IR still show hydroxyl bands. This implies that two of the six oxygen atoms in fusidic acid belong to hydroxyl groups, two to the carboxyl group, and two to an acetoxyl group and the compound is consequently:

$$C_{28}H_{42}\begin{cases}OH\\OH\\OCOCH_3\\COOH\end{cases}$$

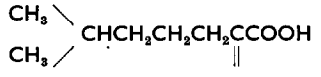
[1] W. O. Godtfredsen, S. Jahnsen, H. Lorck, K. Roholt and L. Tybring; *Nature, Lond.* **193**, 987 (1962).

Double bonds

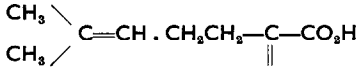
Fusidic acid on catalytic hydrogenation over palladium on calcium carbonate takes up one mole of hydrogen. The resulting dihydrofusidic acid (V), $C_{31}H_{50}O_6,\frac{1}{2}H_2O$ (m.p. 182–183°) shows in the UV an absorption maximum at 220 mμ (ε 8300), characteristic of a fully substituted α,β-unsaturated carboxylic acid, and the difference between the UV spectra of I and V (λ_{max}^{EtOH} 204 mμ (ε 4200)) shows that the chromophore, which was hydrogenated, is a trisubstituted isolated double bond. On hydrogenation of V over platinum oxide in acetic acid another mole of hydrogen is absorbed, and the tetrahydrofusidic acid (VI) $C_{31}H_{52}O_6$ (m.p. 172–173·5°) thus obtained shows no selective absorption in the ultra-violet. Provided that fusidic acid does not contain double bonds resistant to hydrogenation (and this is not likely, since VI gives no colour with tetranitromethane) it follows from the empirical formula that the compound contains a tetracyclic ring system.

The side chain

Ozonolysis of the methyl ester VII, $C_{32}H_{52}O_6$, of dihydrofusidic acid in methylene chloride–pyridine[2] followed by reduction with Zn–acetic acid cleaves the molecule into two fragments: A liquid (VIII), $C_9H_{18}O_3$ (b.p. 95°/10 mm), and a solid (IX) $C_{23}H_{32}O_5$ (m.p. 211·5–212·5°). The IR spectrum of VIII (CS_2) contains bands at 3580 cm^{-1} (OH), 1746 cm^{-1} (ester) and a doublet at 1370–1390 cm^{-1} characteristic of an isopropyl group. The α-ketoester primarily formed on the ozonolysis has evidently been reduced to the corresponding hydroxyester during the subsequent reduction. The most probable structure of this compound is, in view of the empirical formula and the IR spectrum, that of 2-hydroxy-6-methylheptanoic acid methyl ester. This compound has not previously been described, but may be synthesized from the methyl ester of the known 6-methylheptanoic acid[3] by bromination followed by treatment of the resulting α-bromoester with sodium carbonate and re-esterification with diazomethane. The IR spectra of this compound and VIII are identical, and the side chain in dihydrofusidic acid has consequently the structure:



In order to decide whether the isolated trisubstituted double bond in fusidic acid is located in the side chain or in the ring system, the ozonolysis was repeated with dideuteriofusidic acid (obtained on catalytical deuteration of fusidic acid) instead of VII. The fact that the isolated 2-hydroxy-6-methylheptanoic acid methyl ester contains deuterium (bands at 2190 and 2230 cm^{-1} in the infra-red), whereas no deuterium could be detected in the solid ozonolysis product, indicates that the isolated double bond is located in the side chain, and since it is trisubstituted, this side chain possesses the structure:



In accordance with this, ozonolysis of fusidic acid in acetic acid, followed by steam distillation, yields acetone, isolated and characterized as its 2,4-dinitrophenylhydrazone. The structure of the solid, obtained on ozonolysis of VII, is discussed later.

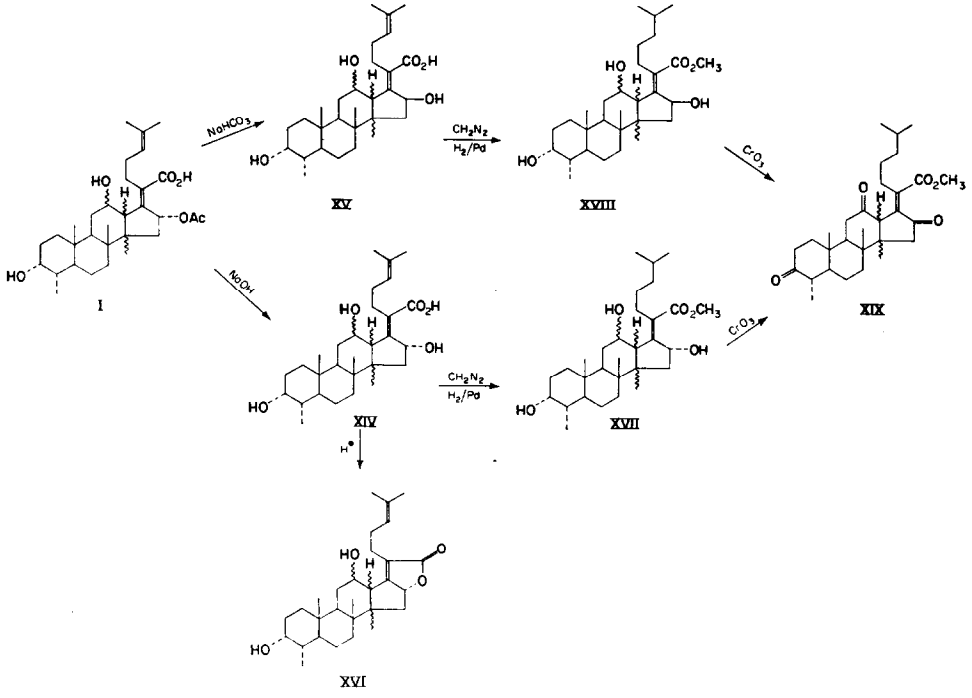
[2] G. Slomp, Jr. and J. L. Johnson, *J. Amer. Chem. Soc.* **80,** 915 (1958).
[3] A. H. Milburn and E. V. Truter, *J. Chem. Soc.* 3344 (1954).

The character of the functional groups

Oxidation of VI with chromium trioxide in acetic acid gives a diketo acid X, $C_{31}H_{48}O_6$ (m.p. 127·5–129°), which on alkaline hydrolysis is converted into a mixture of two isomeric diketo-monohydroxyacids, $C_{29}H_{46}O_5$: XI, m.p. 231–233°, and XII, m.p. 275–277°.* Compound XI is reconverted into X on acetylation with acetic anhydride–pyridine, and therefore represents the corresponding deacetylated compound. The IR spectrum (KBr)of the sodium salt of XI has bands at 1575 (carboxylate) and 1710 cm^{-1} (six-ring ketone), indicating that the two hydroxyl groups in fusidic acid are secondary and located in six-membered rings.

Chromium trioxide oxidation of XI affords a triketo acid XIII, $C_{29}H_{44}O_5$ (m.p. 191·5–192°), the sodium salt of which in the IR has bands at 1585 (carboxylate), 1710 (six-ring ketone), and 1735 cm^{-1} (five-ring ketone). This implies that the acetoxyl group is secondary and located in a five-membered ring.

Alkaline hydrolysis of fusidic acid yields a mixture of two isomeric trihydroxy acids, $C_{29}H_{46}O_5$: XIV, m.p. 106–108°, and XV, m.p. 199–199·5°. Compound XIV lactonises very readily on acidification,† but may be converted into a stable methyl ester on esterification with diazomethane. This acid is the main product when fusidic acid is hydrolysed in concentrated solution with strong alkali. When hydrolysed in weakly alkaline dilute solution, XV, which shows less tendency to lactonization, is the main product.



* XI and XII give different acetates on acetylation and different triketones on chromium trioxide oxidation, and are therefore believed to be C_{13}-epimers. On prolonged treatment with strong alkali, XI is transformed into XII. A similar transformation has recently been reported.[4]

† The lactone XVI, $C_{29}H_{44}O_4$ (m. p. 158–159°) shows an absorption max at 224 mμ with an extinction coeff. (13·300) corresponding to a *transoid* α,β-unsaturated ester.

[4] F. G. Fischer and N. Seiler, *Liebigs Ann.* **644**, 146 (1961).

Hydrogenation of the methyl esters of XIV and XV over palladium on calcium carbonate in ethanol yields the corresponding dihydro esters XVII, $C_{30}H_{50}O_5$ (m.p. 144·5–145·5°) and XVIII, $C_{30}H_{50}O_5$ (amorphous). These give on oxidation with chromium trioxide in acetic acid the same triketoacid methyl ester XIX, $C_{30}H_{44}O_5$ (m.p. 133·5–135·5°) and they are consequently OH-epimers. The UV absorption spectrum of XIX shows an absorption maximum at 247 mμ (ε 9000) indicating that one of the keto groups is α to the double bond, and since the diketo ester XX, $C_{32}H_{48}O_6$ (m.p. 139–139·5°), obtained on chromium trioxide oxidation of VII, shows an UV spectrum similar to VII, this keto group must originate from the acetylated hydroxyl group in fusidic acid, which therefore is in allylic position to the double bond and γ to the carboxylic group. This agrees with the strong tendency to lactonization shown by XIV.

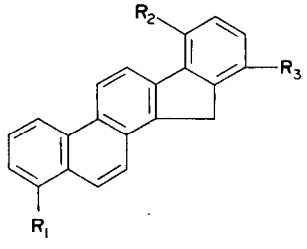
The experiments described show that the two hydroxyl groups in fusidic acid are secondary in six-membered rings, and that the acetoxyl group is secondary and located in a five-membered ring allylic to the double bond which is conjugated with the carboxylic group. This implies that this double bond is attached to the same five-membered ring as the acetoxyl group.

The ring system

The character of the tetracyclic ring-system in I, was determined by a selenium dehydrogenation. Extraction of the dehydrogenation mixture with ether followed by chromatography of the extract on alumina afforded three compounds:

(a) 1,2,5,-Trimethylnaphthalene, characterized as its picrate (m.p. 136–137·5°), and trinitrobenzene adduct, $C_{19}H_{17}N_3O_6$ (m.p. 156–157°). These melting points as well as the UV spectrum are in good agreement with the reported values.[5,6]

(b) 1,8-Dimethylphenanthrene, the IR spectrum of which is indistinguishable from the spectra of authentic samples.*



XXIa $R_1 = CH_3$, $R_2 = H$, $R_3 = CH(CH_3)_2$

XXIb $R_1 = CH_3$, $R_2 = CH(CH_3)_2$, $R_3 = H$

* Samples of 1,8-dimethylphenanthrene were kindly submitted by Professor F. A. King, Nottingham and Dr. Carruthers, Exeter.

[5] D. H. R. Barton and K. H. Overton, *J. Chem. Soc.* 2639 (1955).

[6] J. D. Cocker and T. G. Halsall, *J. Chem. Soc.* 4262 (1956).

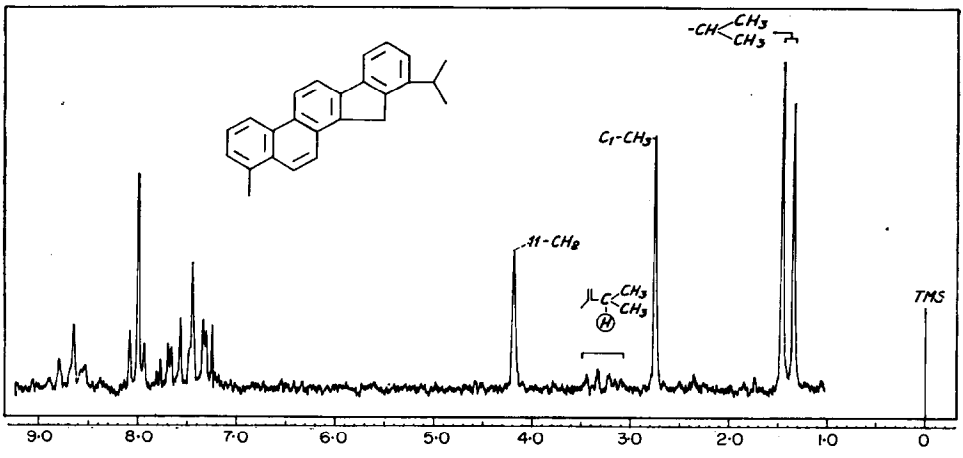


FIG. 1

(c) A solid (XXI), $C_{25}H_{22}$ m.p. 250–254°, the UV spectrum of which is characteristic of the 11 H-naphtho (2,1-a) fluorene ring-system,[7]* and since the ring system in I is tetracyclic, a ring closure during the dehydrogenation must take place. When considering the side chain and the other two dehydrogenation products either structure XXI a or b of this compound is highly probable, and the NMR spectrum (Fig. 1) is consistent with these formulae. Evidence presented below shows that the side chain is attached at C_{17} in the cyclopentenoperhydrophenanthrene ring system and XXIa is, therefore, probably the correct formula.

On the basis of the selenium dehydrogenation, it is concluded that fusidic acid contains the cyclopentenoperhydrophenanthrene ring system, and thus belongs to the steroid class. The formation of 1,8-dimethylphenanthrene suggests the presence of methyl groups at C_4 and C_{14}, and the formation of 1,2,5-trimethylnaphthalene is regarded as an indication of the presence of a methyl group at C_8 or C_9.[10] Since, however, 1,2,8-trimethylphenanthrene, which is a normal product from dehydrogenation of tetracyclic triterpenes,[11] could not be isolated, the C_{13}-methyl group, present in most steroids and tetracyclic triterpenes, is probably absent in fusidic acid.†

The location of the side chain and the functional groups

The compound IX, $C_{23}H_{32}O_5$ (ν_{KBr} 1710, 1755 and 1770 cm^{-1}), obtained on ozonolysis of VII in addition to 2-hydroxy-6-methylheptanoic acid methyl ester, has an acetoxyl group and three keto groups, since the two hydroxyl groups in VII have been oxidized during the ozonolysis. The bands at 1755 and 1770 cm^{-1} are best

* Hydrocarbons with similar UV spectra have been obtained on dehydrogenation of Lanosterol[8] and Helvolic acid.[9]

† The triterpenes of the dammarane group show this unusual feature too.

[7] W. V. Mayneord and E. M. F. Roe, *Proc. Roy. Soc.* **A152,** 299 (1935).

[8] L. Ruzicka, Ed. Rey and A. C. Muhr, *Helv. Chim. Acta* **27,** 472 (1944).

[9] D. J. Cram and N. L. Allinger, *J. Amer. Chem. Soc.* **78,** 5275 (1956).

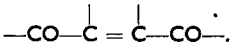
[10] D. H. R. Barton, J. F. McGhie, M. K. Pradhan and S. A. Knight, *J. Chem. Soc.* 876 (1955).

[11] G. Ourisson and P. Crabbé: *Les Triterpènes Tétracycliques* pp. 88 and 140–144. Hermann, Paris (1961).

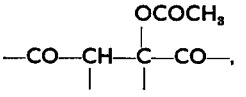
interpreted as a five-ring ketone adjacent to an acetoxyl group,[12] and since none of the hydroxyl groups in VII are located in a five-membered ring, the five-ring ketone must have been formed during the ozonolysis. This involves that the side chain is attached to ring D, as already deduced above, and, in view of the formation of a naphthofluorene on selenium dehydrogenation, at C_{16} or C_{17}.

As IX shows no selective absorption in the UV apart from low-intensity bands due to isolated carbonyl groups, the presence of enolizable α- or β-diketones can be ruled out.

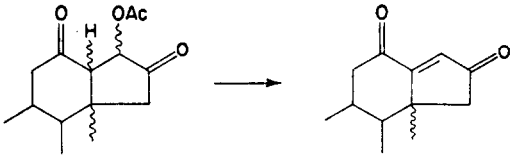
On treatment with strong alkali, acetic acid is rapidly eliminated with the formation of a yellow compound, XXII, $C_{21}H_{28}O_3$ (m.p. 192.5–193°), the UV spectrum of which (λ_{max}^{EtOH} 261 mμ (ε 8100)) is characteristic of the ene-dione system:



This indicates that IX contains the grouping

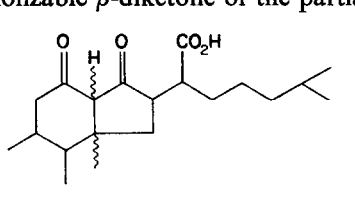


and since the acetoxyl group is secondary and located in ring D, this requirement can only be fulfilled if IX and XXII have the following partial structures:*

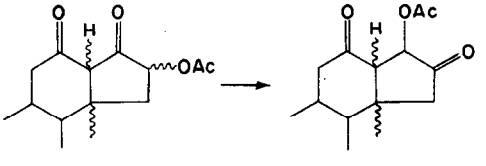


IX XXII

It is, however, unlikely that the side chain in fusidic acid is attached to C_{16}, both for biogenetical reasons and because the triketone XIII does not show absorption in the UV corresponding to an enolizable β-diketone of the partial structure:



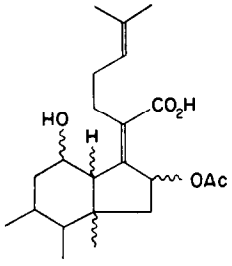
An explanation of this apparent paradox is that the transformation:



* The fact that ozonolysis of VII-monoacetate, $C_{34}H_{54}O_7$ (m.p. 147–152°) gives a product, which on treatment with alkali yields an ene-dione too, indicates that the six-ring ketone in XXII, which forms part of the ene-dione chromophore, originates from the non-acetylateable hydroxyl group in VII.

[12] R. N. Jones and G. Roberts, *Chem. & Ind.* 1269 (1957).

has taken place during the ozonolysis or the subsequent reduction,* and fusidic acid must, in view of these considerations, be assigned the partial structure:



It is *a priori* to be expected that the remaining hydroxyl group in fusidic acid is located at C_3, since practically all natural steroids and tetracyclic triterpenes have an oxygen function in this position. From the following, it will appear that this is actually the case:

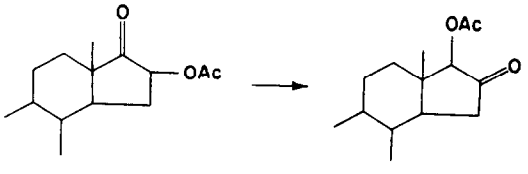
The NMR spectrum of II clearly shows the presence of a secondary C-methyl group (doublet centered at $\delta = 0{\cdot}90$), and since this, in view of the results of the selenium dehydrogenation of I, must be located at C_4, the usual 4-*gem*-dimethyl group can be ruled out. In the compounds XIX and XX (in which the two hydroxyl groups are oxidized to keto groups), the center of the doublet has shifted to $\delta = 1{\cdot}07$, and it is obvious that only the C_3-position can be responsible for this shift.

The location of the methyl groups

The NMR spectra of IX and XXII (Figs. 2 and 3) shows the presence of three tertiary and one secondary methyl groups, two of which (C_4 and C_{14}) already have been accounted for. The location of the two remaining tertiary methyl groups at C_8 and C_{10} is biogenetically plausible and in accordance with the formation of 1,2,5-trimethylnaphthalene on selenium dehydrogenation.

The absence of a methyl group at C_9 can, furthermore, be demonstrated in the following way: The tetrahydroderivative XXIII, $C_{29}H_{48}O_4$ (m.p. 147–148°) of the lactone XVI was acetylated and the resulting 3-acetate XXIV, $C_{31}H_{50}O_5$ (m.p. 195–196°) oxidized with chromium trioxide to the corresponding 12-ketone XXV, $C_{31}H_{48}O_5$ (m.p. 236–237°). The fact that dehydrogenation of XXV with selenium dioxide gives a compound XXVI, $C_{31}H_{46}O_5$ (m.p. 226–228°) with an UV spectrum (λ_{max}^{EtOH} 244 mμ (ε 7350)) characteristic of a $\Delta^{9,11}$-12-ketone, rules out the presence of a methyl group at C_9.

* Similar acyl migrations are known. For example, Fishman[13] recently observed that the transformation:



proceeds under mild conditions.

[13] J. Fishman, *J. Amer. Chem. Soc.* **82,** 6143 (1960).

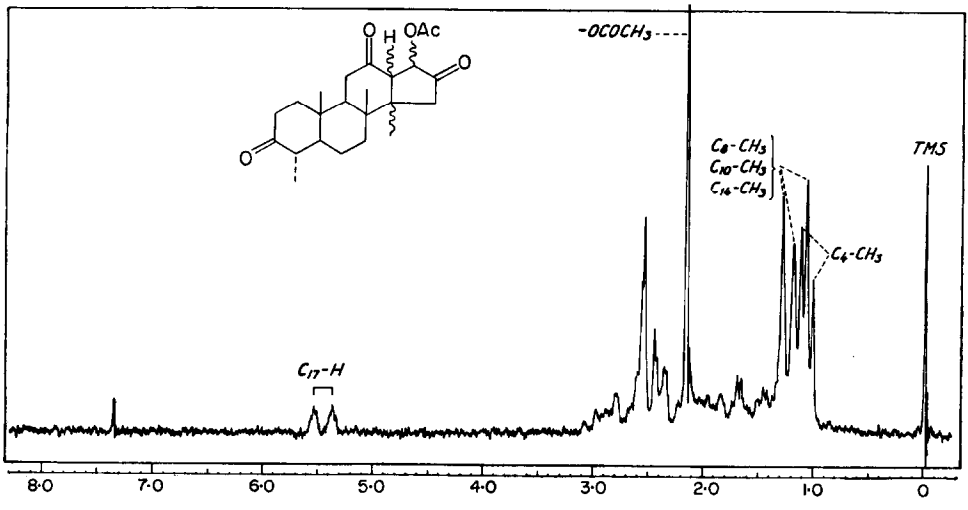


FIG. 2

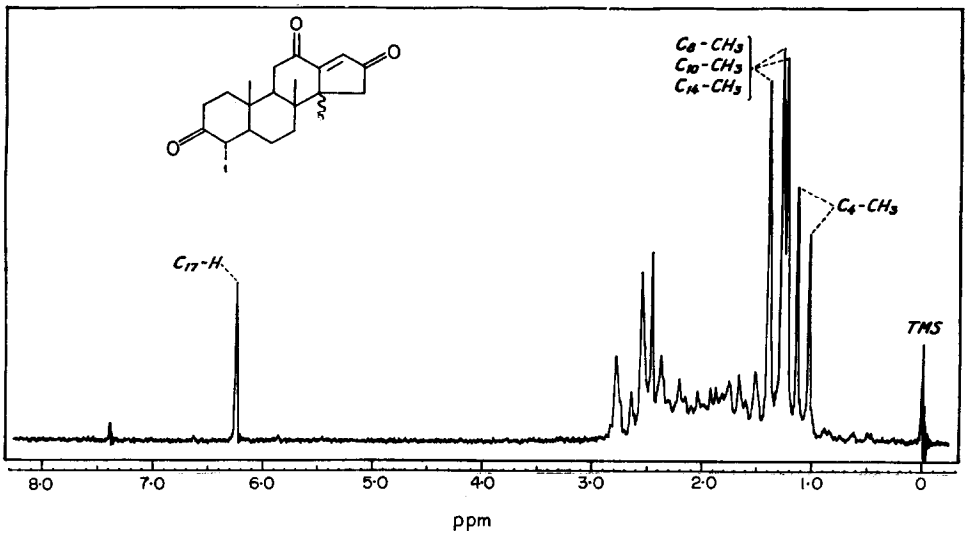


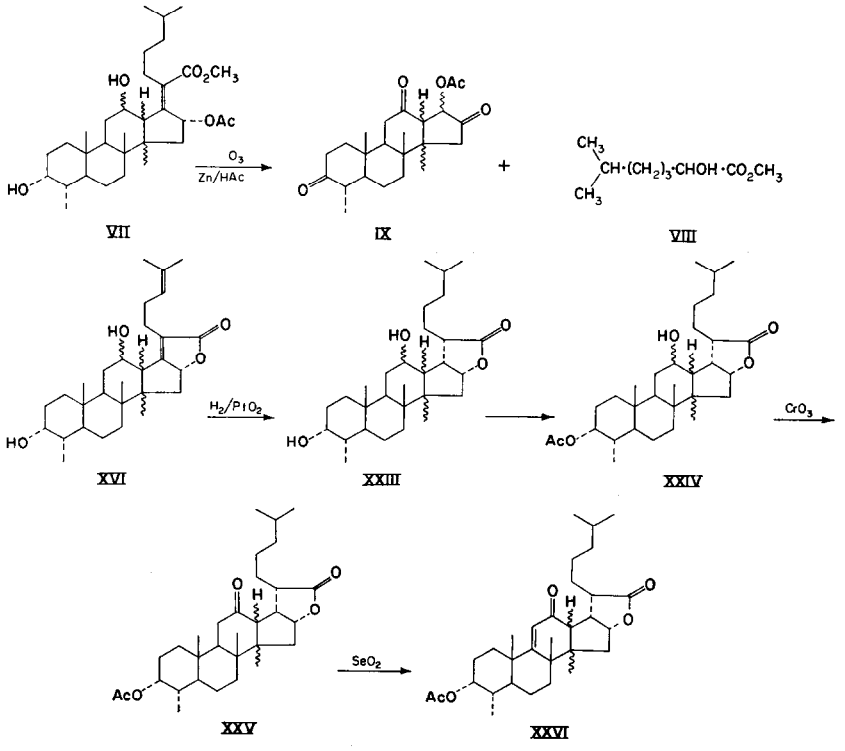
FIG. 3

Stereochemistry

The hydroxyl group at C_3. Chromium trioxide oxidation of dihydrofusidic acid 3-acetate (XXVII), $C_{33}H_{52}O_7,H_2O$ (m.p. 139–141°) gives a monoketone, XXVIII, $C_{33}H_{50}O_7$ (m.p. 191–192°), which on reduction with sodium borohydride is reconverted into XXVII in high yield, indicating that the 12-hydroxyl group is not epimerized during these operations. The fact that a similar reduction of the diketone XXIX, $C_{31}H_{46}O_6$ (m.p. 206–207°), obtained on chromium trioxide oxidation of dihydrofusidic acid, gives XXX, $C_{31}H_{50}O_6$ (m.p. 211°) with a lower R_F-value* than

* Three solvent systems described by Bush[14] (A,B_1, and B_5) were found well suited for paper chromatography of fusidic acid and many of its derivatives.

[14] I. E. Bush, *Biochem. J.* **50**, 370 (1952).



dihydrofusidic acid, shows that the 3-hydroxyl group in fusidic acid is α and axial. This is confirmed by the differences between the molecular rotations of dihydrofusidic acid 3-acetate and dihydrofusidic acid (−94), and between XXX-3-acetate and XXX (+51), which are consistent with 3α and β-steroids, respectively, containing at least one methyl group at C_4.[15]

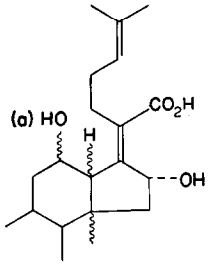
The hydroxyl group at C_{12}. The hindered nature of this hydroxyl group together with the fact that it is not epimerized on oxidation followed by sodium borohydride-reduction suggests an axial orientation, which is confirmed by NMR studies: By comparing the spectra of the dihydrolactone XXXI, $C_{29}H_{46}O_4$, m.p. 157–158° (obtained on hydrogenation of XVI), its 3-acetate, and its 3,12-diacetate (obtained on acetylation with acetic anhydride-pyridine and acetic anhydride–toluene sulphonic acid, respectively) a signal at $\delta = 4{\cdot}38$ in the spectrum of XXXI may be attributed to the proton at C_{12}. The width of this signal rules out the presence of an axial-axial coupling between this proton and a neighbour, as expected if the hydroxyl group is equatorial and the C_{12} proton axial. The hydroxyl group is thus axial.

The acetoxyl group at C_{16}. This group is assigned the α-configuration because of the negative value (−180) of the molecular rotation difference between tetrahydrofusidic acid (VI) and the corresponding de-acetylated compound XXXII, $C_{29}H_{50}O_5$ (m.p. 244–245.5°).[15,*] The following considerations support this assignment: Inspection of

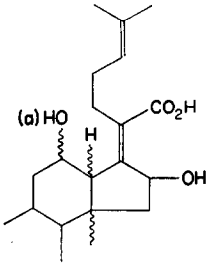
* The possibility that epimerization at C_{16} had taken place during the alkaline hydrolysis of VI may be eliminated since VI and XXXII on acetylation with acetic anhydride–toluene sulphonic acid gives the same triacetate XXXIII, $C_{35}H_{56}O_8$ (m.p. 169–170°).

[15] W. Klyne and W. M. Stokes, *J. Chem. Soc.* 1979 (1954).

Dreiding models of the two 16-epimeric compounds, obtained an alkaline hydrolysis of fusidic acid, shows that the 16-hydroxyl group must be α-orientated in XIV, which readily lactonizes, and β-orientated in XV, which shows a far lesser tendency to lactonization. Compound XV has, however, at C_{16} the opposite configuration of fusidic acid, since acetylation yields a diacetate XXXIV, $C_{33}H_{50}O_7$ (m.p. 168–170°) different from fusidic acid 3-acetate (III),* and the configuration of the 16-hydroxyl group in XIV therefore corresponds to that of the acetoxyl group in fusidic acid.



XIV

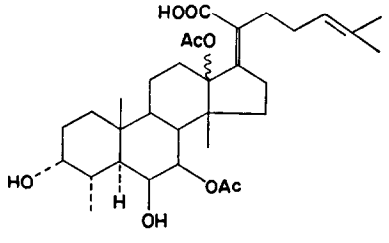


XV

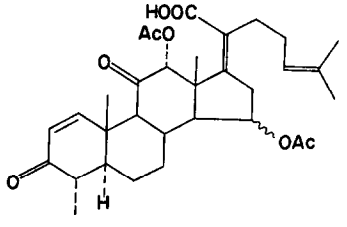
The A/B-*junction.* To decide whether rings A and B are *cis-* or *trans*-fused the 3-monoketone, XXXV, $C_{31}H_{50}O_6$ (m.p. 135–137°) of tetrahydrofusidic acid was synthesized by the following sequence: Ketalization of the diketo-acid X (by the dioxolane method[16]) gave the corresponding 3-ethylene ketal, XXXVI, $C_{33}H_{52}O_7$ (m.p. 157–158°), which on reduction with sodium borohydride, followed by acid catalyzed cleavage of the ketal, gave XXXV.† The rotatory dispersion curve of this compound shows a positive Cotton effect indicating that rings A and B are *trans*.[17]

The methyl group at C_4. This group is assigned the equatorial α-configuration for the following reasons:

Prolonged treatment of the diketone XXIX with strong alkali gives, after acidification, the same lactone XXXVII, $C_{29}H_{42}O_4$ (m.p. 169–171°) which is obtained on oxidation of the dihydro lactone XXXI with chromium trioxide. If the C_4-methyl-group is axial, then prolonged treatment with alkali would transform it into its more stable equatorial isomer.



XXXVIII



XXXIX

* Acetylation of XIV causes lactonization and leads to the 3-acetate of the lactone XVI.

† That XXXV is a 3-ketone and not a 12-ketone follows from the fact that catalytic hydrogenation affords a high yield of VI. If XXXVI is a 12-ketal, the sodium borohydrate reduction, followed by acid-catalysed cleavage of the ketal, would lead to a 3β-hydroxy-12-ketone, and this would not give VI on catalytic reduction.

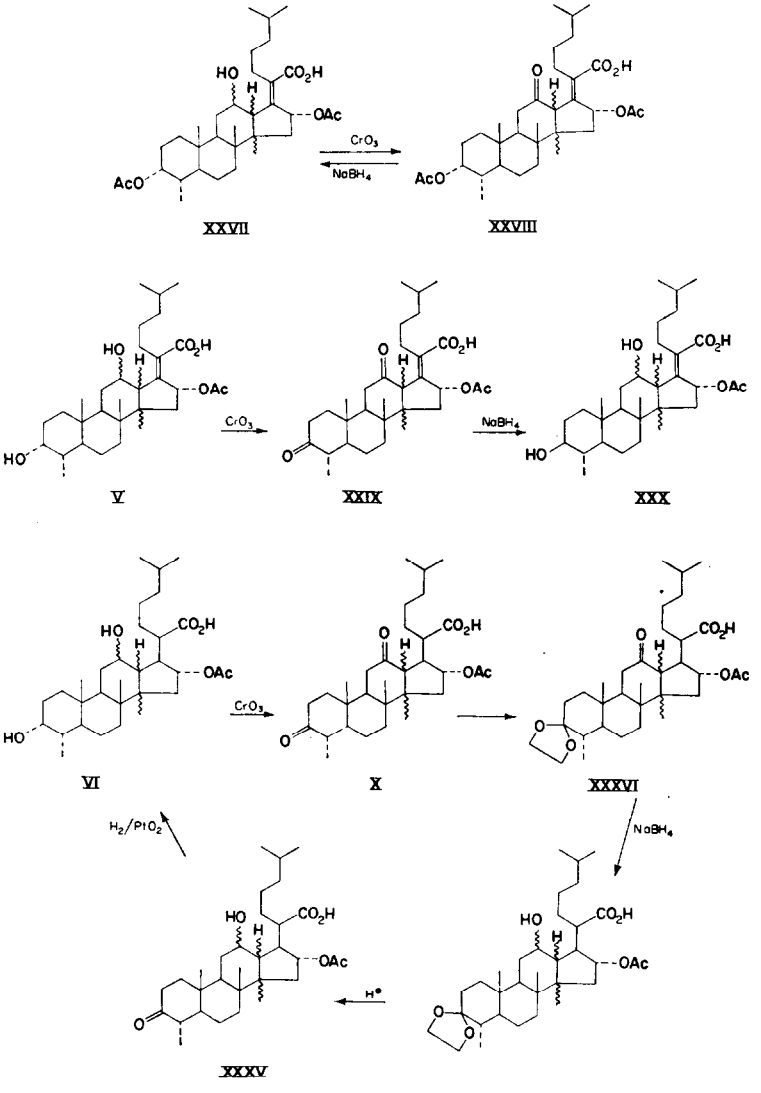
[16] G. Rosenkranz, M. Velasco and F. Sondheimer, *J. Amer. Chem. Soc.* **76**, 5024 (1954).

[17] C. Djerassi, *Optical Rotatory Dispersion* p. 49. McGraw-Hill, New York (1960).

The remaining stereochemical problems, e.g. the B/C- and C/D-junctions will be the subject of further investigations.

Recently, the structures XXXVIII and XXXIX have been proposed for the antibiotics Cephalosporin P_1[18] and Helvolic acid,[19] respectively.

It will be seen that both of these substances have many features in common with fusidic acid.



EXPERIMENTAL

All m.p. are corrected. Optical rotations, unless otherwise stated, were measured in chloroform and UV spectra in 96% ethanol solution. The IR spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer with a sodium chloride prism. The NMR spectra were taken in $CDCl_3$ using

[18] B. M. Baird, T. G. Halsall, E. R. H. Jones, and G. Lowe. *Proc. Chem. Soc.* 257 (1961).
[19] N. L. Allinger and J. L. Coke. *J. Org. Chem.* **26**, 4522 (1961).

a Varian Associates spectrometer Model A-60 Mc/sec. 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). Numbers in parentheses represent the number of protons found by integration. Microanalyses were by Mr. G. Cornali and Mr. W. Egger.

Isolation of fusidic acid (I)

Clarified fermentation broth (500 l.) containing about 250 g fusidic acid (determined by the agar cup method using Staphylococcus aureus as test organism) was adjusted to pH 6 and extracted with isobutyl methyl ketone (2 × 50 l.). The resulting organic phase was extracted with sodium hydroxide aq (15 l.) at pH 11, and immediately after the separation the aqueous phase was adjusted to pH 9·5 by addition of NaH_2PO_4 and washed with ligroin (5 l.). Benzene (3 l.) was added and while stirring dil hydrochloric acid was added slowly to pH 6·3 (1 hr). After stirring for further 3 hr the precipitate was filtered off, washed with water followed by benzene, and dried to yield 204 g crude benzene solvate of fusidic acid. The crude product, dissolved in a mixture of ethanol (200 ml) and benzene (800 ml), was treated with decolorizing carbon (10 g) for 30 min. After removal of the carbon, the filtrate was diluted with benzene (800 ml) and concentrated *in vacuo* to about 800 ml to remove the ethanol. Filtration and washing with benzene gave 180 g of a colourless benzene solvate which sintered at about 95° (loss of solvent) and melted at 191–192°. Solvent-free fusidic acid was obtained as follows: A solution of purified benzene solvate (100 g) in ethanol (100 ml) was evaporated to a syrup *in vacuo*. Ether (500 ml) was added, and the evaporation repeated. The residue was dissolved in ether (500 ml) and after standing overnight the crystalline precipitate was collected, washed with ether and dried to yield 78 g pure solvent-free fusidic acid with the constant m.p. 192–193°. $[\alpha]_D^{20}$ −9°. UV: λ_{max} 204 mμ (ε 9900); $\lambda_{shoulder}$ 210 mμ (ε 9400). IR (KBr): 3420 and 3510 cm^{-1} (OH), 1697 and 1725 cm^{-1} (carbonyl), and 1260 cm^{-1} (acetate). (Found: C, 72·03; H, 9·36. $C_{31}H_{48}O_6$ (M = 516·7) requires: C, 72·06; H, 9·36%). Electrometric titration in 50% (v/v) ethanol gave a pK-value of 6·35 (corresponding to a pK of approximately 5·35 in water), and an eqt wt of 519. Fusidic acid is readily soluble in chloroform, ethanol, dioxane and pyridine, but sparingly soluble in water and hexane. From benzene, acetone and methanol it crystallizes in solvated forms. The sodium salt was prepared as follows: A solution of the benzene solvate (6 g) in a mixture of methanol (3 ml) and acetone (9 ml) was carefully neutralized with 33% aqueous sodium hydroxide (Bromphenol Blue indicator). After addition of acetone (10 ml) crystallization was induced on scratching, and in 1 hr further 60 ml of acetone was added with stirring. Filtration and washing with acetone gave 4·9 g sodium salt, containing ½ mole of water.

Fusidic acid methyl ester (II)

To a suspension of fusidic acid (25 g) in ether (75 ml) an ethereal solution of diazomethane was added until the yellow colour persisted. The resulting solution was evaporated to dryness under red press, and the residue crystallized from ether–hexane to yield 24·7 g methyl ester, m.p. 151–153°. Recrystallization from ether raised the m.p. to 153·5–154°. $[\alpha]_D^{20}$ −14°. (Found: C, 72·30; H, 9·50. $C_{32}H_{50}O_6$ requires: C, 72·41; H, 9·50%).

Fusidic acid 3-*acetate* (III)

Fusidic acid (1 g) was dissolved in a mixture of acetic anhydride (3 ml) and pyridine (3 ml). After standing for 16 hr at room temp water was added, and the amorphous precipitate hereby obtained was collected, washed with water and dried. Crystallization from methanol–water afforded 0·75 g, m.p. 108–111° raised by two further recrystallizations from the same solvents to 121–123°. $[\alpha]_D^{20}$ −23°. (Found: C, 68·77; H, 9·09; acetyl, 15·06. $C_{33}H_{50}O_7$, H_2O requires: C, 68·72; H, 9·09; acetyl 14·95%).

Fusidic acid methyl ester 3-*acetate* (IV)

Acetylation of fusidic acid methyl ester (1 g) in the usual way gave 0·9 g of the 3-acetate, m.p. 118–119°. Recrystallization from ether–hexane raised the m.p. to 119·5–120°. $[\alpha]_D^{20}$ −26°. IR (KBr): 3500 cm^{-1} (OH). (Found: C, 71·25; H, 9·20. $C_{34}H_{52}O_7$ requires; C, 71·29; H, 9·15%).

Dihydrofusidic acid (V)

A solution of fusidic acid (7·5 g) in 96% ethanol (50 ml) was shaken at room temp under one atm hydrogen in the presence of 5% palladium on calcium carbonate (1·5 g). In 40 min, 370 ml hydrogen were absorbed and the consumption ceased. The catalyst was removed, and the filtrate precipitated with water to yield 7·4 g, m.p. 182–184°. For analytical purposes a sample was recrystallized from benzene and finally from ether, m.p. 182–183°. $[\alpha]_D^{20}$ ±0°. UV: λ_{max} 220 mμ (ε 8300). (Found: C, 70·48; H, 9·76, $C_{31}H_{50}O_6$, $\frac{1}{2}H_2O$ requires: C, 70·55; H, 9·74%).

Tetrahydrofusidic acid (VI)

A solution of dihydrofusidic acid (V; 15·6 g) in acetic acid (200 ml) was shaken at room temp under one atm hydrogen in the presence of platinum oxide (1·3 g). In 100 min, 750 ml hydrogen were absorbed and the consumption of hydrogen ceased. The catalyst was removed, and the filtrate evaporated to dryness *in vacuo*. The residue was crystallized from ethanol–water to afford 11·5 g, m.p. 171–173°. Recrystallization from acetonitrile gave the analytical sample, m.p. 172–173·5°. $[\alpha]_D^{20}$ −64°. UV: No selective absorption above 200 mμ. (Found: C, 71·37; H, 10·00. $C_{31}H_{52}O_6$ requires: C, 71·50; H, 10·07%).

Dihydrofusidic acid methyl ester (VII)

This substance was obtained on esterification of dihydrofusidic acid (V) with ethereal diazomethane in the usual way. Two recrystallizations from ether–hexane afforded material with a constant m.p. of 150–150·5°. $[\alpha]_D^{20}$ −2°. UV: λ_{max} 222 mμ (ε 8400). (Found: C, 72·10; H, 9·92. $C_{32}H_{52}O_6$ requires: C, 72·14; H, 9·84%).

Dideuteriofusidic acid methyl ester

A solution of fusidic acid methyl ester (II; 2·13 g) in ethanol (30 ml) was shaken at room temp under 1 atm deuterium gas (obtained on electrolysis of heavy sulphuric acid) in the presence of 5% palladium on calcium carbonate (1 g). In 60 min 110 ml of deuterium were absorbed. The catalyst was removed, and the filtrate evaporated to dryness *in vacuo*. The residue was recrystallized from ether–hexane to yield 1·84 g, m.p. 149–150° alone, or in admixture with VII. IR (KBr): 2185 and 2230 cm^{-1} (C—D).

Ozonolysis of dihydrofusidic acid methyl ester (VII)

Ozonized oxygen was bubbled through a solution of VII (1064 mg) in dry methylene chloride (25 ml) containing dry pyridine (0·25 ml) at −75° in 5 hr. (It was necessary to pass about 10 mmoles of ozone through the solution to complete the reaction). Zinc dust (1·25 g) and acetic acid (2·5 ml) were added, and after stirring for 90 min at 25°, the precipitate was filtered off and washed with methylene chloride. The combined filtrate and washings were washed successively with water, aqueous $NaHCO_3$, and water. After drying and removal of the solvent *in vacuo* the residue crystallized from ether to yield compound IX (360 mg), m.p. 194–200° raised by 2 recrystallizations from ethanol to 211·5–212·5°. $[\alpha]_D^{20}$ ±0°. UV: No selective absorption above 200 mμ. IR (KBr): 1710, 1755, and 1770 cm^{-1}. NMR spectrum: δ = ca. 5·45/d (1); 2·16/s (3); 1·28 + 1·20 + 1·06/3s (each 3); 1·06/d/J = 6 cps (3). (Found: C, 70·94; H, 8·26; acetyl, 11·06. $C_{23}H_{32}O_5$ requires: C, 71·10; H, 8·30; acetyl, 11·08%).

The ethereal filtrate from IX was diluted with hexane and the ether removed *in vacuo*. An amorphous precipitate was removed by filtration and the filtrate concentrated to a colourless oil. Distillation *in vacuo* afforded 150 mg of 2-hydroxy-6-methylheptanoic acid methyl ester (VIII), b.p. 95°/10 mm. IR (CS_2): 3580 (OH), 1746 (ester), 1370 and 1390 cm^{-1} (isopropyl). (Found: C, 62·09; H, 10·39. $C_9H_{18}O_3$ requires: C, 62·04; H, 10·41%).

2-Hydroxy-6-methylheptanoic acid

6-Methylheptanoic acid was prepared by Clemmensen reduction of methyl 4-keto-6-methylheptoate (b.p. 100–104°/10 mm), obtained from isobutylbromide and β-carbomethoxypropionylchloride by the method described for methyl 4-keto-7-methyloctoate.[20] 6-Methylheptanoic acid was brominated in the usual way[21] to afford a 72% yield of 2-bromo-6-methylheptanoic acid

[20] J. Cason, *J. Amer. Chem. Soc.* **64**, 1106 (1942).

[21] S. M. Birnbaum, J. F. Shou-Cheng, and J. P. Greenstein, *J. Biol. Chem.* **203**, 333 (1953).

(b.p. 122°/2 mm). A mixture of 2-bromo-6-methylheptanoic acid (16 g), sodium carbonate (16 g) and water (150 ml) was refluxed for 2 hr. After cooling the solution was acidified with hydrochloric acid and extracted repeatedly with ether. The extract was washed with water, dried, and evaporated to dryness, after which the residue was crystallized from hexane to yield 7 g, m.p. 59–60°. The analytical sample, obtained after 2 recrystallizations from hexane, had m.p. 60–61°. (Found: C, 59·77; H, 10·07. $C_8H_{16}O_3$ requires: C, 59·98; H, 10·07%).

2-Hydroxy-6-methylheptanoic acid methyl ester

This was obtained on esterification of the acid with diazomethane in the usual way. B.p. 96°/10 mm. (Found: C, 61·85; H, 10·55. $C_9H_{18}O_3$ requires: C, 62·04; H, 10·41%). The IR spectrum was identical with the spectrum of VIII.

Ozonolysis of fusidic acid

Ozonized oxygen (containing about 6 mmoles of ozone) was passed through a solution of fusidic acid (2 g) in acetic acid (25 ml) at room temp in 3 hr. Water (25 ml) was added, and the solution was steam-distilled into a solution of 2,4-dinitrophenylhydrazine (800 mg) in a mixture of methanol (40 ml) and conc hydrochloric acid (1 ml). The precipitate was filtered off and washed with ethanol to yield 900 mg of acetone dinitrophenylhydrazone (m.p. 115–120°). Two recrystallizations from ethanol raised the m. p. to 123·5–124·5°, and this was not depressed by admixture with an authentic sample. The IR spectra were identical.

Oxidation of tetrahydrofusidic acid (VI) *to the corresponding diketo-acid* (X)

To a solution of VI (1042 mg) in acetic acid (10 ml) 10% chromium trioxide in 95% acetic acid (2·7 ml) was added with stirring. After standing for 30 min, water (50 ml) was added, and the precipitate was taken up in ether. The ethereal solution was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was recrystallized from ethanol–water to afford 710 mg, m.p. 99–110°. Two recrystallizations from acetone-hexane raised the m.p. to 127·5–129°. $[\alpha]_D^{20}$ +71°. (Found: C, 72·07; H, 9·36. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%).

Saponification of X

(a) A suspension of X (6 g) in a mixture of water (1000 ml) and 2N NaOH (30 ml) was heated on the steam-bath for 2 hours. After cooling the resulting solution was acidified and the precipitate collected. Recrystallization from ethanol afforded 2·8 g XI, m.p. 215–225°. Two recrystallizations from acetone followed by one recrystallization from dioxane raised the m.p. to 231–233°. $[\alpha]_D^{20}$ +60° (pyridine). (Found: C, 73·01; H, 9·75. $C_{29}H_{46}O_5$ requires: C, 73·38: H, 9·77%). The sodium salt of XI was obtained on careful neutralization of a methanolic solution of the acid with methanolic sodium hydroxide. The solvent was removed *in vacuo* and the residue crystallized from acetone. IR (KBr): 1575 (carboxylate) and 1710 cm^{-1} (six-ring ketone).

(b) To a solution of X (250 mg) in hot ethanol (5 ml) 2N NaOH (1 ml) was added, and the mixture was refluxed for 60 min. Upon cooling and acidification with 4N HCl (0·6 ml) the reaction product crystallized. It was collected and recrystallized from ethanol to yield 150 mg of XII, m.p. 265–266°. Two further recrystallizations from ethanol raised the m.p. to 275–277°. $[\alpha]_D^{20}$ −149° (pyridine). (Found: C, 73·36; H, 9·75. $C_{29}H_{46}O_5$ requires: C, 73·38: H, 9·77%). The sodium salt (prepared as the sodium salt of XI) showed in the IR bands at 1575 (carboxylate) and 1712 cm^{-1} (six-ring ketone).

Acetylation of XI

Performed in the usual way (pyridine–acetic anhydride) it gave a product, m.p. 127–129° alone and in admixture with X. The IR spectra were identical.

Acetylation of XII

Performed in the usual way (pyridine–acetic anhydride it gave a product, which after recrystallization from acetone–hexane had m.p. 164–165°. $[\alpha]_D^{20}$ −114°. (Found: C, 71·94; H, 9·44. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%).

Oxidation of XI *to the triketoacid* XIII

Compound XI (1·2 g) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (5 ml) in the usual way. The crude product (0·8 g, m.p. 186–188°) was purified by recrystallization from ether to the constant m.p. 191–192°. $[\alpha]_D^{20}$ +136°. (Found: C, 73·51; H, 9·43. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%). The sodium salt, obtained in the same way as the sodium salt of XI, showed in the IR (KBr) bands at 1585 (carboxylate), 1710 (six-ring ketone), and 1735 cm^{-1} (five-ring ketone).

Oxidation of XII

Compound XII (120 mg) in acetic acid (5 ml) was oxidized with 1% chromium trioxide in acetic acid (1·8 ml) in the usual way. Recrystallization from ethanol yielded 86 mg, m.p. 220–224°. The analytical sample, obtained from further recrystallizations from ethanol, had m.p. 222·5–225°.$[\alpha]_D^{20}$ −89°. (Found: C, 73·55; H, 9·48. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%).

Conversion of XI *into* XII

A solution of XI (100 mg) in a mixture of methanol (10 ml) and 33% aqueous sodium hydroxide (1 ml) was refluxed for 5 hr. The hot solution was acidified with hydrochloric acid and cooled, whereby crystals, m.p. 269–272°, separated. Recrystallization from ethanol raised the m.p. to 271–275° and this was not depressed on admixture with XII. The IR spectra were identical.

16-*Deacetylfusidic acid* (XIV)

Fusidic acid sodium salt (20 g) was dissolved in water (200 ml). 33% aqueous sodium hydroxide (20 ml) was added, and the solution was refluxed for 15 min, whereby the crystalline sodium salt of XIV separated. It was collected, washed with water followed by acetone, and dried to yield 15 g sodium salt, which was suspended in a mixture of ether (500 ml) and water (200 ml). Dil hydrochloric acid was added in small portions while shaking, until 2 clear phases resulted. The ether phase was separated, washed with water and, after drying, evaporated to dryness *in vacuo*. The residue was redissolved in ether (40 ml), and upon scratching, the acid crystallized. Yield: 10·5 g, m.p. 106–108°. Recrystallization from ether did not raise the m.p.; however, a satisfactory analysis could not be obtained. UV: λ_{max} 230 mμ (ε 8250).

16-*Epi-deacetylfusidic acid* (XV)

To a solution of fusidic acid sodium salt (30 g) in water (1000 ml) a few drops of phenolphthalein-indicator was added. The mixture was refluxed, and aqueous sodium bicarbonate was added drop by drop at such a rate that the red colour was just maintained. When the colour was permanent, the solution was refluxed for a further 30 min. After cooling, the solution was acidified with hydrochloric acid and extracted with ether (2 × 150 ml). The ether phase was extracted with 0·5N NaOH (150 ml), and the alkaline aqueous extract thereby obtained was acidified with dil hydrochloric acid and again extracted with ether. After washing with water and drying, the solvent was removed *in vacuo*, and the residue recrystallized from ether to afford 12·0 g, m.p. 198–199°. Another recrystallization from the same solvent raised the m.p. to 199–199·5°.$[\alpha]_D^{20}$ −57°. UV: λ_{max} 230 mμ (ε 8750). (Found: C, 73·27; H, 9·64. $C_{29}H_{46}O_5$ requires: C, 73·38; H, 9·77%).

16-*Deacetylfusidic acid lactone* (XVI)

Sodium salt of XIV (1·5 g) was dissolved in acetic acid (10 ml) and heated on the steam-bath for 30 min. Water was added, and upon scratching, the lactone crystallized. Recrystallization from methanol–water afforded 1·2 g m.p. 157–158°, raised by recrystallization from acetonitrile to 158·5–159·5°. $[\alpha]_D^{20}$ + 58°. UV: λ_{max} 224 mμ (ε 13300) IR. (KBr): 1700, 1750 cm^{-1}. (Found: C, 76·31; H, 9·89. $C_{29}H_{44}O_4$ requires: C, 76·27; H, 9·71%).

The methyl ester of XIV

This was obtained on esterification of XIV with ethereal diazomethane in the usual way. After removal of the solvent the residue was crystallized from ether–hexane to yield material with m.p. 163–166°, raised by recrystallization from acetonitrile to 168–168·5°. $[\alpha]_D^{20}$ ±0°. UV: λ_{max} 230–233 mμ (ε 9200). (Found: C, 73·78; H, 9·94. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%).

The methyl ester of XV

This was obtained on esterification of XV with ethereal diazomethane. The crude product, m.p. 163–164°, was purified by recrystallization from acetonitrile to the constant m.p. 165–166°. $[\alpha]_D^{20}$ −74°. UV: λ_{max} 230–232 mμ (ε 9650). (Found: C, 73·75; H, 10·01. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%).

16-*Deacetyldihydrofusidic acid methyl ester* (XVII)

The methyl ester of XIV (7·3 g), dissolved in ethanol (100 ml), was shaken under 1 atm hydrogen at room temp in the presence of 5% palladium on calcium carbonate (2 g). When 1·1 mole of hydrogen had been absorbed (30 min) the consumption stopped. After removal of the catalyst, water was added, and upon scratching, the product crystallized. The crude product was recrystallized from ether–hexane to afford 5·0 g, m.p. 143–144°. Another recrystallization from ether–hexane yielded the analytical sample, m.p. 144·5–145·5°. $[\alpha]_D^{20}$ −13°. UV: λ_{max} 232 mμ (ε 8700). (Found: C, 73·65; H, 10·15. $C_{30}H_{50}O_5$ requires: C, 73·43; H, 10·27%).

16-*Epi-deacetyldihydrofusidic acid methyl ester* (XVIII)

The methyl ester of XV was hydrogenated in the same way as the methyl ester of XIV. The consumption of hydrogen stopped when the calculated amount of hydrogen had been absorbed. However, it was not possible to obtain the product in a crystalline form.

Oxidation of XVII *to the triketoester* XIX

A solution of XVII (980 mg) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (4 ml) in the usual way. The crude amorphous product was purified by chromatography on florisil (elution with benzene–ether 9:1), whereby 170 mg of crystalline material m.p. 129–133°, was obtained. Several recrystallizations from acetone–hexane raised the m.p. to 133·5–135·5°. $[\alpha]_D^{20}$ +184°. UV: λ_{max} 247 mμ (ε 9000). IR (KBr): 1645, 1707 and 1730 cm^{-1}. (Found: C, 74·43; H, 9·08. $C_{30}H_{44}O_5$ requires: C, 74·34; H, 9·15%).

Oxidation of XVIII *to the triketoester* XIX

A solution of XVIII (980 mg) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (4 ml) in the usual way. With some difficulty the crude product was crystallized from methanol–water to afford 375 mg, m.p. 130–133°. Recrystallizations from methanol–water and ether–hexane raised the m.p. to 133·5–135·5°, alone and in admixture with the product obtained on oxidation of XVII. The IR spectra of the two products were identical.

Oxidation of dihydrofusidic acid methyl ester (VII) *to the diketo ester* XX

A solution of VII (1064 mg) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (2·7 ml) in the usual way. Two recrystallizations of the crude product from ether–hexane gave a product with a constant m.p. of 139–139·5°. $[\alpha]_D^{20}$ +124°. UV: λ_{max} 220 mμ (ε 7900). (Found: C, 72·59; H, 9·27. $C_{32}H_{48}O_6$ requires: C, 72·69; H, 9·15%).

Selenium dehydrogenation of fusidic acid

Fusidic acid (24 g) was heated *in vacuo* (0·1 mm Hg) to 200° for 2 hr, whereby the substance gradually melted under evolution of gas. After cooling the residue (ca. 20 g) was mixed intimately with powdered selenium (30 g) in a mortar. The mixture was heated to 280–300°, at which temp a vigorous evolution of gas began. The temp was gradually raised to about 330° and kept there for 48 hr. After cooling, the flask was crushed and the contents extracted with ether in a Soxhlet extractor for 2 hr. The ether was evaporated leaving a yellow-red oil (6 g) which was chromatographed on alumina (50 g). The column was eluted with hexane (fractions 1–11, each 25 ml) and hexane–benzene 9:1 (fractions 12–23, each 25 ml).

Fractions 1 and 2 (2·2g) were combined and distilled *in vacuo* to yield 1 g of a pale yellow liquid (b.p. 140–150°/10 mm). Refractionation gave 0·3 g 1,2,5-trimethylnaphthalene, b.p. 143–145°/10 mm. UV: λ_{max} 226, 231, 278, 289, 300 (infl), 310, and 325 mμ (log ε 4·76, 4·89, 3·72, 3·77, 3·62, 3·11, and 2·97). (Found: C, 91·35; H, 8·59. $C_{13}H_{14}$ requires: C, 91·71; H, 8·29%). The picrate had, after 2 recrystallizations from ethanol, m.p. 136–137·5°. The 1,3,5-trinitrobenzene adduct melted after two

recrystallizations from ethanol at 156–157°. (Found: C, 59·72; H, 4·63; N, 10·84. $C_{19}H_{17}N_3O_6$ requires: C, 59·53; H, 4·47; N, 10·96%).

Fractions 3–5, which had similar (phenanthrenoid) UV spectra, were combined and rechromatographed on alumina (20 g). The column was eluted with hexane, and fractions of 25 ml were collected. The crystalline residues from fractions V–XI (110 mg) were combined and recrystallized from methanol, whereby 30 mg, m.p. 165–175°, were obtained. Sublimation at 100°/0·001 mm afforded 1,8-dimethylphenanthrene, m.p. 178–180°. UV: λ_{max} 214, 225, 251, 258, 281, 292, 305, 320 (infl), 328, 336, 344 and 352 mμ (log ε 4·37, 4·22, 4·61, 4·70, 4·00, 4·09, 4·17, 2·68, 2·55, 2·58, 2·45, and 2·39). In spite of the rather low m.p. (pure 1,8-dimethylphenanthrene has m.p. 191–192° [22] the IR spectrum was indistinguishable from that of an authentic sample.

Fractions 14–23, which had similar UV spectra, were combined and evaporated to give 607 mg of a pale yellow semicrystalline solid, which was crystallized from ether to yield 82 mg, m.p. 232–240°. Two recrystallizations from acetone afforded 35 mg of compound XXI, m.p. 250–254°. UV: λ_{max} 218, 228 (infl), 234 (infl), 277, 286, 296, 302, 308, 317, 329, 340, 348, and 366 mμ (log ε 4·54, 4·41, 4·34, 4·74, 4·81, 4·61, 4·60, 4·56, 4·40, 3·03, 2·81, 3·09, 2·97). NMR spectrum: δ = 4·18/s (2); ca. 3·25/m (1), 2·75/s (3), 1·45 + 1·34/2s (each 3). (Found; C, 92·91; H, 6·79; M, 316 (Rast). $C_{25}H_{22}$ requires: C, 93·12; H, 6·88; M, 322).

Elimination of acetic acid from IX *with the formation of* XXII

To a suspension of IX (2 g) in hot ethanol (40 ml) 2N NaOH (10 ml) was added. The resulting orange solution was immediately cooled to room temp, and after 2 min acidified with 4N HCl. The yellow ene-dione, which crystallized on addition of water, was collected, washed with water, and dried to yield 1380 mg, m.p. 180–184°. This product was purified by chromatography on acid-washed alumina (Woelm). Elution with benzene–ethanol (98:2) afforded 1030 mg, m.p. 186–190°. Recrystallization from ether gave the analytical sample, m.p. 192·5–193°. UV: λ_{max} 261 mμ (ε 8100). IR (KBr): 1662, 1705, 1725 cm^{-1}. NMR spectrum: δ = 6·23/s (1); 1·38 + 1·26 + 1·22/3s (each 3); 1·07/d/J 6·5 cps (3). (Found: C, 76·87; H, 8·62. $C_{21}H_{28}O_3$ requires: C, 76·79; H, 8·59%).

16-*Deacetyltetrahydrofusidic acid lactone* (XXIII)

A solution of XVI (457 mg) in acetic acid (8 ml) was shaken at room temp under 1 atm hydrogen in the presence of platinum oxide (50 mg). In about 5 hr 62 ml hydrogen were absorbed and the consumption ceased. The catalyst was removed, and the filtrate evaporated to dryness under red press. The residue was crystallized from acetone–hexane to yield 185 mg of XXIII, m.p. 144·5–146°, raised by several recrystallizations from the same solvents to 147–148°. $[\alpha]_D^{20}$ −32°. IR (KBr): 1765 cm^{-1} (five-ring lactone). (Found: C, 75·50; H, 10·59. $C_{29}H_{48}O_4$ requires: C, 75·60; H, 10·50%).

16-*Deacetyltetrahydrofusidic acid lactone* 3-*acetate* (XXIV)

This was obtained on acetylation of XXIII with acetic anhydride–pyridine in the usual way. Recrystallizations from methanol–water and acetone–hexane gave material with a constant m.p. of 195–196°. $[\alpha]_D^{20}$ −36·5°. (Found: C, 74·18; H, 9·95. $C_{31}H_{50}O_5$ requires: C, 74·06; H, 10·03%).

Oxidation of XXIV *to the corresponding* 12-*ketone* (XXV)

A solution of XXIV (2·4 g) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (3·3 ml) in the usual way. Recrystallization of the crude product (2·0 g, m.p. 235–236·5°) from methanol gave the analytical sample, m.p. 236–237°. (Found: C, 74·12; H, 9·60. $C_{31}H_{48}O_5$ requires: C, 74·36; H, 9·66%).

SeO_2-*Dehydrogenation of* XXV *to* XXVI

To a solution of XXV (3·0 g) in acetic acid-t. butanol (1:1) (25 ml) selenium dioxide (660 mg), moistened with water, was added, and the mixture was refluxed for 16 hr. After cooling, the selenium (270 mg) was removed by filtration and the filtrate evaporated to dryness. The residue crystallized on addition of ether to yield 1·7 g XXVI, m.p. 226–229°. After 2 recrystallizations from ethanol the

[22] F. E. King and T. J. King *J. Chem. Soc.* 1373 (1954).

m.p. was 226–228°. UV: λ_{max} 244 mμ (ε 7400). IR (KBr): 1662, 1702 (shoulder), 1735, and 1765 cm^{-1}. (Found: C, 74·40; H, 9·50. $C_{31}H_{46}O_5$ requires: C, 74·66; H, 9·30%).

Dihydrofusidic acid 3-*acetate* (XXVII)

Dihydrofusidic acid (1·0 g) was acetylated in the usual way to yield 0·9 g of XXVII, m.p. 138–140°. Recrystallizations from methanol–water and ether–hexane gave the analytical sample, m.p. 139–141°. $[\alpha]_D^{20}$ −17°. (Found: C, 68·41; H, 9·59. $C_{33}H_{52}O_7,H_2O$ requires: C, 68·48; H, 9·40%).

Oxidation of XXVII *to* XXVIII

A solution of XXVII (1·16 g) in acetic acid (10 ml) was oxidized with 10% chromium trioxide in acetic acid (1·4 ml) in the usual way. Recrystallization of the crude product from methanol–water gave 0·58 g, m.p. 184–186°, raised by further recrystallizations from the same solvents to 191–192°. $[\alpha]_D^{20}$ +51°. (Found: C, 70·89; H, 9·06; $C_{33}H_{50}O_7$ requires: C, 70·93; H, 9·02%).

Sodium borohydride reduction of XXVIII

Compound XXVIII (560 mg) was dissolved in a mixture of methanol (5 ml), water (5 ml) and saturated aqueous sodium bicarbonate (5 ml). Sodium borohydride (200 mg) was added, and the solution was left standing for 10 min at room temp. After acidification and extraction with ether, the extract was washed with water, dried and evaporated to dryness. The amorphous residue (580 mg) showed only one spot, with the same R_F-value as XXVII, on paper chromatography. Recrystallization from methanol–water gave 435 mg, m.p. 139–141°, the IR spectrum of which was indistinguishable from that of XXVII.

Oxidation of dihydrofusidic acid (V) *to the corresponding diketoacid* (XXIX)

Dihydrofusidic acid (10·4 g) in glacial acetic acid (50 ml) was oxidized with 10% chromium trioxide in acetic acid (26·6 ml) in the usual way to yield (after recrystallization from methanol–water) 5·7 g XXIX, m.p. 204–205°. The analytical specimen, m.p. 206–207°, $[\alpha]_D^{20}$ +123°, was obtained after recrystallization from acetonitrile. (Found: C, 72·24; H, 8·95. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%).

3-*Epi-dihydrofusidic acid* (XXX)

Compound XXIX (5·7 g) was suspended in methanol (20 ml) and neutralized with aqueous sodium bicarbonate. A solution of sodium borohydride (1·0 g) in water (5 ml) was added, and the mixture was left standing for 1 hr. Water (75 ml) was added, and after acidification and extraction with ether, the extract was washed with water, dried and evaporated to dryness to yield an amorphous residue, which crystallized from ether to afford 4·2 g of XXX, m.p. 207–208°. Recrystallization from acetonitrile–methanol gave the analytical sample, m.p. 211–211·5°. $[\alpha]_D^{20}$ +14° (pyridine). (Found: C, 71·53; H, 9·60. $C_{31}H_{50}O_6$ requires: C, 71·78; H, 9·72%).

3-*Epi-dihydrofusidic acid* 3-*acetate*

This was obtained on acetylation of XXX with acetic anhydride–pyridine in the usual way. The crude product crystallized from methanol–water. Recrystallization from ether–hexane afforded material of the constant m.p. 190–190·5°. $[\alpha]_D^{20}$ +22°. (Found: C, 70·63; H, 9·42. $C_{33}H_{52}O_7$ requires: C, 70·68; H, 9·35%).

16-*Deacetyldihydrofusidic acid lactone* (XXXI)

A solution of XVI (9·14 g) in methylcellosolve (100 ml) was shaken at room temp under 1 atm hydrogen in the presence of 10% palladium-on-carbon catalyst (1·5 g). When 525 ml hydrogen had been absorbed (in about 1 hr), the hydrogenation was interrupted, and the catalyst was removed by filtration. The filtrate was evaporated to dryness, and the residue crystallized from methanol-water to yield 7·5 g XXXI, m.p. 153–155°. Two recrystallizations from ether–hexane raised the m.p. to 157·5–158°. UV: λ_{max} 224 mμ (ε 13300). NMR spectrum: δ = 4·92/m (1); 4·38/m (1); 3·72/m (1). (Found: C, 75·93; H, 10·04. $C_{29}H_{46}O_4$ requires: C, 75·94; H, 10·11%).

Compound XXXI-3-*acetate*

This was obtained on acetylation of XXXI with acetic anhydride–pyridine in the usual way. Recrystallization of the crude product from methanol–water gave material with m.p. 182–183°, raised by recrystallization from ether–hexane to 182·5–183·5°. NMR spectrum: $\delta = 4{\cdot}90/m$ (2); 4·38/m (1). (Found: C, 74·33; H, 9·65. $C_{31}H_{48}O_5$ requires: C, 74·36; H, 9·66%).

Compound XXXI-3,12-*diacetate*

Compound XXXI (1·0 g) was dissolved in 5 ml of a mixture of acetic acid (40 ml), acetic anhydride (20 ml), and *p*-toluenesulphonic acid (10 g). After standing for 20 min water was added, and the resulting oil was triturated with water to yield an amorphous solid, which crystallized on treatment with methanol. The crude product (1·1 g) was recrystallized twice from methanol to yield material with a constant m.p. of 178–179°. $[\alpha]_D^{20}$ +60°. NMR spectrum: $\delta = 5{\cdot}26/m$ (1); $4{\cdot}90/m$ (2). (Found: C, 72·90; H, 9·37. $C_{33}H_{50}O_6$ requires: C, 73·03; H, 9·29%).

16-*Deacetyltetrahydrofusidic acid* (XXXII)

A solution of tetrahydrofusidic acid (VI; 1042 mg) in a mixture of 2N NaOH (5 ml) and ethanol (5 ml) was heated on the steam-bath for 2 hr. After cooling and acidification the precipitate was collected (800 mg, m.p. 190–225°) and recrystallized from acetic acid to yield 350 mg, m.p. 242–243°. Recrystallization from methanol–ether afforded the analytical sample, m.p. 244–245·5°. $[\alpha]_D^{20}$ −32° (pyridine). (Found: C, 72·57; H, 10·50. $C_{29}H_{50}O_5$ requires: C, 72·76; H, 10·53%).

Tetrahydrofusidic acid-3,12-*diacetate* (XXXIII)

(a) Tetrahydrofusidic acid (VI; 1·0 g) was dissolved in 5 ml of a mixture of acetic acid (40 ml), acetic anhydride (20 ml), and *p*-toluenesulphonic acid (10 g). After standing for 20 min water was added, and the resulting oil was triturated with water to yield an amorphous solid, which after drying crystallized from hexane to yield 720 mg of XXXIII, m.p. 168–170°. Recrystallization from hexane yielded the analytical sample, m.p. 169–170°. $[\alpha]_D^{20}$ −39°. (Found: C, 69·40; H, 9·38. $C_{35}H_{56}O_8$ requires: C, 69·50; H, 9·33%).

(b) XXXII (250 mg) was dissolved in 1 ml of a mixture of acetic acid (40 ml), acetic anhydride (20 ml) and *p*-toluenesulphonic acid (10 g). After standing for 20 min water was added to precipitate an amorphous solid, which after drying crystallized from hexane, (m.p. 164–166°). Recrystallization from hexane raised the m.p. to 168–169°, and this was not depressed on admixture with the sample (m.p. 169–170°) described under (a) above. The IR spectra of the two products were identical.

16-*Epi-fusidic acid*-3-*acetate* (XXXIV)

16-Epi-deacetylfusidic acid (XV; 2 g) was dissolved in a mixture of acetic anhydride (8 ml) and pyridine (8 ml). After 3 hr at room temp the mixture was poured on ice, and the resulting semi-crystalline precipitate was dried and treated with ether to yield 650 mg of a product (m.p. 245–252°) which is believed to be the 3-acetate of 16-epi-deacetylfusidic acid lactone. Recrystallization from acetonitrile raised the m.p. to 261–266°. $[\alpha]_D^{20}$ −66°. (Found: C, 74·62; H, 9·23. $C_{31}H_{46}O_5$ requires: C, 74·66; H, 9·30%).

The ethereal filtrate from this substance was evaporated to dryness and the residue crystallized from ether–hexane to yield 700 mg of XXXIV, m. p. 95–110°. Two recrystallizations from methanol–water yielded 525 mg with a constant m.p. of 168–170°. $[\alpha]_D^{20}$ −78°. (Found: C, 71·09; H, 9·06. $C_{33}H_{50}O_7$ requires: C, 70·93; H, 9·02%).

Ketalization of X *to the monoketal* (XXXVI)

A solution of X (1·0 g) and *p*-toluenesulphonic acid (40 mg) in butanone ethyleneketal (10 ml) was refluxed for 10 min. After cooling, ether (25 ml) was added, and the resulting solution was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was crystallized from methanol–water to yield 0·65 g XXXVI, m.p. 152–154°. Recrystallization from ether–hexane raised the m.p. to 157–158°. $[\alpha]_D^{20}$ +26°. (Found: C, 70·49; H, 9·43. $C_{33}H_{52}O_7$ requires: C, 70·68; H, 9·35%).

Reduction and hydrolysis of XXXVI *to the monoketone* XXXV

A solution of XXXVI (4·0 g) in methanol (15 ml) was neutralized with 2N NaOH. 20% aqueous sodium borohydride (5 ml) was added, and after standing for 15 min the solution was acidified with hydrochloric acid and heated for 10 min on the steam-bath. After cooling, water (100 ml) was added, the oily precipitate extracted with ether, and the extract washed with water, dried, and evaporated to dryness. The residue was recrystallized from ether–hexane to yield 2·8 g XXXV, m.p. 130–135°. Another recrystallization from ether–hexane gave the analytical sample, m.p. 135–137°. RD in methanol (*c*, 0·12), positive Cotton effect with peak at $[\alpha]_{305}$ +468°. (Found: C, 71·78; H, 9·75. $C_{31}H_{50}O_6$ requires: C, 71·78; H, 9·72%).

Reduction of XXXV *to* VI

A solution of XXXV (600 mg) in acetic acid (5 ml) containing three drops of conc hydrochloric acid was shaken at room temp under 1 atm hydrogen in the presence of prereduced platinum oxide (50 mg). In 50 min 30 ml hydrogen were absorbed and the consumption stopped. The catalyst was removed, and the filtrate evaporated to dryness *in vacuo*. The residue was crystallized from acetonitrile to yield 340 mg of crystalline material m.p. 158–162°, raised by several recrystallizations from acetonitrile to 170–172°. The IR spectrum was identical with that of tetrahydrofusidic acid.

Diketolactone XXXVII

(a) A solution of XXXI (4·6 g) in acetic acid (30 ml) was oxidized in the usual manner with 10% chromium trioxide in acetic acid (13·3 ml). The crude product was recrystallized from acetone–hexane to yield 2·7 g XXXVII, m.p. 169–171. $[\alpha]_D^{20}$ +221°. (Found: C, 76·57: H, 9·37. $C_{29}H_{42}O_4$ requires: C, 76·61; H, 9·31%).

(b) A mixture of XXIX (1·0 g), water (20 ml), and 2N NaOH (5 ml) was heated on the steam-bath for 5 hr. The resulting clear solution was acidified, and the amorphous precipitate was filtered off, dissolved in acetic acid (5 ml) and heated for 1 hr on the steam-bath. The semi-crystalline product, obtained on addition of water, was recrystallized from methanol to yield 350 mg XXXVII, m.p. 162–170°. Further recrystallizations from methanol raised the m.p. to 169–172°, undepressed on admixture with the compound mentioned above under (a).

Paper chromatography. The solvent systems: A: Light petroleum (b.p. 60–80°)–methanol–water (10:8:2) B_1: Heptane–benzene–methanol–water (5:5:7:3) and B_5: Benzene–methanol–water (10:5:5) described by Bush[14] were used for descending paper-chromatography of fusidic acid and many of its derivatives. The chromatograms were run at 34°, after equilibration for 16 hours, on Whatman No. 1 paper. For relatively non-polar substances (esters and lactones) system A was used, while compounds containing a free carboxylic group were chromatographed in systems B_1 or B_5. The spots were detected by spraying with a saturated solution of $SbCl_3$ in chloroform followed by drying at 100° for 2 min. Compounds containing free or acylated hydroxyl groups at C_3 or C_{12} gave red or violet spots, while mono- or diketones gave pale yellow spots, visible in UV light. Quantities ranging between 10 and 40 μg could be detected by this method.

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