chain relative to the diphenyl ether bond has been examined recently by Jorgensen and Reid[20a] and Shiba, Höfer, and Cahnmann.[20b] Jorgensen and Reid found that compounds in which the side chain of $T_3$ was placed *ortho* or *meta* to the diphenyl ether bond were inactive in the prevention of thiouracil-induced goiters.

The reduction in thyromimetic activities caused by the introduction of an $\alpha$-methyl group into the side chain of $T_4$ is further evidence for the importance of the unmodified alanine moiety for maximum biological response. The reduction in activity seen with Ib may be a consequence of impaired binding to transport proteins or to sites of biological action which in turn may lead to more rapid metabolism (degradation) and/or excretion. Another less attractive possibility, since currently there are few data to substantiate such an event,[21] is that $\alpha$-methyl-$T_4$ is incapable of being converted to an "active form" of the hormone.

Nonetheless, whatever the specific reason(s), it seems apparent from this and previous studies that the position and nature of the side chain in thyroxine-like substances help to determine the character and potency of the biological response evoked.[22]

**Acknowledgment.**—We wish to thank Dr. W. L. Holmes and Mr. N. W. Di Tullio for the cholesterol studies and members of the Analytical and Physical Chemistry Section, Smith Kline and French Laboratories, for elemental analyses and paper chromatography studies.

(20) (a) E. C. Jorgensen and J. A. W. Reid, *J. Med. Chem.*, **7**, 701 (1964); (b) T. Shiba, A. Höfer, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3171 (1964).

(21) E. C. Wolff and J. Wolff in "The Thyroid Gland," Vol. I, R. Pitt-Rivers and W. R. Trotter, Ed., Butterworth and Co. Ltd., London, 1964, p. 239.

(22) After this manuscript had been prepared it was noted that compounds Ia, Ib, and IX had been disclosed in the Eire Patent 362/65 (May 5, 1965).

# Fusidic Acid Derivatives. I. Relationship between Structure and Antibacterial Activity

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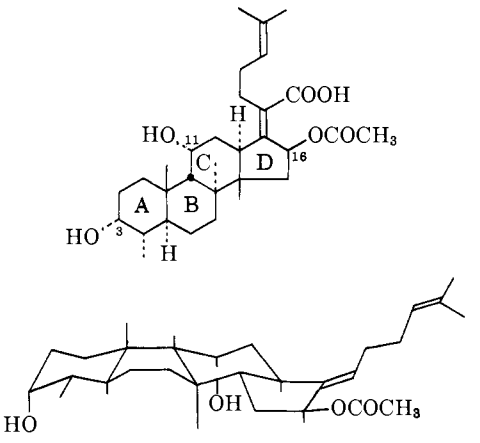
A series of derivatives of the antibiotic fusidic acid has been prepared and their antibacterial activities have been determined. The relationship between structure and antibacterial activity of this group of compounds is discussed.

It has recently been shown[1-3] that fusidic acid (**1a**), an antibiotic formed by *Fusidium coccineum*,[4] has the structure and stereochemistry depicted below. One of the most remarkable features of this structure is the unusual stereochemistry of the cyclopentanoperhydrophenanthrene ring system which differs fundamentally from that of other tetracyclic triterpenes and sterols. In contrast to the usual *trans,anti,trans* arrangement of rings A, B, and C, we find in fusidic acid a *trans,syn,-trans* arrangement of these rings which forces ring B into the boat conformation as illustrated in the perspective formula.

The elucidation of this unusual structure stimulated our inherent interest in studying the influence of structural modifications on the antibacterial activity of this antibiotic which has found a well-established use in the treatment of staphylococcal infections in man.[5,6] In this paper the effect of a number of variations concerning both the two carbon–carbon double bonds and the functional groups at C-3, C-11, C-16, and C-21 on the inhibitory activity against a number of bacteria will be discussed. Some of the compounds studied have previously been described in connection with the structural work, whereas others are new.




**1a**

**Chemistry.**—The two carbon–carbon double bonds in fusidic acid (**1a**) can be hydrogenated stepwise.[1a,4] Hydrogenation over a palladium catalyst yields 24,25-dihydrofusidic acid (**2a**) while reduction of the latter over a platinum catalyst in acetic acid (Chart I) affords a tetrahydrofusidic acid (**3a**) in which the side chain is $\alpha$-orientated. A tetrahydrofusidic acid with a $\beta$-orientated side chain has not been obtained so far.

When a solution of fusidic acid (**1a**) in ethanol or dioxane was irradiated with ultraviolet light from a medium-pressure mercury lamp (Hanovia, Type 509),

(1) (a) W. O. Godtfredsen and S. Vangedal, *Tetrahedron*, **18**, 1029 (1962); (b) D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Marquet, and A. Melera, *Experientia*, **19**, 521 (1963); (c) D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Melera, and S. Vangedal, *ibid.*, **20**, 344 (1964); (d) W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *Tetrahedron*, in press.

(2) A. Cooper, *ibid.*, in press.

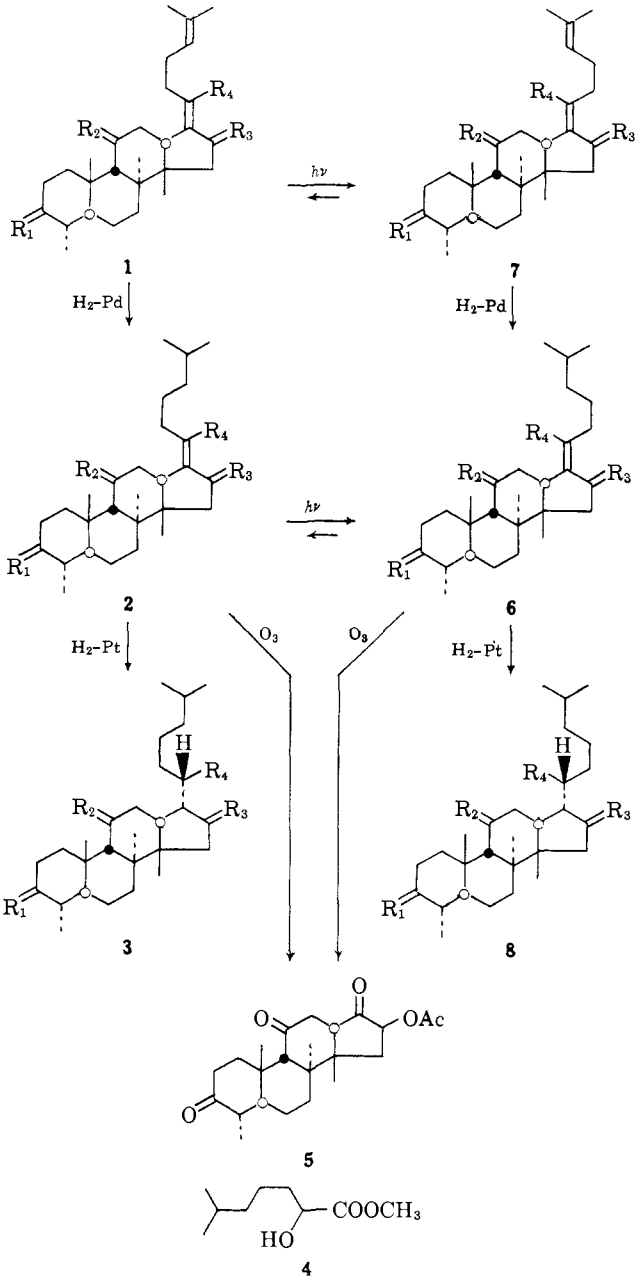
(3) R. Bucourt, M. Legrand, M. Vignau, J. Tessier, and V. Delaroff, *Compt. rend.*, **257**, 2679 (1963).

(4) W. O. Godtfredsen, S. Jahnsen, H. Lorck, K. Roholt, and L. Tybring, *Nature*, **193**, 987 (1962).

(5) M. Barber and L. P. Garrod, "Antibiotic and Chemotherapy," E. and S. Livingstone Ltd., Edinburgh, 1963, pp. 85–87.

(6) G. T. Stewart, *Pharmacotherapia*, **2**, 136 (1964).

CHART I




a, $R_1 = R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
b, $R_1 = R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOCH_3$
c, $R_1 = H$, α-OAc; $R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
d, $R_1 = R_2 = H$, α-OAc; $R_3 = H$, β-OAc; $R_4 = COOH$
e, $R_1 = R_2 = O$; $R_3 = H$, β-OAc; $R_4 = COOH$
f, $R_1 = H$, β-OH; $R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
g, $R_1 = H$, β-OAc; $R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
h, $R_1 = H$, α-OH; $R_2 = O$; $R_3 = H$, β-OAc; $R_4 = COOH$
i, $R_1 = -OCH_2CH_2O-$; $R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
j, $R_1 = O$; $R_2 = H$, α-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
k, $R_1 = O$; $R_2 = H$, β-OH; $R_3 = H$, β-OAc; $R_4 = COOH$
l, $R_1 = O$; $R_2 = H$, β-OAc; $R_3 = H$, β-OAc; $R_4 = COOH$
m, $R_1 = R_2 = R_3 = H$, α-OH; $R_4 = COOH$
n, $R_1 = R_2 = H$, α-OH; $R_3 = H$, β-OH; $R_4 = COOH$
o, $R_1 = O$; $R_2 = H$, α-OH; $R_3 = H$, α-OAc; $R_4 = COOH$
p, $R_1 = R_2 = H$, α-OH; $R_3 = H$, β-OH; $R_4 = CH_2OH$
q, $R_1 = R_2 = H$, α-OAc; $R_3 = H$, β-OAc; $R_4 = CH_2OAc$

the compound was partly converted into a more polar compound, as revealed by paper or thin layer chromatography. The new compound, which in the following is called lumifusidic acid, could be separated from unreacted fusidic acid on chromatography and crystallized, although with difficulty, from methanol–water in a solvated form. A solvent-free methyl ester was obtained on treatment of lumifusidic acid with ethereal diazomethane. The elementary analysis of this compound indicates that lumifusidic acid and fusidic acid are isomeric.

On hydrogenation over a palladium catalyst lumifusidic acid consumes 1 mole of hydrogen with formation of a dihydrolumifusidic acid which also could be obtained on irradiation of **2a**. The infrared and n.m.r. spectra of the corresponding methyl ester (obtained on esterification with diazomethane) are very similar to those of **2b**, whereas the ultraviolet spectra of the two compounds (Figure 1) are different. Therefore, it was

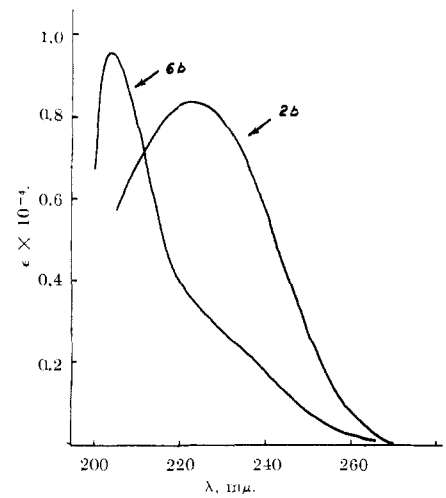



Figure 1.—Ultraviolet absorption spectra of 24,25-dihydrofusidic acid methyl ester (**2b**) and 24,25-dihydrolumifusidic acid methyl ester (**6b**).

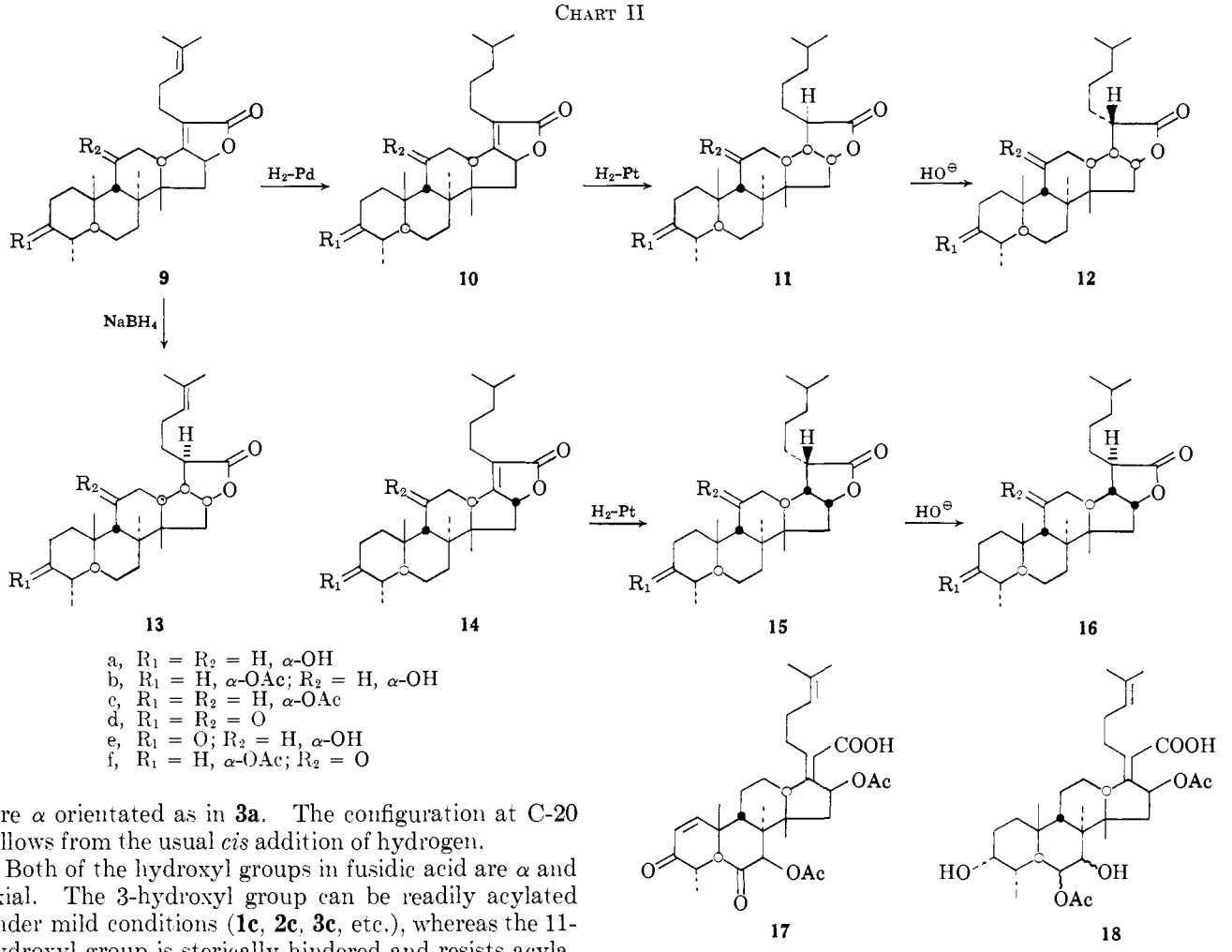
originally thought that a migration of the $\Delta^{17(20)}$ double bond had taken place during the irradiation, but the fact that dihydrolumifusidic acid methyl ester on ozonolysis gave the same two compounds (**4** and **5**) as were obtained by a similar treatment of **2a** indicates that the two compounds are *cis–trans* isomers, and therefore, dihydrolumifusidic acid has the structure shown in **6a**, whereas lumifusidic acid is the corresponding $\Delta^{24}$-unsaturated compound **7a**.

The conversion of fusidic acid into lumifusidic acid is not quantitative. An equilibrium mixture of lumifusidic acid and fusidic acid, in which lumifusidic acid is predominant, is obtained, and when a solution of pure lumifusidic acid is irradiated, the same mixture results. The situation is, however, complicated by the fact that, in addition to lumifusidic acid, other products are formed during the reaction. The structures of these have not been determined.

The *trans* arrangement of the carboxyl group and the 16-acetoxyl group in **6a** is reflected in the fact that the 16-hydroxy derivative **6n**, obtained on alkaline hydrolysis of **6a** does not lactonize on treatment with acids, in contrast to 16-deacetylfusidic acid (**1n**) which lactonizes with great ease.[1a]

On hydrogenation over platinum in acetic acid dihydrolumifusidic acid (**6a**) took up 1 mole of hydrogen with formation of a tetrahydrofusidic acid different from **3a**. The assignment of structure **8a** to this compound is due to the fact that the corresponding 16-deacetylated compound **8n**[7] does not lactonize on treatment with acid as expected (*cf.* ref. **1d**) in the case of a β-orientated side chain. The side chain is there-

CHART II




a, $R_1 = R_2 = H$, $\alpha$-OH
b, $R_1 = H$, $\alpha$-OAc; $R_2 = H$, $\alpha$-OH
c, $R_1 = R_2 = H$, $\alpha$-OAc
d, $R_1 = R_2 = O$
e, $R_1 = O$; $R_2 = H$, $\alpha$-OH
f, $R_1 = H$, $\alpha$-OAc; $R_2 = O$

fore $\alpha$ orientated as in **3a**. The configuration at C-20 follows from the usual *cis* addition of hydrogen.

Both of the hydroxyl groups in fusidic acid are $\alpha$ and axial. The 3-hydroxyl group can be readily acylated under mild conditions (**1c**, **2c**, **3c**, etc.), whereas the 11-hydroxyl group is sterically hindered and resists acylation under normal conditions (acid anhydride–pyridine). In the tetrahydrofusidic acid series, 3,11-diacetylated compounds (such as **3d**) could be obtained on acetylation with a mixture of toluenesulfonic acid, acetic anhydride, and acetic acid, but 3,11-diacetates of fusidic acid and 24,25-dihydrofusidic acid could not be obtained by this method, because these more drastic acetylation conditions caused undesirable reactions in other parts of the molecule.

The 3,11-diketo derivatives **1e**, **2e**, and **3e** were obtained on oxidation of the corresponding diols with chromium(VI) oxide in acetone or acetic acid. Whereas sodium borohydride reduction of 11-keto derivatives yields 11$\alpha$-hydroxy derivatives as the main products,[8] a corresponding reduction of the 3-keto group yields 3$\beta$-hydroxy compounds almost exclusively.[1a] Thus, sodium borohydride reduction of the 3-mono- and 3,11-diketo derivatives **1e**, **1j**, **2e**, and **2j** gave the 3-*epi* derivatives **1f** and **2f**. The 3-keto group in 3,11-diketo derivatives can, however, be selectively reduced to a 3$\alpha$-hydroxyl group on catalytic reduction over platinum oxide in acetic acid containing a trace of hydrogen chloride. In this way the 11-monoketone **2h** was obtained. The structure of this compound follows from the fact that sodium borohydride reduction yields 24,25-dihydrofusidic acid (**2a**).

The 11-keto group is relatively unreactive, and it is, for example, possible to prepare 3-monoketals (**2i** and **3i**) from 3,11-diketo derivatives.[1a,d] This selectivity was utilized in the preparation of the 3-monoketones **2j** and **3j** which were obtained on sodium borohydride reduction of the 3-monoketals **2i** and **3i** followed by acid-catalyzed cleavage of the ketal group.

The 3-mono- and 3,11-diketo derivatives of fusidic acid (**1e**, **j**) are formed in small amounts by the fusidic acid producing strain of *Fusidium coccineum* and have been isolated by chromatography of the mother liquor from the crystallization of fusidic acid.[9]

The 16-acetoxyl group in fusidic acid and 24,25-dihydrofusidic acid can be hydrolyzed with or without inversion. When the hydrolysis is performed in dilute solution with a weak base, such as sodium bicarbonate, the 16$\alpha$-hydroxy compounds **1m** and **2m** are the main products, whereas hydrolysis with strong base in concentrated solution leads to the 16$\beta$-hydroxy compounds **1n** and **2n**.[1a] The latter show a strong tendency to lactonize, and attempts to acylate them at C-16, even under mild conditions, led to the formation of the lactones **9b** and **10b**, respectively (Chart II). The 16$\alpha$-hydroxy compounds **1n** and **2n** *can* lactonize (*e.g.*, on heating with acetic acid), but their tendency to do so is

(7) Since epimerization at C-16 has been observed on alkaline hydrolysis of the 16-acetoxyl group in fusidic acid and some of its derivatives,[1a] it was considered mandatory to check that the hydrolysis in this case proceeded with retention of the configuration at C-16. This was demonstrated by the fact that acetylation of **8n** afforded the same diacetate (**8c**) as was obtained on acetylation of **8a**.

(8) Sodium borohydride reduction of **2i** gave, in addition to the corresponding 11$\alpha$-hydroxy compound, a small amount (less than 5%) of the 11$\beta$ compound which on acid-catalyzed cleavage of the ketal group afforded **2k**. In contrast to **2j**, this compound is readily acetylated at C-11.[1d]

(9) W. O. Godtfredsen, unpublished observations.

TABLE I

ANTIBACTERIAL ACTIVITY OF FUSIDIC ACID DERIVATIVES: $IC_{50}$ (μg./ml.)[a]

| Compd. | *Corynebacterium xerosis* NCTC 9755 | *Sarcina lutea* PDA 1001 | *Bacillus subtilis* ATCC 6633 | *Staphylococcus aureus*[b] I | *Staphylococcus aureus*[b] II | *Staphylococcus aureus*[b] III | *Staphylococcus aureus*[b] IV | *Salmonella typhosa* NCTC 5760 | *Klebsiella pneumonia* ATCC 4208 | *Klebsiella pneumonia* ATCC 7380 | *Escherichia coli* LeoHA2 | *Pseudomonas aeruginosa* ATCC 7700 | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cephalosporin $P_1$ | 0.068 | 1.2 | 16 | 0.19 | 2.3 | >100 | >100 | >100 | >100 | 85 | >100 | >100 | *c* |
| Helvolic acid | 0.048 | 1.1 | 20 | 1.5 | 15 | 93 | >100 | >100 | >100 | 12 | >100 | >100 | *d* |
| Fusidic acid (1a) | 0.008 | 0.23 | 0.49 | 0.058 | 0.85 | 49 | 190 | 350 | 420 | 5.5 | 440 | >1000 | 1a,4 |
| 1b | *50* | *71* | *>300* | *250* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1a,4 |
| 1c | 2.2 | 2.8 | 35 | 25 | 160 | 200 | 220 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 1e | 0.25 | 1.3 | 35 | 4.0 | 63 | 1100 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | |
| 01f | .35 | 0.89 | 22 | 6.3 | 71 | 100 | >1000 | >1000 | >1000 | 790 | >1000 | >1000 | |
| 1j | 0.022 | 0.40 | 10 | 0.32 | 5.0 | 160 | 560 | >1000 | >1000 | 71 | >1000 | >1000 | 9 |
| 1m | 0.63 | 0.71 | 79 | 13 | 180 | 450 | 450 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 1n | 1.6 | 1.8 | 25 | 20 | 50 | 56 | 50 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 1p | *200* | *250* | *>300* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | |
| 1q | *>300* | *>300* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | |
| 2a | 0.009 | 0.20 | 0.50 | 0.071 | 0.71 | 18 | 110 | 400 | 500 | 5.0 | 790 | 790 | 1a,4 |
| 2b | 40 | *100* | *350* | *100* | *500* | *>300* | *>300* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1a |
| 2c | 1.8 | 5.6 | 18 | 22 | 89 | 130 | 160 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 2e | 0.35 | 1.3 | 25 | 5.6 | 50 | 560 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 2f | 0.50 | 1.35 | 16 | 5.0 | 45 | 25 | 350 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 2g | 1.4 | 4.0 | 1.4 | 13 | 50 | 16 | 71 | >1000 | >1000 | 100 | >1000 | >1000 | 1a |
| 2h | 0.032 | 0.50 | 5.0 | 0.22 | 5.0 | 56 | 320 | >300 | >300 | 100 | >300 | >300 | |
| 2i | 32 | 79 | 100 | 140 | 350 | 350 | 400 | >1000 | >1000 | 500 | >1000 | >1000 | 1d |
| 2j | 0.050 | 0.63 | 7.1 | 0.56 | 5.6 | 100 | 250 | >1000 | >1000 | 130 | >1000 | >1000 | 1d |
| 2k[c] | 0.32 | 4.5 | 25 | 2.8 | 35 | >300 | >300 | >300 | >300 | >300 | >300 | >300 | 1d |
| 2l | 5.0 | 79 | >100 | 63 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 1d |
| 2m | 1.1 | 1.6 | 50 | 14 | 160 | 200 | 350 | >1000 | >1000 | >1000 | >1000 | >1000 | 1d |
| 2n | 4.0 | 2.5 | 13 | 16 | 35 | 25 | 32 | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | |
| 2o | 40 | 40 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 1d |
| 3a | 50 | 110 | 50 | 79 | 140 | 130 | 140 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a,4 |
| 3c | 2.0 | 20 | 25 | 16 | 50 | 50 | 50 | >300 | >300 | >300 | >300 | >300 | |
| 3d | 13 | 40 | 16 | 25 | 35 | 35 | 35 | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | 1a |
| 3e | 400 | 500 | 500 | 500 | 790 | 630 | 630 | >1000 | >1000 | >1000 | >1000 | >1000 | 1a |
| 3i | 79 | 140 | 71 | 140 | 160 | 160 | 160 | >300 | >300 | >300 | >300 | >300 | 1a |
| 3j | 140 | 220 | 140 | 160 | 250 | 160 | 220 | >300 | >300 | >300 | >300 | >300 | 1a |
| 3u | *320* | *500* | *500* | *500* | *500* | *500* | *500* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | 1a |
| 6a | 160 | 160 | 160 | 250 | 350 | 280 | 280 | >1000 | >1000 | >1000 | >1000 | >1000 | |
| 6n | 220 | 500 | 500 | 450 | 500 | 500 | 500 | >1000 | >1000 | >1000 | >1000 | >1000 | |
| 7a | 18 | 180 | 160 | 71 | 450 | 450 | 450 | >1000 | >1000 | >1000 | >1000 | >1000 | |
| 8a | 5.6 | 3.2 | 7.9 | 16 | 16 | 16 | 16 | >1000 | >1000 | >1000 | >1000 | >1000 | |
| 9a | 4.0 | 5.9 | 5.4 | 24 | *33* | *38* | *54* | *>300* | *>300* | *>300* | *>300* | *>300* | 1a |
| 9b | *>300* | *>300* | *500* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | |
| 10a | 1.5 | 8.5 | *140* | *45* | *56* | *56* | *63* | *>300* | *>300* | *>300* | *>300* | *>300* | 1a |
| 10b | *200* | *200* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1a |
| 10c | *100* | *160* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>300* | *>300* | 1a |
| 10d | *320* | *100* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | 1a |

| Compd. | *Corynebacterium xerosis* NCTC 9755 | *Sarcina lutea* PDA 1001 | *Bacillus subtilis* ATCC 6633 | *Staphylococcus aureus*[b] I | *Staphylococcus aureus*[b] II | *Staphylococcus aureus*[b] III | *Staphylococcus aureus*[b] IV | *Salmonella typhosa* NCTC 5760 | *Klebsiella pneumoniae* ATCC 4208 | *Klebsiella pneumoniae* ATCK 7380 | *Escherichia coli* Leo11A2 | *Pseudomonas aeruginosa* ATCC 7700 | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10e | *160* | *300* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | |
| 10f | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | *>300* | 1d |
| 11a | *63* | *160* | *160* | *630* | *500* | *630* | *500* | *>300* | *>300* | *>300* | *>300* | *>300* | 1a |
| 11b | *130* | *130* | *1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1a |
| 11c | *160* | *>300* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>300* | *>300* | |
| 12a | *130* | *63* | *>1000* | *500* | *500* | *500* | *630* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1d |
| 13a | *100* | *100* | *500* | *320* | *400* | *400* | *500* | *>300* | *>300* | *>300* | *>300* | *>300* | |
| 14a | *100* | *100* | *500* | *630* | *630* | *630* | *790* | *>300* | *>300* | *>300* | *>300* | *>300* | 1d |
| 14b | *250* | *200* | *>1000* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>300* | *>300* | 1d |
| 15a | *40* | *100* | *320* | *>1000* | *>1000* | *>1000* | *>1000* | *>300* | *>300* | *>300* | *>1000* | *>300* | 1d |
| 16a | 130 | 200 | 1000 | 1000 | 1000 | >1000 | >1000 | >300 | >300 | >300 | >1000 | >300 | 1d |

[a] Figures in italics were read after seeding on agar plates. [b] Strain I is a typical fusidic acid sensitive clinical strain (phage type 3A). II, III, and IV are variants of I representing decreasing levels of sensitivity. [c] H. S. Burton and E. P. Abraham, *Biochem. J.*, **50**, 168 (1951). [d] E. Chain, H. W. Florey, M. A. Jennings, and T. I. Williams, *Brit. J. Expll. Pathol.*, **24**, 108 (1943). [e] This product is contaminated with about 15% of **2j** which could not be removed by recrystallization or chromatography. Bioautography of paper chromatograms of the mixture using *Corynebacterium xerosis* as test organism revealed that this contamination is responsible for practically all of the activity.

far less pronounced, and 16$\alpha$-acyloxy derivatives, such as **2o**, are readily obtained on acylation with acid anhydrides in pyridine.

A remarkable property of the lactones **9** and **10** is that the $\Delta^{17(20)}$ double bond is reducible with sodium borohydride in contrast to the $\Delta^{17(20)}$ double bond in fusidic acid derivatives containing a free or esterified 21-carboxyl group. The two tetrahydrolactones **11a** and **15a**, obtained on catalytic reduction of the dihydrolactones **10a** and **14a**, respectively, are readily epimerized at C-20 with the formation of **12a** and **16a** on treatment with base.[1d]

The carboxyl group in fusidic acid and its derivatives is not very reactive, but a series of methyl esters (**1b**, **2b**, **3b**, **6b**, and **7b**) was readily obtained on esterification with ethereal diazomethane. The methyl ester of fusidic acid (**1b**) does not react with amines or hydrazine under ordinary conditions but could be reduced to the tetrol **1p** with lithium aluminum hydride. On acetylation this compound was converted into its 3,16,21-triacetate **1q**.

**Antibacterial Activity.**—Table I states the antibacterial activities of 51 derivatives of fusidic acid in addition to those of fusidic acid and the related antibiotics helvolic acid and cephalosporin $P_1$[10,11] against a number of gram-positive and gram-negative bacteria.

The activities were determined by the serial-dilution method in nutrient broth seeded with approximately $10^4$ organisms/ml. The results were read after 20 hr. of incubation at 36° and are expressed as the concentrations in micrograms per milliliter required for 50% inhibition of growth ($IC_{50}$). Derivatives containing a free carboxyl group were added as aqueous solutions of their triethylamine salts, whereas neutral compounds were added as 2% solutions in dioxane. Where clear solutions could not be obtained, the $IC_{50}$ values were determined after seeding on agar plates from the tubes. The indicated values are geometric means of at least two determinations performed at different dates. Prior to the testing the purity of all derivatives was checked by thin layer and paper chromatography.

## Discussion

It will appear from Table I that none of the derivatives of fusidic acid described in this work exhibit an antibacterial activity greater than that of fusidic acid, and that only one derivative, 24,25-dihydrofusidic acid (**2a**), is as active as fusidic acid itself. All other derivatives show a somewhat or much lower activity, and many of them are almost completely inactive.

The part of the molecule least sensitive to structural modifications seems to be the side chain. Thus, reduction of the $\Delta^{24}$ double bond generally leads to compounds with activities similar to those of the parent compounds, and it cannot be ruled out that it is possible to synthesize compounds with other side chains,

(10) Structure **17** for helvolic acid was proposed by Professor S. Okuda at the Symposium on the Chemistry of Natural Products, Nagoya, Oct. 1964; *cf.* Abstracts of Papers, p. 192. The structure of cephalosporin $P_1$ is believed to be as shown in formula **18** (private communication from Professor Sir Ewart R. H. Jones).

(11) The authors are indebted to Professor S. Okuda, Institute of Applied Microbiology, the University of Tokyo, for a sample of helvolic acid and to Dr. A. H. Campbell, Glaxo Research Ltd., England, for a sample of cephalosporin $P_1$.

which would be even more active than fusidic acid. In this connection it is worth mentioning that the 24,25-dihydro derivative of the related antibiotic cephalosporin $P_1$ (which has the same side chain as fusidic acid but a different substitution pattern of the ring system)[10] exhibits a similar antibacterial activity as cephalosporin $P_1$ itself, whereas its tetrahydro derivative is inactive.[12]

The low activity shown by the two tetrahydrofusidic acids **3a** and **8a** as well as by lumifusidic acid (**7a**) and its 24,25-dihydro derivative **6a** demonstrates the importance of the presence and correct geometry of the $\Delta^{17(20)}$ double bond. It should, however, be noted that with the exception of the tetrahydrolactones **15** and **16**, tetrahydro derivatives with a $\beta$-orientated side chain have not been obtained so far. It is remarkable that in the lumifusidic acid series the tetrahydro derivative **8a** is the most active one and is considerably more active than the C-20 epimeric tetrahydrofusidic acid (**3a**).

It will be seen that acetylation or epimerization of the hydroxyl group at C-3 in **1a** and **2a** is accompanied by a considerable loss of activity, whereas **1j** and **2j**, in which this group has been replaced by a keto group, retain a substantial part of the original activity. The reason for the relatively high activities of these derivatives may well be that the microorganisms dispose of oxidoreductases capable of reducing the keto group to an $\alpha$-orientated hydroxyl group. In this connection it should be noted that the antibiotics cephalosporin $P_1$ and helvolic acid contain an $\alpha$-orientated hydroxyl group and a keto group, respectively, at C-3.

Conversion of the 11-hydroxyl group in **2a** to a keto group (**2h**) causes likewise a moderate decrease of the activity, and when both hydroxyl groups are oxidized to keto groups, as in **1e** and **2e**, the activity drops to about 1% of the original values.

The 16-acetoxyl group seems to be as important as the hydroxyl groups. Hydrolysis, with or without inversion, yields compounds with a much reduced activity (**1m** and **1n**), and that epimerization of this acetoxyl group is accompanied by a substantial loss of activity is seen by comparing the activities of **2j** and **2o**.

The importance of a free carboxylic group appears from the almost complete lack of activity shown by the derivatives in which this group has been modified in some way (**1b**, **1p**, **1q**, and **2b**).

Among the lactones listed in Table I, only the simple 16-deacetylfusidic acid lactone (**9a**) and its 24,25-dihydro derivative **10a** show reasonable activities. Here again, epimerization at C-16 involves loss of activity (compare **10a** and **14a**).

The antimicrobial spectra of compounds of the fusidic acid series (**1a–n**) and the 24,25-dihydrofusidic acid series (**2a–n**) are qualitatively similar, and it will be noted that all of these derivatives show a lower activity against the fusidic acid resistant strains of *Staphylococcus aureus* than against the normal fusidic acid sensitive strain. This fact, which also is true for the structurally related antibiotics cephalosporin $P_1$ and helvolic acid, points to the same or a similar mechanism of action.[13]

In contrast, tetrahydro derivatives such as **3a** and **8a** as well as the lactones **9a** and **10a** are approximately as active against the fusidic acid resistant strains as against the fusidic acid sensitive strain, and therefore, it must be assumed that a deviating mode of action is operating for these compounds.

(12) H. S. Burton, E. P. Abraham, and H. M. E. Cardwell, *Biochem. J.*, **62**, 171 (1956).

## Experimental Section

All melting points are corrected. Optical rotations, unless otherwise stated, were measured in chloroform ($c$ 1), and ultraviolet spectra in 96% ethanol solution. The infrared spectra were obtained with a Perkin-Elmer 21 spectrophotometer with a NaCl prism. The n.m.r. spectra were taken in $CDCl_3$ using a Varian Associates spectrometer, Model A-60. The line positions are given in $\delta$ values with tetramethylsilane as internal standard. To characterize the signals the following abbreviations are used: s (singlet), d (doublet), t (triplet), m (multiplet), and b (broad, ill-defined signal). Microanalyses were performed by Mr. G. Cornali and Mr. W. Egger.

**Oxidation of Fusidic Acid (1a).**—To a cold (10–15°) solution of fusidic acid–benzene adduct[14] (1.5 g.) in acetone (distilled from permanganate) (200 ml.), Jones's reagent (3 ml.)[14] was added. After standing for 5 min., water (200 ml.) was added, and most of the acetone was removed in a rotatory evaporator. The suspension was extracted with ether, and the extract was washed with water, dried, and evaporated to dryness. The residue (1.47 g.) crystallized on treatment with ether to yield 1020 mg. of **1e**, m.p. 197–198°. Recrystallization from ether did not raise the melting point. Paper chromatograms of this product, when subjected to bioautography on agar plates inoculated with *Corynebacterium xerosis*, showed only one zone of inhibition. No traces of fusidic acid or its monoketo derivatives could be detected; $[\alpha]^{20}D$ +136°.

*Anal.* Calcd. for $C_{30}H_{44}O_6$: C, 72.62; H, 8.65. Found: C, 72.33; H, 8.59.

**3-*epi*-Fusidic Acid (1f).**—The 3-keto derivative **1j** of fusidic acid[9] (200 mg.) was dissolved in 2% aqueous $NaHCO_3$ (5 ml.). Sodium borohydride (50 mg.) was added in small portions, and the resulting solution was allowed to stand at room temperature for 20 min. On acidification with acetic acid 180 mg. of crude **1f**, m.p. 195–197°, crystallized. Several recrystallizations from acetone raised the melting point to 211–211.5°. Paper chromatograms of this product, when subjected to bioautography on agar plates inoculated with *C. xerosis*, showed only one zone of inhibition with an $R_f$ value lower than that of fusidic acid; $[\alpha]^{20}D$ +9.8° (pyridine); $\nu^{KBr}$ 1710, 1725, and 3450 cm.$^{-1}$.

*Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.89; H, 9.48.

**Reduction of Fusidic Acid Methyl Ester (1b).**—A solution of **1b** (10.0 g.) in a mixture of dry ether (100 ml.) and dry dioxane (20 ml.) was added with stirring to a solution of $LiAlH_4$ (3 g.) in ether (200 ml.). After the addition was completed (1 hr.), the mixture was refluxed for 3 hr. Excess $LiAlH_4$ was destroyed by addition of ethyl acetate, and sufficient water was thereafter added to hydrolyze the product complex with formation of a granular mass (lithium aluminate) which was removed by filtration and washed with ether. The combined filtrate and washings were washed with water, dried, and evaporated to dryness. The residue crystallized from ethyl acetate to yield 3.5 g. of **1p**, m.p. 170–175°. Several recrystallizations from the same solvent raised the melting point to 181–184°, $[\alpha]^{20}D$ −65°. The infrared spectrum had no bands in the carbonyl region.

*Anal.* Calcd. for $C_{31}H_{52}O_4$: C, 75.60; H, 10.50. Found: C, 75.50; H, 10.66.

**Acetylation of 1p to the Triacetate 1q.**—Tetrol **1p** (300 mg.) was dissolved in a mixture of acetic anhydride (2.5 ml.) and pyridine (2.5 ml.). After standing for 16 hr., water was added to precipitate an oil which was crystallized from methanol–water to afford 330 mg. of **1q**, m.p. 105–107°. Further recrystallizations

(13) The mode of action of fusidic acid has been investigated by C. L. Harvey, C. J. Sih, and S. G. Knight (Bacteriological Proceedings, American Society for Microbiology, Atlantic City, N. J., April 1965).

(14) This reagent was prepared according to Djerassi, *et al.*,[15] by dissolving chromium(VI) oxide (26.72 g.) in concentrated $H_2SO_4$ (23 ml.) and adding water to a volume of 100 ml.

(15) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

from methanol–water raised the melting point to 108–109°, $[\alpha]^{20}D$ −11.5°.

*Anal.* Calcd. for $C_{35}H_{54}O_7$: C, 71.64; H, 9.28. Found: C, 71.50; H, 9.13.

**Hydrogenation of the 3,11-Diketo Derivative 2e.**—A solution of **2e** (3.09 g.) in acetic acid (30 ml.) containing 6 drops of concentrated HCl was hydrogenated in the presence of platinum oxide (50 mg.). When 170 cc. of hydrogen was consumed (160 min.), the hydrogenation was stopped, and the catalyst was removed. The filtrate was evaporated *in vacuo*, and the residue was dissolved in ether. The ethereal solution was repeatedly washed with water, dried, and evaporated to yield 2.80 g. of an amorphous residue which could not be crystallized. Paper chromatography revealed that this product was contaminated with traces of starting material and 24,25-dihydrofusidic acid (**2a**). It was purified by chromatography on Florisil. Elution with benzene–ethanol (94:6) gave fractions which, according to paper and thin layer chromatography, contained **2h** only. A crystalline product could not be obtained; $[\alpha]^{20}D$ +59°.

*Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.35; H, 9.52.

**24,25-Dihydrofusidic Acid (2a).**—To a solution of **2h** (280 mg.) in methanol (3 ml.) 3% aqueous sodium bicarbonate (6 ml.) followed by $NaBH_4$ (100 mg.) was added. After standing for 10 min. at room temperature, the solution was acidified with dilute HCl and extracted with ether. The extract was repeatedly washed with water, dried, and evaporated *in vacuo*. The residue crystallized from ether to afford 220 mg. of a product with m.p. 172–175°. Recrystallization from ether raised the melting point to 180–182°, alone or mixed with an authentic sample of **2a**. The infrared spectra were identical.

**16-Deacetyl-24,25-dihydrofusidic Acid (2n).**—24,25-Dihydrofusidic acid[1a] (2.0 g.) was dissolved in a mixture of methanol (25 ml.) and 33% aqueous NaOH (5 ml.), and the resulting solution was refluxed for 30 min. After cooling, water (50 ml.) was added to precipitate the sodium salt of **2n** which is sparingly soluble in water. The salt was filtered off, washed with water, and suspended in a mixture of water (50 ml.) and ether (50 ml.). Dilute HCl was added drop by drop to the stirred suspension until two clear phases resulted. The ethereal phase was separated, washed with water, dried, and evaporated to dryness. The residue crystallized from ether–hexane to yield 1480 mg. of **2n**, m.p. 139–139.5°. Recrystallization from the same solvents did not raise the melting point; $[\alpha]^{20}D$ −7.6°.

*Anal.* Calcd. for $C_{29}H_{48}O_5 \cdot H_2O$: C, 70.41; H, 10.19. Found: C, 70.58; H, 10.31.

**Tetrahydrofusidic Acid 3-Acetate (3c).**—Tetrahydrofusidic acid (**3a**) (300 mg.) was dissolved in a mixture of acetic anhydride (2.5 ml.) and pyridine (2.5 ml.). After standing for 16 hr., water was added to precipitate an oil which was extracted with ether. The extract was repeatedly washed with water, dried, and evaporated *in vacuo* to a foam which failed to crystallize; $[\alpha]^{20}D$ −36°.

*Anal.* Calcd. for $C_{33}H_{54}O_7$: C, 70.43; H, 9.67. Found: C, 70.19; H, 9.72.

**Lumifusidic Acid (7a).**—A solution of fusidic acid–methanol adduct (5 g.) in dry, peroxide-free dioxane (50 ml.) was irradiated in a quartz tube with ultraviolet light from a medium-pressure mercury lamp (Hanovia, Type 509) at 15°. During the irradiation, samples were subjected to paper chromatography [solvent system heptane–benzene–methanol–water (5:5:7:3), spray reagent a saturated solution of $SbCl_3$ in $CHCl_3$] which revealed that fusidic acid ($R_f$ 0.40) was partly converted into a more polar compound, lumifusidic acid ($R_f$ 0.18), in addition to other, even more polar compounds. Optimal conversion into lumifusidic acid was achieved after irradiation for about 18 hr. The solvent was removed *in vacuo*, and the residue was dissolved in benzene, which again was removed *in vacuo*. This was repeated until all traces of dioxane were removed. Finally, the residue was dissolved in benzene (25 ml.), and the solution was kept in the refrigerator overnight. Most of the unreacted fusidic acid separated as its benzene adduct which was removed by filtration (1.5 g.). The filtrate was evaporated *in vacuo* to leave 3.5 g. of an amorphous residue which was chromatographed on Florisil (150 g.). Elution with benzene–ethanol (90:10) gave fractions which, according to paper chromatography, contained lumifusidic acid only. These were combined and evaporated *in vacuo*. The residue crystallized from methanol–water to afford 1.6 g. of **7a**, m.p. 115–125°. Recrystallization from the same solvents raised the melting point to 118–125°. This product is a solvated form of **7a**, and a satisfactory microanalysis could not be obtained; $[\alpha]^{20}D$ −63°, $\lambda_{max}$ 204 mμ (ε 14,700), $\nu^{KBr}$ 1665 and 1725 cm.$^{-1}$.

**Lumifusidic Acid Methyl Ester (7b).**—Lumifusidic acid (1.0 g.) was esterified with ethereal diazomethane in the usual way. The residue crystallized from ether–hexane to yield 600 mg. of crystals, m.p. 148–150°. Recrystallization from the same solvents raised the melting point to 150–150.5°; $[\alpha]^{20}D$ −33°; n.m.r., δ 5.65 (d, CH-16), 5.05 (b, CH-24), 4.31 (b, CH-11), 3.72 (s, $COOCH_3$), 3.65 (b, CH-3), and 2.03 (s, $OCOCH_3$).

*Anal.* Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.39; H, 9.50.

**24,25-Dihydrolumifusidic Acid (6a). A. Hydrogenation of Lumifusidic Acid (7a).**—A solution of lumifusidic acid (5.1 g.) in 96% ethanol (40 ml.) was hydrogenated under 1 atm. of hydrogen in the presence of 5% palladium on calcium carbonate (0.5 g.). In 3 hr. 250 cc. of hydrogen was absorbed, and the consumption ceased. The catalyst was removed, and the filtrate precipitated with water to yield 4.5 g. of **6a**, m.p. 141–144°. Recrystallization from ethanol–water did not raise the melting point; $[\alpha]^{20}D$ −66.5°, $\lambda_{max}$ 204 mμ (ε 10,100).

*Anal.* Calcd. for $C_{31}H_{50}O_6 \cdot 0.5H_2O$: C, 70.55; H, 9.74. Found: C, 70.65; H, 9.61.

**B. Irradiation of 24,25-Dihydrofusidic Acid (2a).**—A solution of **2a** (1.0 g.) in dry, peroxide-free dioxane (10 ml.) was irradiated in a quartz tube with ultraviolet light from a medium-pressure mercury lamp (Hanovia, Type 509) at 15° for 24 hr. The mixture which, according to paper chromatography [solvent system heptane–benzene–methanol–water (5:5:7:3), spray reagent $SbCl_3$-saturated $CHCl_3$], contained **6a** and unreacted **2a**, in addition to other minor components, was evaporated *in vacuo*, and the residue was chromatographed on Florisil. Elution with benzene–ethanol (90:10) gave fractions which, according to paper chromatography, contained **6a** only. These were combined and evaporated *in vacuo*. The residue crystallized from ethanol–water to yield 220 mg. of crystals, m.p. 140–143°, alone or mixed with a sample of **6a** prepared as described under A above. The infrared spectra were identical.

**24,25-Dihydrolumifusidic Acid Methyl Ester (6b).**—To a solution of **6a** (500 mg.) in ether (5 ml.) an ethereal solution of $CH_2N_2$ was added until the yellow color persisted. The solvent was removed *in vacuo*, and the residue crystallized from ether–hexane to yield 325 mg. of **6b**, m.p. 146–146.5°. Recrystallization from the same solvents raised the melting point to 147–147.5°; $[\alpha]^{20}D$ −31°; n.m.r., δ 5.65 (d, CH-16), 4.28 (b, CH-11), 3.71 (s, $COOCH_3$), 3.65 (b, CH-3), and 2.03 (s, $OCOCH_3$).

*Anal.* Calcd. for $C_{32}H_{52}O_6$: C, 72.14; H, 9.84. Found: C, 72.19; H, 9.85.

**Ozonolysis of 6b.**—Ozonized oxygen was bubbled through a solution of **6b** (4.0 g.) in dry methylene chloride (40 ml.) containing dry pyridine (0.4 ml.) at −78° in 7 hr. Zinc dust (2.0 g.) and acetic acid (4 ml.) were added, and after stirring for 2 hr. at 25°, the precipitate was filtered off and washed with $CH_2Cl_2$. 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 was extracted with hexane. The extract was concentrated to a colorless oil which on distillation *in vacuo* afforded 170 mg. of a liquid, b.p. 95° (10 mm.), the infrared spectrum of which was identical with that of an authentic sample of 2-hydroxy-6-methylheptanoic acid methyl ester (**4**).[1a] The residue from the hexane extraction crystallized from ether to yield 240 mg. of colorless crystals, m.p. 190–195°. Several recrystallizations from ethanol raised the melting point to 211–212°, alone or mixed with a sample of **5** obtained on ozonolysis of 24,25-dihydrofusidic acid methyl ester.[1a] The infrared spectra of the two products were identical.

**Hydrolysis of 6a to 6n.**—A solution of **6a** (540 mg.) in a mixture of ethanol (5 ml.) and 2 *N* NaOH (10 ml.) was heated on the steam bath for 1 hr. After cooling, the mixture was acidified with dilute HCl and extracted with ether. The extract was washed with water, dried, and evaporated to dryness. The residue crystallized from ethanol–water to afford 360 mg. of colorless crystals, m.p. 200–210°. Several recrystallizations from the same solvents gave pure **6n**, m.p. 218–219.5°, $[\alpha]^{20}D$ +26° (pyridine), $\lambda_{max}$ 204 mμ (ε 10,000).

*Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found: C, 73.05; H, 10.23.

**Conversion of 7a to 1a.**—A solution of pure lumifusidic acid (1 g.) in dry, peroxide-free dioxane (10 ml.) was irradiated in a quartz tube with ultraviolet light from a medium-pressure mercury lamp (Hanovia, Type 509) at 15° for 18 hr. Paper

chromatograms of the reaction mixture, when subjected to bioautography on agar plates inoculated with *C. xerosis* showed a zone of inhibition with an $R_f$ value corresponding to that of fusidic acid. The solvent was removed *in vacuo* and the residue was dissolved in benzene (5 ml.) and seeded with fusidic acid–benzene solvate. After standing overnight, a crystalline solid was collected. The crude product (120 mg.) was recrystallized from benzene to yield 95 mg. of fusidic acid–benzene solvate identical in every respect with an authentic sample.

**Hydrogenation of 6a to 8a.**—A solution of **6a** (520 mg.) in acetic acid (5 ml.) was hydrogenated under 1 atm. of hydrogen in the presence of $PtO_2$ (50 mg.). In 3 hr. the theoretical amount of hydrogen was absorbed, and the consumption ceased. The catalyst was removed, and the filtrate was precipitated with water to yield a semicrystalline solid which was collected and dissolved in ether (20 ml.). The ethereal solution was washed with water, dried, and evaporated. The residue crystallized from ether to afford 440 mg. of colorless crystals, m.p. 180–183°. Recrystallization from acetonitrile gave pure **8a**, m.p. 184.5–185°, $[\alpha]^{23}D$ −39°. No selective absorption occurred in the ultraviolet spectrum above 200 mμ.

*Anal.* Calcd. for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07. Found: C, 71.44; H, 10.09.

**Hydrolysis of 8a to 8n.**—A solution of **8a** (350 mg.) in a mixture of ethanol (10 ml.) and 2 *N* NaOH (10 ml.) was refluxed for 5 hr. After cooling and acidification, most of the ethanol was removed under reduced pressure. Working up through ether gave a product which crystallized from methanol–water to yield 300 mg. of **8n**, m.p. 189–192°. Recrystallization from the same solvents gave 270 mg., mp. 184–186°, $[\alpha]^{23}D$ −66°.

*Anal.* Calcd. for $C_{29}H_{50}O_5 \cdot 0.5H_2O$: C, 71.41; H, 10.54. Found: C, 71.51; H, 10.44.

**Acetylation of 8a.**—Compound **8a** (200 mg.) was dissolved in a mixture of acetic anhydride (1 ml.) and pyridine (1 ml.). After standing for 3 hr., water was added to precipitate an oil which was extracted with ether. The extract was repeatedly washed with water, dried, and evaporated to a foam which failed to crystallize: $[\alpha]^{26}D$ −38°.

*Anal.* Calcd. for $C_{33}H_{54}O_7$: C, 70.43; H, 9.67. Found: C, 70.13; H, 9.76.

**Acetylation of 8n.**—Compound **8n** (100 mg.) was acetylated as described for **8a**. The infrared spectrum (KBr) of the product was identical with that of the compound obtained on acetylation of **8a**.

**Acetylation of 9a to 9b.**—16-Deacetylfusidic acid lactone (200 mg.) was dissolved in a mixture of acetic anhydride (2 ml.) and pyridine (2 ml.). After standing for 16 hr. the product was precipitated with water. The crude product was recrystallized from ether–hexane to yield crystals with m.p. 191–192°, $[\alpha]^{20}D$ +40°.

*Anal.* Calcd. for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30. Found: C, 74.51; H, 9.18.

**Acetylation of 11a to 11c.**—Compound **11a** (1.0 g.) was dissolved in 5 ml. of a mixture of acetic acid (40 ml.), acetic anhydride (20 ml.), and *p*-toluenesulfonic acid (10 g.). After standing for 23 min., water was added to precipitate an amorphous solid which crystallized from methanol–water to yield 900 mg. of **11c**, m.p. 202–203°. Recrystallization from methanol raised the melting point to 203–204°, $[\alpha]^{25}D$ −24°.

*Anal.* Calcd. for $C_{35}H_{52}O_6$: C, 72.75; H, 9.62. Found: C, 72.97; H, 9.51.

**Sodium Borohydride Reduction of 9a to 13a.**—To a solution of compound **9a** (200 mg.) in methanol (10 ml.) 5% aqueous $NaBH_4$ (1 ml.) was added. After standing for 30 min., the solution was acidified with acetic acid and precipitated with water to yield 150 mg. of crude **13a**, m.p. 168–171°. Two recrystallizations from ether–hexane raised the melting point to 174–176° (sintering at 136–138°); $[\alpha]^{26}D$ −42°; $\lambda_{max}^{MeOH}$ 203 mμ (ε 5200); $\nu^{KBr}$ 1580 (w), 1655 (w), and 1750 (s) cm.$^{-1}$.

*Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.84; H, 10.26.

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# Biologically Active Guanidines and Related Compounds. II. Some Antiinflammatory Aminoguanidines[1]

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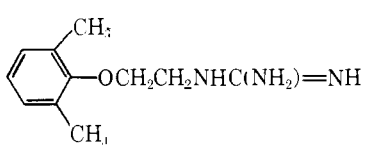
A series of N-amino-N-substituted guanidines have been synthesized and evaluated for antiinflammatory activity.

In a previous publication,[1] we discussed a series of phenoxyalkylguanidines and related compounds which exerted a potent blocking action on the sympathetic nervous system. Of particular interest was 2-(2,6-xylyloxy)ethylguanidine (I), which may be considered as combining the structural features of choline 2,6-



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xylyl ether bromide[2] and guanethidine [2-(octahydro-1-azocinyl)ethyl]guanidine sulfate.[3] Further investigations have shown that compounds in this series are also active in certain assays for antiinflammatory activity, and in an extension of this work structurally related aminoguanidines have been synthesized, some of which have proved to be active in a range of tests for antiinflammatory activity.

**Chemistry.**—1-Amino-3-substituted guanidines (III) were prepared by the reaction of 2-methyl-2-thioisosemicarbazide with phenoxyalkylamines[4] (see Scheme I). However, as it is laborious and often difficult to separate the products (III) from tetrazine derivatives[5] and other products[6] formed by the alkaline decompo-

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